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ROLE OF TESTOSTERONE IN HOST-PARASITE INTERACTION DURING MURINE EXPERIMENTAL INFECTION OF *SCHISTOSOMA JAPANICUM*

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Abstract: We analyzed roles of testosterone on susceptibility to infection during murine experimental schistosomiasis japonica. Male C57BL/6 mice infected with *Schistosoma japonicum* showed marked reduction in serum level of testosterone compared with infection-free male control mice, suggesting that *S. japonicum* infection suppressed testosterone level in host animals. Potential roles of testosterone in the susceptibility to schistosomiasis were tested by preparing mice in which testosterone levels were artificially manipulated. At 45 days after infection, we observed that male mice with reduced testosterone level showed significantly higher worm burden ($p<0.05$). The area occupied by granulomas in liver was increased in mice of low circulating testosterone level possibly due to the high worm burden. High level of testosterone also inhibited, to some extent, fecundity of the female parasites. Treatment of infected mice with an inhibitor for testosterone receptor did not alter worm burden, and this suggests that testosterone could have direct effects on the parasites.

Key Words: *Schistosoma japonicum*, testosterone, susceptibility, egg granuloma, fecundity

INTRODUCTION

It is a rather common observation in epidemiological studies that prevalence of schistosomiasis in males are higher that in females in human populations (Butterworth *et al*., 1984; Rose *et al*., 1997; Ministry of Health, China, 1998). The exact reason for such observation is not clear, however, biological as well as behavioral factors have been discussed (Chandiwana and Woolhouse, 1991). Gender-dependence in intensity of parasitic infection has been analyzed, and some of the studies demonstrated that hormonal circumstances affect both incidence and intensity of parasitic infections (Kamis and Ibahim, 1989). Male sex hormones, including testosterone (Te), are known to have regulatory roles in immune responses of vertebrate animals (Fulford *et al*., 1998). Effects of Te in susceptibility to parasitic infections are still controversial, however, accumulated results indicate a critical role(s) of Te in host-parasite interaction (Wunderlich *et al*., 1998; Benten *et al*., 1992).

In murine experimental *Schistosoma mansoni* infection, Te was reported to have a resistant function for the infection (Eloi-Santos *et al*., 1992; Nakazawa *et al*., 1997). Similarly, hormone balance might modify innate or acquired resistance to schistosome infections in humans, and some biological analysis was attempted for *S. mansoni* infection (Hagan *et al*., 1998). On the other hand, little information is still available for schistosomiasis japonica. We, therefore, extend a further study testing the situation of Te during murine experimental infection of *S. japonicum*. In this study, we measured Te level in infected host mice, and examined the effects of Te on worm burden, fecundity, and pathological lesions due to the parasite eggs. Furthermore, we also treated mice with an inhibitor for testosterone receptor during *S. japonicum* infection to test a possibility that Te has direct effects on the worms.

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MATERIALS AND METHODS

Mice and parasites: Six week old male C57BL/6 mice were purchased from Japan SLC (Hamamatsu, Japan), and were infected percutaneously with 25 cercariae of *S. japonicum* of a Chinese isolate kept in Human Institute of Parasitic Diseases, China.

Te measurement: Forty five days after the infection, blood was taken from the heart, and Te levels in sera of mice infected with or without *S. japonicum* were determined by a radio-immunoassay (RIA) kit for Te (DPC, Tokyo, Japan) in line with the manufacture's instruction.

Manipulation of Te level: Male C57BL/6 mice at 6 weeks old (SLC) were devided into four groups of which Te-levels were artificially manipulated; castration alone as a low-Te group (Group Ⅰ), injected with Te after castration as high-Te mice (Group Ⅱ), injected with flutamide (Sigma, St. Louis, USA) which is an inhibitor for Te receptor (Walker *et al.*, 1994) (Group Ⅲ), and sham operation as control-Te mice (Group Ⅳ). Castration or sham castration was performed 3 weeks before experimental infection under sodium pentobarbital anesthesia. Group Ⅱ mice received 5 mg Te propionate (Teikoku Hormone, Tokyo, Japan) daily by injecting alternately into the right and left thigh muscles beginning 2 weeks prior to infection and continuing until the mice were sacrificed. Mice of Group Ⅲ were injected with flutamide at the dose of 25 mg/kg subcutaneously in the same time-schedule as the case of Group Ⅱ. Mice were infected percutaneously with 25 cercariae of a Hunan isolate of *S. japonicum*.

Evaluation of worm burden and egg count in host mice: All mice were sacrificed at 45 days after infection with *S. japonicum*, and parasites were recovered from the portal venous system of the infected mice using a conventional perfusion technique. Adult male and female worms were counted separately by using a stereomicroscope. Same parts of liver from each group mice were resected, weighed, and then digested for 12-18 hr with 4% KOH for egg enumeration (Cheever *et al.*, 1980). The entire gut of each mouse was also digested for egg counting. Te levels of the four groups were measured by RIA at a commercial clinical laboratory (SRL, Hachioji, Japan). Because the RIA system in this case was different from that of the former description, Te levels from the two different testing were not comparable (Table 1).

Histopathological examination. The liver was dissected and immidiately fixed in 10% buffered formalin for morphometric analysis. Liver sections were embedded in paraffin and stained with hematoxylin and eosin. We assessed the size of granulomas formed around a single egg containing a mature miracidium by using a video micrometer (VM-30; Olympus, Tokyo, Japan). The average of granuloma size was determined by examining more than ten granulomas in each mice. We also evaluated the mean percentage of granulomatous area in 1mm^2 liver sections.

Statistical analysis: Results from the different groups of mice were compared one-to-one by use of two-tailed student's t-test. Regression analyses were performed by Spearman rank correlation for nonparametric regression tests and Pearson's correlation coefficient test (Neter, 1985). All results are expressed as mean±SD.

Table 1 Worm recovery and testosterone levels in C57BL/6 mice in infection of *S. japonicum*

	Mouse group			
Experiment 1	Infection(-) (N=5)	*S. japonicum*-infected (N=7)		
Te level (pg/dl)	8,413±5,134	270±114**		
Total worms	(-)	10.0±1.8		
Mature intestinal eggs/ female worm	(-)	155.6±20.4		
Experiment 2	Group Ⅰ (N=9)	Group Ⅱ (N=6)	Group Ⅲ (N=9)	Group Ⅳ (N=8)
Te level (ng/dl)	21.1±7.89**	5,366±2,147**	72.3±79.8	189±277
Total worms	14.9±3.2**	6.5±2.1	9.7±2.3	9.8±3.2
(Female worms)	(7.7±1.4**)	(2.5±1.9)	(4.2±1.2)	(4.3±2.4)
Intestinal egg count	24,398±1,499**	2,258±536*	2,593±246	3,287±612
Mature intestinal eggs/ female worm	1,018±461*	276±127	125±35.6	188±41.9
Mature intestinal eggs/ total intestinal egg (%)	32.0±0.0**	19.2±3.8	20.3±0.0**	24.3±0.0
Total liver egg count	38,606±5,800*	22,614±18,486	26,158±10,682	22,782±16,806

*$p<0.05$, and**$p<0.01$, vs. Group Ⅳ or infection-free mice.

RESULTS AND DISCUSSION

Te levels of four groups are shown in Table 1. We observed that there was marked reduction of Te level in the infected mice compared with infection-free animals (Table 1), and the difference in those Te levels was statistically significant in the Student's t-test ($p<0.01$). Under such different Te levels, numbers of recovered worms also were different according to Te level. Low-Te group had increased number of infected worms than did high- or control-Te groups ($p<0.01$) (Table 1). On the other hand, there was no significant defference in worm recovery when flutamide-treated mice were compared with sham castrated ones. Comparison of worm recovery and Te levels in 29 mice examined showed a significant inverse relationship ($r=-0.374$, $p<0.05$) (Fig. 1).

The mean numbers of intestinal eggs are shown in Table 1. Intestinal eggs in Group I were increased in number than of high- as well as control-Te groups. Furthermore, the percentage of mature eggs per total intestinal eggs in flutamide-treated mice was significantly lower than that in control group ($p<0.01$). And the mean number of mature intestinal eggs per female worm and the percentage of mature intestinal eggs in Group I was signficantly increased ($p<0.01$), suggesting the presence of an inhibitory role of Te for fecundity of female parasites and egg maturation.

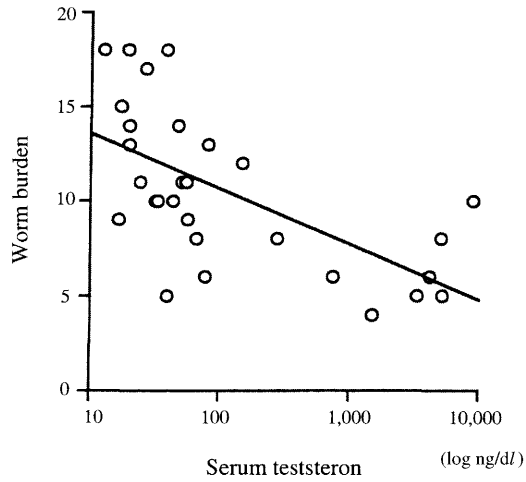

Figure 1 Correlation between serum testosterone levels and worm burdens in mice infected with 25 *S. japonicum* cercariae. Of 29 mice with *S. japonicum* infection examined, mice with lower testosterone level showed higher worm burden, and association between the two parameters was statistically significant ($r=-0.374$; $p<0.05$).

When we assessed the effect of Te for granuloma formation in liver, there was a more widespread granulomatous lesion in low-Te group than in control-group (Table 2). The mean percentege of the area occupied by granulomas was significantly different between Group Ⅰ and Group Ⅳ ($p<0.05$), although there was no significant difference in mean diameter of granulomas formed around a single egg of *S. japonicum* among any of groups tested here. Total liver eggs in Group Ⅰ were significantly increased in comparison with control Group Ⅳ mice ($p<0.05$) (Table 1). These data suggest that Te levels have modulatory effects on hepatic lesion during murine schistomiasis japonica not through the enlargement of granuloma size, but through the increase of fecundity.

It is not clear how Te level affects the susceptibility of murine hosts to *S. japonicum* infection. One of the possiblities is immunological environment being manipulated by Te. Th1 cells are supposed to have important roles in protective immunity to *S. mansoni* in mice (Sher *et al.*, 1989). In our preliminary study, we did not detect evidence that Te level was tightly associated with the serum level of cytokines of Th1 and Th2 during murine schistosomiasis japonica (data not shown). Our finding that flutamide did not change the disease profile also suggests that Te-mediated alternation in the host function can not fully explain the reduced worm burden observed in the present study, although we tested function of flutamide at only a single dose. Together with those, Te seems to have somehow direct inhibitory effects on the worm survival in host animals. It is needed to study whether schistosomes express specific receptors for Te produced by host animals.

Although previous report testing schistosomiasis mansoni concluded that egg production per a female worm did not change even in reduced Te-level (Eloi-Santos *et al.*, 1992), our present observation suggests that Te inhibits fecundity of female worm of *S. japonicum*. It might be possible that difference in the species of parasites and/or in the strain of host mice, of difference in conditions for experimental infection could provide such difference.

We have to consider about discordance of gender-dependent susceptibility to schistosomiasis between mice and humans. In humans, prevalence of schistosome infection in males is much higher than in females even when females have more frequent water-contact (Butterworth *et al.*, 1984). It is not clear whether male population has also Te−driven higher resistance than females as was observed in murine infection, and detailed epidemiological analysis considering Te levels is essential to conclude the role of Te during human schistosomiasis.

As conclusion, Te level has significant effects on susceptibility to *S. japonicum* in mice. Murine hosts of higher

Table 2 Conparisons of granuloma size and density in livers

	Mouse group			
	Group Ⅰ (N=9)	Group Ⅱ (N=6)	Group Ⅲ (N=9)	Group Ⅳ (N=8)
Percentage granulomas	40.5±9.7*	9.6±8.1	12.9±4.9	18.8±8.6
Diameters of granuloma (μm)	499.6±136.5	387.7±97.6	512.9±210.8	493.7±146.2

*$p<0.05$, vs. Group Ⅳ

Te level seem to be resistant against *S. japonicum* infection, although relatively small number of mice was tested. Results in our present study are not inconsistent with other reports testing schistosomiasis mansoni, and Te seems to be resistant factor for schistosome infection. Based on such observation, new therapeutics and prophylaxis could be developed for schistosomiasis through a Te-mediated resistance. Roles of Te in humans are still not conclusive, and further analysis is required for developing a new strategy for controlling human schistosomiasis japonica.

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REFERENCES

1) Benten, W.P., Wunderlich, F. and Mossmann, H. (1992): Testosterone-induced suppression of self healing *Plasmodium chabaudi*: an effect not mediated by androgen receptor? J. Endocrinol., 135, 407-413
2) Butterworth, A.E., Dalton, P.R., Dunne, D.W., Mugambi, M., Ouma, J.H., Richardson, B.A., Siongok, T.K. and Sturrock, R.F. (1984): Immunity after treatment of human schistosomiasis mansoni. 1. Study design, pretreatment observations and the results of treatment. Trans, Roy. Soc. Trop. Med. Hyg., 78, 108-123
3) Chandiwana, S.K. and Woolhouse, M.E. (1991): Heterogenecities in water contact patterns and the epidemiology of *Schistosoma haematobium*. Parasitol., 103, Pt3, 363-370
4) Cheever, A.W., Duvall, R.H. and Minker, R.G. (1980): Quantitative parasitological finding in rabbits infected with Japanese and Philippine strains of *S. japonicum*. Am J. Trop. Med. Hyg., 29, 1307-1315
5) Eloi-Santos, S., Olsen, N.J., Correa-Oliveira, R. and Colley, D.G. (1992): *Schistosoma mansoni*: mortality, pathophysiology, and susceptibility differences in male and female mice. Exp. Parasitol, 75, 168-175
6) Fulford, A.J.C., Webster, M., Ouma, J.H., Kimani, G. and Dunne, D.W. (1998): Puberty and age-related changes in susceptibility to schistosome infection. Parasitol. Today, 14, 23-26
7) Hagan, P., Ndhlovu, P.D. and Dunne, D.W. (1998): Schistosome immunology: more questions than answers. Parasitol. Today, 14, 407-412
8) Kamis, A.B. and Iblahim, J.B. (1989): Effects of testosterone on blood leukocytes in *Plasmodium berghei*-infected mice. Parasitol, Res., 75, 611-613
9) Nakazawa, M., Fantappie, M.R., Freeman, G.L., Eloi-Santos, S., Olsen, N.J., Kovacs, W.J., Secor, W.E. and Colley, D.G. (1997): *Schistosoma mansoni*: Susceptibility differences between male and female mice can be mediated by testosterone during early infection. Exp. Parasitol., 85, 233-240
10) Neter, J. (1985): Applied linear statistical models: Regression, analysis of variance, and experimental designs, 2nd ed., Irwin, Richard D.
11) Office of Endemic Diseases Control, Ministry of Health Expert Advisory Committee on Schistosomiasis, Ministry of Health, China (1998): A nation-wide sampling survey on schistosomiasis in 1995 in China. *In* Epidemic status of schistosomiasis in China, Shanghai: Press of Chinese Journal of Schistosomiasis Control, 1-17.
12) Ross, A.G., Yuesheng, L., Sleigh, A.S., Yi, L., Williams, G. M., Wu, W.Z., Xinsong, L., Yongkang, H. and McManus, D.P. (1997): Epidemiologic features of *Schistosoma japonicum* among fishermen and other occupational groups in the Dongting Lake region (Human Province) of China. Am. J. Trop. Med. Hyg., 57, 302-308
13) Sher, A., James, S.L., Correa-Oliveria, R., Hieny, S. and Pearce, E. (1989): Schistosome vaccines: current progress and future prospects. Parasitol., 98, s61-s68
14) Wunderlich, F., Mossmann, H., Helwig, M. and Schillinger, G. (1988): Resistance to *Plasmodium chabaudi* in B10 mice: influence of the H-2 complex and testosterone. Infect. Immum., 56, 2400-2406
15) Walker, S.E., Besch-Williford, C.L. and Keisler, D.H. (1994): Accelerated deaths from systemic lupus erythematosus in NZB×NZW F1 mice treated with the testosterone-blocking drug flutamide. J. Lab. Clin. Med., 124, 401-407

Jpn. J. Trop. Med. Hyg., Vol. 29, No. 1, 2000, pp. 5-85

PROCEEDINGS OF THE 41ST ANNUAL MEETING OF JAPANESE SOCIETY OF TROPICAL MEDICINE

10-11 November 2000, Tokyo

President

Hiroshi Ohtomo
Professor, Department of Tropical Medicine,
Jikei University School of Medicine

CONTENTS

Watanabe, J. *et al.*

5 Invasive forms of protozoan parasites have a positive charge at their contact site with host cells — Akaki, M. *et al.*

6 Bacteria expressing single-chain immunotoxin inhibit malaria parasite development in mosquitoes — Yoshida, S. *et al.*

7 Expression pattern of mitochondrial Complex II (succinate-ubiquinone oxidoreductase) in the erythrocytic stage cells of *Plasmodium falciparum* — Mi-ichi, F. *et al.*

8 Effect of jasplakinollide on the growth and actin cytokeleton of *Plasmodium falciparum* — Mizuno, Y. *et al.*

9 A malaria patient infected with *Plasmodium falciparum* and *P. malariae* — Ohnishi, K. *et al.*

10 Two cases of falciparum malaria with atypical clinical course — Yoshikawa, K. *et al.*

11 A case of severe falciparum malaria with prolonged immunohemolytic anemia — Tanaka, Y. *et al.*

12 Chronic auto-immune thrombocytopenia following recovery from severe falciparum malaria — Kato, Y. and Masuda, G.

13 A case of clinically chloroquine resistant vivax malaria infection — Kasai. D. *et al.*

14 A case of falciparum malaria successfully treated with intravenous Artesunate — Yasuoka, C. *et al.*

15 A case recovering completely from severe falcipurum malaria complicated with high levels of multiple organ failure — Aizawa, M. *et al.*

16 A case of severe falciparum malaria with hyper-bilirubinemia and nephropathy — Hashizume, K. *et al.*

17 The use of the atovaquone/proguanil combination (Malarone®) — Hitani, A. *et al.*

18 Analysis of severe and complicated falciparum malaria in Japanese patients caused by delay of diagnosis and treatment — Ohtomo, H. *et al.*

19 On the WHO's Guidelines for Severe Malaria, 2000 — Kimura, M. *et al.*

20 Problems of local transport system for anti-malaria drugs — Ishiwata, K. *et al.*

21 Determination of plasma levels of prostaglandins and thromboxane in falciparum malaria patienes in Thailand with GC/MS/SIM — Nakano, Y. *et al.*

22 Detection of *Plasmodium vivax* from clinical specimens by the use of *ICT Malaria P.f/P.v* kit — Abe, K. *et al.*

23 An evaluation of *ICT Malaria P.f/P.v* in imported malaria in Japan — Ohtomo, H. *et al.*

24 A community-based malaria control program in Palawan, the Philippines — Kano, S. *et al.*

25 Changes of the spleen rate on Aneityum Island — Kaneko, A. *et al.*

26 Active case detection surveys on malaria in recent Laos, with special reference to the comparative efficacy of Giemsa staining and Dipstick method on detection of malaria infection — Kobayashi, J. *et al.*

27 Molecular epidemiological evaluation of pyrimethamine/sulfadoxine efficacy in *Plasmodium falciparum* patients from Vanuatu archiplago — Mita, T. *et al.*

28 Population genetics and epidemiology: Malaria in Vanuatu — Lum, J.K. *et al.*

29 Repetitive dosing of artemisinin and quinine against *Plasmodium falciparum in vitro*: a simulation of the *in vivo* pharmacokinetics — Bwijo, A.B. *et al.*

30 Gene analysis of pfmdr 1 in mefloquine-resistant *Plasmodium falciparum* — Nagai, Y. *et al.*

31 Mechanisms of parasiticidal activity of tetracyclines on *Plasmodium falciparum*: a possibility of the plastid as the drug target — Lin, Q.H. *et al.*

32 Antimalarial activiyty and potent enhancement of the sensitivity of *Plasmodium falciparum* to chloroquine by the bisbenzylisoquinoline alkaloid cepharanthin — Haruki, K. *et al.*

33 The development of new antimalarial drugs- *in vitro* and *in vivo* antimalarial activity of endoperoxides — Ono, K. *et al.*

34 A potent antimalarial activity of hot-water extract of *Hydrangea macrophylla* var. *otaksa* leaves against *Plasmodium yoelii* 17XL in ICR mice — Ishih, A. *et al.*

35 Fractionation of antimalarial principle from *Hydrangea macrophylla* var. *otaksa* leaves and its activity against *Plasmodium yoelii* 17XL in ICR mice — Takezoe, H. *et al.*

36 A potent antimalarial activity of hot-water extracts of plants belonging to the family *Saxiragaceae* against *Plasmodium yoelii* 17XL in ICR mice — Sakai, M. *et al.*

37 An antimalarial activity of hot-water extract of *Dichroa febrifuga* leaves or roots against *Plasmodium yoelii* 17XL in ICR mice — Fujii, K. *et al.*

B-1 Three cases of creeping disease due to larval hookworm infection — Nakamura-Uchiyama, F. *et al.*

2 A case of urinary schistosomiasis with macrohematuria — Miki, K. *et al.*

3 Four imported cystic echinococcosis cases in Japan confirmed serologically — Ito, A. *et al.*
4 A severe case of venomous ophathalmia caused by spitting cobra in Central Kalahari — Nishiyama, T. and Osaki, M.
5 Diagnosis of kala-azar by ELISA using urine samples — Itoh, M. *et al.*
6 Evaluation of dot-ELISA for the immunodiagnosis of trematode infections — Araki, K. *et al.*
7 A sero-immunological studies on cysticercosis — Ichikawa, H. *et al.*
8 Usefulness of Em18-ELISA in monitoring effect of remedies against alveolar echinococcosis: case report on long-term chemotherapy with albendazole — Ishikawa, Y. *et al.*
9 Effect of cytochalasin D on the growth, encystation and multinucleation of *Entamoeba invadens* — Makioka, A. *et al.*
10 Inhibition of encystation of *Entamoeba invadens* by antitublin drug oryzalin — Makioka, A. *et al.*
11 Molecular cloning and characterization of peroxiredoxin from *Entamoeba moshkovskii* — Cheng, X.-J. *et al.*
12 Modifications of staining procedures for the preparations of *Entamoeba histolytica* — Kumagai, M. *et al.*
13 Epidemiological study of an epidemic amebiasis in institutions in Japan — Takeuchi, T. *et al.*
14 Congenital *Toxoplasma* infection at placenta resulting in intrauterin growth retardation — Yano, A. *et al.*
15 Intractable recurrent toxoplasmic retinochoroiditis — Norose, K. *et al.*
16 Four cases of cryptosporidiosis — Shiota, T. and Arizono, N.
17 Sero-epidemiological survey of cryptosporidiosis at Sumbawa Island, Indonesia — Yanagi, T. *et al.*
18 Genetic characterization of *Cryptosporidium parvum* isolates from 14 cattle and 22 patients with diarrhea — Yagita, K. *et al.*
19 Complex II (succinate-ubiquinone reductase/quinol-fumarate reductase) of *Trypanosoma cruzi* mitochondria — Takashima, E. *et al.*
20 Characterization of cyanide insensitive oxidase of *Trypanosoma brucei brucei* expressed in *Escherichia coli* — Kawai, K. *et al.*
21 Expression and properties of dihydroorotate dehydrogenase in *Trypanosoma cruzi* — Nara, T. *et al.*
22 In BALB/c mice, immune responses and the course of *Leishmania major* infection were controlled by cytokine expression plasmids delivery with the gene gun — Li, Y. *et al.*
23 Th2 cytokines play different roles during the course of infection with *Leishmania major* — Nashed, B.F. *et al.*
24 The effects of meglumine antimoniate (Clucantime®) against *L. major* and *L. amazonesis* co-cultured with macrophages — Kasem, K.M.A. *et al.*
25 Leishmaniasis in Equador, with special reference to mucocutaneous forms and man-biting sand flies, *Lutzomyia* spp. in the Amazonian endemic areas — Hashiguchi, Y. *et al.*
26 Pulmonary infection caused by *Rhodococcus equi* in HIV-infected patients: Report of six patients from northern Thailand — Watanabe, H. *et al.*
27 Influence of HIV infection against community-acquired pneumonia in adults in Uganda — Yoshimine, H. *et al.*
28 Genetic susceptibility to re-infection of *Schistosoma japonicum* in China — Kikuchi, M. *et al.*
29 A population study of the intermediate host of *Schistosoma mansoni* and the application of remote sensing — Mohamed, F.Y. *et al.*
30 A questionnaire study to quantify the human behavior at river infested with *Schistosoma mansoni* — Kisu, T. *et al.*
31 Where do *Schistosoma japonicum* come from? — Agatsuma, T. and Iwagami, M.
32 The difference of the echogenic patterns among schistosomiasis japonica and schistosomiasis mekongi? — Otake, H. *et al.*
33 Localization of type IV collagen in granuloma formation and liver fibrosis due to schistosomiasis japonica — Yoshizaki, M. *et al.*
34 Influence of ultraviolet rays on infection of *Schistosoma mansoni* — Ohwatari, N. *et al.*
35 Usefulness and limitation of COPT in low endemic area of schistosomiasis — Ohmae, H. *et al.*
36 Urine cytology in an endemic area of schistosomiasis haematobia in Kenya — Ohki, T. *et al.*
C-1 Hepatic penicilliosis marneffei in Northern Thailand — Toriyama, K. *et al.*
2 A unique drug-susceptibility pattern of *Vibrio cholerae* Ol in Laos — Iwanaga, M. *et al.*
3 Two infant cases infected with typhoid fever in the Philippines — Ozaki, A. *et al.*
4 Two cases of melioidosis in Japan — Kunishima, H. *et al.*
5 Measles serosurveillance study during mass immunization campaign in Malawi: antibody prevalence and serological responses using particle agglutination method — Takechi, M. *et al.*

Prize Winner's lecture

JSTM (Japanese Society of Tropical Medicine)
Young Investigator Award

A SINGLE-CHAIN ANTIBODY FRAGMENT SPECIFIC FOR THE *PLASMODIUM BERGHEI* OOKINETE PROTEIN PBS21 CONFERS TRANSMISSION-BLOKADE IN THE MOSQUITO MIDGUT

SHIGETO YOSHIDA

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Pbs21 is a protein expressed on the surface of macrogamete, zygote, ookinete and oocyst stages of the rodent malaria parasite, *Plasmodium berghei,* as they develop in/on the mosquito midgut. It has been reported that anti-Pbs 21 mAbs effectively block the development of *P. berghei* ookinetes *in vitro* and oocysts in *Anopheles stephensi* mosquitoes. The antigen has thus been identified as an important target in transmission-blocking immunity and is a model for development of transmission-blocking vaccines. Ideally, transmission-blocking vaccines should induce high titre, long-lasting transmission-blocking antibodies after single immunization. However, since the vaccine candidate antigens of Pfs25/28 family are expressed by parasites in the mosquito stage but not in vertebrate host, boosting of the immune response following a natural infection has never been expected. Nonetheless the induction of transmission-blocking antibodies in patiences in combination with anti-malarial drugs could be of considerable importance to prevent the spread of the drug-resistant parasites.

To examine the properties and potential uses of a single-chain antibody fragment (scFv) for blocking transmission of malaria parasites to the mosquitoes, we have cloned and sequenced the genes encoding variable regions of the immunoglobulin heavy and light chains (V_H and V_L) of mAb 13.1. The V_H and V_L genes were assembled as an scFv gene, and expressed in a baculovirus expression system. Following purification of 13.1 scFv, Western blotting and inhibition ELISA assays confirmed that 13.1 scFv retained the binding specificity of the parent mAb 13.1 for Pbs21. Furthermore, 13.1 scFv bound to the surface of *P. berghei* ookinete, and blocked oocyst development in mosquito midgut by at least 93%, as assessed by oocyst counts in mosquitoes. We suggest that the 13.1 scFv gene could be useful not only in studying for the mechanism of transmission-blockade but also in generating, by mosquito germline transformation, a model system to evaluate the production of mosquitoes refractory to malaria.

President's lecture

RECENT ADVANCE OF SUPPRESSIVE TREATMENT AGAINST FALCIPURUM MALARIA IN JAPAN

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The treatment of malaria is chemotherapy that acts specific for the stage of parasite and results in improvement of symptoms with suppression of fever. The treatment of falcipalum malaria has been changing by appearance of drug-resistant strains of this parasite and supply of orphan drugs from the study group supported by Japanese Ministry of Public Health and Welfare. Although many kinds of anti-malarial drug has been developed for the treatment of drug-resistant falciparum malaria, only two drugs obtained approval for general use from Japanese government, namely quinine powder as a classic anti-malaria drug and Fansidar registered in 1987. Under these situations, the study group has supported the therapy of imported malaria by some orphan drugs. During 1970's to early 1980's the period when imported malaria started to be paid attention in Japan, single or combined administration of MP tablet, quinine and chloroquine were frequently used.

Fansidar had been distributed from 1980 by the study group and had been made a main choice for the treament. Mefloquine has been imported by the study group since 1991. The frequency of choice of this drug has been gradually increasing and reached to 77% of falcipalum malaria cases in 1999. Tetracycline or artemisinin and its derivatives are used as a drug for combined administration with mefloquine. Recently, severe falcipalum malaria has been frequently found. 29 to 37% of these patients during 1995 to 1999 were treated with slow intravenous infusion of quinine dihydrochloride or quinine gluconate contributing effectively for their life saving. Moreover, severe falcipalum malaria has been also treated with combined administration with quinine and artemisinin or its derivatives from 1999, or Malarone (atvaquon+proguanil), new drug having been distributed by the study group from 1998.

Special lecture

1 PRINCIPLES AND PRACTICE OF TRAVEL MEDICINE: EXPERIENCES OF A MAJOR TRAVEL CLINIC IN GERMANY

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What is Travel Medicine?

Travel Medicine it not Tropical Medicine: Tropical Medicine is specialised for health risks to the indigenous population and for infectious diseases. Travel Medicine is specialised for health risks to our population travelling to developing world.

