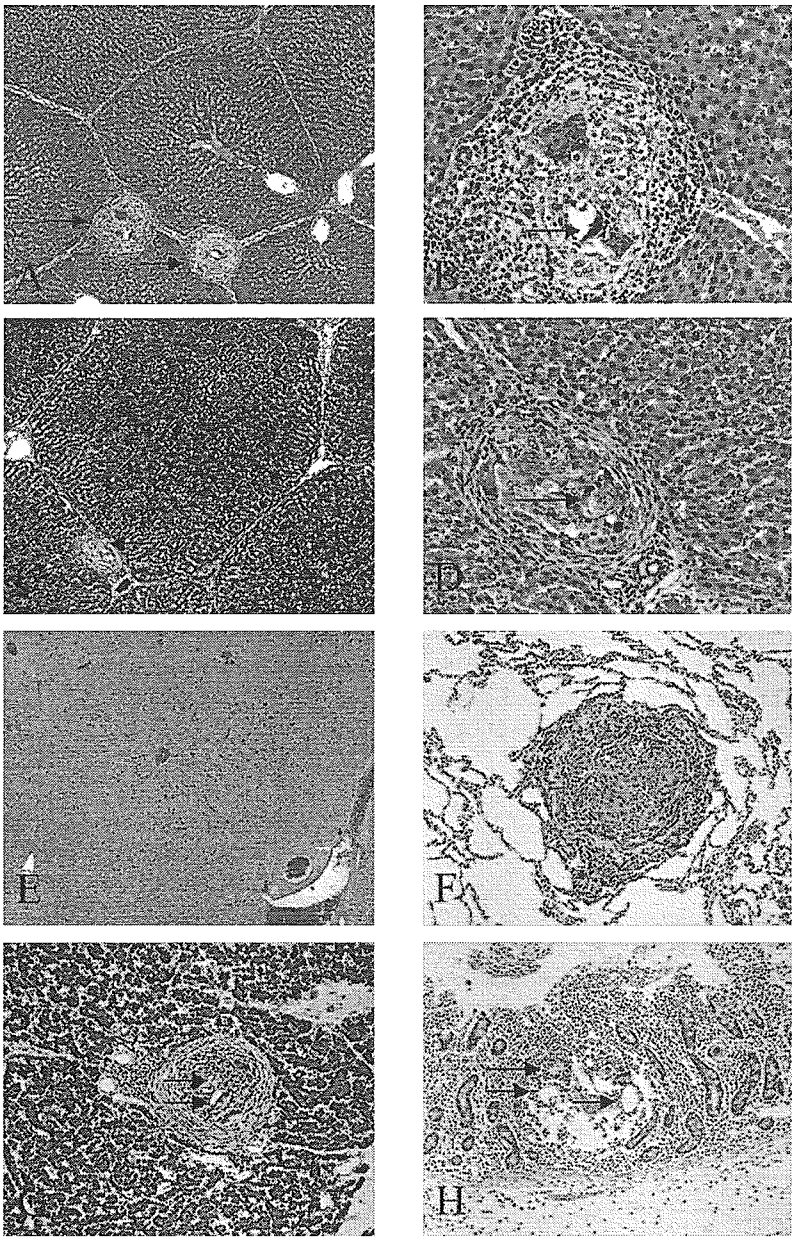


Fig. 3. (a) Liver (C-1), Masson's trichrome stain, × 100 magnification. There were two granulomas in the portal areas (arrowed). The eggs are associated with a granulomatous reaction and fibrotic changes. (b) Liver (C-1), Hematoxylin–eosin stain, × 200 magnification. The eggs (arrowed) were surrounded by epithelial cells, fibers and eosinophils. The eggshells still remained visible. (c) Liver (C-2), Masson's trichrome stain, × 100 magnification. There was a granuloma in the portal areas (arrowed). (d) Liver (C-2), Hematoxylin–eosin stain, × 200 magnification. The eggs (arrowed) were surrounded by mainly epithelioid cells and fibers. There was no infiltration of eosinophils and lymphocytes. The size of the granuloma was relatively small compared to C-1 (Fig. 2a). (e) Liver (naïve, non-infected miniature pig liver) Masson's trichrome stain, × 100 magnification. (f) Lung (C-1), Hematoxylin–eosin stain, × 200 magnification. Foreign body granuloma formation in the interstitium. An egg might be in the center of the granuloma. (g) Pancreas (C-1), Hematoxylin–eosin stain × 100 magnification. The eggs (arrowed) were surrounded by epithelial cells and fibers. (h) Intestine (C-1), Hematoxylin–eosin stain, × 100 magnification. Foreign body granulomas within the lamina propria. Arrows show the eggshells.