Travel Medicine means: development of expertise; new discipline; diverse background of practitioners; subject not taught in medical schools; no formal training. There are different possible backgrounds: Preventive Medicine/Public Health (global impact of travel-impact of travel on host countries), for infectious diseases (tropical medicine), for gastroenterology (diarrhoea-hepatitis), for internal medicine/ family medicine and for surgery (accidents-infected wounds).

The Practice of Travel Medicine crosses many disciplines: For example, with endocrinology/internal Medicine (insulin dosing in patient travelling Rome-Hongkong), with obstetrics/gyn (pregnancy and travel), with pediatrics (children/infants and travel), and with internal medicine (chronic diseases and travel).

Problems in Travel Medicine

Travel Medicine meets some problems: There are difficulties due to the host countries, with climate and infrastructure, and with different culture too. For new travel styles: last minute/last second booking, adventure travel, sports activities (climbing, diving). Travel industry is a problem too, because of negligence and ignorance. In the medical profession, we can also find as problems ignorance, contradictory information and excessive restriction of vaccinations. Problems are due to the traveller too: lack of information, refusal of recommendations. As obstacle for preventive therapy, there is resistance of pathogens, costs, rumours and media reports. For new destinations: lack of information and exotic, high risk areas.

Role of the Travel Medicine Clinic

The travel clinic has to address all health needs of the international traveler:

- -avoidance of enteric infection, mosquito/insect borne diseases and vaccine preventable illness
- -avoidance of blood borne diseases such as hepatitis B and HIV
- -protection from sun
- -coping with jet lag
- -provision and documentation of all necessary vaccinations
- -supply or prescription of appropriate medications (e.g. antimalarials, antidiarrheals, antibiotics)
- -instructions for the self treatment
- -specific health problems of individual traveller
- -provision of referral information (e.g. dialysis in India)

Need for Specialisation:

Travel Medicine is a dynamic discipline

- -rapidly changing information
- -need for surveillance
- -diseases/epidemic/vaccination requirement changes
- -need to develop data base on health risks in order to be able for individual risk assessment
- -monitor governmental and regulatory agencies (MOH, WHO)
- -medical literature/speciality journals/computer based services
- -network of travel medicine specialist and conferences

How to establish and run a travel clinic?

- -Examples from Munich

2 CHEMOTHERAPY OF MALARIA IN SOUTH EAST ASIA

TRAN TINH HIEN, M.D.

Center for Tropical Diseases Ho Chi Minh City, Viet Nam

Malaria is still the most common infectious cause of mortality and morbidity in Viet Nam as it is in other South East Asian countries. The presence of resistance to available antimalarials and compliance in the target population are factors that influence the choice of drugs and regimens. The loss of antimalarial drugs to resistance may be one of the greatest threats to the control of malaria in the tropic countries.

Several clinical trials have been conducted to develop an optimal treatment for drug-resistant falciparum malaria in patients with the disease in different settings. The results of these trials suggest that a combination of artemisinin (or its derivatives) and other antimalarial drugs (mefloquine or lumefantril or piperaquine) is the most effective, safe and practical treatment for acute non-complicated malaria due to multidrug resistant *Plasmodium falciparum*. Concerning severe and complicated malaria, parenteral or rectal multidoses of artemisinin or analogues are recommended due to their rapid parasite clearance time and other possible anticytoadherence effects. The rectal administration of qinghaosu drugs in patients with disease-related nausea and/or vomiting would obviously provide a useful therapeutic advance, particularly in areas where parenteral administration is difficult. It is simple and can be done by unskilled persons. The suppository formulation can provide emergency treatment of patients at periphery until the patient can be referred to a health facility where parenteral treatment is possible. It also avoids the risk of parenteral administration such as hepatitis or HIV infection.

There are evidences that with its rapid parasite clearance, very early treatment of uncomplicated cases with oral or suppository formulation of artemisinin and derivatives, applicable at a primary health care level will help to prevent the development of complications, consequently reducing the overall malaria mortality rate.

Concerning *P. vivax* malaria, chloroquine has been effective in SEA region and its management is less complicated. However, recently, a study in Thailand has shown that *P. vivax* was more common in women having their first baby and was associated with maternal anemia and a significant reduction in birth weight which increased the risk of infant mortality and suggest that the use of antimalarial drug against *P. vivax* in pregnancy may be justified

3 A PERSPECTIVE ON THAILAND'S HIV/AIDS EPIDEMIC AND ITS PREVENTION AND CONTROL MEASURES

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Background:

The first case of full-blown AIDS was reported in Thailand in August 1984. Up-to-date, the Royal Thai Ministry of Public-Health (MoPH) has estimated, as based on sentinel surveillance, about 1.3 million Thais were infected with Human Immunodeficiency Virus-1 (HIV-1). Accumulated numbers of AIDS cases and HIV-symptoms patients in Thailand from 1985-2000 was reported as 142,027 and 55,635, respectively. Up to June 2000, the infection rate has been estimated about 329 with a death rate of about 75 per 100,000 populations.

Epidemiology:

Few years after the first full-blown AIDS case was reported in Bangkok on August 1984, HIV-1 infection has been spread among homosexual gay men or males who have sex with males, male commercial sex workers (MCSWs) and injection drug users (IDUs). During the first round of sentinel serosurveillance conducted by the Royal Thai Ministry of Public Health (MoPH) in 1989, an unexpected high prevalence rate (44%) was detected in direct female commercial sex workers (FCSWs) in Chiang Mai Province, northern part of Thailand. This finding was reported as a first wave of HIV epidemic of HIV heterosexual transmission in Thailand. Later on, it was disclosed that the "epicenter" of HIV infection was located in six upper northern provinces. Later, HIV have found their ways to spread

into their clients, mostly Thai males who have had risk-behaviors of having sex with FCSW and came to attend MoPH's STD clinics after developing STD symptoms and signs. This risk-group is called "male STD clinic users" or "male STD clinic attendees". Subsequently, the infection in male STD clinic users has been transmitted to their family, wives and/or partners. This leads to an episode of a current "wave" of the epidemic that is a "mother to child" transmission. In parallel to the sentinel serosurveillance among high risk groups which was initiated by MoPH in 1989, a routine HIV testing of pregnant women attended the MoPH's Ante-Natal-Clinics (ANCs), of blood donors from blood banks across the nation, and male military conscripts have been included in the survey.

Results obtained from the sentinel serosurveillance among high-risk groups revealed that MCSWs from three tourist provinces showed median prevalence of 10%, ranged from 9.25% to 12.4% (between 1991-1999). Infection rates among IDUs have remained extremely high -at 31% to 43% - and are still rising in Bangkok, the Central region, and the south. National HIV prevalence among direct and indirect FCSWs (between 1989-1999) were 16.7%, (range: 16% to 44.7%), and 6.6% (range: 1.6% to 10.1%), respectively. Prevalence among male STD clinic users (between 1989-1998) was 8.3% (range: 2% to 9%), with an increasing trend. Results obtained from additional HIV testing population showed that a median prevalence among pregnant women was 1.76% (range: 0.8% to 2.3%), and is elevating. HIV among blood donors was 0.44% and trend is static. HIV prevalence among male conscripts of 21-year-old was 1.6% (range: 0.5% to 4.0%), with a decreasing trend.

Molecular Epidemiology:

In the early phase of the epidemic, HIV-1 circulating in infected homosexuals and IDUs was identified as B subtype or B′subtype. At the early phase of the first "wave" of heterosexual transmission in FCSWs, HIV E subtype was identified in majority and B′subtype in minority with a current extensive molecular studies, newly isolates of subtype E from Thailand is now called "Circulating Recombinant Form 01_AE" or "CRF01_AE".

Prevention and Control:

After an explosive epidemic outbreak of HIV infection in IDUs and FCSWs was disclosed in 1989, the Thai government has responded decisively and comprehensibly launching a control program to reduce a scope of epidemic through FCSWs. At the early phase of the epidemic, approaches to control the transmission was targeted on public health problem only. However, in a later phase, a societal issues related to HIV infection have been included. The control program could be discernable in four aspects. Firstly, for legislative action, announcement to include AIDS in the list of notifiable diseases was amended in Communicable Diseases Control Act. Secondly, for administrative action, the National Advisory Committee on AIDS was established in 1989. Later, it was called the National Commission fo Prevention and Control of AIDS. This Commission is chaired by the Prime Minister. The responsibilities of the Commission are to setting up a policy for national AIDS control program, to provide a recommendation about annual budget allocation for AIDS control to the government; to co -ordinate, co-operate, and to facilitate with organizations/institutions dealing with prevention and control of AIDS. Lastly, is to give advice, to provide recommendation for researchers in conducting biomedical and behavioral intervention researches submitted for the Commission to review and approval. Thirdly, for technical action, the MoPH in 1989 initiated HIV serosurveillance, which has been considered to be a more efficient effort of monitoring HIV transmission across the country. Fourthly, for implementing or practical action, "short-term" and "medium-term" action plans to control AIDS have been developed. The extensive outreach HIV health education, HIV counseling, and the promotion of 100% condom use in brothels and sex establishment have been considered to be the most effective and successful tool to slow down the epidemic.

Conclusion:

AIDS arrived in Thailand around 1984, but the national response was silent. When the infection was explosive among IDUs and FCSWs, the national response was very vigorous. The national responses that contributed to the success of the control included national leadership, political commitment, strong epidemiological surveillance, extensive outreach HIV health education and counseling, and the promotion of 100% condom use in sex establishments. This national response has shown a slow down of the epidemic.

Invited lecture

CLINICAL EXPERIENCE WITH CURDLAN SULFATE IN *PLASMODIUM FALCIPURUM* MALARIA

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Faculty of Tropical Medicine, Mahidol University, Thailand[3] and
Ajinomoto Co., Inc., Tokyo, Japan[4]

Curdlan Sulfate a sulfated 1,3,β-D-glucan (CRDS) has been shown in pre-clinical studies as being effective in inhibiting *P. falciparum in vitro*, *P. berghei in vivo* and *Babesia cannis* infection in dogs. CRDS has been shown to be synergistic with chloroquine, quinine and artesunate, to down modulate the immune response in decreasing both TNF and NO and direct nonspecific effect on cytoadherence and rosetting may be predicted as has been described previously with other sulfated polysaccharides, e.g. heparin. Thus CRDS is a potential candidate as an adjunct medication for treatment of severe/cerebral malaria. The clinical studies have been initiated in four phases to address safety, efficacy and potential interaction *in vivo* of CRDS with classical antimalarials.

Phase A -randomised, double blind, crossover, placebo controlled study (CRDS used alone) in patients with asymptomatic malaria.

Phase B -randomised, double blind, placebo controlled study in patients with mild malaria as adjunct medication to chloroquine.

Phase IIB -randomised, double blind, placebo controlled study in patients with severe malaria as adjunct medication to artesunate.

Phase C -randomised, double blind, placebo controlled study in patients with cerebral malaria as adjunct medication to artesunate.

The two arms of treatment in all studies showed similar results for all haematological, biochemical and urine analysis results. In all studies only adverse events recorded during CRDS treatment arm was an increase in APTT. This adverse effect is well documented with CRDS and can be easily monitored with the subsequent adjustment of dosing.

There is an indication of the positive effect of CRDS on cytoadherence as recorded in phase A where a slight increase in parasitaemia has been observed after CRDS treatment without subjects presenting with malaria symptoms. In phases B, IIB and C CRDS facilitates clearance of parasite and fever clearance time (e.g. severity of disease). In addition, CRDS showed no recrudescence in patients treated with chloroquine in phase B. CRDS seems beneficial in cerebral malaria in patients where there is no organ damage present (renal failure and pulmonary oedema).

In conclusion CRDS was well tolerated in all studies. CRDS seems to augment disease process along the line of the results obtained from pre-clinical studies. It seems that the group of patients, which will benefit most are sever/cerebral cases with no additional clinical complications such as renal failure and pulmonary oedema. These complications are rare in children. This group represents the majority of deaths recorded in Africa.

Symposium: Travel to tropical nations and vaccination

1 IMMUNIZATION FOR INTERNATIONAL TRAVELERS IN JAPANESE RED CROSS MEDICAL CENTER

TOMOYOSHI SONOBE[1], K. TSUCHIYA[1], JUNKO KOMATSU[2] AND MASAE ORITSU[2]

Department of Pediatric Health Care[1] and Department of Health Care[2],
Japanese Red Cross Medical Center

Our Pediatric Health Care Clinic consists of Well baby clinic, Psychological counseling and Immunization service. Immunization service is provided to all children including sick travelers and international children. Proportion of international travelers was about 2% of all visitors in these services in 1999. Age distribution less than 1 year of age, 1 to 9 years and over 9 years were 66%, 27% and 7%, respectively. Regarding Area distribution of destination, Asia, North America, Australia, Europe and Africa was 72%, 24%, 1%, 1%, 1% and 1%, respectively. Among 6,876 immunizations 461 (7%) were given to international travelers: DPT 122 (5%), DT 2 (4%), Measles 21 (3%), Rubella 25 (6%), Mumps 7 (2%), BCG 30 (24%), Polio 52 (22%), HB 140 (43%), JE 39 (5%), Varicella 11 (2%), Rabies 7 (100%), Tetanus 2 (100%), IG 3 (100%). The parenthesis is the proportion to total number. Immunizations up to 4 kinds were given simultaneously when needed. Six visitors were only for consultation without vaccination.

Adult immunization was also provided at our Health Care Clinic. Total number of immunization to international travelers from January to June 2000 was 351. Destination were as follows; Asia 56%, North America 39%, Europe 2%, South America 1%, Mid East 1%, Africa 1%. Given vaccine was as follows; HB 65, HA 59, Rabies 47, Tetanus 35, DT 35, Measles 30, Rubella 26, Mumps 25, Polio 10, JE 10, Varicella 4, IG 3, Cholera 2.

The main reason for international travel was job transfer both in adult and children. They tended to have little knowledge of immunization. Most children had enough time but most adult travelers did not have enough time before their departure.

2 DEFFERENCE IN VACCINATION SCHEDULES AND PLANS OF VACCINATION FOR TRAVELLERS

NAOHIDE TAKAYAMA

Department of Pediatrics, Tokyo Metropolitan Komagome Hospital

The vaccination schedule of Japan is not standard when seeing in the world. The characteristics of the Japanese vaccination schedule are as follows; The kinds of vaccines injected to infants and young children are fewer in kind than many other developed countries, a few kinds of polyvalent mixed vaccines are adopted, and simultaneous injections of 2-3 kinds of vaccines on the same day are not generally accepted.

The vaccines necessary for those who are going abroad are divided into 2 categories; namely vaccines demanded for entering a country and vaccines to preserve one's health. The former is only a yellow fever vaccine. Travellers should complete immunization against vaccine-preventable diseases before their departure. The vaccines that should be at least given are tetanus toxoid and hepatitis A vaccine. The yellow fever vaccine should be injected when going to the yellow fever endemic area, even if it is not demanded for entering a country. The vaccination schedule should be planed to complete injection of many kinds of vaccines before leaving the country by simultaneously injecting 2-3 vaccines on the same day.

The post-exposure prophylaxis against rabies is added as a special case of vaccination. To the person who was bitten by possibly rabid animals in the rabies endemic regions, the post-exposure prophylaxis should be given.

3 PROBLEMS ON THE POLIO VACCINE: IS IT NEED TO CONTINUE POLIO VACCINE?

YUZURU DOI, HITOSHI HORIE AND SO HASHIZUME

Japan Poliomyelitis Research Institute

(Abstract not received on time)

4 VACCINATION AT QUARANTINE STATION IN JAPAN

EMIKO IWASAKI, KIGEN ISHIZUKA, NOBUYUKI YAGI AND SHUN-ICHI INAGAKI

Sendai Quarantine Station, Ministry of Health, Labor and Welfare

The first Quarantine Station in Japan was established about 130 years ago. Now the Quarantine Station are set up at major international ports and airports to prevent pathogenic organs from entering country through travelers, imported foods and cargos. Due to the advancement of transportation and the increased number of travelers, the focus for "Quarantine" is changing.

One third of Japanese traveling overseas are in 20's, and some of them are backpackers without appropriate protection and knowledge for preventing infectious disease. In addition, travelers in 60's and 70's, who have weaker immune system, are increasing.

Currently, Japanese Quarantine Stations are focusing not only on the prevention of the IHR designated infectious diseases like cholera, yellow fever and plaque but also on controlling of the infectious disease by vaccination and education to international travelers before departure to adjust travelers to various health conditions. Therefore, since 1999 Japanese Quarantine is giving vaccination not only for cholera, yellow fever and plaque but also polio, diphtheria, hepatitis A, rabies, Japanese encephalitis, tetanus and measles.

5 TRAVEL VACCINES NOT MARKETED IN JAPAN

ATSUO HAMADA, YUTAKA UJITA AND EIICHI OKUZAWA

Japan Overseas Health Administration Center, Labor Welfare Corporation

Recently many Japanese people who travel and stay abroad have started to take preventative immunization. Results of a survey we conducted on 5,338 Japanese expatriates staying in developing countries showed that 46.9% of them were vaccinated against hepatitis A, hepatitis B, tetanus, rabies, etc. These vaccines are marketed in Japan. We also noted that some Japanese expatriates were inoculated with vaccines not available in Japan. These include vaccines against yellow fever, typhoid fever, meningococcal meningitis and tickborn encephalitis. In some developed countries, injectable purified polysaccharide vaccine and oral live vaccine are licensed for use against typhoid fever. These two vaccines are moderately effective and have fewer side effects. For prevention of meningococcal meningitis, bivalent and quadrivalent purified polysaccharide vaccines are marketed in some developed countries for travelers to the endemic area. For tickborn encephalitis, injectable inactivated vaccine is available in endemic areas such as East Europe and Russia. These vaccines are difficult to administer legally in Japan, although yellow fever vaccine can be legally administered in Quarantine Stations as special case. However, Japanese physicians can personally import these vaccines to inoculate Japanese travelers. They must first obtain temporary permission from the Japanese Ministry of International Trade and Industry and the Japanese Ministry of Health and Welfare. It is important to make Japanese physicians more aware of this system. We must also encourage the Japanese government to grant approval to travel vaccines not marketed in Japan.

6 THE OPTIMAL VACCINATIONS FOR TRAVELERS IN JAPAN

TOMOYOSHI SONOBE

Department of Pediatrics, Japanese Red Cross Medical Center

The vaccine givers are required to have the latest knowledge of travel medicine to give travelers the optimal vaccinations. It became easy to obtain the latest information of endemic diseases by Internet recently. Nowadays, however, many routine vaccines are also recommended for travelers other than vaccines for endemic diseases. Thus the vaccine givers are required to have the sufficient knowledge of routine vaccinations not only for adults but also for children. To make good vaccination schedule, vaccine givers have to realize that Japan is one of the worst countries to prevent vaccine-effective diseases. To the contrary USA had almost eradicated those diseases. Because USA has an excellent vaccination system based on the strict risk control policy to save the health and life and property of the people. Until the consensus of good vaccination system is established in Japan, vaccination schedule or method of USA may be used. To promote vaccination for travelers, the vaccine service should be friendly and convenient even for busy travelers. For example, the service should be available even at night and on Sunday. Now we are requested to establish our own good vaccination system for the Japanese people including travelers.

Mini symposium:

1 SITUATION OF MATERNAL AND CHILD HEALTH IN CAMBODIA AND THE MCH PROJECT

HIDECHIKA AKASHI, SEIKI TATENO AND TAKAKO YAMADA

International Medical Center of Japan, Bureau of International Cooperation

Health situation in Cambodia: The Maternal Mortality Rate (MMR) in Cambodia was 500, Infant Mortality Rate was 115 (UNIECF 1995). More than 90% of deliveries are occurred at home in rural area. Many births are attended by family members or Traditional Birth Attendant (TBA)s. HIV infection rate is estimated to be around 4%. Blood transfusion system is weak and there is no ambulance system nationally. Many health facilities, including health centers (HCs), district hospitals, provincial hospitals and national hospitals are old, and many medical equipments are broken. About health personnel, usually TBAs are illiterate, some nurses and midwives cannot measure blood pressure, nor read graphs, nor calculate, and some doctors do not understand anatomy, physiology and pathology. The senior staffs do not know how to transfer their knowledge and technique to juniors in practice, and it seems to be difficult to expect for them to refer the patient to the other health facilities.

Maternal and Child Health (MCH) Project: Japan International Cooperation Agency (JICA) started the MCH project in April, 1995, and its Overall Goal is to reduce the MMR in Cambodia, and the Project Purpose is to improve National MCH Center (NMCHC) on management, clinical and training. The new NMCHC was built by Grant Aid from Japan opened in 1997, and several new systems in Cambodia were introduced, such as user fees, exemption for the poor, patient registration, independence of nursing division on management. On clinical, partograph and magnesium treatment were introduced as new techniques, and case conference were started. Also trainings of HC midwives were started, and provincial hospital midwife or doctor trainings, hospital management seminar and so on were also performed. In addition, materials were provided such as doppler fetal heart beat detector to district hospitals, TBA kits to NGOs, and motorcycles to provincial health departments.

Results: The number of patients is increasing after the opening of the new NMCHC, and user fees with exemption system has high reputation from the Ministry of Health. More than 180 HC midwives were trained and around 20% of HCs have been covered, and also other donor agencies sent their midwives in their project areas to the training courses in the NMCHC. However, many problems including low government salary and low staff motivation are relating to the national level problems and it is difficult only for the project to change everything.

2 NATIONAL TB CONTROL PROJECT, CAMBODIA: STRUGGLE IN THE TRANSMISSION OF HEALTH POLICY AND AID POLICY

IKUSHI ONOZAKI

JICA Project Chief Advisor, Chiba Anti-TB Association

[Setting] Situation of TB in Cambodia might be the worst in Asia in prevalence and incidence. Directly Observed Treatment with Short Course Therapy (DOTS) was introduced as a national policy in 1994, and it has been successfully implemented through public hospital network since then. In 1999, 145 TB units in all 23 provinces across the nation provided DOTS, when National TB Control Project by JICA launched.

[Problems] The project extensively reviewed the situation and found that the magnitude of the TB problem exceeded the capacity of TB services despite the primary success of DOTS; HIV/AIDS epidemic may increase burden of TB even double in near future. While National TB Program has been enjoying strong vertical structure supported by international agencies, Health Sector Reform left TB program out of new system: DOTS is not available in newly established health centers network. Many poor patients in villages are dying with TB without visiting hospitals in town.
[Target of the project] The project aims to expand TB services into the community level through new health system.

[Constrains] Low motivation of the civil servants due to low salary is one of major constrains. Aid agencies often seek outcome in short span, and recipients seek instant benefit such as cash flow. Those behaviors make us difficult to set a long-term goal.

Shift of qualified staff from public sector to private including NGOs is also an obstacle to sustain a program.

[Recommendation] Inter-agency coordination is essential. To ensure the best use of resources, we should be flexible including reallocation of current budgeting.

3 PHC PROJECT THROUGH HUMAN RESOURCES DEVELOPMENT FOCUSED ON MCH AND HIV/AIDS PROGRAM

SATOKO KONO, MARI SATO, TAKASHI SAWADA, YUKO MORIMOTO,
HARUHISA NISHINA AND TORU HONDA

Services for Health in Asian & African Regions

Since 1988, we SHARE have been implementing primary health program in Cambodia. Especially since 1998 we have PHC project that aims for human resources development through health education focused on MCH and HIV/AIDS in Sreu-sentou Province located north of Phnom Penh.

Activities to achieve such objects are as below.

A. Health education and community empowerment targeted to mothers.
   1) Health education: To be acquired accurate knowledge about basical health problems by mothers, health education was performed in 4 villages. The curriculum includes personal hygiene and sanitation, diarrhea, nutrition, immunization, local environmental hygiene, emergent medication, the body and health of female, antenatal care, birth spacing, HIV/AIDS, etc.
   2) Organization of mother group: We followed up the villages that finished health education, and facilitated to organize mother groups. Several mothers were chosen as leaders of their group. They were strengthened to manage their group having group discussion about health topics that were interested for them.

B. Training for health staffs and TBA (traditional birth attendance).
   1) TBA training: TBA carries 60 percent of all deliveries in Cambodia. We held TBA training to improve and activate local MCH situation. Our training covered 86 percent of TBA in our target area. TBA showed active attitude to attend the group discussion and role-playing. We developed and supplied appropriate support information/education materials and teaching methods. After training, referral cases from TBA to hospital increased.
   2) According to increase of HIV/AIDS patients in target area, the need of training for health staffs is also increasing. We held HIV/AIDS training for health staffs in our target area. Under insufficient situation of health system in Cambodia, HIV/AIDS patients undergo health care in their home by their families. Health education by health staff to villagers, education of health care skill for HIV/AIDS patient's families who care patients, and development of health system to support health staffs are needed.

C. Health education of HIV/AIDS prevention.
   1) Health education of HIV/AIDS prevention at junior high school: For junior high school teachers, training about health education (trainers' training) was carried. The teachers who attended training managed health education to their students.
   2) Support to Provincial HIV/AIDS Committee: HIV/AIDS seminar for village mayors, management of HIV/AIDS Day's festival, etc. were carried.

4 PRIMARY HEALTH CARE PROJECT IN CAMBODIAN RURAL VILLAGES

KEIKO SUWA

Primary Health Care Project, 24hTV Charity Committee-Cambodia
(Abstract not received on time)

5 PROMOTION OF MCH THROUGH TBA SUPPORT IN PREYKABAS DISTRICT OF TAKEO PROVINCE

SATOSHI TSUKAMOTO, MUTSUMI KATO, RYU MORITA, KAZUKO MIYAMOTO, SATOKO YANAGISAWA AND TOMOYUKI IGARI

Japan Overseas Christian Medical Cooperative Service (JOCS)

Since 1997, JOCS has been implementing PHC project in Preykabas District of Takeo Province covering about 14,000 people in 18 villages with a focus on MCH through TBA support in collaboration with Kampeng Health Center.
Problems:

There is no referral center for MCH nearby the villages in case of emergency. Patients have to pay much for transportation and consultation for referral. Malnutrition is commonly seen among pregnant women and children. Traditional birth attendants (TBAs) in the community lack a good knowledge and skills.
JOCS project:

14 TBAs are identified as key persons for MCH. JOCS has been doing some activities with TBA such as TBA meeting, TBA training or TBA material credit. TBA meeting is held every month for providing learning opportunities for TBAs by sharing their own activities. Through TBA training, TBAs are expected to improve necessary knowledge and skills so that TBA may be able to find dangerous sign of pregnant women, do health education in early stage of pregnancy. Aim of TBA Maternal Credit is to purchase/manage TBA's materials for delivery support such as disposal plastic grove, eye medicine and antiseptic solution. TBA cooperative committee has been established to purchase/manage materials by themselves. This is functioning well. There are good outcomes through these TBA activities. TBAs have improved their knowledge and skills. A good relationship is being developed among TBAs and between TBA and Kampeng Health Center midwife.
Future perspective and remaining problems:

Management of TBA meeting is being handed over to Kampeng Health Center, and that of TBA Material Credit to TBA themselves. In the next phase of TBA training, JOCS expects that TBA would be able to do health check for pregnant women in cooperation with Kampeng Health Center midwife. How can JOCS support villagers to take an ownership of activities? Cooperation with community development organizations is a way to improve villager's conditions in life and health. Another need is an establishment of referral system and mutual finance support system for poor people to borrow money in case of referral.

6 PRESENT STATUS OF INFECTIOUS DISEASE IN CAMBODIA

KIICHI SATO

Kurobe Onsen Hospital

Author was staying in Phnom Penh from Feb/1998 to Mar/1999 as one of JICA staff for investigation of infectious disease in Cambodia. The National Center for Parasitology Entomology and Malaria Control (NMC), which was attached to Ministry of Health was author's office. The operation of NMC was consulted by specialist of WHO/ Phnom Penh. During one year, author had been investigated, what kinds of infectious disease occupied in Cambo-

dia and how to prevent and medication for patients. Just the year of 1998 was occupied by outbreak of Dengue fever and 342 children were died. Results of our investigation was as followed. The whole number of reported infectious patients were about 1,500,000. Among them, respiratory infection, including pneumonia and severe influenza was largest patients (498,781: occupied 33.3%). Secondary was unknown caused diarrhoea (297,610). Next was malaria (about 200,000) and bacterial/amoebic dysentery (196,350) was the fourth. These four kinds of infectious diseases was occupied 80% of all infection, excluding the parasite, filariasis and AIDS. Remained 20% of the infectious diseases were gynecologic infection (puerperal sepsis, etc. 46,524), sexual transmittened disease (45,845), Dengue fever (16,228), tuberculosis (12,838), cholera, measles, meningitis and tetanus (95) and so on. The number of HIV(+)/AIDS infected patients was estimated 120,000 (up to 1997) by WHO. However the number of parasite positive patients and filariasis were unknown. The prevention of malaria and Dengue fever was continuing by guidance of NMC, regularly. The purpose of prevention was "*Kill the mosquitoes*", which mears "delivering mosquito net and spraying of Abate" (kill powder for larva). These works were supported by many kinds of NGO's group. The prevention of parasite and filariasis did not yet perform, especialy for local people. In Phnom Penh, the staffs of NMC delivered the helminthague for infected children, directly. The problem of Tbc will be improve very soon, due to the earnest activities of Dr. ISHIKAWA, Dr. ONOZAKI and his expert group, supported by JICA.

General presentation

A−1 MOLECULAR ANALYSIS OF ALPHA-THARASSEMIA IN NEPAL: CORRELATION WITH MALARIA ENDEMICITY

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Thalassemia is a prevalent hereditary disorder characterized by impaired synthesis of globin chains. It has been suggested that the high frequency of thalassemia might reflect heterozygote advantage due to reduced susceptibility to malaria. In Nepal, malaria has often occurred in places below the altitude of 1,200 m. We carried out a microepidemiological study on thalassemia in two neigboring populations in Kabhrepalanchok District in Nepal, the Danuwar and the Tamang. Settlements of the Danuwar are located below the limit of the malarial zone in Mahadevstan VDC (1,200 m in altitude), whereas those of the Tamang are found in malaria-free uplands in Anaikot VDC. Three heterozygotes for hemoglobin E (HbE) were observed in the Danuwars. We detected one type (-alpha 3.71) of alpha+-thalassemia that involves a deletion of 3.7 kb, leading to a loss of one of two alpha-globin genes, in the Danuwars, at a high gene frequency of 63%, while the gene frequency in the Tamang was only 5%. Four different haplotypes were associated with the type of alpha+-thalassemia in the Danuwars. Nucleotide sequences of the D-loop region in the mitochondrial DNA of the two populations indicated similar nucleotide diversity in each population. The fixation index, FST, representing the degree of genetic differentiation estimated from mitochondrial DNA diversities (FST, 0.05), was smaller than that obtained from the gene frequencies of alpha-thalassemia (FST, 0.55). If we assume neutral molecular evolution in the D-loop region of mitochondrial DNA, these results suggest that the high frequency of alpha+-thalassemia may be due to biological adaptation to the malarial environment rather than to events such as a bottleneck.

A−2 MOLECULAR ANALYSIS OF G6PD VARIANTS IN SOUTHEAST ASIA

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We investigated glucose-6-phosphate dehydrogenese (G6PD) variants in Southeast Asian countries (Indonesia, Malaysia, Laos and Myanmer). Blood samples were mainly collected on malaria survey sites in the malaria endemic area. Some samples were collected in hospitals. Blood of participants was examined for malaria parasites and G6PD activity with two rapid tests, which were developed by Kawamoto (1992) for malaria and by Hirono (1998) for G6PD. Rate of G6PD deficiency in male was 5.6%, which was similar rate with previous studies.

After taking informed consent from people of G6PD deficiency, we extracted genomic DNA from 0.1 m*l* of peripheral blood and amplified DNA coding G6PD by PCR. Each exon of G6PD was analyzed with single strand conformation polymorphism (SSCP) method. Some exons were read by DNA sequencer. Ten variants were detected among sixty-three G6PD deficient subjects. They were G6PD Gaohe, G6PD Vanua Lava, G6PD Mahidol, G6PD

Coimbra, G6PD Viangchan, G6PD Chatham, G6PD Union, G6PD Canton and G6PD Kaiping, respectively. A noble variant was found from a 18 year-old man in Surabaya, Indonesia, who had Chinese ancestor. The variant was at cDNA 1,291 from G to A (Val→Met). We named this new variant as G6PD Surabaya.

A−3 MICROSATELLITE POLYMORPHISM IN THE HEME OXYGENASE-1 GENE PROMOTER IS ASSOCIATED WITH CEREBRAL MALARIA IN MYANMAR

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Malariais one of the most important tropical diseases caused by *Plasmodium falciparum*. When the infected RBCs were lysed, the parasites and hemoglobin were released into blood stream. Following the hemoglobin is resolved into heme and globin, the heme is resolved into the biliverdine, iron and carbon monoxide by the inductive heme oxygenase, (HO-1). The biliverdine is immediately reduced to the bilirubin by the biliverdine reducutase. The accumulation of the catalytic products of heme degradation, such as bilirubin, carbon monoxide and iron may affect the clinical course of malaria A (GT)n dinucleotide repeat in the 5'-flanking region of human HO-1 gene shows length polymorphism and is reported to modulate the level of gene transcription. In this study, we examined the relation between cerebral malaria and HO-1 polymorphism, in Karen. The frequency of the allele of more than 30 GT repeats was significantly decreased in the cerebral malaria patients than in the uncomplicated patients ($P<0.05$, OR 3.26), indicating that the longer (GT) repeat showed resistant against malaria.

A−4 TRIAL TO DEVELOP NOVEL DNA VACCINE USING FULL-LENGTH-CDNA LIBRARY FROM *PLASMODIUM BERGHEI* ANKA

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Previously, we have produced a full-length cDNA library from the erythrocytic stage parasites of *Plasmodium falciparum* and analyzed the nucleotide sequences. In the present study we further explored a potential application of this library as a DNA vaccine which was constructed from the lethal murine malaria parasites, *Plasmodium berghei* ANKA.

Library construction: The erythrocytic stage of *P. berghei* was propagated by intraperitoneal injection into rats. Blood was collected by cardiocpuncture. White blood cells were removed using Plasmodipur filter. Purified parasites were used as a source of mRNA to construct a full-length cDNA library. The vector pCE-FL that contains EF321 (strong promoter of elongation factor) and CMV-IE enhancer en-

sures efficient expression of malaria proteins within murine cells.
Immunization: Plasmids were divided into five groups each representing 2,000 independent clones. The plasmids were propagated in *E. coli* and purified using the Endotoxin-free QIAGEN Megaprep kit. As a control, the plasmid vector without inserts were used. For each group, eight female mice (BALB/c at fifth week) were injected with purified DNA (50 μg/50 μl saline) initially directly into the spleen, followed by two subsequent intramuscular injections with one week interval.
Challenge infection: At one week after the last injection, 50,000 eryhrocytes infected with *P. berghei* were injected intraperitoneally. Mice were observed daily and tail blood was collected on the every second day for monitoring the parasitemia.
Results: The five groups were pooled together for statistical analyses because they followed the similar course of the disease. Unexpectedly, the survival rate of the vaccinated groups was lower than that of the control group (P=0.053, Kaplan-Meyer). There was no significant difference in parasitemia between the two groups. There was no increase in antibody titer in both groups.
Discussion: Our results unexpectedly indicate that DNA vaccination had adverse effects on the survival of infected mice. Fur bristling, shivering, and convulsions were observed which coincided with the time of death. These observations suggested a possibility that vaccination has a reverse effect on the cellular immunity resulting in the development of severe malaria in BALB/c mice which do not develop cerebral malaria under normal conditions.

A−5 INVASIVE FORMS OF PROTOZOAN PARASITES HAVE A POSITIVE CHARGE AT THEIR CONTACT SITE WITH HOST CELLS

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It is known that host membrane is, in general, negatively charged. To determine whether surface charges of invasive forms of protozoa contribute to invasion or not, we examined the surface charges of *Plasmodium falciparum*, *Toxoplasma gondii*, *Leishmania amazonensis*, *Trypanosoma cruzi* and *Entamoeba histolytica* by atomic force microscopy (AFM) and surface portential spectroscopy.

We used an AFM (BioScope AFM, Digital Instruments, Santa Barbara, CA) equipped with an Extender Electronics Module (Digital Instruments), which can measure surface potential. As a sample, indochina-1 strain of *P. falciparum*, RH strain of *T. gondii*, LV78 strain (MPRO/BR/73/M1845) of *L. amazonensis*, Tulahuen strain of *T. cruzi*, and HM1: IMSS strain, clone 6 of *E. histolytica* were examined. During examination, they were on indium tin oxide coated glass slides (Evers, Osaka, Japan).

As a result, merozoites of *P. falciparum*, tachyzoites of *T. gondii* were positively charged at their apical ends. Promastigotes and amastigotes of *L. amazonensis*, and amastigotes of *T. cruzi* were positively charged at their flagella. These results presented that the specific part of the protozoa which makes initial contact with the host cell is positively charged. This indicates that the positive charge at the site of contact facilitates binding of the invasive protozoa to negatively charged host cells. This leads to conclude that the charge difference between the contact sites of protozoa and host cell initially brings them together before a ligand-receptor interaction occurs.

While trophozoites of *E. histolytica* did not have any positive surface charges. The amebae are highly motile and can easily change their form without orientation. They phagocytose, not penetrate, host cells. A charge difference may not be necessary for their interaction with host cells.

This finding strongly suggests that the initial contact sites of other protozoa may also be positively charged, with the electric charge possibly playing a major role in host cell invasion.

A−6 BACTERIA EXPRESSING SINGLE-CHAIN IMMUNOTOXIN INHIBIT MALARIA PARASITE DEVELOPMENT IN MOSQUITOES

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Single-chain immunotoxins are ideal tools to selectively kill infectious agents. In applying this technology to block transmission of malaria parasites in the mosquito vector, we have constructed a single-chain immunotoxin composed of a single-chain antibody fragment (scFv) directed to Pbs21 on the surface of *Plasmodium berghei* ookinetes linked to a lytic peptide, Shiva-1. The single-chain immunotoxin was expressed in *Escherichia coli*, and the protein was purified by a Ni-NTA column. The single-chain immunotoxin was initially shown to exhibit greater killing properties for *P. berghei* ookinetes *in vitro* compared with the scFv or synthetic Shiva-1 peptide alone. In an attempt to block malaria transmission by genetically engineered bacteria, recombinant *E. coli* harboring the single-chain immunotoxin gene were introduced into the mosquito midgut by membrane feeding. The number of infected mosquitoes and their oocyst densities were significantly reduced when the mosquitoes were subsequently allowed to feed on *P. berghei*-infected mice. These results indicate not only that a single-chain immunotoxin with enhanced parasiticidal activity could form a basis for the development of more effective malaria therapeutic agents, but also that introduction of genetically engineered bacteria into anopheline mosquitoes may offer a practical approach to the regulation of malaria transmission.

A−7 EXPRESSION PATTERN OF MITOCHONDRIAL COMPLEX Ⅱ (SUCCINATE-UBIQUINONE OXIDOREDUCTASE) IN THE ERYTHROCYTIC STAGE SELLS OF *PLASMODIUM FALCIPARUM*

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Since energy metabolisms of *Plasmodium* is quite different from that of the mammalian host, the enzymes of energy transducing pathway of the parasite are promising target of anti-malarial drugs. Significant advances have been made in our understanding of the importance of *Plasmodium* mitochondria, although main energy metabolism in the erythrocytic stage cells of *Plasmodium falciparum* was considered to be ATP production by the glycolytic pathway. However, much more information on the *Plasmodium* mitochondria is required to develop new anti-malarials.

Complex II (succinate-ubiquinone reductase/quinol-fumarate reductase) is well known marker enzyme of mitochondria in the eukaryotic cell. It is one of the members of the TCA cycle enzymes as well as electron transport chain, a direct link between major systems for energy metabolism in aerobic organisms. In addition, complex II functions as quinol-fumarate reductase in the anaerobic respitatory chain, NADH-fumarate reductase system, of parasitic protozoas and helminths such as *Ascaris suum*. Thus, complex II changes its function to adapt various environmental conditions.

Generally, complex II is composed of four subunits, and we have cloned the genes for *Plasmodium* Fp and Ip subunits which form a catalytic portion of complex II. Antisense DNA for *Plasmodium* Ip showed an inhibitory effect on the growth of cultured *P. falciparum* suggesting that complex II is essential for the survival of *Plasmodium* in the red blood cell.

In the present study, expression pattern of complex II in mRNA and protein level in the life cycle was analyzed. From Northern blot analysis, expression of Fp and Ip of was highest in schizonts and lowest in ring forms. Immuno blot analysis using antibody against Ip showed similar expression pattern. Intense signal of the Ip (33 kDa band) was ob-

served in schizonts and weak one in ring forms and trophozoites. Transcripts of Fp and Ip were also highly expressed at sexual stage gametocytes.

This expression pattern is similar to those of two mitochondrial proteins, subunits of cytochrome *c* oxidase and cytochrome *b* encoded on tandem-repeated 6 kb mitochondrial DNA. On the other hand, the expression pattern of another mitochondrial protein dihydroorotate dehydrogenase (DHODH) was different from that of complex II. The mRNA for DHODH was highly expressed in trophozoites and hardly detected in schizonts.

A−8 EFFECT OF JASPLAKINOLIDE ON THE GROWTH AND ACTIN CYTOKELETON OF *PLASMODIUM FALCIPARUM*

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The actin filaments in eukaryotic cells are responsible for cell motility. To elucidate microfilament functions, actin-modifying agents have been used extensively.

Jasplakinolide, a naturally occurring cyclic peptide from the marine sponge, *Jaspis* sp. is a membrane permeable, actin-polymerizing and filament-stabilizing drug. The effect of jasplakinolide on the growth and actin cytoskeleton of *Plasmodium falciparum* was examined in synchronized culture *in vitro*. Jasplakinolide inhibited the growth of *P. falciparum* strain FCR-3 in a time- and concentration-dependent manner, where inhibition was observed after 2 days of culture with doses greater than 0.3 μM of drug. The inhibitory effect of jasplakinolide on growth was reversed by removal of the drug. Although development of ring-forms to schizonts and liberation of merozoites were observed on slide smears of jasplakinolide-treated cultures, no further growth to ring-forms occurred. This demonstrates the inabiliy of treated merozoites to invade red blood cells. Electron microscopy showed the formation of cytoplasmic cavities in the treated merozoites, but not the apical extensions which were reported previously in tachyzoites of *Toxoplasma gondii* treated with the drug. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analysis revealed that the jasplakinolide treatment led to an increase of F-actin polypeptide. Thus, our results indicate that jasplakinolide inhibits the growth of *P. falciparum* by blocking the invasion of merozoites into red blood cells and, therefore, the drug may become a useful tool for studies on the relationship between the actin cytoskeleton and the process of invasion of the red blood cell.

A−9 A MALARIA PATIENT INFECTED WITH *PLASMODIUM FALCIPARUM* AND *P. MALARIAE*

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Although malaria is not endemic to Japan, the number of malaria patients has increased in this country. But cases of malariae malaria, especially in patients coinfecetd with falciparum malaria, are very rare. We treated a patient coinfected with malariae malaria and falciparum malaria, as reported below.

A 36-year-old Indian man was admitted to our hospital on May 17, 2000, because of a 9-day of fever and 6 days of diarrhea. He reported he had never had malaria. He had been in Japan for 10 years, and had traveled in India from March 30 to April 28, 2000, then returned to Japan. Laboratory examination revealed thrombocytopenia (platelet count: $5.5\times10^4/mm^3$) and prothrombin time was 42.8%. Peripheral blood smears revealed *Plasmodium malariae*

and *P. falciparum*, and the parasitemia of *Plasmodium* sp. was 1.0%. He was treated with Quinimax® in an intravenously drip May 17 and then oral administration of Mephaquin® May 18. Neither *P. malariae* nor *P. falciparum* were identified in his peripheral blood smear since May 19.

Malariae malaria has a spotty presence in tropical and subtropical areas and this may explain why Japanese physicians are unfamiliar with the disease. Although mixed infection is rare, it is important to investigate whether other kinds of malaria coinfect in malaria patients.

A−10 TWO CASES OF FALCIPARUM MALARIA WITH ATYPICAL CLINICAL COURSE

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The first case is a 42-year-old Japanese male who had traveled on business to Myanmar from March 12 to March 30 without malaria chemoprophylaxis.

He had a high fever (39.6℃) on April 8 and was admitted to our hospital. On admission, he was diagnosed with *P. falciparum* malaria on blood smear (parasitemia 0.7%) and was treated with artesunate and mefloquine. Parasites had disappeared on the next day. But he had a delirium on the 3rd hospital day. His blood examination showed AST 2,816 IU/*l*, ALT 1,067 IU/*l*, T-Bil 3.6 mg/d*l*, CK 4,777 IU/*l*, myoglobin 1,100 ng/m*l*. We think that they were cerebral malaria, severe hepatocellular damage and rhabdomyolysis and were caused by the malaria infection. His body tempareture was normalized after the 10th hospital day. He was discharged on the 36th hospital day after a fully recovery of all abnormal laboratory data.

The second case is a 52-year-old Japanese male who had traveled on business to Zimbabwe from April 27 to May 7 without malaria chemoprophylaxis.

On May 18, he rolled downstairs and was admitted to orthopedics at our hospital, then he was diagnosed with compound fracture of ankle joint. On admission, body temperature was 35.8℃ and he had a disturbance of consciousness, oliguria, watery diarrhea and dehydration. Urgent laboratory data showed thrombocytopenia (Plt $25\times10^3/\mu l$), renal damage (Cr 1.3 mg/d*l*), hyponatremia (Na 123 mmol/*l*), and hyocalcemia (Ca 7.3 mg/d*l*). On the next day, *P. falciparum* malaria were detected in his blood smears (parasitemia 4.93%). He was diagnosed severe falciparum malaria with algid malaria, cerebral malaria and acute renal failure. He was treated with intravenous quinine infusion, oral artesunate, fluid therapy and hemodialysis (2 times). Parasites had disappeared on the 4th hospital day. He was discharged on September 4 after a recovery of compound fracture and abnormal laboratory data.

A−11 A CASE OF SEVERE FALCIPARUM MALARIA WITH PROLONGED IMMUNOHEMOLYTIC ANEMIA

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(Abstract not received on time)

A−12 CHRONIC AUTO-IMMUNE THROMBOCYTOPENIA FOLLOWING RECOVERY FROM SEVERE FALCIPARUM MALARIA

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A 49-year-old Japanese man made a two-week visit to Gambia. He took no malaria prophylaxis. When he returned to Japan, he became febrile and was admitted to our hospital. The temperature was 38.0°C, the pulse was 90, and the respirations were 30. The blood pressure was 108/72 mmHg. On the examination, he was lethargic and jaundiced. His gums were bleeding and liver was palpable at 2 cm below the right costal margins. Thin and thick blood films showed *Plasmodium falciparum* trophozoites; 3% of the red cells were parasitized. The platelet count was 10,000/mm^3 and fibrinogen level 123 mg/d*l*. The fibrin-degradation products were 40 μg/m*l*. The diagnosis of severe falciparum malaria complicated with disseminated intravascular coagulation (DIC) was made. Quinine (600 mg every 12 hrs) and minocycline (100 mg every 12 hours) were given intravenously followed by orally. Transfusions of platelet concentrations and fresh frozen plasma were administered to prevent bleeding. On the third day of treatment, he got afebrile and *P. falciparum* was not seen in the blood smear. He was discharged on day 28 with a platelet count of 104,000/mm^3.

Two weeks later, the platelet count fell to 56,000/mm^3. The peripheral-blood smear showed only thrombocytopenia and the bone marrow examination was normal. Blood coagulation test revealed no abnormalitity for DIC. Ultrasonography revealed no splenomegaly. The platelet-associated IgG (PAIgG) level was elevated to 11.96 ng/10^6 cells (normal 0.9-2.5 ng/10^6 cells). He did not have detectable antiplatelet antibodies in his serum. A test for antibodies to the human immunodeficiency virus was negative. Antinuclear antibodies and antiphospholipid antibodies were negative. The mild thrombocytopenia persisted and the PAIgG level remained high at 18 months after discharge.

This case was considered a chronic auto-immune thrombocytopenia following recovery of severe falciparum malaria. Various virus infections including rubella, varicella and infectious mononucleosis, are known to induce acute auto-immune thrombocytopenia. About 10% of these cases develop chronic auto-immune thrombocytopenia. The mechanism of thrombocytopenia in malaria remains unknown. But this case suggested that malaria induced auto-immune thrombocytopenia.

A−13 A CASE OF CLINICALLY CHLOROQUINE RESISTANT VIVAX MALARIA INFECTION

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A 49-year-old Japanese man who had traveled Iriyanjaya, Indnesia since 2 Mar. 2000 till 20 Mar. 2000, hospitalized with complaint of fever and general malaise. We diagnosed him as vivax malaria infection by smear of peripheral blood. Although we prescribed him chloroquine 3 days, high fever still continued and the rate of malaria infected red blood cell did not decrease. We concluded as chloroquine resistant vivax malaria infection and prescribed Fansidar, so clinical symptom got better. At Iriyanjaya, chloroquine resistant vivax malaria infection is widespreded and this is the case with it.

A−14 A CASE OF FALCIPARUM MALARIA SUCCESSFULLY TREATED WITH INTRAVENOUS ARTESUNATE

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A Nigerian man, who was presumed to be infected in Nigeria, developed malaria during his visit in Japan. The patient showed fever with chills, arthralgia and diarrhea. The blood test revealed thrombocytopenia and anemia. Ring forms of 0.03% of his RBCs and *ICT™MalariaP.f/P.v* test was also positive for *Plasmodium falciparum.* We prescribed mefloquine to him, but the number of the parasites in his peripheral blood did not decrease, and, in fact, they came to increase (maximum 6.66%) 20 hr after the drug treatment. As clinical condition of malaria were liable to change seriously, intravenous Artesunate (a qinghaosu derivative) was decided to be given additionally to the patient. Artesunate was prescribed to him by intravenous injection in three doses (120 mg given followed by 60 mg 24 hr and 48 hr later; total dose was 240 mg). Consequently the parasites disappeared in 20 hr from his blood but fever still continued in low grade possibly because of cholecystitis. At the same time of Artesunate treatment, hemoglobinuria started and anemia worsened partly because of his G6PD deficiency. All pending problems were improved by the time he left Japan and those parasited were finally found to be susceptible for mefloquine by the *in vitro* susceptibility test (IC_{100}=100 nmol/m*l*, IC_{50}=7.86 nmol/m*l*). This is the first reported case of falciparum malaria successfully treated with intravenous Artesunate in Japan.

A−15 A CASE RECOVERING COMPLETELY FROM SEVERE FALCIPURUM MALARIA COMPLICATED WITH HIGH LEVELS OF MULTIPLE ORGAN FAILURE

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A 56-year-old male, who had returned from Ginbabue in Africa, was admitted with consciousness disturbance (JCS, 100) after three days history of high fever. Soon after his travel history was revealed, he was diagnosed as *Plasmodium falciparum* malaria by the preparation of peripheral blood smear with the parasitemia of 49%. Despite intravenous gluconate quinine infusion and artesnate (administration), his consciousness state, respiratory, renal and hepatic function, deteriorated progressively. Although he was treated with respirator, plasma exchange, hemodialysis, continuous hemodiafiltration in the intensive care unit, he fell into shock state on the 4th day of hospitalization. He recovered from shock by using electric defibrillator and from acute respiratory distress syndrome by methylprednisolone pulse therapy. On the 9th day blood preparation showed no parasitemia and multiple organ failure gradually improved. On the 26th day his consiousness was clear, and on the 117th day he was discharged without any impairments of life quality. We would like to emphasize the importance of supportive and general care as well as the importance of the loading-dose IV quinine in the treatment of severe falciparum malaria.

A−16 A CASE OF SEVERE FALCIPARUM MALARIA WITH HYPER-BILIRUBINEMIA AND NEPHROPATHY

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The patient was a 46-year-old male who returned from a 10-day trip to central Africa on January 4, 1998. The patient felt chill and developed fever of 38.6℃ on the following day, and visited a physician. The patient was referred to our hospital and admitted on January 9. On peripheral blood test, white blood cell and platelet were low: 2,300/μ*l* and 22,000/μ*l*, respectively. Blood chemistry revealed increases in total bilirubin (T. Bil) (2.7 mg/d*l*) and LDH (1,240 IU/*l*). Splenomegaly was observed on abdominal ultrasonography. On January 12, protozoan were demonstrated in red blood cells in blood smears (infection rate: 1.4%), and the patient was diagnosed as having falciparum malaria. Mefloquine was administrated on the same day (the eighth day after the onset), but the protozoan infection rate in red blood cells has increased to 13.7% on the following day. Therefore, quinine hydrochloride was administrated, and parasitemia rapidly improved. However, on January 17, T. Bil and direct bilirubin (D. Bil) were 58.5 mg/d*l* and 38.1 mg/d*l*, respectiely, indicating that jaundice with predominating D. Bil worsened, and creatinine (Cr) was 6.5 mg/d*l*, indicating aggravation of nephropathy. Intrahepatic biliary stasis was suspected for jaundice, and steroid pulse therapy and plasma pheresis were performed. For acute renal failure, hemofiltration was performed. Sub-

sequently, T. Bil and Cr gradually decreased. Liver biopsy performed on February 17 revealed bilirubin pigmentation in hepatocytes, bile thrombus in capillary in blood vessels, and Küpffer cells phagocytosing malarial pigments in the sinusoid. We encountered a patient with severe falciparum malaria accompanied by severe jaundice and nephropathy.

A−17 THE USE OF THE ATOVAQUONE/PROGUANIL COMBINATION (MALARONE®)

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Malarone® is a fixed-dose combination of 250 mg of atovaquone and 100 mg of proguanil hydrochloride. The continuing spread of drug-resistant malaria emphasizes the need for new antimalarial drugs. Atovaquone is a new broad-spectrum antiprotozoal drug with a novel mechanism of action, *i.e.,* via inhibition of parasite mitochondrial electron transport. The combination of atovaquone and proguanil is synergistic *in vitro,* and clinical studies demonstrated enhanced efficacy of the combination compared to either drug alone for treatment of malaria. Malarone® is available in many countries for treatment of acute, uncomplicated falciparum malaria. It is reported that at the recommended dose (in adults, four tablets once daily for three days), the overall cure rate was >98%. Adverse events (abdominal pain, anorexia, nausea, vomiting, diarrhea and coughing) occurred with similar frequency as in patients treated with other drugs, such as mefloquine, amodiaquine, and chloroquine.

We reviewed our experiences of Malarone® between July 1999 and August 2000. This study enrolled 4 patients with acute uncomplicated falciparum malaria and 3 patients with vivax malaria, 4 of whom were native Africans. All patients did not take any antimalarial drugs as prophylaxis. Initial parasitemia ranged from 100 to 25,000/μl. All patients were treated with Malarone® at the recommended dose. The patients with vivax malaria took primaquine at a standard dose after treatment with Malarone®.

Efficacy: Parasitemia cleared in six patients within seven days and no recrudescence developed. Mean PCT (parasite clearance time) was 72 hr. The cure rate was 100%. One patient with vivax malaria came back to Madagascar after treatment and the long-term follow-up was not possible. So far other 2 patients with vivax malaria have had no recurrence.

Adverse events: One patient had a non-serious adverse event (urticaria) that necessitated switching of Malarone® to mefloquine. One patient had a non-serious, mild itching. One case showed slightly elevated levels of ALT and AST that continued after the clearance of parasitemia.

It has been reported that Malarone® is safe and effective for treatment of malaria caused by *Plasmodium falciparum*, and limited data indicate that this combination is also active against the blood stage of *P. vivax, P. ovale,* and *P. malariae*. Therefore, Malarone® is an important new alternative for treatment of malaria. However, only limited data are available with Japanese patients and further evaluation should be needed.

A−18 ANALYSIS OF SEVERE AND COMPLICATED FALCIPARUM MALARIA IN JAPANESE PATIENTS CAUSED BY DELAY OF DIAGNOSIS AND TREATMENT

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As the incidence of imported malaria increased in Japan, fatal of severe Japanese falciparum malaria cases were reported every year. Analyzing case-reports, which were collected by the Research Group for Development of Chemotherapeutic Agents against Tropical Parasitic Diseases (supported by the Japanese Ministry of Health and Welfare) and the Research Group for Clinical Evaluation of Orphan Drugs against Imported Tropical and Parasitic Diseases (supported by the Human Science Promotion Foundation), the relationship between the severity of disease and delays of the treatment were examined. The criteria of severe and complicated malaria in this study were cerebral malaria, severe anemia (Hb<5 g/d*l*), pulmonary edema, acute respiratory distress syndrome, and renal failure (Cre>3.0 mg/d*l*). Seventy-four Japanese falciparum malaria case-reports were collected from 1991 to 1999. Seventeen cases were severe and complicated, including five fatal cases, and 57 cases were uncomplicated. Forty-two percents of patients began to get anti-malarial treatment on the day when they visited to doctors. Twenty-five percents began on the next day, and 18% began on the third day or the forth day from the first visits to doctors. When the treatment had began on the day or the next day, the rate of severe cases was 10% (5 in 48 cases). On the third day or the forth day, the rate was 39% (5 in 27 cases). On the fifth day or later, the rate reached to 55% (6 in 11 cases). The delays of the treatment usually depended on the misdiagnosis. The later the treatment began from the first visits to doctors, the more the rate of severe cases increased. Concerning the days between the onset of symptoms and the beginning of the treatment, the same tendency was shown. When the treatment began on the third day or earlier from the onset of symptoms, the rate of severe cases was 4% (1 in 25 cases). On the forth day, the fifth day, or the sixth day from the onset, the rate was 19% (5 in 27 cases). On the seventh day or later, the rate reached to 50% (11 in 22 cases). In summary, delays of the treatment cause severe and complicated malaria. Therefore, we should diagnose malaria promptly and treat it as soon as possible.

A−19 ON THE WHO'S GUIDELINES FOR SEVERE MALARIA, 2000

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As the clinical experiences of severe malaria are limited in our country, guidelines proposed by authoritative bodies are highly needed. The WHO's guidelines for severe malaria are one of those and have just been revised 10 years after the last edition appeared. We investigated their contents caring about the differences compared to the old one. Overall, there are no fundamental changes in the new edition; however, some treatments are recommended more enthusiastically than were before. The importance of the loading-dose IV quinine is stressed because otherwise, the optimal blood concentration could not be attained even under hepatic or renal disturbances. The use of artemisinin derivatives especially that of IM artemether is recommended although showing a concern that it could retard the recovery from comatose state. The oral artesunate may be useful for patients with high parasitemia that can otherwise develop to severe malaria. Steroids and mannitol for cerebral malaria, steroids, heparin and aspirin in DIC-like hem-

orrhagic complication are not recommended or regarded even harmful as in the old guidelines. The acute renal insufficiency is divided into two types, one occurring at the acute stages and the other during convalescence with poorer and favorable outcomes, respectively. It is stressed that the complication is often caused by dehydration, necessitating fluid infusion before the patients are injected with diuretics or dopamines. The indications for hemodialysis include hyperkalemia, metabolic acidosis, fluid overload and the serum creatinine rising rapidly by 2.5-3 mg/dl/day. As for the treatment of pulmonary edema/ARDS, the importance of maintaining CVP between 0-5 cm H_2O is again stressed. Exchange transfusion should be considered in >30% parasitemia or even in >10% if there are complications such as cerebral malaria, acute renal insufficiency and pulmonary edema/ARDS, although comparative studies which show its efficacy are not available. The treatment of metabolic acidosis with sodium bicarbonate does not seem to be the most ideal due to the possible deterioration of tissue acidosis in the brain and the induction of sodium overload that can precipitate pulmonary complications. Alternatives include THAM and DCA; however data of their efficacy and safety are limited. It is thus revealed that many still remain to be known regarding the appropriate treatment of severe malaria.

A−20 PROBLEMS OF LOCAL TRANSPORT SYSTEM FOR ANTI-MALARIA DRUGS

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Malaria is the most important imported tropical disease in Japan nowadays. About 100 new cases have been recorded every year. Nevertheless, some critically important drugs to treat malaria or other tropical diseases are not available through ordinary drug distribution system in Japan. At the moment, under authorizaton of the Ministry of Health and Welfare such drugs are provided through the study group, "Orphan drugs for the imported tropical diseases and parasitic disesases", supported by the Human Science Foundation, Japan. As a member of this study group, we provided anti-maralia drugs to two hospitals in Kyushu to treat severe falciparum malaria patients in Spring 2000. Here we describe our experience of the drug delivery.