3.3. Antibody responses against schistosome adult worm antigens (SWA) and schistosome soluble egg antigens (SEA)

In order to examine the serological response of the host against *S. japonicum* infection, the antibody levels against egg and adult worm antigens were measured (Fig. 4). Specific IgG against SWA (Fig. 4a) began to increase significantly at 4 weeks p.i. In C-2, the overall IgG response against SWA and SEA, shown in Fig. 4, was lower than that of C-1. Specific IgG against SEA (Fig. 4b)

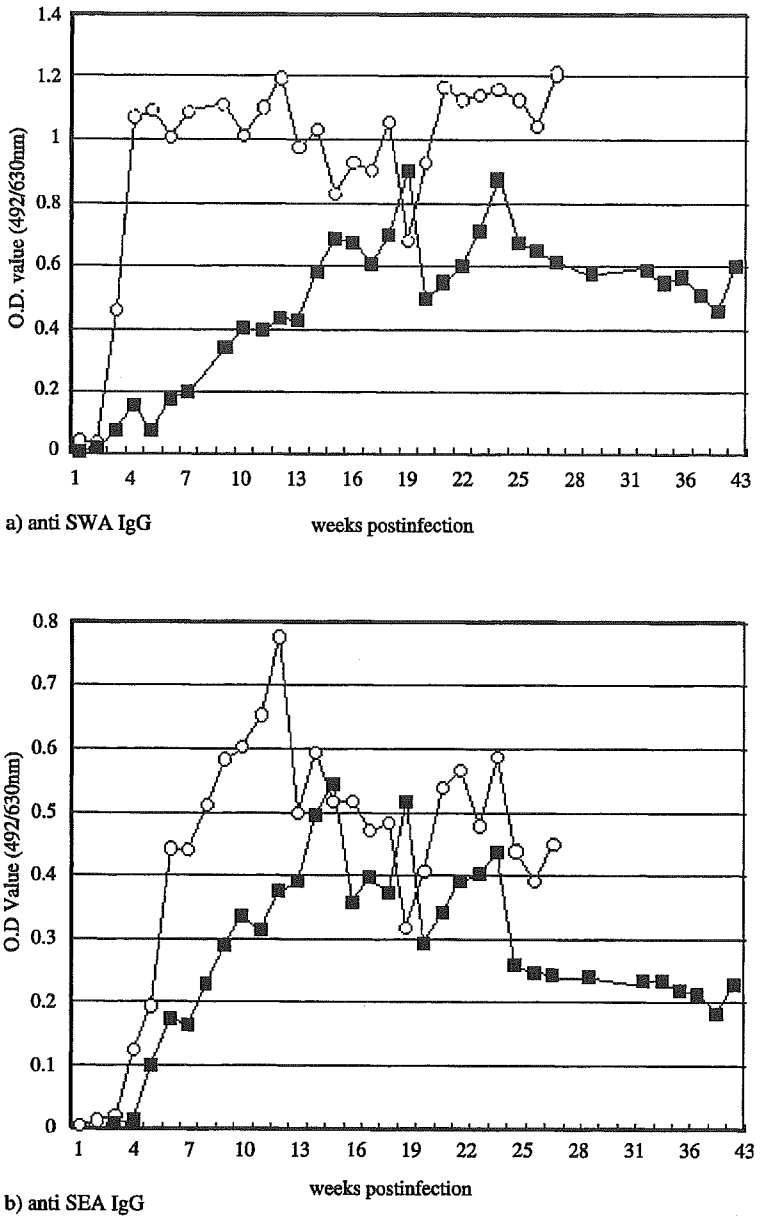


Fig. 4. IgG antibody response against adult worm antigen (SWA) and egg antigen (SEA). The serum diluted with blocking buffer (400-fold) was applied to an ELISA. Open circle, data from C-1; and closed square, data from C-2.

began to increase at 5 weeks p.i. prior to eggs being excreted into the feces. These responses persisted during the course of the experiment.

4. Discussion

In this study, we demonstrated that the CLAWN miniature pig is highly susceptible to *S. japonicum*. The EPG values were higher than those reported in experiments that used domestic pigs [7]. Marked egg excretion persisted until approximately 20 weeks p.i., with many adult worms able to be recovered by portal perfusion even at 27 and 47 weeks p.i.