Case 1: 30-year-old female. She and her father visited Madagascar Island on March 10-24, 2000. Although they had taken prophylactic tablets for 3 days after returned to Japan, both of them developed fever on April 2-3. Her father's course was rush and, inspite of being diagnosed as having *P. falciparum* infection on April 6, he died of cerebral malaria with pulmonary edema and renal failure. She also developed signs and symtoms of severe malaria and was transferred to the ICU, Kumamoto University Hospital. Because this is our first emergency case to provide anti-malaria drugs, we tried to find out the quickest way to deliver drugs. We found that although we could charter a helicopter or an ambulance though the local government, it would take more than 4 hrs to arrange under permission. Eventually Qunimax for two i.v. injections were delivered by our official school car with the driver, and the Plasmotrim and Mephaquin for follow-up treatment were delivered by high-way bus cargo. In both ways, the drugs were delivered within 4 hr. The patient was successfully cured by treatment with these drugs.

Case 2: 30-year-old female. She visited Solomon Island on April 29-May 6, 2000. She did not take any prophylactic tablets. She felt malaise and developed fever since May 17. She was diagnosed as having falciparum malaria and was admitted to the Dept. of Internal Medicine, Kagoshima University Hospital. She was treated with Plasmotrim followed by Mephaquin which were delivered by high-way bus cargo. The patient was also successfully cured by treatment with these drugs.

In the present study, the father of Case 1 died within a week after the onset of the disease. Blood smear examinations with suspicion of malaria must be done as early as possible when physicians saw patients developed fever shortly after their return from the endemic areas.

In Kyushu, the orphan drugs for tropical diseases including anti-malaria drugs are deposited in three places; 1) Dept. of Parasitology, Miyazaki Medical College 2) Clinical Division, Inst. Trop. Med., Nagasaki University, and 3) Infectious Diseases Center, Fukuoka Children's Hospital. After our experience of two cases, we found that basically drugs can be delivered to any part of Kyushu from one of these institutions within 4 hrs via high-way bus cargo. In emergency cases, official cars of the institutions can be utilized to shorten the time to deliver drugs.

A−21 DETERMINATION OF PLASMA LEVELS OF PROSTAGLANDINS AND THROMBOXANE IN FALCIPARUM MALARIA PATIENTS IN THAILAND WITH GC/MS/SIM

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We monitored the plasma levels of prostaglandins (PGs) and thromboxane B2 (TXB2) in falciparum malaria patients in Thailand. We collected samples from 36 normal adults (NA) and 51 falciparum malaria patients before treatment (MP) and 10 falciparum malaria patients who had recovered with treatment. The plasma levels of prostanoids were measured by using simultaneous analysis of gas chromatography-mass-spectrometry-selected ion monitoring method (GC/MS/SIM). TXB2 decreased significantly ($p<0.001$) and PGF2α, 8-epi PGF2α, PGE1 and PGE2 were significantly increased in MP compared with those in NA, respectively.

A−22 DETECTION OF *PLASMODIUM VIVAX* FROM CLINICAL SPECIMENS BY THE USE OF *ICT MALARIA P. F/P. V* KIT

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The "gold standard" for both the detection and identification of the malaria pathogen, *Plasmodium* sp. is a microscopic examination of Giemsa stained blood specimens, this parasitic infection is sometimes difficult to diagnose because the physicians and medical technologists in our country are recently not very familiar with these organisms. This time, we examined the *ICT Malaria P. f/P.v* kits for identification of *Plasmodium vivax* in vivax-malaria patients' whole blood specimens because we previously reported this kit has only low positive ratio for diagnosis of vivax-malaria.

The *ICT malaria P. f/P.v* kit is a kit which has been developed to identify *P. falciparum* and *P. vivax* by means of detecting the malaria specific antigen. Our results in using this kits are as follows; (1) in the *P. vivax* positive patients' whole blood, 12 out of 19 (63.2%) were positive for *P. vivax*. (2) when specimens with very low parasitemia (infected RBC by *P. vivax* < 0.01%) were excluded from the total, the positive results for *P. vivax* was as high as 80.0% (12 out of 15). (3) we had difficulties in distinguish it's positive band when specimens with low parasitemia. In summary, this kit is considered to be useful as a supportive tool for the detection and identification *P. vivax* from whole blood specimens, but we must pay attention to its falsepositive result in clinical laboratory.

A−23 AN EVALUATION OF *ICT MALARIA P.F/P.V* IN IMPORTED MALARIA IN JAPAN

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ICT Malaria P.f/P.v (ICT) is a rapid immunochromatographic test for the detection of circulating antibodies of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) in whole blood. Two separate lines have been immobilized across a test strip. Line1 is specific for the histidine-rich protein 2 antigen (HRP 2) of Pf and line 2 is specific for a common antigen to both Pf and Pv. A test diagnosis of Pf and Pv malaria were respectively made if line 1 was positive (+) with or without line 2 and only line 2 was +. We evaluated the test for the diagnosis in the imported malaria in Japan, comparing with microscopic detection and *Para*Sight F (PF test). A total of 145 people enrolled in the study were 122 people returned from malarial country who attended or were consulted Jikei University or the University of Tokyo for about a year from August 1998 to August 1999, and 35 malaria cases (freezed blood was used) who were in hospital of Jikei University previously. Microscopically, they were 35 Pf malaria, 1 mixed infection of Pf and Pv, 30 Pv malaria, 2 *P. ovale* (Po) malaria, 1 *P. malarie* (Pm) malaria and 76 cases free from malaria parasites. ICT was sensitive (100%, 35/35) similar to PF test. Both lines were + for 27 Pf cases with relatively high parasitemia, while only line1 was + for 8. ICT was sensitive (87%, 26/30) also for Pv. Both lines were + for the mixed infection, which may reflect the antigens of 2 species.

One Po case was ICT negative (−) and the other 1 was line 2 +. The common antigen may be expressed also by Po. One Pm case was −. Three of 76 cases microscopically free from the parasites were both ICT line 1 and PF test +. They had been treated for Pf malaria or treated by herself with antimalarial for fever, and thus remaining HRP 2 after cure would be detected. Except them, ICT was specific for both Pf (100%, 106/106) and Pv (99%, 110/111, false + in 1 Po case). There were 5 cases with PF test + but ICT − who neither had history of having antimalarial nor became malaria afterwards. These PF test + must be false, and ICT probably has fewer false + than PF test. ICT is thus useful for the diagnosis of imported malaria.

A−24 A COMMUNITY-BASED MALARIA CONTROL PROGRAM IN PALAWAN, THE PHILLIPPINES

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Malaria has been considered to be one of the major health threats to every Palaweno for a long time. For years, different methods and strategies have been used in the control of malaria in Palawan Island, the Philippines, but there were problems in sustaining these efforts.

In 1992, we conducted an epidemiologic study of malaria in Barangay (village) Mangingisda, Puerto Princesa City. A unique feature of the study was the active participation of the Barangay Health Committee members in the promotion and implementation of Community-Based Ma-

laria Control (CBMC). In 1993, a community-based health program, "Alayka Palawan", became a testimony of this island's commitment to community empowerment.

In 1996, the Provincial Health Office successfully obtained support from Japan International Cooperation Agency for its implementation. Since then, many barangays have been mobilized for the control of malaria. The strategy aimed to increase the awareness of the people and partner agencies of their potential role in health care. The Barangay Health Committee and barangay health workers played an important role in its activity. They were responsible in providing appropriate information on the control and prevention of malaria through organized community action.

At last, August 10, 1999 was declared Malaria-Free Day and during which the malaria control campaign under the Governor was launched. The activities included free blood smears, lectures, poster exhibits, and displays of insecticide-impregnated mosquito nets and larvivorous fish for biological control.

Palawan is the island most severely affected by malaria in the Philippines. Thus, the CBMC activities so far constitute the inception phase for the future malaria eradication in the island. Now the world malaria control is integrated under the WHO's initiative, "Roll Back Malaria (RBM)". Soon the implementation phase in Palawan will commence under the RBM banner in the West Pacific Region. Dedicated effort has to be made through the partnership of people in Palawan Island, WHO and other agencies until they will enjoy the true freedom from malaria.

A−25 CHANGES OF THE SPLEEN RATE ON ANEITYUM ISLAND

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Vanuatu consists of 80 inhabited islands in Melanesia, with hypo- to mesoendemic malaria and suitable conditions for sustained parasite elimination. Weekly mass drug administration (MDA) of chloroquine, pyrimethamine/sulfadoxine and primaquine was conducted on the entire population of 718 inhabitants of Aneityum Island for nine weeks in 1991 prior to onset of the rainy season. Simultaneously permethrin-impregnated bednets were distributed to the entire population and have been re-impregnated yearly. Larvivorous fish were also introduced into several identified breeding sites of *Anopheles farauti.* Periodic blood surveys for the past nine years have showed complete absence of *Plasmodium falciparum* after the MDA and *P. vivax* from 1996 onwards, with the exception of two instances of imported infections (one mixed infection in 1993 and one *P. vivax* infection in 1999), suggesting that malaria can be eliminated if the intervention is conducted over the whole area of transmission and the risk of importation of malaria is controlled within a framework of community consent and participation (Kaneko *et al.,* Lancet, 2000, 356, 1560-1564).

Throughout this period we have also continued to monitor the changes of splenomegaly in children on Aneityum Island. Two additional islands of Vanuatu, one with (Malakula) and one without (Futuna) malaria transmission have been monitored for comparison. The spleen surveys were conducted for the children aged two to 12 years old. AK assessed spleen size in the recumbent or standing position according to the Hackett's method with calculation of spleen rate (SR) and average enlarged spleen (AES). Thirteen surveys were conducted in Aneityum from January 1991 to June 1999: two before, two during and nine after the intervention. Seven surveys were conducted in Malakula from February 1988 to March 1998, and two surveys were conducted in Futuna (February 1992 and July 1997).

The SRs in Aneityum (48%) and Malakula (36%) with AES of around 1.6 indicated a meso-endemic situation on both islands before the intervention. In Futuna, the SR was around 5% with AES of 1.00, although no parasitaemia was detected in the two surveys. Since 1996 the SRs in Aneityum (about 4% with AES of 1.00) have been comparable to those in Futuna without malaria transmission. In Malakula, the SRs oscillated between 30% and 50%. The observed changes of SRs well correlated with those of parasite rates. Spleen survey is a simple and useful method to estimate the geographical differences and the chronological changes of malaria endemicity in given localities with or

without of control activities. It is necessary to train local health workers to standardise the method of spleen examination.

A−26 ACTIVE CASE DETECTION SURVEYS ON MALARIA IN RECENT LAOS, WITH SPECIAL REFERENCE TO THE COMPARATIVE EFFICACY OF GIEMSA STAINING AND DIPSTICK METHOD ON DETECTION OF MALARIA INFECTION

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Lao PDR has been known to be a seriously endemic country for malaria in Southeast Asia. The exact prevalence of malaria in Laos, however, is not well known because the surveillance system to obtain actual prevalence of malaria among the inhabitants has insufficiently functioned. An extensive surveys to obtain exact information on malaria prevalence, therefore, has been desired for future malaria control not only in Laos but also in the neighboring countries. Since 1995, the authors have conducted active case detection surveys on malaria among the villagers in a southern province, Khammouane Province, and the results obtained in 1999 were reported in the presentation.

The surveys were conducted in 20 villages where the estimated total population was 5,857. A total of 3,597, accounting for 61.4% of total population, were examined for malaria infection by microscopical examination using Giemsa-stained blood smears and by Dipstick method using ICT malaria kit (AMRAD Australia) to detect *P. falciparum* antigen in peripheral blood. A part of blood samples collected on filter papers were also examined by the nested-PCR method to evaluate the efficacy of the two methods. The malaria infection was detected in all villages surveyed and the positive rates by Giemsa staining in 18 villages ranged from 8.0 to 58.2% (mean=23.8%). *P. falciparum* malaria occupied 87.8% of malaria species detected and the positive rates of P.f. were 7.5-57.7% (mean=21.3%). On the other hand, the mean positive rates of P.f. by Dipstick method in the 20 villages were 22.3%, ranging from 8.5% to 64.5%. The positive rates were generally higher by Dipstick method than Giemsa staining, but the difference was consistently small. Among the samples, 136 (3.8%) were false-negative in Dipstick method, in which positive results were obtained only in Giemsa staining. On the other hand, 5.4% of the samples gave positive results only in Dipstick method. When the samples positive only by Dipstick method were further examined by the PCR method, the number of samples in which malaria infection could not be confirmed even by the PCR method were extremely few in the present study. These results indicate that malaria infection is seriously prevalent among the inhabitants in southern Laos and Dipstick method may be a useful tool for rapid diagnosis of malaria infection in active case detection survey.

A−27 MOLECULAR EPIDEMIOLOGICAL EVALUATION OF PYRIMETHAMINE/SULFADOXINE EFFICACY IN *PLASMODIUM FALCIPARUM* PATIENTS FROM THE VANUATU ARCHIPLAGO

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Plasmodium falciparum resistance to cycloguanil and pyrimethamine has been associated with specific point mutations in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes. Vanuatu consists of 80 islands in Melanesia with seasonal and unstable malaria endemicity. The first-line drug for malaria treatment in children aged less than five years old was changed in 1991 from chloroquine to pyrimethamine/sulfadoxine (PS). We have studied *in vivo* drug susceptibility of 40 *P. falciparum* patients to PS on two islands of Vanuatu (Gaua, Santo) and also investigated the distribution patterns of the *dhfr* and *dhps* genotypes in 140 *P. falciparum* infections on 4 islands (Gaua, Santo, Pentecost and Malakula) by direct sequencing. A total of 137 out of 140 *P. falciparum* infections had the same mutations at codon 108 (Ser→Asn) and at codon 59 (Cys→Arg) in *dhfr*. The remaining three isolates in Gaua had the same two mutations, a change not previously reported in the literature at codon 51 (Asn→His) and another at codon 108 (Ser→Asn). In contrast to *dhfr*, no isolate had a mutation in the *dhps* gene. Although all 40 isolates had mutations in the *dhfr* gene at codon 108 (Ser→Asn) and at codon 59 (Cys→Arg), *in vivo*, 39/40 patients responded well to single dose of PS. This is in contrast to many *in vitro* studies which have demonstrated that isolates with mutations in the *dhfr* gene at codon 108 (Ser→Asn) and at codon 59 (Cys→Arg) are normally associated with moderate resistance to *dhfr*-inhibiting drugs. Thus, our *in vivo* study in Vanuatu clearly demonstrates the clinical efficacy of PS on *P. falciparum* infections with the putatively resistant *dhfr* gene. The reason for the discrepancy between *in vitro* and *in vivo* studies is as yet unclear, however, it is consistent with acquired immunity to host malaria parasite infection.

A−28 POPULATION GENETICS AND EPIDEMIOLOGY: MALARIA IN VANUATU

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The correlated patterns of mutations within populations and disease endemicity serve to implicate genes involved in immunity and resistance to pathogens. In addition, population founder events and gene flow biased by proximity or cultural factors can also influence the distributions of alleles within populations. Here we compare the distributions of alleles implicated in malarial resistance [glucose-6-phosphate dehydrogenase (G6PD)] and variable drug metabolism [cytochrome P450 (CYP) 2C19 (CYP2C19)] with neutral loci [mitochondrial DNA (mtDNA) and autosomal short tandem repeats (STRs)], geographic proximity, and linguistic relationships within Vanuatu to evaluate the contributions of disease selection and gene flow to patterns of diversity. Our analyses indicate that malarial selection has shaped the frequencies of G6PD deficiency within the archipelago. In contrast, distributions of CYP2C19 alleles involved in the metabolism of a wide range of pharmacologically important compounds are correlated with geography, but not malarial prevalence or neutral autosomal genetic variation. These data suggest CYP2C19 has been regionally selected. Our comparisons of functional and neutral genetic loci provide an unambiguous demonstration of genetic selection.

A−29 REPETITIVE DOSING OF ARTEMISININ AND QUININE AGAINST *PLASMODIUM FALCIPARUM IN VITRO*: A SIMULATION OF THE *IN VIVO* PHARMACOKINETICS

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Plasmodium falciparum (F32) parasites were exposed to artemisinin and quinine for 3 and 4 hr respectively, once or twice daily for 3, 5 or 7 days. Between the peaks the parasites were exposed to through concentrations. Continuous drug exposure was also assessed for comparison. After drug exposure, the cultures were extended for an observation period of up to 30 days to assess the viability of the parasites remaining after the drug exposure. For artemisinin, a critical threshold concentration of 3×10^{-8} M was required for growth inhibition. Dosing twice daily for at least 5 days was also critical. Prolonging the duration of drug exposure to 7 days further increased the efficacy. For quinine the results were quite different. The concentration dependency of the efficacy was more gradual. On the other hand, dosing once daily appeared to be nearly as effective as twice daily and radical clearance was obtained even after 3 days of exposure at peak concentrations of 10^{-5} M. A concentration of 10^{-6} M provided the same effect if the duration was extended to 7 days. There was a strong similarity between estimated concentrations of free unbound drug required for radical clearance *in vitro* and those empirically required for clinical efficacy *in vivo*. This suggests that the *in vitro* model represents an appropriate model for estimating drug efficacy and pharmacodynamics if the *in vitro* system is adapted to simulate the *in vivo* pharmacokinetics.

A−30 GENE ANALYSIS OF PFMDR 1 IN MEFLOQUINE-RESISTANT *PLASMODIUM FALCIPARUM*

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Although mefloquine-resistance in *Plasmodium falciparum* is increasing, the molecular mechanism remains unclear. Recent reports have shown that the Asn-86, Ser-1034, Asn-1042 and Asp-1246 alleles of the pfmdr 1 gene of *P. falciparum* is associated with decreased sensitivity of the anti-malarials mefloquine.

To investigate the association between drug sensitivity and sequence polymorphisms in the pfmdr 1 gene, first we tryed to get mefloquine-resistante parasite. Isolate was obtained from mefloquine resistant patient (523a strain). The 523a strain was cultured under mefloquine pressure, after two years, we succeed to get the parasite which can grow in 1.5×10^{-7} M mefloquine concentration (523a R strain). The parasite was cultured under the same condition without drug as control (523a S strain). Second, using these strains we measured the half-maximal inhibitiory concentration

(IC_{50}) for several anti-malarials. As a result, in 523a S strain the IC_{50} value of mefloquine is 6.1×10^{-9} M, in 523a R strain, is 6.2×10^{-8} M. The 523a R strain have about 10 times activity than the 253a S strain. Moreover, 523a R strain increased resistance to artemisinin and halofantrine and increased sensitivity to chloroquine. Third, we analyzed amino acid sequence by Dye terminator method after particular domain amplyfied by PCR. Both strains have Tyr-86, Ser-1034, Asn-1042 and Asp-1246 of the pfmdr 1 gene. There is no change between 523a R and 523a S. The FCR-3 also have the same sequences.

In conclusion, although the IC_{50} value of mefloquine is higher in 523a R strain than in 523a S strain, but amino acid sequences are not differrent. 523a R strain, we get in this study, is not associated with drug resistance and sequence polymorphisms in pfmdr 1 gene. We think that perhaps another mechanism work for drug-resistance.

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We wish to thank Dr. Mikio Kimura and Dr. Takahisa Furuta of University of Tokyo for offering 523a strain to us.

A−31 MECHANISMS OF PARASITICIDAL ACTIVITY OF TETRACYCLINES ON *PLASMODIUM FALCIPARUM*: A POSSIBILITY OF THE PLASMID AS THE DRUG TARGET

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A plastid-like organelle, apicoplast, was recently discovered in the protozoan parasites of the phylum Apicomplexa, including *Plasmodium.* The plastids of *P. falciparum* carries a 35-kb circular genome, which encodes large and small subunit rRNAs, tRNAs, ribosomal proteins, subunits of a eubacterial RNA polymerase, a translation elongation factor and other open reading frames. The fact that the plastid contains its own RNAs and prokaryotic proteins associated with transcription and translation suggests an essential role of the plastid for malaria parasite survival, although the function of the plastid remains unknown. The prokaryotic feature of the plastid also makes this organelle a target for drug development.

We previously reported that tetracycline and minocycline showed a delayed death activity on *P. falciparum in vitro.* In the present study, we examined effects of these antibiotics on the plastid activity of the parasites. We used an assay of plastid-encoded RNA polymerase mRNA levels by reverse transcriptase-polymerase chain reaction (RT-PCR) (McConkey *et al.,* J. Biol. Chem., 272, 1997), because of the absence of a direct measurement system for the protein synthesis in the plastid. Since the plastid DNA contains three subunits of a eubacterial RNA polymerase genes, such as rpoB, rpoC1 and rpoC2 and these genes are polycistronically transcribed, the synthesis of the rpoB/C mRNA was compared to a nuclear-encoded merozoite surface antigen (MAS1) mRNA. As a control, a nuclear-encoded small subunit rRNA (ssu rRNA) was also amplified because the ssu rRNA synthesis is unaffected by antibiotics. The rpoB/C RT-PCR products was remarkably decreased by the treatment with tetracycline at 100 mg/m*l* for 14 hr, but not for 8 hr. Minocycline at 60 mg/m*l* had a similar effect on the decay of the mRNA. A similar effect was also observed with the treatment with thiostrepton and ripampin, which appears to be a selective inhibitor of plastid protein synthesis and an inhibitor of prokaryotic RNA polymerase, respectively. However, there is no effect of these antibiotics on the levels of nuclear-encoded MSA1 mRNA nor ssu rRNA.

These results suggest that tetracycline antibiotics may inhibit the protein synthesis in the plastid, leading the inhibition of transcriptional activity of the organelle, although tetracycline might target mitochondorial protein synthesis.

A−32 ANTIMALARIAL ACTIVITY AND POTENT ENHANCEMENT OF THE SENSITIVITY OF *PLASMODIUM FALCIPARUM* TO CHLOROQUIN BY THE BISBENZYLISOQUINOLINE ALKALOID CEPHARANTHIN

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Cepharanthin is an extract of *Stephania cepharantha,* widely used in Japan for clinical use. Major components of Cepharanthin (CP) are Isotetrandrin (IT), Cepharanthine (CE), Berbamine (BE), Homoaromoline (HO), Cepharanoline (CO) and Cycleanine (CY). We investigated antimalarial activity of CP and component alkaloids with chloroquine resistant strain K1. And also enhancement of the sensitivity to chloroquine was determined in resistant and sensitive strains of *Plasmodium falciparum* with CP and components. CP and component alkaloids exhibited moderate antimalarial activity, with IC 50 in the range of 170 to 3,700 nM (CP 338, IT170, CE1,020, BE485, HO1,490, CO1,015 and CY 3,700 nM respectively). CP enhanced the activity of chloroquine against resistant clones by a factor of 15 at a concentration of only 200 nM (120 ng/m*l*). It is 50 times more potent than verapamil, the standard resistance modulator, and 3 times more potent than the sum of its individual alkaloids. Combination of component alkaloids acted synergistically to sensitize the parasite to chlorquine, possibly explaining the enhanced potency of CP. CP differed from verapamil in that it further sensitized clones that are considered to be fully susceptible, improving the baseline activity of chloroquine. Potent sensitization of parasites to chloroquine *in vitro* coupled with low toxicity suggests that coadministration of CP might extend the clinical utility of chloroquine.

A−33 THE DEVELOPMENT OF NEW ANTIMALARIAL DRUGS- *IN VITRO* AND *IN VIVO* ANTIMALARIAL ACTIVITY OF ENDOPEROXIDES

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A number of medicines such as chloroquine and quinine are available for treatment of malaria, but the rapid development of drug resistance are a serious problem. Medicinal agents based on novel mechanisms of action are, therefore, required to overcome emergence of resistance and to control an ever-increasing number of epidemics caused by the malaria parasite.

Now, we have confirmed the potential of 1, 2, 4, 5-tetraoxacycloalkanes as a new class of simple peroxide antimalarial drugs. In the preliminary study, some 1, 2, 4, 5-tetraoxacycloalkanes have been found to be available for chloroquine-sensitive and -resistant *P. falciparum in vitro.* Especially, 1, 2, 6, 7-tetraoxaspiro [7.11]nonadecane (N-89) has a potent antimalarial activity for *P. falciparum* (FCR-3 strain) that the IC_{50} value is 2.5×10^{-8} M and (KI strain) is 2.6×10^{-8} M, in contrast, the 50% inhibitory concentration of against mouse mammary FM3A cells was 8.0×10^{-6} M, demonstrating that the selective toxicity (>300) was also remarkable. It should be noticed that these compounds are similar effective of artemisinin (the IC_{50}'s against *P. falciparum* and FM3A cells were 1.8×10^{-8} M and 1.0×10^{-5} M, respectively). An *in vitro* test, morphological changes of malaria parasites during the treatment of N-89 against *P. falciparum* were studied. In these results, we have observed a condensation of parasite nuclei, shrinked cytosol in trophozoites and inhibition of schizont formation by microscopy after treatment with 100 times the EC_{90} value of N-89 (2×10^{-6} M) for 24 hr. The morphological changes in parasites induced by N-89 may be a consequence of several phenomena including difference in antimalarial activity *in vivo.*

In addition, we investigated the *in vivo* antimalarial activities of N-89 by 4-day suppressive test. The ED_{50} value of the compound has shown that 12 mg/kg/day (ip), which was required to cause 50% suppression of *P. berghei* in mice. In the experiments, the dose of 50 mg/kg/day of N-89 causes cure (for 4 mice in 5 tested mice), in which malaria parasites were not observed in circulating blood after 60 days. Furthermore, no side effects such as diarrhea, body weight loss and mortality were observed during treatment with N-89 at doses by 1,600 mg/kg (ip). As a control, mice treated with artemisinin (50 mg/kg/day, ip) were not cured and mice died due to *P. berghei* infection. Next, the effect of treatment of N-89 for three consecutive days *in vivo* was assayed. Parasitemia declined have been found within 24 hr after drug administration. We used 5 mice for this test, 4 mice have been cured and only 1 mouse was died. On the other hand, in treatment with artemisinin, parasitemia declined have been found after 24 hr of drug administration, but after 72 hr after drug administration, parasitemia become increased again. Our studies indicate that N-89 has a strong and continuous antimalarial activity.

Our results may be helpful in the design of chemotherapeutic 1, 2, 4, 5-tetraoxacycloalkanes in the worldwide fight against malaria.

A−34 A POTENT ANTIMALARIAL ACTIVITY OF HOT-WATER EXTRACT OF *HYDRANGEA MACROPHYILA* VAR. *OTAKSA* LEAVES AGAINST *PLASMODIUM YOELII* 17XL IN ICR MICE

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Malaria is still one of the major health problems in the world. The emergence and spread of chloroquine-resistant *Plasmodium falciparum* has stimulated the development of new effective treatments against malaria. There is now a new interest in investigation of folk medicine exhibiting a potent antimalarial activity. We investigated experimentally an antimalarial activity of *Hydrangea macrophylla* var. *Otaksa* with rodent malaria models. Leaves of this plant were collected in the campus of Hamamatsu University School of Medicine in August, 1999. Five gram of air-dried leaves put in a herbal bag was added with 500 m*l* of distilled water and boiled. After removing the leaves the extract fluid was finally concentrated to 50 m*l*. Hot-water extract was stored at 4℃ unitl use. Outbred male ICR mice, 8 weeks old, were infected intraperitoneally with 1×10^5 *P. yoelii* 17XL-parasitized erythroytes. Starting on day 3 after injection of parasitized erythrocytes, mice were orally given a hot-water extract at 75 μ*l*/10 g body weight in the treated group and distilled water in the untreated, infected one, respectively, twice a day for 5 consecutive days. Non-treated control mice died with a gradual body weight loss and a rapid increase of parasitemia in the bloodstream from 6 to 7 after infection, but mice given the leaf extract survived during the experiment. Mice treated with leaf extract showed low parasitemia levels during administration. Following a transient recrudescence of malaria parasites in the bloodstream of the treated mice, no parasites could be detected by a microscopic examination. Leaf extract seems to have 2 different types of effects on rodent malaria parasite, *P. yoelii* 17XL, *in vivo*.

A−35 FRACTIONATION OF ANTIMALARIAL PRINCIPLE FROM *HYDRANGEA MACROPHYLLA* VAR. *OTAKSA* LEAVES AND ITS ACTIVITY AGAINST *PLASMODIUM YOELII* 17XL IN ICR MICE

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Malaria is still one of the most important tropical diseases in the world. One of the reasons is the emergence of drug resistant parasites. Thus it is important to develop new antimalarials quickly. In 1972 artemisinin was isolated from the herb *Artemisia annua* L. used in the traditional Chinese medicine. Today artemisinin and its derivatives have become one of the antimalarials for severe malaria. We have already reported that the hot-water extract of *Hydrangea macrophylla* var. *Otaksa* leaves had a high *in vivo* antimalarial activity against *Plasmodium yoelii* 17XL in mice. In this study we investigated the antimalarial activity of the fractions isolated from the hot-water extract. Four different fractions were prepared from the hot-water extract of the leaves in the usual manner for obtaining alkaloid fractions. Male ICR mice, 8 weeks old, were infected intraperitoneally with 1×10^5 *P. yoelii* 17XL-parasitized erythrocytes. Starting on day 3 after injection of parasitized erythrocytes, mice were orally treated twice a day for 5 consecutive days with the hot-water extract (5 g leaves/50 m*l*) or each fraction at two dosages equivalent to 10 g leaves/50 m*l* and 100 g leaves/50 m*l* of the hot-water extract. Only the less polar alkaloid fraction at higher dosage showed the same antimalarial activity as the hot-water extract (5 g leaves/50 m*l*) showed, though other fractions had no antimalarial activity. The NMR spectra of the less polar alkaloid fraction showed that the signal patterns and chemical shifts of compounds in the fraction were closely similar to those of febrifugine and isofebrifugine. However, the fraction was 20 times less active than the hot-water extract.

A−36 A POTENT ANTIMALARIAL ACTIVITY OF HOT-WATER EXTRACTS OF PLANTS BELONGING TO THE FAMILY *SAXIRAGACEAE* AGAINST *PLASMODIUM YOELII* 17XL IN ICR MICE

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We showed that the hot-water extract of *Hydrangea macrophylla* var. *Otaksa* leaves had a high *in vivo* antimalarial activity against *Plasmodium yoelii* 17XL. It is therefore of interest to evaluate the antimalarial activity of related plants of the Family Saxifragaceae from a practical viewpoint of folk medicines. The plants specimens such as *H. macrophylla, H. macrophylla* subsp. *serrata* var. *acuminata, H. involucrata, H. hirta, H. paniculata, Cardiandra alternifolia* and *Clerodendron trichotonmum* were evaluated. Five gram of air-dried leaves or 20 g of air-dried roots put in a herbal bag was added with 500 m*l* of distilled water and boiled. After removing the materials the extract fluid was finally concentrated to 50 m*l*. Hot-water extract was stored at 4℃ until use. Outbred male ICR mice, 8 weeks old, were infected intraperitoneally with 1×10^5 *P. yoelii* 17XL-parasitized erythrocytes. Starting on day 3 after injection of parasitized erythrocytes, mice were orally given a hot-water extract at 75 μ*l*/10 g body weight in the treated group and distilled water in the untreated, infected one, respectively, twice a day for 5 consecutive days. The extract of *H. macrophylla* leaves showed an antimalarial activity, although other extracts showed no effects. Mice

treated with leaf extract of *H. macrophylla* showed low parasitemia levels during administration. Following a transient recrudescence of malaria parasites in the bloodstream of the treated mice, no parasites could be detected by a microscopic examination. Furthermore, the leaf extract of *H. macrophylla* showed a higher antimalarial activity than that of *H. macrophylla* var. *Otaksa* in respect of suppression of malaria parasite increase at a recrudescence and of mouse body weight loss.

A−37 AN ANTIMALARIAL ACTIVITY OF HOT-WATER EXTRACT OF *DICHROA FEBRIFUGA* LEAVES OR ROOTS AGAINST *PLASMODIUM YOELII* 17XL IN ICR MICE

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We showed that the hot-water extract of *Hydrangea macrophylla* var. *Otaksa* leaves had a high *in vivo* antimalarial activity against *Plasmodium yoelii* 17XL though the extract of roots had no activity. Febrifugine and isofebrifugine having antimalarial activity were isolated from the roots of *Dichroa febrifuga*. It is therefore of interest to examine these principles within leaves of *D. febrifuga*. Old leaves and roots of *D. febrifuga* and young leaves were collected in December, 1999, and in June, 2000, respectively. Five gram of air-dried leaves or air-dried roots put in a herbal bag was added with 500 m*l* of distilled water and boiled. After removing the material the extract fluid was finally concentrated to 50 m*l*. Hot-water extract was stored at 4℃ until use. Outbred male ICR mice, 8 weeks old, were infected intraperitoneally with 1×10^5 *P. yoelii* 17XL-parasitized erythrocytes. Starting on day 3 after injection of parasitized erythrocytes, mice were orally given a hot-water extract at 75 μ*l*/10 g body weight in the treated group and distilled water in the control, respectively, twice a day for 5 consecutive days. Mice in the non-treated control and group given the old leaf extract (5 g/50 m*l*) died with a gradual body weight loss and a rapid increase of parasitemia in the bloodstream. Root extract (5 g/50 m*l*) showed an antimalarial activity. All mice and 50% of mice given the youg leaf extract at a dose of 5 g/50 m*l* and of 2.5 g/50 m*l*, respectively, died without multiplication of malaria parasite and thus the death seemed due to the adverse effects of extract. But mice given young leaf extract at a dose of 1.25 g/50 m*l* survived during the experiment. Present results suggested the seasonal changes of alkaloids' content within leaves and also indicated the important ideas for material collection.

B−1 THREE CASES OF CREEPING DISEASE DUE TO LARVAL HOOKWORM INFECTION

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Larvae of the dog and cat hookworms, *Ancylostoma caninum* and *A. brasiliensis* are known to cause creeping disease. Recently three cases of creeping disease due to larval hookworm infection on abroad were referred to our laboratory. Two of them were young women (twenties) traveled Thailand or Malaysia for several weeks. They walked around the bush/seashore on bare feet or lay on the beach. Creeping disease developed shortly after outdoor episodes. The remainder was an 1-year-old Cambodian boy adopted an American living in Japan. He had a history of creeping disease just before coming to Japan. The disease reappeared in January 2000. Skin biopsy was performed on all three cases, but sections of worms morphologically determined *Ancylostoma* spp. larvae were found in the superficial skin epidermis of only one case. In other cases, just small tunnel with eosinophil infiltration in epidermis layer considered to warm trail was found in the sections.

Although *Gnathostoma* sp. and *Spirurina* larvae are famous causative spices of the creeping disease in Japan, we should be aware of animal hookworm larvae cause the disease. Especially the patient developed a creeping disease with rapid onset after outdoor episodes on abroad.

B−2 A CASE OF URINARY SCHISTOSOMIASIS WITH MACROHEMATURIA

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A 27-year-old Japanese man admissioned our hospital with dyspnea after six-month journey to Africa. In the biginning he was treated by the diagnosis of the eosinophilla. He had been presented to the Department of Urology, because of macrohematuria.

Several eggs of schistosomiasis were seen in urinalysis and many white protuberances were also seen on the mucous membrane of urinary bladder. We diagnosed this patient as a schistosomiasis haematobium by the urinalysis and his typical clinical progress.

He was treated with insectfuge for two days, and macrohematuria disappeared three days after this therapy. After one month of initial therapy, no eggs of schistosomiasis were presented in urinalysis but cystoscopy showed several irregular changes on mucous membrane of urinary bladder. We biopsied some of those through the cystoscope and found the eggs of schistosomiasis under the mucous membrane but no malignant changes were seen histologically.

Some of urinary bladder carcinoma related to this disease were reported in several papers, so we try to follow up this patient very carefully.

B—3 FOUR IMPORTED CYSTIC ECHINOCOCCOSIS CASES IN JAPAN CONFIRMED SEROLOGICALLY

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From 1st April 1999, echinococcosis, either alveolar (AE) or cystic (CE) is included in the parasitic diseases in category 4 of the New Law of Prevention of Infectious Diseases in Japan. Two AE cases were reported from Honshu (the main island) based on or referred to the ongoing serodiagnosis carried out at the Hokkaido Institute of Public Health. However, one was fascioliasis, the other was CE but not AE. However, mass-media reported that the killer parasite had already invaded into Honshu Island based on these misdiagnosed cases. So far, there is no official message that these two were not AE even from the Project group on the control of AE in Japan sponsored by Ministry of Health and Welfare, Japan. Under the new law, AE should be confirmed after surgical resection of the lesions. Asahikawa group has already established differential serodiagnosis without surgical confirmation for zoonotic cestodiases including AE, CE and neurocysticercosis, highly appreciated internationally.

Last four years (1997-2000), we experienced four CE cases in Japan: one Nepali, one Jordanian, one Chinese and one Japanese. These all were serologically easily confirmed to be CE when we carried out immunoblot analysis using crude cyst fluid and purified antigen B subunit (8 kDa) of *Echinococcus granulosus* and crude and purified Em18, specific to AE. These all cases recognized the antigen B subunit without any response to Em18 and were concluded to be CE serologically. After serological analysis, we asked the clinicians to show us the image data. The image data from US and CT also showed typical patterns for CE. However, CE is absolutely uncommon in Japan and almost no clinicians have any experience to observe such image data before. It is pointed out that CE with very unique image data is not indigenous in Japan but imported cases should be more common in the future. Curative treatment for these three zoonotic cestodiases differs critically. Therefore, it is essential and urgent to establish better resolution for confirmation serodiagnosis before surgical treatment. We stress that it is easy to obtain critical diagnosis to differentiate these three diseases from other parasitic or non-parasitic diseases and we better shift to introduce a confirmation serodiagnosis of AE, CE and NCC using more reliable antigens and technology without surgery.

B—4 A SEVERE CASE OF VENOMOUS OPHATHALMIA CAUSED BY SPITTING COBRA IN CENTRAL KALAHARI

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We researched about health status of the Central Kalahari Bushman (Gwi and Gana Bushmen) in Republic of Botswana. In this research, a first case of venomous of ophathalmia caused by spitting cobra was experienced. Sixty-five-year old male, whose job is leather processing, lived in New Xade (Ghantsi District, Botswana). He had been sputtered at both eyes, in March 1975 and had intense eyes pain, eye mucous and palpebral edema, and when he had attacked, he could not wash his eyes because it was a dry season, and then he lost his eyesight soon.

At the latter, he consulted an ophathalmologist of general hospital at Gaborone (the capital of Botswana), but he could not recovered. His physical signs were as follows: The pupils were symmetric and their diameters were about 3 mm and their conjunctivas were without jaundice and anemia. The light reflection of these pupils was not found. And other physical signs were almost nothing particular.

In conclusion, according to Branch (1998), several species of venomous snakes are known in southen part of Africa. Especially, the venomous snakes, which can sputer

the venom, are Mozambique Spitting Cobra (*Naja mossambica*) and Black-necked Spitting Cobra (*N. nigricollis*). But, in the Central part of Kalahari Desert in Botswana, these cobras have not been investigated, yet. In this research, we collected the materials of the spitting cobra in the Central Kalahari, black color Spitting Cobra is not found but brown Spitting Cobra was sometime seen in this area. Therefore, we thought that this case was attacked Mozambique Spitting Cobra. Thus this is a first report that the case of visual disorder by spitting cobra stays in the Central Kalahari.

B−5 DIAGNOSIS OF KALA-AZAR BY ELISA USING URINE SAMPLES

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For the diagnosis of visceral leishmaniasis (kala-azar), detection of the causative parasite is the most accurate method. Detecting anti-*Leishmania donovani* antibodies in serum samples is an alternative method. In this study, we developed a diagnostic method to detect anti-*L. donovani* IgG in urine samples. Anti-*L. donovani* IgG, IgA, IgM, and total immunoglobulins (total Igs; IgG, IgM and IgA) in serum and urine samples were measured with ELISA. Among 48 urine samples from kala-azar patients, 43 (90%) or 41 (85%) samples were judged positive with IgG or total Igs ELISA respectively, using cut off points obtained with Japanese samples (mean+3SD). Anti-*L. donovani* IgM and IgA titers in urine from kala-azar patients were very low and the sensitivities were 67% and 38%, respectively. All urine samples from healthy people in Bangladesh were negative. IgG ELISA using serum samples gave 29 positive results (88%) out of 33 kala-azar patients. The IgG ELISA with urine samples was similar in sensitivity. As collection of urine is much easier than serum, this method will be useful for epidemiological studies and laboratory diagnosis of patients.

B−6 EVALUATION OF DOT-ELISA FOR THE IMMUNODIAGNOSIS OF TREMATODE INFECTIONS

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In recent years, dot-ELISA has been used for the immunodiagnosis of parasitic diseases. However, the cross-reactivity caused by the crude antigens used has not been examined sufficiently. We have already reported that an improved dot-ELISA is very useful for the immunodiagnosis of Japanese paragonimiases, amoebiasis and human toxo-

cariasis. In the present study, a total of 58 serum samples from patients infected with 4 different trematodes, *Paragonimus* sp., *Fasciola* sp., *Schistosoma japonicum* and *S. mansoni*, were examined. One microgram of crude antigens extracted from *S. japonicum*, *S. mansoni*, *P. miyazakii*, *P. westermani*, *Fasciola* sp. and *Clonorchis sinensis* adult worms were absorbed onto a nitrocellulose membrane. In addition, *S. japonicum* egg antigen was also absorbed to compare the reactivity with *S. japonicum* adult antigen.

Clear dark purplish spots against *P. miyazakii* and *P. westermani* antigens were recognized in all 15 cases of imported paragonimiasis. No big difference in the spot intensity of both *Paragonimus* antigens was recognized at 1: 200 serum dilution. The cross-reactions against other trematode antigens were less than those by ELISA. Therefore, dot-ELISA is useful for the differential diagnosis between paragonimiasis and other respiratory diseases. It is not always necessary to decide the endemic *Paragonimus* species, because various kinds of *Paragonimus* exist in China and Southeast Asia. Similar results were obtained for 14 schistosomiasis japonica and 12 schistosomiasis mansoni. Clear spots were recognized against *S. japonicum* egg, *S. japonicum* adult and/or *S. mansoni* antigens. *S. japonicum* adult antigen is also available for the immunodiagnosis of schistosomiasis japonica, although egg antigen has a higher specificity than the adult antigen. 8 out of 17 fascioliasis showed strong cross reactions against *P. miyazakii* and *P. westermani* antigens. In these cases, ELISA or other serological tests are desirable to obtain a definitive diagnosis.

Our results suggest that the dot-ELISA is very useful for the immunodiagnosis of imported trematode infections. Further investigations will be needed for the immunodiagnosis of nematode and cestode infections by dot-ELISA, as well as trematode infections.

B—7 A SERO-IMMUNOLOGICAL STUDIES ON CYSTICERCOSIS

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Immunoserological study of cysticercosis carried out on sera from patients, healthy chinese, endemic inhabitant and non-endemic inhabitant, using the enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunotransfer blot assay (EITB) in Jilin Province, China.

For the evaluate of suitable antigen, we prepared the 2 materials designated as cyst fluid (CF) and whole cyst (WC) obtained from pig. A total 450 sera, including 134 healthy sera, 86 patients sera, 115 non-endemic inhabitants, 95 endemic inhabitants sera obtained from Changchung in China. In patients (clinically suspected) sera, 82 (95.4%) out of 86 were positive. On the other hand, 4 (3.0%) of 134 healthy chinese sera showed positive. Sixteen (13.9%) out of 115 sera from non-endemic area were positive. On the other, 33 (34.7%) of 95 sera of endemic area showed positive. In comparison with ELISA value among sera from healthy chinese, non-endemic and endemic area inhabitants, clealy significant difference were observed.

For the detection of specific antigen by immunoblot assay, we compared with healthy inhabitant, patient and endemic inhabitant sera which obserbed positive reaction on ELISA. Among the sera from patient or endemic inhabitant, some of the bands were recognized on the molecular weight 15.5, 17.5, 18.5, 20, 22, 26.5, 27, 28, 32, 35, 37, 40, 48, 56, 62, 82, and 97 kDa of WC and CF antigens. On the other hand, the few of healthy inhabitant sera were very thinly band recognized on molecular weight 48 and 97 kDa of WC and CF antigens. We can't find specific antigens which diagnosis for cysticercosis until now. But we will tray with our vest to find the useful ELISA antigen fractions for the diagnosis of cysticercosis.

B—8 USEFULNESS OF EM18-ELISA IN MONITORING EFFECT OF REMEDIES AGAINST ALVEOLAR ECHINOCOCCOSIS: CASE REPORT ON LONG TERM CHEMOTHERAPY WITH ALBENDAZOLE

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We have reviewed the correlation between clinical courses and immunological responses in two patients with alveolar echinococcosis (AE) involving the liver, the lungs and the bone. We operated on both of the patients for the bone AE and have administered Albendazole for several years. Case report: A 39-year-old woman from Kitami City in Hokkaido was admitted to Asahikawa Medical College Hospital in August 1989. She had suppurated discharge through a fistula on the right hip with a dull pain. Around September 1987, she only had a dull pain but later she felt a subcutaneous nodular lesion. The lesion was about the size of her fist without bulge or hyperemia by March 1988.

Percutaneous cutting from a softened part of the lesion was perfomed at Kitami Red Cross Hospital in April 1988. Persistive pyorrhea followed the treatment, and she then underwent curettage against suppurative osteomyelitis in the right iliac bone three months later. After the total clinical investigation, she was diagnosed as having tuberculous osteomyelitis, but anti-tuberculotics had no effect. The second curettage performed in November 1988, did not heal the wound and severe pain brought her to Asahikawa Medical College. We detected not only the specific tissue fragments of AE in a tissue specimen obtained in the previous surgery but also multiple findings of hepatic AE by ultrasonography (US). Physically she looked well except for the discharging fistula on the right hip. We finally diagnosed the illness as AE involving the right iliac bone, the liver, and the lungs. We started chemotherapy with Albendazole on October 18 as radical surgery was thought to be difficult to perform. We felt the effectiveness of the chemotherapy was confirmed because the intractable discharge stopped 6 weeks after the administration of Albendazole. The third operation was perfomed on the right iliac AE on December 19, 1989. Since that time, she has undergone surgical treatment twice against recurrent development of the iliac AE. However, we have detected no remarkable progression in the hepatic and the pulmonary AE for the last eleven years. We have made regular examinations through the use of US, CT, MRI, common laboratory tests and Em 18-ELISA. Movement of Em 18-ELISA value and clinical activity of AE, e.g. recurrence of iliac focus have almost correlated with each other. We will make another case presentation concerning the correlation between the course of AE and Em 18-ELISA value.

Conclusions: 1. Albendazole has been effective for both hepatic and pulmonary AE, whereas, the bone foci have not necessarily been suppressed to full satisfaction. 2. Em 18-ELISA is a useful indicator to evaluate the activity of AE that may be difficult to detect using common laboratory tests or imaging devices. 3. Em 18-ELISA may be applicable not only to detect residual AE after surgery or a recurrence but also, to help make decisions for treatment or a complete cure.

B−9 EFFECT OF CYTOCHALASIN D ON THE GROWTH, ENCYSTATION AND MULTINUCLEATION OF *ENTAMOEBA INVADENS*

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The effect of cytochalasin D, a specific inhibitor of microfilaments, on the growth, encystation, and multinucleation of *Entamoeba invadens* was examined. Cytochalasin D blocked the growth of axenic *E. invadens* strain IP-1 in a dose-dependent manner, which suggests that the drug is effective against this species of *Entamoeba* as well as against *E. histolytica* strain HM-1: IMSS as previously demonstrated. Encystation of *E. invadens* as induced *in vitro* was also inhibited by cytochalasin D. This is the first evidence of the participation of microfilaments in the encystation process.

Concentrations of cytochalasin D effective for the inhibition of encystation were lower than those effective for the inhibition of growth. Trophozoites grown with cytochalasin D became multinucleate; more than three nuclei per cell were observed in 71% of trophozoites grown in the presence of the drug as opposed to only 5% of those grown in the absence of the drug. Also, trophozoites grown with cytochalasin D produced multinucleate cysts following their transfer to encystation medium. Encystation with cytochalasin D was more strongly inhibited among trophozoites grown in the presence of the drug than among those grown in the absence of the drug. Also, encystation without cytochalasin D was less frequently observed among trophozoites grown in the presence of the drug than among those grown in the absence of the drug. Thus, the multinucleation of trophozoites induced by cytochalasin D had an inhibitory effect on their encystation.

B−10 INHIBITION OF ENCYSTATION OF *ENTAMOEBA INVADENS* BY ANTITUBLIN DRUG ORYZALIN

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We have previously demonstrated that the antimicrotubule drug oryzalin inhibits the growth of *Entamoeba invadens* as well as *E. histolytica*, the former being more resistant to the drug than the latter. The aim of the present study was to examine the effect of oryzalin on the encystation of *E. invadens* using an axenic encystation system *in vitro*. Oryzalin inhibited the encystation of *E. invadens* strain IP-1 in a dose-dependent manner. The addition of oryzalin after the induction of encystation was also inhibitory for encystation. Trophozoites incubated for 1 day in encystation medium with oryzalin did not encyst after removal of the drug. Although trophozoites grown in the presence of 300 μM oryzalin for 2 days did not encyst after their transfer to encystation medium containing the same concentration of drug, a number of trophozoites survived for at least 3 days. In contrast, trophozoites grown in the absence of oryzalin neither survived nor encysted after their transfer to encystation medium supplemented with the drug, which suggests that pretreatment of trophozoites with oryzalin contributes to their continued survival as trophozozites, without their transforming into cysts, in encystation medium. Trophozoites grown with oryzalin did encyst after their transfer to encystation medium without the drug.

Accumulation of trophozoites in the mitotic phase was observed after culture with oryzalin. When cysts prepared at day 1 of encystation, most of which were mononucleate, were reincubated in the presence of oryzalin for an additional 2 days, inhibition of their maturation was observed.

Thus, oryzalin is a potent mitotic-phase inhibitor of *E. invadens* and may become a useful tool for studies on the relationship between the cell cycle and encystation of this parasite.

B—11 MOLECULAR CLONING AND CHARACTERIZATION OF PEROXIREDOXIN FROM *ENTAMOEBA MOSHKOVSKII*

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Recently, the term "peroxiredoxin" has been proposed for a family of antioxidant enzymes which includes thioredoxin peroxidase and alkyl hydroperoxidase. The peroxiredoxin of *Entamoeba histolytica* seems to be important in protection from oxidative attack by activated host phagocytic cells during the ameba's invasion of host tissue and/or in protection against its own metabolically produced hydrogen peroxide. The nonpathogenic *E. moshkovskii* is morphologically indistinguishable from *E. histolytica*, but usually is isolated from polluted waters. As a step towards clarifying the biological significance of peroxiredoxin in both pathogenic and nonpathogenic *Entamoeba*, we report here the molecular cloning and characterization of *E. moshkovskii* peroxiredoxin.

Trophozoites of *E. moshkovskii* Laredo were axenically cultured in BI-S-33 medium. A cDNA library was constructed from poly (A) RNA of *E. moshkovskii* and then screened with a 352 bp probe prepared from genomic DNA by PCR amplification using primers p1 and p3 for *E. histolytica*. The clone containing the longest insert was subcloned into a pUC19 vector and then sequenced. The open reading frame of a cloned cDNA encoded a polypeptide of 217 amino acids with a calculated molecular mass of 24 kDa. When the deduced amino acid sequence of *E. moshkovskii* peroxiredoxin was compared with that of *E. histolytica* (233 amino acids) via the FastA Program, identities were 81% on the overlapping 215 amino acids. A distinctive difference was that the cysteine-rich N-terminus sequence of *E. moshkovskii* peroxiredoxin was shorter than that of the *E. histolytica* peroxiredoxin.

The cDNA encoding peroxiredoxin of *E. moshkovskii* was subcloned into expression vector pET19b. Recombinant peroxiredoxin was expressed as a fusion protein with a histidine tag in *Escherichia coli*. The recombinant protein was purified by affinity chromatography and then examined for its ability to remove exogenously added H_2O_2 in an *in vitro* assay. The peroxiredoxin was capable of catalyzing the removal of H_2O_2, in the presence of dithiothreitol, in a concentration-dependent manner. Its activity was comparable with that of *E. histolytica*. Immunofluorescent staining by polyclonal antibodies, prepared against the recombinant peroxiredoxin of *E. moshkovskii*, localized the protein in the nucleus and cytoplasm of trophozoites. The *E. moshkovskii* peroxiredoxin seems to function in protection against its own metabolically produced H_2O_2.

B—12 MODIFICATIONS OF STAINING PROCEDURES FOR THE PREPARATIONS OF *ENTAMOEBA HISTOLYTICA*

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Preparation of stained smears of *Entamoeba histolytica* has several drawbacks. The iron hematoxylin staining and the trichrome staining, which have been most widely used, for example, employ highly toxic mercuric chloride. We therefore tried to develop simple staining procedures by modifying Kohn's chlorazol black E staining and the

trichrome staining. Amoebae fixed in suspension with either the basic solution of Kohn's staining or Bouin's fixative were clearly stained with Kohn's stain and the trichrome stain, and can be examined as wet mounts.

Permanent preparations were made by processing for mounting. Amoebae in fecal smears fixed in these fixatives were also stained with these stains. These procedures were easier when the basic solution and trichrome stain were used. Erythrocyes ingested by trophozoites, however, can not be stained with these stains after fixation in the basic solution, but were stained after Bouin's fixation. Further the staining can be combined with a cyst-concentration technique, which uses the basic solution (instead of formalin) and ether. Consequently, without highly toxic mercuric chloride, wet mounts and permanent preparations can be made using permanent stains and preserved cysts can be stained after concentration.

B−13 EPIDEMIOLOGICAL STUDY OF AN EPIDEMIC AMEBIASIS IN INSTITUTIONS IN JAPAN

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(Abstract not received on time)

B−14 CONGENITAL *TOXOPLASMA* INFECTION AT PLACENTA RESULTING IN INTRAUTERIN GROWTH RETARDATION

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Fetal infections by *Toxoplasma gondii* can result in neuological sequelae, congenital malformations, and ocular disorders. The severity of human congenital toxoplasmosis and its importance as a public health concern are now recognized by many scientists and physicians in European countries, USA, and more recently in Japan. Early diagnosis by seroconversion and clinical diagnosis of fetuses followed by early chemotherapy have been shown to improve the prognosis.

We report a case of IUGR (intrauterin growth retardation) delivered from the mother infected by *T. gondii* during her pregnancy. Malformation and hypofunction of the placenta were observed, and the infection of *T. gondii* was confirmed with pathologic and immuno-pathologic diagnosis together with PCR diagnosis. No evidence of *T. gondii* infection in the newborn, however, was obtained by serological and chinical diagnosis. Thus, we propose a new classification of congenital toxoplasmosis by adding a pivotal concept of "congenital toxoplasmosis at placenta" in which only IUGR of fetuses occurred without *T. gondii*-fetal infection in utero. Further validation of the up-dated techniques for measuring fetal growth will undoubtedly enhance the ability to diagnose congenital toxoplasmosis prenatally.

B−15 INTRACTABLE RECURRENT TOXOPLASMIC RETINOCHOROIDITIS

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Toxoplasmic retinochoroiditis is the most common manifestation of congenital *Toxoplasma gondii* infection. Many issues remain unsolved regarding the mechanism of the recurrence and the treatment of ocular toxoplasmosis. Here we report two cases of retinochoroiditis caused by congenital toxoplasmosis.

Case 1: This 14-year-old boy visited out-patient clinic due to a decreased and blurred vision in his right eye. He was born in Senegal. He was apt to have fever and skin abscesses in the upper part of the body in his infancy and childhood. He came back to Japan when he was 3 years old. He was pointed out that his visual acuity was poor when he was 6 years old. Inactive multiple chorioretinal scars suggestive of toxoplasmosis were present in both maculas, involving the left fovea but not the night. The latex aggulutination titer was positive (1:10,240) for toxoplasmosis. In April 2000, when he was first examined, preretinal hemorrhages and retinal edema were present in the right eye. He was treated with clindamycin and corticosteroid. During the tapering off the corticosteroid, active infection recured while the patient was still receiving clindamycin. The vitreous opacity and retinal edema increased. Although the dose of corticosteroid was increased again, he is not under medical control.

Case 2: This 7-month-old boy visited out patient clinic due to a esotropia. The patient had focal chorioretinal lesion with pigmentation at the both macular areas. The latex aggulutination titer was positive (1:10,240) for toxoplasmosis. Computed tomographic scan showed an intracerebral calcification. The patient was treated with acetilspiramycine. His father, mother, and elder sister were healthy and had no abnormal findings in their eyes, although the latex aggulutination titers were all positive (1:10,240) for toxoplasmosis. He was clinically free of evidence of recurrent disease throughout the 11 years follow up.

The disease recurred repeatedly at long or short irregularly spaced intervals in spite of the treatment. Medical treatment of toxoplasmosis may not be effective always. We discussed with regard to current policies in treatment.

B−16 FOUR CASES OF CRYPTOSPORIDIOSIS

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The signs and symptoms of cryptosporidiosis caused by *Cryptosporidium parvum* (Cp) can easily be confused with other intestinal disturbances. In this report we described the clinical course of cryptosporidiosis in 4 Japanese with severe diarrhea.

Case 1: A 24-year-old Japanese male who had traveled to India was referred to our institute because of severe prolonged watery diarrhea. Cp oocysts (3.5×10^5/g) were detected in his stool. Stool examinations were negative for other intestinal protozoa. Bacterial cultures were also negative for pathogenic agents. He was treated with sulfamethoxazole/trimethoprim for 6 days and he soon recovered. In immunocompetent individuals cryptosporidial diarrhea usually subsides within a 2 to 3 week period of time. Therefire, it may be that the timings of natural recovery and treatment were the same.

Case 2: A 25-year-old Japanese male who had traveled to Kenya was referred to our institute because of high fever and severe diarrhea. *Plasmodium falciparum* was detected in his blood smears and also Cp oocysts in his stool specimens. He was treated with pyrimethamine/sulfadoxine and quinine and he soon recovered from malaria as well as from cryptosporidiosis.

Case 3: A 27-year-old Japanese female who had traveled to Malaysia was referred to our institute because of severe prolonged watery diarrhea. Cp oocysts and *Blastocys-*

tis hominis were detected in her stool specimens. No treatment was done and her symptoms subsided spontaneously after 13 days-prolonged diarrhea.

Case 4: A 44-year-old Japanese male was referred to our institute because of severe 5-months-prolonged watery diarrhea which resulted in a 20 kg body-weight loss. Laboratory data showed positive for anti-HIV antibodies, CD4/CD8=0.009 and 100 CD4T cell count/μl in his peripheral blood. Cp oocysts were detected in his stool specimens. CMV showed positive in his large intestinal biopsy specimens. *Pneumocystis carinii* (Pc) were detected in his tracheal specimens. He was treated with acetylspiramycin against Cp, ganciclovir against CMV and pentamidine isethionate against Pc, but diarrhea continued and respiratory failure appeared. Then he died one month after his condition was diagnosed. The signs and symptoms of cryptosporidiosis can be easily mistaken for other intestinal disturbances -i.e., infections caused by other protozoas, bacteria, viruses or food poisoning. Physicians must include cryptosporidiosis in their differential diagnosis when examining patients with severe diarrhea of unknown origin.

B−17 SERO-EPIDEMIOLOGICAL SURVEY OF CRYPTOSPORIDIOSIS AT SUMBAWA ISLAND, INDONESIA

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We carried out the serological test to detect antibodies against *Cryptosporidium parvum* in sera of people in three villages of Sumbawa Island. Serum samples were collected from villagers selected by random sampling, i.e., 68 from desa Labanka IV, 110 from desa Penyaring and 107 from desa Stowe Berang. Indirect IFA was used for antibody detection. Oocysts obtained from SCID mice infected with *C. parvum* were used as antigens. Each serum was diluted with PBS at 10% and used for the test. IgG, IgM and IgA antibodies were examined separately. The rate of people who had antibodies at least in one class of immunoglobulin was 42.6% in Labanka, 52.3% in Stowe Berang and 57.3% in Penyaring. The very characteristic differences were found when the positive rates of different class of immunoglobulin were compared between villages. For example, the positive rate of IgG in Labanka, Penyaring and Stowe Blang was 35.3%, 31.8% and 28.0% respectively; that of IgM was 0%, 19.1% and 28.0%; that of IgA was 13.2%, 35.5% and 11.2%. In order to understand the meaning of these differences, we carried out the fecal examination in three villages, but we could not find out any indicative result, although the transmission mode of *Cryptosporidium* has been shown to be completely different from other intestinal parasites. In the present report, we discussed plausible reasons for the differences, considering social, geographical, meteorological and environmental conditions.