Eleven paired worms were recovered at 27 weeks p.i. At that time, the EPG was approximately 100. As the weight of the feces produced per day was approximately 200 g, approximately 20 000 eggs were excreted by the 11 pairs of worms. Therefore, each worm pair produced over 1800 eggs per day. This number is similar to that found in a previous report [5]. Assuming the productivity of eggs per pair is constant during the course of infection, the number of

paired adult worms at 8 weeks p.i., when the EPG was approximately 400, was estimated to be approximately 44. Therefore, after the 200 cercarial challenge infections, half of the inoculated cercariae were assumed to establish infection in this pig. However, because we did not perform the perfusion when they showed the peak EPG, it is not clear how many worms were present. After 35 weeks p.i., EPG became almost nil, but at least four pairs of mature worms were present in the portal vein. Logically, these worms should have produced 7000–8000 eggs per day, resulting in an EPG of at least 30–40. There are at least three possible mechanisms to explain why the EPG declined after 18 weeks p.i.: (1) immunity killed the worms, (2) the worms decreased their egg production, and (3) excretion of eggs through gut mucosa was inhibited. Our observation using two pigs cannot exclude any of those possibilities. Willingham et al. [7] reported that pigs inoculated with a single dose of 2000 cercariae of *S. japonicum* showed markedly reduced EPG from 17 weeks p.i. and had eliminated most of the worms by 24 weeks p.i. Thus, this indicated that the first possible explanation, i.e. that immunity killed the worms, was viable.

Reid and Lichtenberg in 1977 [12] concluded that miniature pigs could not be used as an adequate model of human *S. japonicum* infection, because of the difficulty in establishing infection with extremely low egg numbers (EPG$\leq$4), short duration (up to 14 weeks p.i.) of fecal egg excretion, and mild or moderate pathology in the liver. Although only two pigs were used in this study, the EPG values and recovered worm numbers were higher than those observed in their study. Additionally, there were some differences in experimental conditions between our study and theirs. Because our CLAWN strain is newly established in Japan, the miniature pig itself was different from the strain used in the other study. The size and age of their pigs at the time of inoculation also differed from ours. The miniature pigs they used were 10-week-old pigs, ranging in weight from 8 to 11 kg. The origin of the *S. japonicum* also differed as they used a Japanese strain.

Histological examination disclosed the deposition of *S. japonicum* eggs had a widespread distribution, and were found in the liver, spleen, pancreas, lungs, small intestine, and mesenteric lymph nodes. The deposited eggs were surrounded by foreign body granulomas as a result of host responses. It was rather difficult to recognize where the eggs were deposited at first in these tissues, but the presence of eggs in lymph nodes suggests that the eggs were also spread through lymphatic pathways in addition to the peripheral blood circulation. Although Yason et al. [13] reported that the adult worms were present in blood vessels such as pancreatic and splenic veins and pulmonary arteries, no adult worms were observed macroscopically or microscopically in those organs that were positive for egg deposition in this study. However, further detailed examination of blood vessels might show their presence.

In the liver, occasional bridging–bridging fibrasis were observed. Kardoff et al. [14] reported that the dilation of portal vein in infected pigs were observed in ultrasonography. These facts indicated that egg deposition in the liver affected the portal flow even in pigs. The experiments of repeated infection may reveal the role of egg deposition in the portal hypertension and fibrosis observed in the human case.

An antibody response was detected against both eggs and adult worms. The antibody response against adult worms began at 4 weeks p.i. It may indicate that the cercariae inoculated in the miniature pigs became adult worms before 4 weeks and laid eggs before 5 weeks p.i.

Hurst et al. [15] reported that there were CD4 and/or CD8 positive T cells, B cells, $\gamma\delta$ T cells around the deposited eggs in the liver. Taking it into consideration, the adaptive immunity really occurred against eggs deposition in the pigs liver.

In the present study, we could not reveal any individual variations in the population due to the limited number of available pigs. The closed colony of the CLAWN strain has already reached approximately 75% consanguinity after 27 years of closed matings from the original two kinds of F1. Regarding the SLA (swine MHC), there are several alleles detected and some of those are the majorities [16]. Although we had no data on our pig's SLA, these alleles might have influenced the outcomes of their course of infection.

As far as we know, this is the first report that miniature pigs are highly susceptible to Chinese strain of *S. japonicum* and that it may be a useful animal model for human schistosomiasis.

Acknowledgements

This study was supported in part by 21st Century Center of Excellence Programs of Nagasaki University from Ministry of Education, Culture, Science, and Technology, a Grant for Emerging and Re-emerging Infectious Diseases, from the Ministry of Health and Welfare, Japan (H-12 ShinkoKou-18) and a grant from the Japan - US Cooperative Medical Science Program (1999–2002). RTM was supported by the JSPS Postdoctoral Fellowship for Foreign Researchers (2001–2003). RU was supported by Yoneyama Scholarship of Rotary Club (2002–2004) and by Saitama Medical School (1999–2001).