B—18 GENETIC CHARACTERIZATION OF *CRYPTOSPORIDIUM PARVUM* ISOLATES FROM 14 CATTLE AND 22 PATIENTS WITH DIARRHEA

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Polymerase Chain Reaction combined with *Rsa I* Restriction Fragment Length Polymorphism (PCR-RFLP) analysis of a threonine-rich open reading frame gene from *Cryptosporidium parvum* was applied to 36 specimens collected from patients with cryptosporidiosis associated with two waterborne outbreaks, sporadic human cases and livestock (neonatal calves) from other areas. The sizes of the fragment amplified with the primer pair (Sense: 5'-CTCTTAATCCATCATTACAAC-3'; Anti-sense: 5'-AGCAGCAAGATATGATACCG-3') for *C. parvum* anthroponotic genotype 1 is 521 bp, and for *C. parvum* zoonotic genotype 2 is 518 bp. The genotype 1 fragment contains 2 *Rsa I* restriction sites, and is digested into three DNA fragments (55, 62 and 404 bp). The genotype 2 fragment contains 3 *Rsa I* sites, and is digested into four DNA fragments (55, 62, 128 and 273 bp). Fourteen isolates from neonatal calves were exclusively of genotype 2. Isolates from patients linked to the first waterborne outbreak in Japan in September 1994 were of genotype 2, and those linked to the second waterborne outbreak in June 1996, were of genotype 1. Among isolates from sporadic cases, 2 were of genotype 2, and 10 were of genotype 1. Specimens from 3 patients with sporadic cryptosporidiosis who were all immunologically healthy adults yielded a new genotype. The size of the fragment amplified was 521 bp spanning 2 *Rsa I* sites, and was digested into three fragments (55, 190 and 276 bp). One specimen from a patient with a sporadic case yielded both genotype 1 and the new genotype. The latter two genotypes failed to visibly infect nude mice, demonstrating that there is a strong preference for one host. These findings strengthen the concept of *C. parvum* genetically clustering into subgroups that correlate with infectivity, transmission of infection to humans, and with the resulting clinical manifestations.

B—19 COMPLEX II (SUCCINATE-UBIQUINONE REDUCTASE/QUINOL-FUMARATE REDUCTASE) OF *TRYPANOSOMA CRUZI* MITOCHONDRIA

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In the present study, protocol to isolate active mitochondria from epimastigote of *T. cruzi* was established and succinate-ubiquinone reductase (SQR) was partially purified. Furthermore, a cDNA for Fp subunit, which contains binding sites of flavin and substrates of *T. cruzi* SQR was cloned and characterized.

Complex II is located in the inner mitochondrial membrane where it plays a role in mammalian type mitochondria as a component of the TCA cycle and electron transport chain. In addition, some organisms living in anaerobic condition use complex II as quinol-fumarate reductase (QFR), which catalyzes reverse reaction of SQR. The role of complex II in the *T. cruzi* mitochondria remains unknown, although several reports suggested the presence of fumarate

reductase (FRD) which is a part of QFR activity in the mitochondria.

Mitochondria were isolated from cultured *T. cruzi* epimastigote by using glass-beads treatment and following differential centrifugations. Then complex II was solubilized from the mitochondria with sucrose monolaurate and separated by DEAE-cellulofine in the presence of the detergent. SDH activity was eluted from the column as a single peak with FRD activity. Immunoblotting showed a 70 kDa band was recognized with the anti-bovine Fp antiserum suggesting that this protein band is Fp subunit of *T. cruzi*.

Then, a cDNA for Fp subunit of the enzyme has been cloned by homology proving and RACE. The cDNA with 2,035 bp has a 35-bp spliced leader sequence and the ORF encodes 609 amino acids. Amino acid sequences around FAD binding and substrate binding regions were highly conserved. Southern blotting analysis showed that this gene is single copy in the *T. cruzi* genome. Northern blotting analysis revealed that *T. cruzi* Fp mRNA contains long 3'-noncording region. Amino acid sequence of *T. cruzi* Fp showed higher homology to that of eukaryote rather than that of the bacterial QFR. Amino acid composition of Fp peptide of purified preparation determined by MS analysis was correlated well with that deduced from the cDNA sequence.

B−20 CHARACTERIZATION OF CYANIDE INSENSITIVE OXIDASE OF *TRYPANOSOMA BRUCEI BRUCEI* EXPRESSED IN *ESCHERICHIA COLI*

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African trypanosome is a parasite, which causes African sleeping sickness of human and nagana disease of cattle. Because, at present, any drug against trypanosomes has strong side effect and is not effective for chronic patients, a new effective drug is awaited. We found ascofuranone isolated from *Ascochyta visiae* inhibits specifically mitochondrial respiration of blood stream form of *Trypanosoma brucei brucei* at very low concentration. In addition, we found that the parasite in the blood disappeared quickly by the treatment with ascofuranone in combination with glycerol.

Experimental evidences showed that a target of ascofuranone is cyanide insensitive terminal oxidase localizeded in mitochondrion of blood stream form of *T. b. brucei*. This enzyme is referred to as TAO (trypanosome alternative oxidase) and, unlike cytochrome *c* oxidase of mammalian mitochondria, functions as quinol oxidase. Because mammals don't have TAO that is essential for re-oxidation of reducing equivalent generated during glycolysis in the glycosome, TAO is expected as a candidate of target for chemotherapy against trypanosomes. It is also reported that TAO is highly homologous to alternative oxidase of plant mitochondria, but biochemical analysis has not been carried out due to the difficulty of purification. Therefore, we have been trying to overexpress a recombinant TAO in *E. coli* to characterize TAO. Our final goal is clinical development of ascofuranone.

We constructed FN102/pTAO by transformation of cDNA for TAO into heme-deficient *E. coli*. In this strain, because of the deficiency of heme, cytochrome *bo* complex and cytochrome *bd* complex of *E. coli* don't show quinol oxidase activity, and we can analyze solely quinol oxidase activity of rTAO. Then, we established the large scale culture system on which about 80% of membrane protein is rTAO. Kinetic analysis of rTAO in this membrane indicated that ascofuranone inhibits quinol oxidase activity of TAO competitively with quinol, suggesting that aromatic structure of ascofuranone is recognized by quinol binding site of the enzyme.

B−21 EXPRESSION AND PROPERTIES OF DIHYDROOROTATE DEHYDROGENASE IN *TRYPANSOMA CRUZI*

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Dihydroorotate dehydrogenase (DHOD, E.C.1.3.3.1) is the fourth enzyme of the denovo pyrimidine biosynthetic pathway and thought to be one of possible targets for cancer and infectious diseases. We have previously reported a pyrimidine-biosynthetic gene cluster that contains five genes encoding all six enzymes, including DHOD, of the pyrimidine biosynthetic pathway in *Trypanosoma cruzi*. Phylogenetic relationships among DHOD sequences were shown to be related to their localization and catalytic properties in the cell. The *T. cruzi* DHOD is classified to the cytosolic group utilizing fumarate as an electron acceptor, whereas the animal DHODs are of membrane-bound utilizing oxygen via respiratory chain. In order to characterize the *T. cruzi* DHOD, the recombinant *T. cruzi* DHOD was expressed in *Escherichia coli* and the enzymatic properties were analyzed. The recombinant DHOD tagged with hexahistidine at the N-terminal was recovered in the soluble fraction and highly purified by using the affinity column for the tagged sequence. The *T. cruzi* DHOD displayed the specific absorbance at 368 nm and 455 nm, characteristic to a flavoprotein. The DHOD activity was dependent to fumarate as an electron acceptor and inhibited by a final product, orotate. The kinetic values for dihydroorotate and fumarate were 0.01 mM, 0.11 mM, respectively. These results indicate that the *T. cruzi* DHOD provides the distinct character from that of mammalians, suggesting the possible target of chemotherapy.

B−22 IN BALB/C MICE, IMMUNE RESPONSES AND THE COURSE OF *LEISHMANIA MAJOR* INFECTION WERE CONTROLLED BY CYTOKINE EXPRESSION PLASMIDS DELIVERY WITH THE GENE GUN

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DNA vaccination has been shown to elicit a long-lasting immune response in a variety experimental models. The concept of DNA immunization has been applied recently to gene therapy using vector constructs that encode cytokine gene. The particle-mediated method for gene delivery with a gene gun utilizes a shock wave to accelerate DNA-coated gold particles into target cells or tissues. This gene delivery method is effective in various somatic tissues *in vitro* and *in vivo*. In this report, we investigated the effect of cytokine gene treatment to Th1/Th2 response and the course of *Leishmania major* infection. BALB/c mice were infected with *L. major* subcutaneously into hind footpad. Simultaneously, they were begun to be treated with 4 μg of IL-12 or IL-4 plasmid DNA by gene gun weekly. Mice treated with IL-12 gene developed slighter footpad swelling and parasite burden than did control mice. On day 42 postinfection, mice treated with IL-12 gene were capable of generating more IFN-γ as well as *L. major*-specific IgG2a titers than did control mice, while that of IL-4 and IgG1 was decreased. In contrast, treatment with IL-4 plasmid DNA exacerbated the disease. The results demonstrate that gene gun-based IL-12 plasmid DNA treatment can skew the immune response in a Th1 direction in *L. major* infected BALB/c mice, while IL-4 DNA treatment may promote Th2 response. Further studies are in progress to determine the mechanisms of gene gun-based cytokine plasmid DNA treatment.

B—23 TH2 CYTOKINES PLAY DIFFERENT ROLES DURING THE COURSE OF INFECTION WITH *LEISHMANIA MAJOR*

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Leishmania spp. are dimorphic obligate intracellular parasites. Flagellated promastigotes replicate and differentiate in the gut of the sandfly vector and are transmitted to a vertebrate when the sandfly takes a blood meal. Survival of the parasite within a vertebrate host is dependent on the successful entry into a macrophage and transformation into the amastigote form. They cause a wide range of infectious processes from asymptomatic to cutaneous, mucosal or visceral manifestations, depending on the species of the parasite and the type of immune response induced. The immune responses and clinical outcome in leishmanial infections are dependent in part on the patterns of cytokines produced.

Experimental cutaneous leishmaniasis is a useful model in studying the mechanism regulating immune responses between Th1 and Th2. Mice susceptible to *Leishmania major* infection such as BALB/c are associated with the induction of the disease-promoting Th2 response, while the resistant mice such as DBA/2 develop the protective Th1 response. Genetically determined resistance and susceptibility are clearly related to the development of polarized $CD4^+$ Th1 and Th2 responses, respectively. Cytokines are the most important stimulus in influencing the development of functionally polarized $CD4^+$ Th effector responses.

Adjuvants are known to enhance the immune responses through the combined effect of several factors: prolonged release of antigen, migration of cells, mitogenic effect and so forth. Incomplete Freund's adjuvant (IFA) which is one of the oil adjuvants was used to investigate Th-differentiation in experimental cutaneous leishmaniasis and to study the effector roles of the different Th cytokines (IL-4, IL-5, IL-13, IL-10, IL-9, and IFN-γ) in this immune deviation. Twice immunization using soluble leishmanial antigen (SLA) in IFA (IFA/SLA) can make the resistant mouse strain (DBA/2) susceptible to *L. major* infection with shifting the immune response from Th1 to Th2. This could be attributed to the modulation of the cytokine pattern with marked Th2 cytokine up-regulation by this immunization scheme. IL-4 and IL-13 are considered to be the most important susceptibility factors in mounting the disease-promoting Th2 response in mice susceptible to *L. major* infection. The production of these cytokines is sustained in susceptible mice until the chronic phase of the infection. While the levels of these two cytokines decrease markedly in the resistant strains of mice. However, it is difficult to explain the mechanism that establishes Th2 response with only these two Th2 cytokines, because we could not detect significant difference in the production of IL-4 and IL-13 early after the infection between the healer and the non-healer groups of mice. On the contrary, IL-5 and IL-10 appear to have a significant role in determining the course of the disease early after the infection.

B—24 THE EFFECTS OF MEGLUMINE ANTIMONIATE (GLUCANTIME®) AGAINST *L. MAJOR* AND *L. AMAZONESIS* CO-CULTURED WITH MACROPHAGES

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Meglumine antimoniate (MA) has been widely used for leishmaniasis. Anti-leishmanial effect of MA has been studied on the point antipromastigote activity till date. However, no report has been found about how MA affects the parasite-intake of macrophage. In this study, we examined the parasite activity co-cultured with macrophage un-

der several concentrations of MA.

Promastigotes of *Leishmania major* and *L. amazonesis*, were treated at the concentration ranged from 0%, 0.0001% (0.085 μg/m*l*), 0.01% (8.5 μg/m*l*) and 1% (850 μg/m*l*) of MA. Simultaneously, macrophage (J774) co-culture was incubated with the both strains of *Leishmania* parasites and also exposed to drug as same dosages. The number of parasites was counted by hemocytometer after 48 hr of incubation, stained by neutral red and Giemsa staining. Electron-microscopic study was also performed to investigate the activity of MA against amastigotes, formed inside of macrophages. For which, infection of macrophages with *L. major* promastigotes was done prior to administration of MA at concentration of 20 mg/m*l*.

The number of promastigotes of *L. major* and *L. amazonesis* treated without MA, was proliferated but the reduction in number of promastigotes was observed treated with 1% to 0.0001% concentrations of MA. Both strains of *Leishmania* promastigotes co-cultured with macrophages and treated with 0% to 1% concentrations of MA, revealed the reduction in number of promastigotes. The observation of Giemsa stain, disclosed that macrophages treated with MA, contained many promastigotes outside and a few number of amastigotes inside but macrophages treated without MA, showed the opposite results. The promastigotes of *Leishmania* parasites treated with 0% to 1% concentrations of MA, showed no significant difference of staining within them in neutral red stain. Electron-microscopic study revealed that *L. major* infected macrophages treated with MA contained less amastigotes as compared to cells treated without MA.

Our data suggests that MA seems to have a weak leishmaniacidal effects. As an anti-leishmanial effects, MA inhibit the proliferation of promastigotes only but also may interfere the promastigotes entry into the macrophages.

B—25 LEISHMANIASIS IN EQUADOR, WITH SPECIAL REFERENCE TO MUCOCUTANEOUS FORMS AND MAN-BITING SAND FLIES, *LUTZOMYIA* SPP. IN THE AMAZONIAN ENDEMIC AREAS

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To disclose the epidemiological features of leishmaniasis in Ecuador, intensive field research and laboratory works have been done from 1982 to date. During the period, almost all of the suspectable areas endemic for the disease in that country were surveyed, by performing investigations for patients, vector sand flies, and reservoir animals. Nine provinces of Ecuador are located in the Pacific regions; 2, in the mid-Andes; and 4, in the Amazonian regions. To date, 7 species of the genus *Leishmania*, *L. (L.) mexicana*, *L. (L.) amazonensis*, *L. (L.) major*-like, *L. (V.) braziliensis*, *L. (V.) guyanensis*, *L. (L.) panamensis*, and *L. (V.) equatorensis* (the last species may be belonging to the genus *Endotrypanum*), were identified based on their zymodeme, serodeme, schizodeme and karyodeme analysis. Regarding to vectors and reservoirs, 4 species of the genus *Lutzomyia*, *Lu. tarapidoi*, *Lu. hartmann*, *Lu. gomezi* and *Lu. ayacuchensis*, and 8 species of mammals, anteaters, 2 spp. of sloths, 2 spp. of squirrels, kinkajous, rats and dogs, were incriminated at different endemic areas of Ecuador, respectively. The data mentioned above, however, were mainly obtained from the endemic areas of lowlands and Andean slopes of Pacific regions, and Andean plateau of the country, but not from the Amazonian regions. In the present paper, we mentioned the data from the Amazonian endemic areas, Loreto, Napo, by performing epidemiological surveys including the examinations of patients and the collections and dissections of sand flies. The results obtained revealed that there were considerable numbers of patients with mucocutaneous leishmania-

sis in addition to those with cutaneous one. In the fly collection using protected human baits, 43 in total belonging to 7 species of the genus *Lutzomyia* were collected, and dissected, but none of them were positive for *Leishmania* promastigotes; the man-biting flies were *Lu. tortura*, *Lu. carrerai thula*, *Lu. flaviscutellata*, *Lu. olmeca bicolor*, *Lu. geniculata*, *Lu. yuilli yuilli*, and *Lu. gomezi*. Among these flies collected at the Amazonian leishmaniasis-endemic areas, only the last species, *Lu. gomezi* was already incriminated as a probable vector of the disease at the Pacific lowlands and Andean slopes of Ecuador. Further studies are required in order to disclose the epidemiological and vector entomological features of the disease in the Amazonian regions.

B−26 PULMONARY INFECTION CAUSED BY *RHODOCOCCUS EQUI* IN HIV-INFECTED PATIENTS: REPORT OF SIX PATIENTS FROM NORTHERN THAILAND

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We report six human immunodeficiency virus (HIV) infected patients (5 males and one female, average age, 32.2 years) with pulmonary infection (four with pneumonia and two with lung abscess) caused by *Rhodococcus equi*. These patients who presented with fever and productive cough were admitted to Nakornping Hospital in northern Thailand. Chest roentgenograms showed pulmonary infiltration and/or cavitary lesions. Their conditions were poor because of severe anemia, and transfusion was necessary in 5 of 6 patients. The etiologic microorganisms identified in sputum smears were gram-positive and acid-fast coccobacilli before culture results were available. Only one of the six cases was a mixed-infection by *R. equi* and *Salmonella enteritidis*. The mean CD4 lymphocyte count in 5 of tested patients was 8/mm^3 (CD4/CD8 ratio=0.05). Eight isolates of *R. equi* in 6 patients were sensitive to imipenem, minocycline, erythromycin, clarithromycin vancomycin and teicoplanin (minimum inhibitory concentration, MICs$\leqq$1 μg/m*l*), but resistant to most β-lactam antibiotics. Four isolates were sensitive (MICs$\leqq$1 μg/m*l*) and four resistant (MICs$\geqq$64 μg/m*l*) to rifampicin. Three patients were treated with erythromycin+rifampicin while the other two were treated with anti-tuberculous drugs and penicillinG+gentamicin. However, three patients subsequently died because of respiratory failure due to pulmonary infection caused by *R. equi*.

B−27 INFLUENCE OF HIV INFECTION ON COMMUNITY-ACQUIRED PNEUMONIA IN ADULTS IN UGANDA

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Hospital-based prospective study on 48 patients with community-acquired pneumonia (CAP) has been carried out in Kampala, Uganda from September 1999 to June 2000. We evaluated the bacterial etiologies employing quantitative sputum culture and Toluidin blue-O (TBO) staing, clinical features, and prevalence of rerurrent pneu-

monia. We found high prevalence (81.2%) of HIV-1 infection among enrolled patients. Mean CD4 positive lymphocyte counts of patietns infected and uninfected with HIV-1 was 191.4/μl and 509.5/μl, respectively. Major bacterial pathogen were *Streptococcus pneumoniae* (11 strains), *Branhamella catarrhalis* (9 cases), and *Haemophilus influenzae* (8 strains). One patient infected with HIV-1 had a typical clinical features of *Pneumocystis carinii* pneumonia. He was successfully treated with co-trimoxazole. Five of thirty-three (15%) patients infected with HIV-1 had recurrent pneumonia. Mean CD4 positive lymphocyte counts of these patients decreased from 132.0/μl at the first episode to 61.0/μl at the second episode. The mean period between the first and the second episode was 81.6 days. All of five cases had different bacterial pathogens in each case. Recurrent bacterial infection may progress the decline of cellular immunity in each individual.

B−28 GENETIC SUSCEPTIBILITY TO RE-INFECTION OF *SCHISTOSOMA JAPONICUM* IN CHINA

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Previous field studies showed that resistance to re-infection of *S. mansoni* or *S. haematobium* is positively associated with age, and that the resistance is determined by the balance between the levels of effective anti-adult worm IgE and of blocking IgG4 antibodies. In 1995, we have done a re-infection study of *S. japonicum* in Zhuxi Village near Poyang Lake, Jiangxi Province, where schistosomiasis had been highly endemic (around 40%). In March, the rate and intensity of infection was quantified by modified Kato-Katz methods, then all subjects (n=100) received praziquantel treatment. After the confirmation of 100% cure rate, the water contact investigation by the questionnaire method was carried out during the transmission season. Then, they were re-examined for their fecal eggs in December. The subjects whose water contact index were more than 20, were 94% re-infected and we suspected that those highly exposed persons could get a infection regardless of resistance. Therefore we excluded them from our study. Thirty six persons were re-infected and 50 were not infected. There was no age difference between them. The serum levels of immunoglobulines were positively correlated to intensity of infection, but that looked no relation to resistant to infection. No HLA-DRB1 allele was significantly associated to resistant or susceptible group. However, DRB1*1101 and DRB1*1501, that were associated with protection and susceptibility to Shistosomal fibrosis (Hirayama *et al*., 1999), looked protective and susceptible to re-infection in Zhuxi, respectively. Taken together, T cell immunity that is controlled by host immune-response genes (HLA-class II) seemed to be more important than B cell in the protective immunity to *S. japonicum* infection.

B—29 A POPULATION STUDY OF THE INTERMEDIATE HOST OF *SCHISTOSOMA MANSONI* AND THE APPLICATION OF REMOTE SENSING

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To determine the risk of infection, the density of the intermediate host of *Schistosoma mansoni* was studied for two years at transmission sites in a village at Lower Moshi, Tanzania. The specific objectives are to identify the specific season, condition and site with high risk of infection. Snails were collected with 10-time-scooping, or 5-minute-picking where scooping was not applicable. *Biomphalaria pfeifferi* was found to be widely distributed in a river and irrigation canals of paddy fields. The density of the snail was 10-100 times higher in the river than in the irrigation canals of paddy fields. The snails appear to change in number depending on the stagnation and vegetation of the water, but not on the water temperature. The possibility of the application of remotely sensed variables to determine the risk of infection by analyzing the correlation between the population of the snail and vegetation will be discussed.

B—30 A QUESTIONNAIRE STUDY TO QUANTIFY THE HUMAN BEHAVIOR AT RIVER INFESTED WITH *SCHISTOSOMA MANSONI*

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Quantification of human behavior is essential to determine the risk of *Schistosoma* infection. However the quantification of the behavior is not easy to estimate and it is difficult to relate the behavior to the infection individually. Three hundred eighty three primary school children aged 7-14 were studied for their infection with *Schistosoma mansoni* by a stool examination, for their presence at school and for their water contact behavior by questionnaire in January and February, 2000, at Moshi, Tanzania. The specific objectives were to determine if it is feasible to quantify the degree of individual water contact by questionnaire in a short period and to determine if there is a correlation between the degree of water contact and the intensity of infection individually. Intestinal helminths were also examined quantitatively and school attendance was taken into account as well. Kato-Katz technique was applied for the stool examination. Children were asked if they went to river or not, what for and so on, for 10 successive weekdays. The prevalence of *S. mansoni* and *Ascaris lumbricoides*, hookworm, *Trichuris trichiura* were 49%, 13%, 16%, 25%. The intensities of infection, EPG (geometric mean of egg count per gram), were 10.2, 1.6, 1.0, and 2.0, respectively. The possibility if quantification of water contact activity through questionnaire and the relationship between the intensity of infection and the degree of water contact will be discussed.

B—31 WHERE DO *SCHISTOSOMA JAPONICUM* COME FROM?

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In his hypothesis on coevolution of Asian schistosomes and snails, Davis (1980,1992) implies that the ancestors of the *S. indicum* species group and its associated pulmonate snail hosts (*Indoplanorbis* and *Lymnaea*) were African and arrived in Asia via the Indian plate. However, our recent chromosome analysis using the C-banding pattern suggested that the *S. japonicum* group is the original type, and the African species represents the derived type (Hirai *et al*., 2000). Further, Snyder and Loker (2000) also argued that, on the base of their LSUrDNA data, *Schistosoma* appears to have originated in Asia, colonized and diversified in pulmonate snails in Africa, and then recolonized Asia. The present study revealed that members of the *S. indicum* group have a close affinity with the African human schistosomes and supported a hypothesis of the Asian origin of the genus *Schistosoma*. However, we believe that the *S. indicum* group may have originated in Asia, but not recolonized Asia. Because nucleotide differences in both ITS2 and CO1 regions between the *S. indicum* group and the African group are still considerably large; this should have happened long before domestication of wild animals by man. Therefore, it would be difficult to explain the above hypothesis of "recolonization by early humans activity" as suggested by Barker and Blair (1996). Instead, it could be speculated that an ancestral species of the *S. indicum* group of Asian origin may have been brought to Africa, colonized and diversified in pulmonate snails in Africa. Afterwards, human schistosomes in Africa may have speciated from the diversified schistosomes as suggested by Despres *et al*. (1992). *S. hippopotami* placed in a basal position of the African species could be an ancestral species for the African species, as have been suggested (Despres *et al*., 1995). Therefore, large mammals such as hippopotamus or elephants could be a suitable host for a common ancestral species of the African group and the *S. indicum* group, since they have longer life span and bigger capacity for harborring a number of parasites that enable them to maintain and carry the parasites to wide areas in a long span. Very recently, molecular data of elephant schistosomes belonging to the genus *Bivitellobilharzia* discovered in Sri Lanka suggested that the elephant schistosomes were placed in a more basal position among Asian species of *Schistosoma* than *S. incognitum* (Rajapakse *et al*., 2000). Existence of these schistosomes in Asia including *Orientobilharzia* which was placed among species of *Schistosoma* (Snyder and Loker, 2000) would support the hypothesis of Asian origin of *Schistosoma*.

B—32 THE DIFFERENCE OF THE ECHOGENIC PATTERNS AMONG SCHISTOSOMIASIS JAPONICA AND SCHISTOSOMIASIS MEKONGI?

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Since *Schistosoma mekongi* was first described by Voge *et al*. in 1978. Those two schistosome species were told to be quite resembled each other at the appearance of their eggs. The habitats and morphology of the snail intermediate hosts, *Oncomelania* spp. and γ-*Neotricula aperta* were quite different each other compare with the morphological similarities of the parasites. Even though no new patient was reported with *Schistosoma japonicum* since

1978 when the ultrasonographic investigation was introduced for schistosomiasis cases in Japan. We were able to report the echogenic pattern of the network on 11 young patients, 9 to 14 years old, among elementary school children at the highly endemic areas in Mindanao Island, Philippines. Appearance of the echogenic pattern of the network with *S. japonicum* has been confirmed with not only for adult cases but also for younger group. Our investigation was conducted in 1999 and 2000 at several highly endemic locations inside the two provinces, Stung Treng and Kratie, in Cambodia in order to observe the echogenic pattern of the network with schistosomiasis mekongi for the first time. Totally around 400 adult patients who seriously affected with *S. mekongi* were examined for the abdominal ultrasonography. However, none with the echogenic pattern of the network was found through our investigation. These data suggest the echogenic pattern of the network appeared on only *S. japonicum* among schistosome species even though our investigations could not be performed under ideal circumstance.

B—33 LOCALIZATION OF TYPE IV COLLAGEN IN GRANULOMA FORMATION AND LIVER FIBROSIS DUE TO SCHISTOSOMIASIS JAPONICA

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Type IV collagen is a main component of basement membrance in the liver parenchyma. A significant increase in the levels of serum was reported among *Schistosoma japonicum* infected patients with periportal fibrosis.

Localization of types I, III, and IV collagen concomitant with biochemical serum analysis were performed in murine schistosomiasis japonica. Each mouse was infected percutaneously with 30 cercarie. From five to eleven weeks after the infection, seven mice were sacrificed every two weeks. Before sacrifice, serum levels of types collagen I and IV, and procollagen-III-peptide were examined. Pathological changes of the liver were examined by Hematoxylin and eosin and Masson trichrome stainings. And localization of types I, III and IV collagens were detected by immunopathological methods.

Five weeks after the infection, serum level of type IV collagen showed significant elevation and kept high levels until eleven weeks after the infection. By immunopathological method, type IV collagen was detected along the wall of portal veins in the liver from five weeks to eleven weeks after the infection. But it was not clearly detected in granulloma formation around ovas of schistosomiasis.

Serum levels of type I collagen and procollagen-III-peptide did not show high elevation in the early stage of the infection. As the progress of periportal fibrosis due to schistosomiasis japonica, they became to show high levels. And they were detected in granulloma formation due to schistosomiasis in the liver.

Type IV collagen is detected along the wall of portal vein in the liver from early stage of the infection. The serum level of type IV collagen is more sensitive to periportal changes due to schistosomiasis than the other types of collagen such as types I and III.

B—34 INFLUENCE OF ULTRAVIOLET RAYS ON INFECTION OF *SCHISTOSOMA MANSONI*

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There are reports that ultraviolet rays exposure changes immunology ability. So we examined that the changes of immunology ability induced by artificial ultraviolet (UV-B) exposure influences to infection of *Schistosoma mansoni*.

After ultraviolet rays exposed to DDY mice, the value of CD4/CD8 in the mice decreased gradually for one week and was minimum value which was significant difference compared with non-exposure mice at one week after. Afterwards, it recovered to the normal value for 2 weeks.

The 24 DDY mice separated to three groups of control, skin and lung. Control group had not without UV-B irradiation, and skin and lung groups were irradiated at 400 J/m^2 of UV-B two times for one week interval. Skin group was infected by cercariae of *S. mansoni* at one week after the UV-B irradiation, and lung group was infected by it at one day after the UV-B irradiation. Challenge infections were 500 cercariae with abdominal skin cut hair of all mice under anaesthesia.

On the number of cercariae invaded, it in skin group was significantly many cercariae compared with it in control group and lung group ($p<0.01$), but it was no significant difference between control group and lung group. There were not significant differences among three groups on the egg of *S. mansoni*.

On the number of adult worm recovered, it in lung group was significantly many cercariae compared with it in control group and skin group ($p<0.01$). UV-B exposure was encouraged cercariae invasion into skin, but it was not due to skin damage by UV-B in skin group results. On the other hand, lung group data is suggest that immunology ability about one week after infection, when many cercariae gathered in lung was important to inhibit cercariae growing.

B—35 USEFULNESS AND LIMITATION OF COPT IN LOW ENDEMIC AREA OF SCHISTOSOMIASIS

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In high or low schistosomiasis endemic area, usefulness of immunological tests is different. We compared the changes of prevalence of schistosomiasis detected by Kato-Katz's stool examination and positive rate of circum oval precipitin test (COPT) in a high endemic area, Leyte Island and a low endemic area, Bohol Island, Philippines.

There are about 270 schistosomiasis endemic villages in Leyte Island and the area of snail colonies is very wide. So in Leyte, selective or non-selective mass treatment has been a main measure for control of schistosomiasis and

snail control measures have not been so active. In Leyte, the prevalence of schistosomiasis decreased from 8% in 1986 to 4% in 1996, however, COPT positive rate kept 30-40%.

In Bohol Island, there are eight schistosomiasis endemic villages and the area of snail colonies is limited. Each village has 700-2,000 inhabitants and total population is about 10,000. Through yearly selective mass treatment together with snail control, the prevalence of schistosomiasis japonica had decreased from 4.2% in 1982 to 0.1% in 1996. In three villages, infected snails rapidly disappeared, and no snails have been found since 1990. In these villages, the prevalence of schistosomiasis had decreased from 3.8% in 1982 to 0.8% in 1988. And COPT positive rate had also decreased from 10.1% in 1982 to 2.2% in 1988. In the heavy endemic village, infected snails had been found until 1992. In this village, the prevalence of schistosomiasis decreased from 8.4% in 1982 to 2.6% in 1988, however COPT positive rate showed 21.3% and 14.1%. And six years after the disappearance of infected snails, COPT positive rate became 0% among primary school children in 1996.

By a transmission of schistosomiasis from the snail to human, COPT shows positive reaction and it continues for two years after the treatment with praziquantel. COPT is a good indicator to evaluate frequency of transmissions in low endemic area of schistosomiasis. And in the final stage of control measures COPT is also useful to evaluate elimination of schistosomiasis.

B−36 URINE CYTOLOGY IN AN ENDEMIC AREA OF SCHISTOSOMIASIS HAEMATOBIA IN KENYA

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Urinary schistosomiasis has been linked to bladder cancer since the turn of the 20th century. However, the incidence in the community is still unknown due to a scarcity of a population-based studies. Although reported sensitivity for urine cytology detection of bladder cancer is approximately 40%, urine cytology has been an integral part of bladder cancer screening. And this is technically feasible, relatively inexpensive and readily acceptable by the community. We conducted a urine cytologic survey on the inhabitants of an endemic and non-endemic area of schistosomiasis haematobia in Kenya. The target populations were villagers who were all 20 years and over. Urinary cytology was performed using a slight modification of the standard techniques. The smear reports were classified according to Papanicolaou (1946). Single voided specimens were obtained from 1,516 villagers in an endemic area and from 294 villagers in non-endemic area. The cytological reports were divided into 3 class V, 1 class IV, 6 class III, 22 class II and 1,484 class I. Those of non-endemic area were all class I. The patient age for the 4 cytologically detected bladder carcinomas ranged between 38 and 70 years. One of the patients was male. Squamous metaplasia, RBC, WBC and schistosome eggs were encountered in 2, 2, 2 and 1 of the patients. Cytologically diagnosed tumors included 2 squamous and 2 transitional cell carcinomas. All villagers with class III had squamous metaplasia. Our result is comparable to that from screening bladder cancer in the high-risk group of a rural Egyptian population infected with *S. haematobium* (El-Bolkainy *et al*., 1982).

C−1 HEPATIC PENICILLIOSIS MARNEFFEI IN NORTHERN THAILAND

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Penicillium marneffei, which is the only dimorphic member of the genus *Penicillium*, was first isolated from the liver of a bamboo rat (*Rhizomis sinensis*) in Vietnam in 1956. *P. marneffei* shows globose-to-oval shaped yeast-like cell, which measures 2-3×2-7 μm with obvious septa. *P. marneffei* infection is one of the major opportunistic infections among the HIV infected individuals in Southeast Asia, particullary in Northern Thailand. We performed a clinicopathological study of hepatic penicilliosis marneffei in Chiang Mai, Northern Thailand, examining 30 cases of AIDS patients with disseminated *P. marneffei* infection. Hepatic lesions of the liver biopsy specimens were histologically classified to three types as follows; 1) 10 cases of Diffuse type (D) with diffuse infiltration of histiocytes, which contain a numerous number of *P. marneffei* and without significant cellular reaction, 2) Six cases of Granuloma type (G) with multiple granulomas, which contain a few number of intra- and extracellular *P. marneffei* and with mild to moderate cellular reaction, 3) 14 cases of Mixed type (M), which showed intermediate features between D and G types. Clinically, the highest elevation of AST level was found in D (mean level; 252), followed by M (mean level; 245) and G (mean level; 108). The highest AL-P level was observed in M (mean level; 535), followed by G (mean level; 473) and D (mean level; 389). These findings suggest that D infections cause the hepatocyte injury, the bile ducts are mainly damaged by G infections and M infections show the both of hepatocyte and bile duct injuries and the degrees of immunosuppression of the patients might influence the histological and clinical differences of hepatic penicilliosis marneffei.

C−2 A UNIQUE DRUG-SUSCEPTIBILITY PATTERN OF *VIBRIO CHOLERAE* O1 IN LAOS

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Cholera epidemic due to polymyxin B sensitive El Tor vibrios appeared in southern part of People's Democratic Republic of Lao since 1998.

During the past 7 years (1993-1999) in Laos, we have investigated the changes of drug susceptibilities of *Vibrio cholerae* O1. Until 1996, the susceptibilities were almost as expected and cholera disappeared in 1997. When cholera outbreak resurfaced in 1998, the susceptibilities of isolated *V. cholerae* O1 against tetracycline, sulfomethoxazoltrimethoprim, chrolamphenicol and polymyxin B were quite different from the previously isolated organisms. Minimum Inhibitory Concentrations (MICs) of tetracycline and chrolamphenicol against the isolates in 1998 were 3.13 or 6.25 μg/m*l* which were about 16 times higher than the MICs against the previous isolates. The MICs of sulfomethoxazol-trimethoprim were about 256 times higher than those against the previous isolates, they were mostly 640 μg/m*l* (trimethoprim 32 μg/m*l*, sulfomethoxazol 608 μg/m*l*) or more. Eleven percent of the isolates (11/99) were as sensitive to polymyxin B as the classic cholera vibrios (MIC<2 μg/m*l*). Polymyxin B sensitive strains were isolated from Southern part of the country and the City of Vientiane (central Lao) but not from Northern districts. In 1999, the susceptibility pattern was almost the same with that in 1998 except polymyxin B to which 58% of the isolates (21/36) became sensitive.

In 2000, the stool samples were not collected from the epidemic area in the South but only 3 strains because of traffic disturbance. The 3 strains were sensitive to polymyxin B, whereas the all strains from the North were resis-

tant. The susceptibility pattern to the other drugs was almost the same with those in 1999.

Genetic analysis using PFGE suggested that the clones of the isolates in 1998 and 1999 were closely related regardless of the drug-susceptibilities, but different from the isolates in 1995. Polymyxin B sensitive isolates produced El Tor hemolysin which was neutralized by the corresponding antiserum. Polymyxin B susceptibility test is no longer the tool for biotype classification of *V. cholerae* O1. Ecological and epidemiological consideration should be made for the polymyxin B sensitive El Tor vibrios.

C−3 TWO INFANT CASES INFECTED WITH TYPHOID FEVER IN THE PHILIPPINES

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If an overseas trip returnee (especially returned from developing countries) have had an onset of fever, it is necessary to perform diagnosis and clinical examinations, taking imported infections into consideration. This time we report our experiences of two infant cases with typhoid fever who had infected in the Philippines.

Case 1: Male, infant aged one year. The patient stayed in the Philippines, the homeland of the patient's mother, from January 1999 to late in April 1999. Since April 28, 1999, the patient became febrile and also had an onset of diarrea. Then the parient visited this department on April 30, 1999. The administration of a medicine for intestinal disorders improved diarrea, but the fever persisted and the patient admitted to this department on May 12, 1999. At the admission, the patient had the distention of abdomen. Clinical examination data at admission were WBC 8,900/μl Hb 8.9 g/dl, GOT 354 IU/l, GPT 284 IU/l and CRP 2.5 mg /dl. Abdominal ultrasonography (US) revealed hypertrophy of enteric wall. Blood culture at admission was Salmonella Typhi positive. We started IV of ABPC. Later it was found that the strain of Salmonella Typhi in the patient was ABPC resistive. Therefore, we changed ABPC IV to peroral administration of norfloxacin (NFLX). Two days after, the fever was alleviated. NFLX was administrated perorally for two weeks in total. No recurrence was found thereafter.

Case 2: Male, infant aged eight months. On July 29, 2000, the patient returned from the Philippines where the patient was grown up from birth. Since August 1, 2000, fever of around 38℃ manifested. Since August 5, 2000, the patient had an onset of diarrea, then fever of 38℃ to 40℃ persisted. Then on August 7, 2000, the patient visited this department. The clinical examination were, WBC 9,380/μl and CRP 3.8 mg/dl. Then we doubted bacterial enteritis and we administrated fosfomycin (FOM). However, water ingestion reduced and the patient was admitted to this department on August 10, 2000. At the time of admission, slight distention of the abdomen was found. The feces culture of the day before admission was *Salmonella* O7 group positive. Blood culture of the time of admission was Salmonella Typhi positive. Abdominal US revealed hypertrophy of gallbladder wall. We administered two antimicrobes, cefotaxim (CTX) by IV and NFLX orally. The fever around 39℃ persisted and it took twelve days from the start of the therapy to the complete alleviation of fever. After discharge the patient had no fever and the systemic condition was well, but the feces culture for confirming eradication of Salmonella Typhi bacilli was positive and fever manifested again. Then the patient readmitted to this department because of recurrence. We administered amoxillin (AMPC) perorally. Two days after, the fever was alleviated. No recurrence was found thereafter.

Discussion: The improvement of sanitary conditions has made the infection with typhoid fever in Japan rare. The number of report of typhoid fever in infant in Japan is also rare. However, if a patient have ever been abroad (especially to South East Asia and developing countries) typhoid fever must be considered in case of differential diagnosis. Regarding infantile typhoid fever, general condition is not severely worsened and it often tends to be diagnosed as common cold. Feces and blood culture test are important for the differential diagnosis of typhoid fever. As antimicrobial therapy for typhoid fever, new quinolon type antimicrobial drugs are mainly used recently because of increase of resistive Salmonella Typhi strains for chloramphenicol (CP), ABPC, and ST mixture and consequentry, recurrence of disease. In these two cases, NFLX was effective for the case 1, but recurrence was obserbed in the case 2 in spite of the administration of NFLX. Infants are in disadvantageous

situations regarding the treatment of typhoid fever, for example, tosufloxacin (TFLX) and levofloxacin (LVFX) that are frequently used in adults with typhoid fever has no indication to infants. Therefore we believe that to improve the system for enabling the administration of TFLX and LVFX to infants with typhoid fever in case the benefits of treatment exceed the risk of administration of TFLX and LVFX.

C−4 TWO CASES OF MELOIDOSIS IN JAPAN

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Melioidosis is amphixenosis caused by *Burkholderia pseudomallei*. Its patients have been commonly observed in the Southeast Asia region between latitude 20° N and 20° S. This bacterium can infect from injured skin percutaneously or through the respiratory tract, and form an abscess on any organ of the body. There are few effective medications for it. Both case fatality rate and recurrence rate of this infectious disease are high in Southeast Asia. Reported in this study are two cases of melioidosis observed at our hospital.

(Case 1) A 69-year-old male patient. Chief complaints: fever, breathing difficulty. Medical history: The patient was diagnosed with diabetes in 1979 and has been on a dietary regimen since then.

Case histry: On Jan. 30, 1998, the patient traveled to Myanmar, and several days later, started showing symptoms of common cold. After the fever exceeded 38℃, the patient returned on Feb. 3. Two days later, he visited a nearby hospital and was administered ofloxacin and ceftazidime, but did not recover. On Feb. 18, he visited our hospital. Upon observing water retention in the upper left of the chest on plain films, and a honeycomb pattern on the right lower limb, the patient was admitted to the hospital.

Developments: By the pleural effusion centesis, the patient was diagnosed with empyema. A drainage of the thoracic cavity was performed while administering panipenem/betamiprom 1.0 g/day, and clindamycin 1.2 g/day. This was followed by an incision to discharge pus for the honeycomb pattern. After identifying *B. pseudomallei* at both sites, we diagnosed it as melioidosis. After that, the patient made a rapid recovery.

(Case 2) A 33-year-old male patient. Chief complaints: Abdominal pain on the left side. Personal history: Vietnamese, immigrated to Japan 10 years ago, has never traveled abroad since.

Medical history: The patient was diagnosed with diabetes three years ago, but had received no treatment.

Case history: On Oct. 1, 1999, the patient visited a hospital for abdominal pain on the left side. A conspicuously high level of blood sugar was observed and he was admitted to the hospital for the treatment of diabetes. An abscess on the spleen was suspected after abdomen CT. On Oct. 8, the patient was transferred to our hospital for complete examination and further treatment.

Developments: We treated the patient conservatively by administrating cefazolin 2.0 g/day, but with little improvement. On Oct. 15, drainage of splenic abscess was performed. After identifying *B. pseudomallei* at the same site, we diagnosed it as melioidosis. For antibacterial medication, meropenem 1.0 g/day and minocycline 200 mg/day were administered. After that, the patient made a rapid recovery.

(Discussion) In recent years, researchers have reported a declining tendency in pharmaceutical sensitivity of this bacterium to antibacterial drugs. In the two cases we studied, sensitivity was low on such drugs as the β-lactam antibacterial. Since the bacteria in this study is known to proliferate in a cell, we found it highly possible that the patient in Case 2 was inadvertently infected with the bacterium in Vietnam. It then developed further due to a suppression of the immune system, which had been caused by diabetes. Although this kind of afferent infectious disease is rare in Japan, it is detectable by a general bacteriological examination. As travelers to Southeast Asia are on the rise, it will be necessary to exercise adequate caution to detect this infectious disease.

C−5 MEASLES SEROSURVEILLANCE STUDY DURING MASS IMMUNIZATION CAMPAIGN IN MALAWI: ANTIBODY PREVALENCE AND SEROLOGICAL RESPONSES USING PARTICLE AGGLUTINATION METHOD

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(Objective) To determine age-specific measles antibody prevalence and serological response of vaccination on the first mass campaign against measles in Malawi.
(Design) Cross-sectional study using a questionnaire and a serological particle agglutination (PA) test.
(Settings) Two health centres in Salima district, central Malawi during the national measles immunization week, 1998.
(Participants) Two hundred forty-six children under five years old.
(Results) The enrolled children of 74% (95% confidence interval, 69-80%) were measles PA antibody positive at the vaccination. The antibody positive rate was 17.4% in children aged 8-12 months and gradually increased up to 90% by four years-old, while the age-specific geometric mean titers (GMTs) in 48-59 months-old group were significant lower than those in 24-35 months-old group, suggesting antibody waning after previous vaccination (p=0.0047). Two hundred thirty follow-up specimens were obtained eight weeks after the vaccination. The seroconversion rate was 100% in 58 children seronegative at the vaccination and the GMTs in 172 children seropositive at the vaccination were significantly increased (p<0.001).
(Conclusion) These results indicated that the first national measles immunization campaign successfully immunized the enrolled children or gave a booster response of antibody levels. We also confirmed that the PA test was easy to perform and most suitable for the field condition in developing countries.

C−6 EXPRESSION AND SECRETION OF MUTANT CHOLERA TOXIN AND ITS B SUBUNIT IN AVIRULENT RECOMBINANT *SALMONELLA*: CONSTRUCTION OF COST-EFFECTIVE ORAL VACCINES AGAINST INEFCTIOUS DISEASES

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Recent advances in molecular biology and immunology have led to the development of novel strategies for vaccine production and their delivery method. Examples include DNA vaccines, polypeptide vaccines and vaccines based on recombinant BCG. However, one of the most important parameters required for practical applications of vaccines against many infectious diseases, especially prevalent in developing nations, is the cost of production and their application method. Many infectious diseases such as malaria are widely spread over the areas of tropical and subtropical regions of world where only low-cost vaccines are practically useful.

Immunizations through gastrointestinal tract might provide an effective way of stimulating both local and systemic immune responses. However, oral vaccines, the most convenient way of vaccine antigen delivery method, must be formulated in such a way that vaccine antigens are protected from protein degradations occur in the gastrointestinal tract. Vaccine antigens produced and delivered by avirulent recombinant Salmonella may be provided such protection as they pass through the stomach and intestine. In addition, purification processes are not required for antigens produced in recombinant Salmonella. Salmonella-delivered vaccine antigens are efficiently presented to cells of mucosal immune systems such as Peyer's patches for induction of both humoral and cell-mediated immune responses.

We have expressed nontoxic mutant of cholera toxin (mCT) in avirulent Salmonella and found that the mCT was efficiently secreted to the culture media confirmed by G_{M1}-ELISA method. The mCT molecule may function as a mucosal adjuvant for co-locally produced and/or secreted foreign protein antigens derived from other pathogenic microorganisms such as malaria and Japanese encephalitis virus. We are also interested to find whether such secreted mCT provide mucosal adjuvantisity for co-expressed pathogen-derived antigens. In addition, we are producing Salmonella secreting fusion proteins between mCT and microbial antigens for effective induction of systemic immune responses against fused antigens. Avirulent recombinant Salmonella may provide cost-effective vaccine antigen production and delivery systems for induction of local and systemic immune responses against many life-threatening infectious agents to which new type of low-cost vaccines are need for the improvement of health of humans and domestic animals.

C—7 EVALUATION OF DENGUE DIAGNOSTIC TEST KITS

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Dengue virus infections are a major public health problem in tropical and sub-tropical countries of the world, and dengue is considered as one of the most important re-emerging infectious diseases. There are currently no domestic dengue virus infections in Japan; however, imported dengue cases have been reported. Detection of specific IgM by IgM-capture enzyme-linked immunosorbent assay (ELISA) and of dengue virus genome by reverse transcriptase polymerase chain reaction (RT-PCR) have been widely used as laboratory diagnosis. Commercially available kits for the detection of anti-dengue virus IgM have recently been developed. These standarized assays have greatly enhanced our ability to effectively and efficiently diagnose dengue virus infections. In the present study, we evaluated the dengue diagnostic test kits in comparison with in-house diagnostic assays. Four prototype dengue strains (type 1, Hawaii; type 2, New Guinea C; type 3, H87; and type 4, H 241) were used as the antigens for in-house IgM-capture ELISA. Viruses were grown in the *Aedes albopictus* mosquito cell clone C6/36. Three commercial diagnostic assays, a IgM-capture ELISA (MRL, California, USA), an immunochromatographic card assay and a strip assay (PanBio, Brisbane, Australia) were evaluated for detection of IgM to dengue viruses. The specificity of the in-house ELISA with a flavivirus group specific monoclonal antibody, D1-4G2-4-15 was higher than that with patients' IgG, and lower levels of specific IgM was detected in the serum samples. These results suggest that the modified dengue IgM-ELISA with monoclonal antibody has multiple advantages over the original in-house ELISA. We compared the in-house ELISA with the commercial ELISA kits. Fifty-seven serum samples from confirmed and suspected dengue cases were tested by the in-house and the commercial ELISAs. The results were consistent between the in-house ELISA and the commercial one, except for one sample. The results by the new commercial kits (DEN-25, new type and DEN-25S, strip type) were different from those by the in-house ELISA and the commercial ELISA kit.

C−8 A CLINICAL STUDY ON DENGUE HEMORRHAGIC FEVER IN METRO MANILA, PHILIPPINES

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Dengue hemorrhagic fever (DHF) is characterized by acute fever associated with hemorrhagic diathesis and a tendency to develop shock, which is distinct from dengue fever. However, the mechanisms responsible for bleeding in DHF remain unclear. To elucidate the mechanisms of hemorrhagic diathesis in DHF, we evaluated the clinical features and hematologic data including platelet counts, fibrinogen levels, bleeding time, prothrombin time, fibrin degradation products (EDP) and anti-platelet antibody in sera among 65 patients with virologically confirmed dengue infection. From our preliminary data, we diagnosed dengue fever (DF) (n=38) and DHF (n=27) by the WHO criteria. The mean age is 10.8 and 8.6 years old in DF and DHF, respectively. Bleeding manifestations other than subcutaneous hemorrhage was observed in 48% of patients with DHF. A patient with DHF grade IV died due to prolonged thrombocytopenia and generalized hemorrhage. The platelet counts in DHF were significantly lower than those in DF (42,000 in DF vs. 109,000 /μl in DHF; p value <0.001). The fibrinogen levels in DHF were significantly lower than those in DF. No significant difference was found in prothrombin time between DF and DHF. Disseminated intravascular coagulation (DIC) was found only in 11.1% of patients with DHF and in 5.2% of patients with DF by DIC scores (symptoms and laboratory data described above). Platelet binding antibody was not detected in sera from either patients with DF and DHF. Our data indicate that DHF is closely associated with severe thrombocytopenia and is not frequently associated with DIC. The mechanisms of thrombocytopenia in DHF requires further investigation.

C−9 A CASE OF DENGUE FEVER COMPLICATED WITH PROBABLE ACUTE DISSEMINATED ENCEPHALOMYELITIS

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Dengue fever is mosquito-borne viral infection and primarily a disease of older children and adults in south-east Asia and western Pacific countries. The dengue virus is a Flavivirus and *Aedes aegypti* is a major vector. Recently, several factors including demographic changes and increased chances of air travel contribute to increased epidemic/endemic dengue activity. Forty percent of world population is currently under constant threat of this infection.

We report here a dengue fever case of a 58-year-old Japanese male who lived in Brazil. He had fever, anorexia, and rash on Jan. 15, 2000, and was made diagnosis of dengue fever because of positive IgM antibody to dengue virus. On the 9th day after onset, he experienced neurological symptoms including confusion, bilateral visual disturbance, sensory disturbance and mild paraplegia of lower extremities, which was gradually worsened to complete palsy below Th7 level by the 11 day after onset. He was transferred to our hospital on Feb. 25, 2000.

MRI revealed multiple spots of T2WI high lesions in spinal cord of Th 7～11 without abnormal findings in brain. CSF study showed slightly increased cell number and protein concentration. From his clinical course and MRI, we suspected his neurological symptoms as acute disseminated

encephalomyelitis (ADEM). Thus, 3 cycles of methylprednisolone (1,000 mg/day) for three days were administrated intravenously, and bilateral sensory disturbance and paraplegia were improved step by step after each cycle. After 3 months from the onset, his spinal MRI did not show any abnormal findings, and he could walk with help of a stick, although mild bilateral visual disturbance, dysuria, and dyschezia remained unchanged.

ADEM is an immune-mediated inflammatory demyelinating disease of central nervous system. It usually occurs acutely and exhibits monophasic course about 1 or 2 weeks after viral infection and vaccination. There have been some reports about the involvement of central nervous system in dengue fever and dengue haemorrhagic fever, such as multiple neuropathies, mononeuropathy, and meningoencephalitis. However, we could not find reports of ADEM caused by dengue fever. Thus, this seems to be the first case of ADEM associated with dengue fever.

C−10 DENGUE VECTOR SITUATION ALONG URBAN-RURAL ECOLOGICAL GRADIENT

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Larval survey of dengue vectors were conducted from July 1996 to September 1997 in Chiangmai Province, Thailand. Three study villages at urban (Sri Phoom), semi-urban (Mae Rim) and rural area (Mae Taeng) were selected for the survey to clarify the spatial distribution of *Aedes aegypti* and *Ae. albopictus* along urban-rural ecological gradient. The results of ovi-trap survey showed clear differences in larval density among the study areas. The average density of *Ae. aegypti* larvae was the highest at urban area and intermediate at the rural area. Although we expected the lowest density of *Ae. aegypti* at rural area, it was observed at semi-urban area in this study. The results for *Ae. albopictus* were opposite to *Ae. aegypti*, semi-urban> rural area> urban area. House survey of breeding containers showed significant differences in the number and composition of breeding containers among the study areas. The seasonal pattern of rainfall recorded at the study areas from February-November 1997 did not differ significantly among the study areas. However, there were significant area differences in the average distance between houses, average tree height, and percentage of area with vegetation cover. These results suggested that urbanization is one of important factors influencing density and distribution of dengue vectors. However, there might be additional ecological factors to explain area differences in dengue vector situation.

C−11 MOLECULAR AND *IN VITRO* ANALYSES OF DENGUE-1 VIRUS STRAINS

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The genomic RNA of two dengue-1 virus strains was sequenced and compared with other published strains to identify regions of dengue genome that might contribute to the viral growth characteristics *in vitro*. The growth rate and yields of the mouse-adapted Mochizuki strain (isolated in 1943) in Vero cells were significantly higher and their infectivity decreased slightly slower than those of A88 strain (isolated in 1988) after freezing at −80℃. Analysis of deduced amino acid sequences indicated charge differences, mostly observed at the envelope (E) protein of Mochizuki

strain. The other charge differences were observed at capsid (C), pre-membrane (prM), non-structural NSl, NS4b, and NS5 proteins. A unique amino acid, Ile-69 for Mochizuki at E protein resulted in the loss of an Asn-67-linked glycosylation site. Radical amino acid differences for A88 strain were observed at the cleavage site of C/prM junction, E, NS3, NS4a, and NS5 proteins. These differences might be related to their different growth characteristic *in vitro*.

C−12 RISK OF WEST NILE FEVER AND JAPANESE ENCEPHALITIS IN JAPAN

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A survey of viruses in *Culex tritaeniorhynchus* of Karachi and environs, Pakistan, was conducted from 1985 to 1993. A total of 208,375 *Cx. tritaeniorhynchus* were submitted to virus isolation. The Japanese encephalitis virus (JEV) was not detected but 4 serotypes of West Nile virus (WNV) were isolated from 1,865 *Cx. tritaeniorhynchus* of 20 pools collected on July 6-8, 1986 in Karachi. In the cerebrospinal fluid collected from 24 patients with acute encephalitis in Karachi during February-August 1992, the genome of WNV and JEV were detected in 8 and 1 samples, respectively. The results suggested that WNV but not JEV is present commonly in Karachi, with occasional incidences of JEV infection that is transported by migratory birds coming from Siberia in October. Similarly, the migratory birds come from India or Middle East to Japan in May-June, when the mosquitoes also become more active. The number of birds imported to Japan is estimated to be 420,000 adults and 152,000 young birds per year, and they are not inspected for viral infection. Living mosquitoes are also found in the hold of airplanes, with a possibility of introduction of infected mosquitoes in Japan by flights coming from Africa, Middle East or other parts of the world. The mosquito vectors for WNV or JEV, such as *Cx. tritaeniorhynchus, Cx. pipiens pallens, Cx. pipiens molestus, Aedes albopictus, Ae. japonicus* and *Ae. vexans* are commonly found in Japan. Moreover, in August 1998, the JEV was isolated, in high proportion, from *Cx. tritaeniorhynchus* collected in Uchinada, Ishikawa Prefecture. Besides this, an increase in antibody titer against JEV was detected in sera of pigs during August and September. All these facts suggest a high risk of occurrence of West Nile fever and Japanese encephalitis in Japan in the future, and a continuous surveillance with isolation of virus from birds and wild animals are of significant importance.

C−13 A STUDY ON ANTIGENICITY OF JAPANESE ENCEPHALITIS VIRUS ISOLATES IN VIENTIANE, LAO PDR

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Epidemics of Japanese encephalitis (JE) have not been repored in Lao PDR, the inland country of Indo-china peninsula surrounded by JE epidemic countries such as Thailand and Vietnam. We reported that two JE virus isolates (LaVS 56 and LaVS 145) from slaughtered swine sera in Vientiane belong to Genotype II, distributing from northern Thailand to Cambodia, and that the sera of two JE patients of Vientiane did not neutralize both of Vientiane isolates,

but neutralized Chinese strain (Beijing-1) significantly. In this study antisera against 2 Vientiane strains (LaVS 56, and LaVS 145), Thai Chiang Mai strain (P19, Genotype II) and 2 standard strains, Nakayama (Japan, Genotype I), and Beijing-1 (China, Genotype I) were made in BALB/c mice and used to analyze antigenicity by cross-neutralization test. Anti-Nakayama and anti-Beijing sera neutralized significantly their homologous strains but not P19, LaVS56 nor LaVS145. Neutralizing reactivity of antisera for P19, LaVS 56, and LaVS145 was similar to each other: the three antisera significantly neutralized Nakayama, Beijing-1, LaVS 56, LaVS145, and P19 strains. The results indicate that LaVS56 and LaVS145 are the same antigenicity as P19, but distinct from Nakayama and Beijing strains. Neutralization pattern of 2 JE patients' sera of Vientiane was similar to that of antiserum for Beijing-1. And the neutralization patterns of the Vientiane patients' sera was compared with those of Chiang Mai (North Thailand) and Okinawa (Japan). Chiang Mai patients' sera neutralized JE viruses in the order of Nakayama, Beijing-1> P19 > LaVS56, LaVS145: this pattern was apparently different from that of Vientiane patients'. Okinawan patients' sera significantly neutralized Nakayama and Beijing but not P19, LaVS56 nor LaVS145.

These results suggest (1) The two JE virus Vientiane strains (LaVS56, LaVS145, genotype II) are antigenically the same as P19 which belong to antigenicity of ThCMAr 6793. (2) Two JE patients in Vientiane seemed to have been infected with JE virus that is antigenically the same as Beijing-1 strain. (3) There are JE viruses with at least two distinct antigenicities in Vientiane.

C−14 MUCOSAL IMMUNIZATIONS WITH A JAPANESE ENCEPHALITIS VIRUS ANTIGEN-CHOLERA TOXIN B SUBUNIT FUSION PROTEIN INDUCE VIRUS-NEUTRALIZING ANTIBODIES IN MICE: AN ATTEMPT FOR THE DEVELOPMENT OF EDIBLE VACCINES AGAINST JAPANESE ENCEPHALITIS VIRUS

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Recent advancement of plant genetic engineering technology has made many genetically modified (GM) plants commercially available. Our group is currently constructing several transgenic plants expressing vaccine antigen genes derived from virus and parasitic microorganisms. These GM plants may be used in future for induction of mucosal and systemic protective immune responses against infectious agents. The advantages of the GM plant-based vaccines are their ease of large-scale production and cost-effectiveness in comparison with conventional fermentation-based recombinant protein production systems. In addition, vaccines produced in edible plants may be used as oral vaccines for the induction of protective immune responses. However, protein components without specific affinity for mucosal tissues are generally weak in their immunogenicity.

Therefore, we took advantage of specific affinity for mucosal immune systems exhibited by cholera toxin B subunit (CTB) for more efficient induction of mucosal and systemic immune responses. In this study, we expressed a fusion gene between Japanese encephalitis virus (JEV) E glycoprotein and CTB genes in Agrobacteria. We also conducted immunization experiments for evaluation of the fusion protein as mucosal vaccines.

Experimental methods and Results: A fusion gene (E glycoprotein/CTB) has been constructed and inserted downstream of the cauliflower mosaic virus 35S promoter in a plant expression vector pBI121. The constructed plasmid DNA was introduced into *Agrobacterium tumefaciens* LBA 4404, a bacterial vector used for plant transformation. The transformed bacteria was found to produce the fusion protein retaining specific affinity for G_{M1}- ganglioside, a recep-

tor of cholera toxin. Several groups of mice were immunized with crude extracts of the fusion protein in nasal, oral and intra-peritoneal routs. Immunization was conduced once a week for a total of 4 immunizations. A week after the last immunization, antibodies were analyzed. In contrast with mice immunized intra-nasally and orally, mice immunized intra-peritoneally produced strong anti-bacterial antibodies without anti-fusion protein antibodies. Both groups of mice immunized in nasal and oral routs developed specific antibodies against the fusion protein. We have also immunized mice with commercially available JEV vaccine in nasal and oral routs to determine the mucosal immunogenicity of the vaccine. Mice immunized intra-nasally developed anti-virus antibodies whose immunogenicity was significantly enhanced when the vaccine was mixed with a mucosal adjuvant cholera toxin. However, oral immunizations with the JEV vaccine did not develop detectable levels of virus-specific antibodies even in the presence of cholera toxin. Antisera containing anti-fusion protein or anti-JEV vaccine antibodies were analyzed for their virus neutralization ability *in vitro*. Antisera developed in mice immunized with the JEV vaccine by intra-nasal immunization strongly neutralized the virus *in vitro*. Anti-fusion protein antibodies also exhibited weaker but significant virus-neutralizing capability. This study indicated that E glycoprotein/CTB fusion protein and commercially available JEV vaccine are mucosally immunogenic for induction of virus-neutralization antibodies.

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C−15 WHAT IS THE ROLE OF APOPTOSIS IN FLAVIVIRUS-INDUCED CYTOPATHIC EFFECT?

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Introduction: Japanese encephalitis (JE), St. Louis encephalitis (SLE), Murrary Valley encephalitis (MVE) and West Nile fever (WNF) viruses, are members of the family *Flaviviridae*. These viruses are members of the JE antigenic complex and associated to encephalitis. Flaviviruses produce cytopathic effect in a variety of continuous cell cultures. In recent years, a growing number of viruses have been shown to induce cell death by a mechanism called apoptosis. The purpose of this study was to investigate whether the observed cytopathic effect due to flavivirus infection in a human mononuclear cell line (K562) could be related to apoptosis.

Materials and Methods: C6/36 cell line was used for viral growth and BHK-21 cell line was used for virus titration by focus formation assay. An input m.o.i. of 1 FFU/cell was used and mock infected cells were used as negative control. Cells were harvested at various times post infection (pi) and infected cells were identified by flow cytometry using FITC labeled anti-flavivirus group specific antibody. Cell survival was determined by trypan blue exclusion test. Apoptotic cells were identified by DNA content analysis (hypotonic fluorochrome solution: PI 50 μg/ml in 0.1% sodium citrate plus 0.1% Triton X-100) by flow cytometry and chromatin condensation by Hoechst staining.

Results: Flow cytometry analysis revealed that at 48 hr pi 68% and 69% of cells were infected by WNFV and MVEV, respectively. The percentage of cells infected by JEV and SLEV, however, was above 90%. Seventy two hpi a rise in cell mortality up to 30% was observed for cells infected with JEV, MVEV and SLEV; and 76% for WNFV infected K562 cells. Propidium iodide staining of the nucleus revealed that approximately 50% of K562 cells had subgenomic DNA content (43% for JEV, 44% for MVEV, 49% for SLEV, 50% for WNFV) as compared to mock infected cells (2%). Similar percentages of K562 cells showing chromatin condensation by Hoechst staining was observed for JEV, MVEV and SLEV, although for WNFV this value was above 80%.

Discussion: In this study we demonstrated that the cytopathic effect observed in JEV, SLEV, WNEV and MVEV infection of the human mononuclear K562 cell line progresses with cell shrinkage, chromatin condensation and a rise in the percentage of cells showing subgenomic DNA content. All these, characteristics of cell death mediated by apoptosis.

C−16 EPIDEMIOLOGICAL SURVEY ON THE RELATION BETWEEN HELMINTH INFECTION AND NASAL ALLERGY

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The increase of allergic patients has arisen an issue of the effect of helminth infection in allergy. Our animal experiments demonstarted that helminth infection induced potentiated IgE production and the hyper IgE resulted in blockade of IgE receptors on mast cells. The former is augmenting factor and the latter is suppressing factor for allergy. Many epidemiological studies suggested that helminth infection suppresses onset of allergic diseases. However, these studies were performed mainly in developing countries where bacterial and viral infections were endemic. These infections also interfere allergy.

Therefore, rigid epidemiological study under clean and defined circumstances is necessary. Our survey is conducted to evaluate the prevalence of nasal allergy in humans infected with *Ascaris suum* in Kyushu, Japan. The survey was carried out in southern part of Kyushu with total population of 1,283. 26% of residents of this area were infected with *A. suum*. The infection was determined by anti-*A. suum* IgG ELISA. 273 peoples received clinical examination by otorhinolaryngologists. Anti-cedar IgE was detected in 55% of *A. suum*-infected population and 27% of uninfected population. Cedar pollinosis was diagnosed in 32% of infected and 15% of uninfected population. Anti-house dust IgE was detected in 74% of infected and 34% of uninfected population. Allergic rhinitis by house dust was diagnosed in 53% of infected and 21% of uninfected population. In both cases positive rate of IgE antibodies and the prevalence of nasal allergy in infected population were twice higher than those in uninfected population. These results indicate that *A. suum* infection augments the prevalence of nasal allergy through potentiated IgE antibody production.

C−17 EPIDEMIOLOGICAL SURVEY ON ECHINOCOCCOSIS IN AOMORI PREFECTURE, JAPAN

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Since alveolar echinococcosis has been seriously endemic in Hokkaido, it is suggested that the disease will be spread to Aomori Prefecture near at Hokkaido, most Northern part of Honshu, mainland Japan. In Aomori Prefecture, therefore, the surveillance system is required to detect in its earliest stage of prevalence for echinococcosis. We carried out epidemiological survey for *Echinococcus multilocularis* infection among wild animals and also domestic animals.

1. Survey on *E. multilocular* infection is in wild animals

A. Definitive host: Although from 1997 to 1999, foxes (*Vulpes vulpes japonica*), raccoon dogs (*Nyctereutes procyonoides viverrinus*), martens (*Martes melampus melampus*) and weasels (*Mustela sibirica itatsi* and *M. nivalis namiyei*) were investigated, no animals were found infected.

B. Intermediate host: Since *Microtus montebelli, Apodemus* spp. and *Urotrichus talpoides* in Aomori Prefecture were investigated, since 1990, no animal infected was detected.

2. Survey on *E. multilocularis* infection in domestic animals

A. Pigs: In Aomori Prefecture, nearly 800,000 pigs are going to be killed in a year at the prefectural meat inspection center. During the inspection of 1998, 3 pigs were found infected with *E. multilocularis*. Those pigs were not originated in Hokkaido and confirmed to be bred in Aomori Prefecture. This issue indicated that the infection source such as infected foxes or porcine feed contaminated with *E. multilocularis* eggs were somewhere around the pigpen. It should be stressed that the life cycle of *E. multilocularis* possibly has been established already in Aomori Prefecture. Therefore, epidemiological

survey of the disease in mainland Japan is strongly necessary for prevention of the spread of *E. multilocularis* infection among wild animals.

B. Dogs and Cats: Although no animal was found infected with the parasites, the surveillance on dogs and cats must be intensively carried out for the public health in future.

C—18 PRELIMINARY REPORT OF A SURVEY FOR INTESTINAL PARASITOSIS AT TWO AREAS IN NEPAL

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We carried out a survey of intestinal parasitosis at a wet season, July and August, 2000 in Nepal to clarify actual infectious patterns, especially, of intestinal parasitosis caused by *Protozoa* such as amebiasis, cryptosporidiosis and cyclosporiasis. We chose two areas for the investigation to compare and examine the different environmental factors related to infectious patterns. The survey was done at a slum area along Tripreswor Street in Kathmandu and a rural agricultural village, Khokana, located a few kilometers far from the capital. The populations of the slum and the village were about one thousand and five thousand, respectively. We collected and examined 225 stool and serum samples in the former, and 386 in the latter. The infectious rates in the slum and the village were 32% and 44% with *Ascaridae*, 20% and 11% with *Trichuridae*, 3% and 4% with hookworm, 1% and 2% with *Hymenolepididae*, 0% and 0.2% with *Taeniidae*, 6% and 13% with *Giardia lamblia*, 14% and 6% with *Entamoeba histolytica*, 5% and 6% with *E. coli*, 2% and 1% with *Iodamoeba butschlii*, 2% and 1% with *Cyclospora cayetanensis*, and 1% and 1% with *Blastocystis hominis*, respectively. All parasites, even *Ascaris*, were found out in every age bracket, and any distinctive correlation between parasites and an age group did not exist in both areas. *Cryptosporidium* was never detected in any stool samples from both areas even by a floating method to concentrate oocysts with sucrose. The antibodies to *C. parvum* oocysts were examined with immunofluorescence assay, and then the positive rates of IgM, IgG, and IgA were 0%, 4% and 21% in the slum, and 1%, 0.5% and 6% in the village, respectively. Up to now we did not conclude anything about the survey study from the last data. In the near future we are going to accumulate data more to complete it.

C−19 PLEVALENCE OF ANTI-*TOXOCARA* ANTIBODIES AMONG CHILDREN IN JABOATÃO DOS GUARAPES, PERNAMBUCO, NORTHEAST BRAZIL

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In Brazil, epidemiological surveys on human toxocariasis have been conducted in São Paulo, and Pernambuco, northeast Brazil, and high prevalence rates (40%) have been repored (Virginia *et al*., 1991; Moreira-Silva *et al*., 1998). However, the high prevalence rates were considered to be due to the low specificity of the excretory-secretory (ES) antigen used in the surveys. Recently, the authors developed a recombinant *Toxocara canis* larval antigen with a high specificity for the immunodiagnosis of human toxocariasis. In April and May, 2000, the authors had an opportunity to examine the prevalence of anti-*Toxocara* antibodies using the recombinant *T. canis* antigen in Jaboatão dos Guararapes, near Recife, Pernambuco, northeast Brazil. In the present survey, a total of 215 serum samples from children ages from 1 to 17 years old were examined for the detection of anti-*Toxocara* antibodies by ELISA. Among 215 children, parasite eggs, including the most common species *Ascaris, Trichuris*, hookworms, and *Schistosoma mansoni*, which are endemic to this area, were detected in 163 cases (82%) and none were found in 35 cases (18%).

ELISA was performed by the procedure of Yamasaki *et al.* (2000) except that tetramethylbentzidine was used as a substrate. Anti-*Toxocara* antibodies were detected in 28 out of 215 samples when the recombinant *T. canis* antigen was used. Among these positive cases, 20 were also infected with *Ascaris, Trichuris*, hookworm and so on. No parasite eggs were detected in two cases. The data on parasite infection in the remaining 5 cases were not available. In order to clarify the specificity of the recombinant antigen against other parasitic infections, 16 children who were serologically positive for *Ascaris suum* adult antigen were selected. In tropical and subtropical areas of the world, ascariasis, trichuriasis and ancylostomiasis are the most common parasitic diseases, and crossreactions reduce the reliability of the results when conventional antigens are used. However, no serum sample from ascariasis crossreacted with the recombinant *T. canis* antigen. From these results, it has been confirmed that the recombinant *T. canis* antigen is highly specific and provides more reliable diagnostic results in epidemiological surveys of human toxocariasis.

This study was supported in part by Japan International Cooperation Agency (JICA).

C−20 KILLING OF *ACANTHOCHEILONEMA VITEAE* MICROFILARIAE BY MURINE EOSINOPHILIS

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This paper attempts to elucidate whether murine eosinophils are capable of producing nitric oxide (NO) which is possibly associated with the killing of *Acanthocheilonema viteae* microfilariae *in vitro* and *in vivo*.

A. viteae microfilariae were harvested from the blood of previously infected *Millardia meltada* whereas murine eosinophils from the peritoneal cavity of IL-5 transgenic mice which had been treated with $CdSO_4$. Microfilarial motility was assessed visually and its viability by MTT reduction method (Comley *et al.*, 1989). Production of NO by

eosinophils was assayed by measuring the accumulation of nitrite during culture using Griess reaction (Ding *et al.*, 1988).

When *A. viteae* microfilariae were co-cultured with IFNγ- or LPS-activated-eosinophils, they showed more reduced viability, in terms of morbidity and survival rate, than those co-cultured with non-activated eosinophils. IFNγ-activated eosinophils produced a significantly higher amount of nitrite in the culture supernatants than non-activated eosinophils. The addition of aminoguanidine, inhibitor of NO synthesis, to the assay system caused a recovery of microfilarial viability and a reduced nitrite production by eosinophils. The degrees of NO production and microfilaricidal activity by activated eosinophils are correlated with the numbers of eosinophils used. Moreover, eosinophils were more effectively activated when both IFNγ and LPS were simultaneously present in the culture.

To determine the possible association of eosinophils with microfilaremia *in vivo*, eosinophilic IL-5 transgenic mice and their counterpart normal C3H/HeN mice, were intravenously injected with microfilariae and their peripheral microfilarial levels monitored. Interestingly, IL-5 transgenic mice provoked a rapid reduction in peripheral microfilarial density, with its almost complete disappearance by day 14 postinjection, whereas, in C3H/HeN mice, microfilarial density remained very high up to this time.

These data suggest that eosinophils are involved in the disappearance of *A. viteae* microfilariae from peripheral blood, and also that NO derived from eosinophils could be a candidate effector molecule for killing microfilariae.

C−21 KINETICS OF MYOREGULATORY FACTORS IN THE NURSE CELL FORMATION OF *TRICHINELLA*

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It is well known that establishment of parasitism of *Trichinella* results in transformation of muscle cells leading to the nurse cell formation. This transformation can be confirmed in both capsule forming species (*T. spiralis*) and non-capsule forming species (*T. pseudospiralis*). The basic question resides in why terminally differentiated cells such as muscle cells can differentiate to another phenotype that has never been anticipated before the infection and probably never encoded in host's DNA.

In this study we developed a detection system for myogenic regulatory factors such as MyoD, myogenin and MRF4. Adapting the method we performed a longitudinal analysis of such regulatory factors after infection with *T. spiralis* and *T. pseudospiralis*. MyoD and myogenin, originated from satellite cells, were expressed from the early phase of cystogenesis in *T. spiralis* infection, during which time satellite cells were most activated. The expression returned to the normal level after 18 days from the infection when the cyst was complete, and no satellite cells were activated. In *T. pseudospiralis* infection, they were also expressed from the early phase of cystogenesis, but continuously expressed at least up to 43 days post infection. In this infection, satellite cells were continuously activated. MRF4, originating from muscle cells, was always highly expressed.

C−22 THE PREVALENCE OF MICRO-ALBUMINURIA IN DIABETIC PATIENTS ATTENDING THE MULAGO HOSPITAL DIABETIC CLINIC (MHDC), KAMPALA, UGANDA

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Background: One of the most serious complications of diabetes mellitus is diabetic nephropathy. If detected early, however, this complication can be arrested or even reversed with appropriate intervention. Micro-albuminuria is usually an early marker of this complication and also a signal for intervention. Unfortunately, routine monitoring of micro-albuminuria is not done in the Mulago Hospital Diabetic Clinic (MHDC), and therefore the prevalence of micro-albuminuria among patients attending MHDC is not known. The rationale of this study was to determine the prevalence of micro-albuminuria among patients attending MHDC, to provide some insight into the magnitude of incipient nephropathy, which is a stage of transition to full blown diabetic nephropathy.

Study Objectives: The objectives of this study were to determine the prevalence of micro-albuminuria among diabetic patients attending the MHDC, to find out some of the factors associated with micro-albuminuria, and to estimate the level of glycaemic control among the study patients.

Design: This was a cross-sectional descriptive study involving diabetic patients attending the MHDC.

Setting: The study was carried out in the MHDC located in the medical outpatient department of New Mulago Hospital, Kampala.

Subjects: The study subjects were diabetic patients who attended the MHDC during the months of January and February 1999 and fulfilled the study criteria. One hundred and twenty seven patients selected from the MHDC were enrolled in the study. These patients were randomly selected from among the patients attending the MHDC during the study period. Sixty-four of these were Type I diabetics and sixty-three were Type II diabetics. Their age range was 20 to 79 years, and they were not known to have any overt complications of severe renal disease.

Results: The prevalence of micro-albuminuria was 69%. Overall, the level of glycaemic control was poor among the study patients, with 53% of the patients having haemoglobinA_{1c} of 11% and above, representing very poor control. Ninety four percent of the patients with micro-albuminuria also had raised haemoglobinA_{1c}. Seventy-five (60%) of the patients had mild to moderate hypertension. The majority (68%) of the patients with hypertension also had micro-albuminuria.

Conclusion: Micro-albuminuria was a common finding among the study patients. This may suggest that incipient nephropathy is common among diabetic patients attending the MHDC. The implication of this finding is that if appropriate measures are not put in place, these patients are likely to develop overt diabetic nephropathy.

C−23 HEALTH CARE SURVICE FOR JAPANESE LIVING IN CITIES OF SOUTHEAST ASIA

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Purpose: We planed a survey aiming to understand the needs of medical health service for Japanese staying in Ha Noi (H), Phnom Penh (P), Vientiane (V), and Yangon (Y).

Subjects: Questionnaires were distributed to transient Japanese residents or permanent Japanese residents through each regional association of Japanese Society. One hundred and sixty six families responded from 4 cities, 44 (H), 32 (P), 31(V) and 59 (Y), respectively.

Methods: Questionnaire consists of questions asking family status, about satisfaction of climate and housing, the number of servants, who cook meals, about drinking water, about satisfaction of child life, about their offered medical

services from employee, about local medical services, and about their desired medical services.
Results: The percentage of the multiple member households was highest at Ha Noi (86.3%) and that of the single member household was highest at Yangon (55.5%). The percentage of people who were unsatisfied with hygienic level of their houses at 4 cities were 29.5% (H), 28.1% (P), 45.1% (V) and 62.7% (Y), respectively. Yangon was highest compared with other three places. About 97% of Japanese of either city bought drinking water or boiled their water before drinking. The percentage of people who were unsatisfied with medical services offered from there employee at 4 cities were 36.3% (H), 15.3% (P), 31.8% (V) and 43.1% (Y), respectively. It should be noticed that politically unstable Cambodia was better compared with other three places. The percentage of people who were unsatisfied with local medical services at 4 cities were 38.1% (H), 53.1% (P), 58.0% (V) and 66.1% (Y), respectively. Although the level of unsatisfaction was high at all cities, Ha Noi was lowest compared with other three places. Percentage of people who desired the medical services from Japan at 4 cities were 65.9% (H), 53.1% (P), 77.4% (V) and 71.1% (Y), respectively. The levels were high at all 4 cities and there were no big differences between them. Desired services were obstetric, gynecologic, pediatric and dental cares, which are thought to have less priority to employers.
Conclusion: In conclusion, we surveyed by questionnaire to know the needs of medical services for Japanese living in Ha Noi, Phnom Penh, Vientiane and Yangon. The results suggested that the level of unsatisfaction depended on specific socio-economical and political status of each country, such are economical spurt in Vietnam, politically unstableness in Cambodia, economical retardation in Laos, and economically stagnated because of politically closed to outer world in Myanmar. Japanese living in either country wants more medical services from Japan, especially the areas where employers neglect; those are gynecology, pediatrics, and dentistry.

C−24 EVALUATION OF GIDEON FOR IMPORTED INFECTIONS

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Object: Imported infections are increasing with the number of overseas travelers increases. Quick and accurate diagnosis of these diseases imported infections is crucial. However, the awareness toward imported infections is poor in medical institutions in Japan. Therefore, aggravation of these diseases due to delay of correct diagnosis and not only the death of infected patients but also the risk of secondly infection to those around the infected patients are possible. Under such circumstance, development of aid system that can diagnose imported infections correctly is needed seriously. GIDEON is a comprehensive software developed by C.Y. Informatics Ltd. for information gathering, diagnosis, and treatment of imported infections. The diagnosis module in GIDEON is a computer program that can diagnose patients based on the overseas travel history, the symptoms, and the findings of clinical findings of a patients. We studied the diagnostic accuracy of GIDEON retrospectively, based on its diagnostic results on the real cases.
Materials and Methods: Ninety-two admission cases who have been confirmed diagnosis as imported infections at the Department of Infectious Diseases Yokohama Municipal Citizens Hospital and the Department of Infectious Diseases & Applied Immunology, Tokyo University were eligible for this study. Imported infections were malaria (n=29), typhoid or paratyphoid fever (n=23), dengue fever (n=16), bacillary dysentery (n=12), hepatitis A (n=8), Mediterranean spotted fever (n=2), Lyme disease (n=1), and Lassa fever (n=1).
Results: Of all cases, the accuracy of diagnosis with correct diagnosis ranked any order was 90.2%. Correct diagnosis was ranked first and second to fifth in 51.1% and 29.3%, respectively. The accuracy of diagnosis was highest for typhoid or paratyphoid fever (100%), Mediterranean spotted fever (100%: only two cases), dengue fever (93.8%), malaria (93.1%), and bacillary dysentery (91.7%). As for Lassa fever (the sole case in Japan), the diagnostic result of GIDEON was deniable due to the inflammation of salivary gland but excluding this inflammation from data inputs, the diagnostic result was Lassa fever with 100% of possibility.
Discussion: The problem of GIDEON was that it diagnosed the correct diagnosis as "not deniable but rare" or as "deniable" in some cases. GIDEON diagnosed the cases with

malaria as "deniable" if the cases had sore throat and relative bradycardia. GIDEON also diagnosed the cases with typhoid or paratypoid fever as "deniable" if the cases had hematuria. Decrease of platelets, liver dysfunction, infection in India, Thailand and Viet Nam were judged "rare" by GIDEON for typhoid or paratyphoid fever. In the cases with hepatitis A and bacillary dysentery, GIDEON diagnosed that severe illness and necessity of admission were rare. However, many of these incidents diagnosed "rare" by GIDEON were not rare in reality.

GIDEON was useful in diagnosing imported infections to some extent. However it has various problems. Relying diagnosis only upon GIDEON would be very dangerous, but it was worthy enough in diagnosing patients from broad point of view, with taking much information GIDEON indicates into consideration.

C−25 THIRTEEN-YEARS EXPERIENCE OF THE JICA TUBERCULOSIS CONTROL IN NEPAL

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In Nepal tuberculosis has been a major public health problem among adult population, with the Annual Risk of TB Infection (ARTI) calculated as 2.2%. Dr. Noboru Iwamura, who came to Nepal in early 1960s, was one of the pioneers in this field. To tackle the problem at the national level, technical cooperation by JICA started in 1987. First, the National Tuberculosis Centre (NTC) was constructed in Kathmandu Valley to establish the headquarters for the National Tuberculosis Programme (NTP). Suitable strategies to combat TB with were then explored in the project pilot areas. After the official adoption of the Directly Observed Treatment, Short Course (DOTS) strategy by the NTP in 1995, the project's strategies also shifted to focus on DOTS. At the same time the project provided assistance at central level to strengthen the management of the NTP. To develop human resources, training was provided at the Research Institute of Tuberculosis (RIT) for program managers and laboratory technologists. The RIT also provided long and short-term consultants on TB control to Nepal.

Necessary supplies and equipment, such as TB drugs, microscopes, X-ray machines, vehicles, etc. were also provided for the smooth operation of the project. The goals and the targets of the project were set in accordance with the WHO strategy; 70% case detection rate and 85% cure rate of the new smear positive pulmonary tuberculosis cases. Overall case detection rate reached approximately 60%. Analysis was made to compare ways of supervision of TB patients' drug taking. When the health facility-DOT was compared with the family-DOT and non-DOT, the cure and completion rates were highest in the health facility-DOT. Therefore the strict DOT based at health facilities was chosen as the method for introduction of DOTS in Nepal in 1996. Four pilot areas were selected (National DOTS Demonstration Area) in the plain to introduce strict health facility-based DOTS. All the pilot areas were able to achieve the high cure rate of over 85%. Encouraged by the initial result, the NTP gradually expanded DOTS coverage in the plain. High cure rate was continuously observed in the expansion areas. While the DOTS strategy was successfully introduced in those areas, where the access to health care facilities is relatively good, geographically difficult areas such as hilly and mountainous areas remained uncovered by DOTS. People living in those areas would have to walk for many hours to receive DOT at the nearest health facility.

While hospitalization during the intensive phase is used in some areas, the JICA project utilized the Female Community Health Volunteers (FCHV) as treatment supervisors in those areas. The FCHV are unpaid volunteers, working in the community for the benefit of the community. By spending time and money for transportation on their own, the FCHV collects medicine once a week to give DOT at the patients' residence in their own villages. Perhaps there is no single solution to the problem of DOTS in geographically difficult areas. Suitable methods should be selected in each community, to improve the coverage of DOTS in the country.

C−26 DEVELOPMENT OF URBAN DOTS IN CHITTAGONG CITY OF BANGLADESH

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Tuberculosis is a major public health problem in Bangladesh and effective program called DOTS has recently been successfully implemented in rural areas. However, TB control system has not yet been established in most of the big cities as in many other developing countries. The Chittagong City is the second largest city in Bangladesh, with the population of 3.3 million. 30% of the population is thought to be a slum area. The City has a Chest Clinic/TB Clinic, 32 urban health centers and NGO clinics. Estimated TB patients are 3,000 per year. Before the project, there was no systematic DOTS in the city and majority of the patients used only TB clinic. TB program used to be under the central government program. But due to the decentralization and health sector reform, local government has become fully responsible for TB program. Before new system was started, TB clinic diagnosed nearly 600 patients, of whom only 175 were sputum AFB positive and their treatment completion was only 32%. Thus the service quality was very poor. Our institute started an operational research to demonstrate a model TB control system DOTS for urban area in Bangladesh with central government (DOH), Chittagong City, BRAC and WHO. In the beginning, all components of DOTS was introduced by the initiative of the central governmnet; namely, drugs are supplied by the central government; patients are given drugs under DOT at the nearest clinic, microscopic centers are increased in number, recording/reporting system is standardized, training is given to all concerned staff, and so on. As a research component, the progress has been periodically monitored, and evaluation workshops have been held every 6 months through participatory process. After the operation since 1996, patients diagnosed at the TB clinic were referred back to the nearest city clinic to have a TB drug under DOT. NGO clinics were also involved for DOT. The TB services have been more decentralized. As the outcome, the number of sputum positive patients increased up to above 500; the number of the patients registered at urban city centers is increasing while that in TB clinic is decreasing. And their treatment success rate has been improving above 70%. Achievements are: overall decentralization is going on; treatment success is improving; case finding increased, but about 500 is still low against 3,000 estimated; political commitment by city corporation has increased, but not sufficient. For further development, we recommend to the city and DOH; to form city TB control committee under city mayor and to nominate TB program manager; to renew the development plan; to hold periodic review meetings; and external supports both technical, moral and financial are still needed from central government, WHO and elsewhere.

C-27 IMMUNOHISTOCHEMICAL IDENTIFICATION OF PHENOLYC GLYCOLIPID-1 (PGL-1) IN TISSUES OF BURULI ULCER PATIENTS

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Buruli ulcer is a skin necrotizing disease caused by *Mycobacterium ulcerans*, which is closed to *M. leprae* and *M. tuberculosis*. Although a toxin of the polykritide family has been incriminated in the pathogenesis of the disease, phases of defect in the inflammatory reaction to the mycobacterial infection are highly suspected. Phenolyc glycolipids play crucial roles in the survival of some mycobacteria in phagocytic cells of infected patients. We sought to investigate the immunoreactivity of monoclonal antibodies to PGL-1, which was once believed to be specific to *M.*

leprae, in tissues of patients infected with *M. ulcerans*. Thirty specimens of surgically obtained skin flaps from patients clinically diagnosed as Buruli ulcer, originating from the Ashanti County of Ghana, were classified as follows: Plaqes 3 cases (10%), nodules 10 cases (33%), ulcerated nodules 1 case (3%), deep ulcer bed 7 cases (23%), healing ulcer 9 cases (30%). Eighty-three percent of submitted specimen were histopathologically compatible with infection by *M. ulcerans*. Acid fast bacilli (AFB), were demonstrated by Fite-Faraco and Harada stains in 1 plaque, 5 nodules, 4 deep ulcer beds, 2 healing ulcers and 0 ulcerated nodules. In all AFB positive specimens, PGL-1 was uniformly detected as strongly positive, in a confluent pattern, mimicking clusters of AFB, regardless of the clinical form of the disease. The antigen was detected around extracellular and intracellular AFB (Foamy macrophages, adipocytes) distributed in necrotic dermal tissues, fragmented collagen bundles, fatty lobules, septae and occasionally in wall of blood vessels. These findings suggest at first, the possible role of PGL-1, in phases of Buruli ulcer where the striking absence of reactive inflammatory response has been observed. Secondly, they are another indication of the non specificity of this antigen to a specific member of the Genus *Mycobacterium*.

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CONTENTS

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