References

[1] WHO. Report of the WHO informal Consultation on schistosomiasis control. Geneva: WHO, 1998.
[2] Qingwu J, Shaoji Z, Hongchang Y, Zide L, Genming Z, Brinkmann U. The effect of a combined approach to schistosomiasis control on the transmission of *Schistosoma japonicum* in Xingzi of Poyang Lake Area, China. Southeast Asian J Trop Med Public Health 1996;273:535–41.
[3] Genming Z, Brinkmann UK, Qingwu J, Shaoji Z, Zhide L,

indberg R. The relationship between morbidity and intensity of *Schistosoma japonicum* infection of a community in Jiangxi Province, China. Southeast Asian J Trop Med Public Health 1997;283:545–50.
[4] Von Lichtenberg F, Sadun EH, Cheever AW, Erickson DG, Johnson AJ, Boyce HW. Experimental infection with *Schistosoma japonicum* in chimpanzees. Am J Trop Med Hyg 1971;206:850–93.
[5] Basch PF. Schistosomiasis. Oxford: Oxford University Press, 1991.
[6] Willingham 3rd AL, Hurst M. The pig as a unique host model for *Schistosoma japonicum* infection. 1996;12:132–4.
[7] Willingham 3rd AL, Hurst M, Bogh HO, Johansen MV, Lindberg R, Christensen NO, et al. *Schistosoma japonicum* in the pig: the host–parasite relationship as influenced by the intensity and duration of experimental infection. Am J Trop Med Hyg 1998;582:248–56.
[8] Chen H, Nara T, Zeng X, Satoh M, Wu G, Jiang W, et al. Vaccination of domestic pig with recombinant paramyosin against *Schistosoma japonicum* in China. Vaccine 2000;1820:2142–6.
[9] Hurst MH, Shi YE, Lindberg R. Pathology and course of natural *Schistosoma japonicum* infection in pigs: results of a field study in Hubei province, China. Ann Trop Med Parasitol 2000;945:461–77.
[10] Hurst MH, Willingham AL 3rd, Lindberg R. Tissue responses in experimental schistosomiasis japonica in the pig: a histopathologic study of different stages of single low- or high-dose infections. Am J Trop Med Hyg 2000;621:45–56.
[11] Johansen MV, Bogh HO, Nansen P, Christensen NO. *Schistosoma japonicum* infection in the pig as a model for human schistosomiasis japonica. Acta Tropica 2000;76:85–99.
[12] Reid WA, von Lichtenberg F. Experimental *Schistosoma japonicum* infection in miniature pigs. J Parasitol 1977;632:392–4.
[13] Yason CV, Novilla MN. Clinical and pathologic features of experimental *Schistosoma japonicum* infection in pigs. Vet Parasitol 1984;171:47–64.
[14] Kardorff R, Eriksen L, Nielsen DH, Johansen MV. Validation of ultrasonography for hepatic schistosomiasis using a porcine *Schistosoma japonicum* model. Acta Tropica 2003;85(3):315–23.
[15] Hurst MH, Willingham AL, Lindberg R. Experimental schistosomiasis japonica in the pig: immunohistology of the hepatic egg granuloma. Parasite Immunol 2002;24(3):151–9.
[16] Chardon P, Renard C, Vaiman M. The major histocompatibility complex in swine. Immunol Rev 1999;167:179–92.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Parasitology International 53 (2004) 193–196
www.elsevier.com/locate/parint

Immunogenetic analysis of post-schistosomal liver fibrosis

Kenji Hirayama*

Department of Molecular Immunogenetics, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

Abstract

Schistosomiasis is a major endemic parasitic disease in the world. In China, we have identified two major genes related to the severity of liver fibrosis, one an HLA class II gene, and the other the IL-13 gene. The frequency of the HLA-DRB5*0101 allele and that of the IL-13 promoter A/A (IL-13P- A/A) genotype were elevated in fibrotic patients, although the two genes are located on different chromosomes, chromosomes 6p and 5q, respectively. Subjects with both genotypes had odds ratios (OR = 24.5) much higher than the sum of the ratios for each individual genotype (OR = 5.1, 95% confidence interval 1.3–24.7 for HLA-DRB5*0101, OR = 3.1 95% CI 1.5–6.5 for IL-13P- A/A). Although we have not yet characterized the functional difference between HLA-DRB5*0101 and other alleles, peripheral blood mononuclear cells from IL-13PA/A donors produced much higher amount of mRNA than IL-13PA/B 24 h after the stimulation with PHA. Those findings strongly suggest that the pathogenic Th2 response directly influences the prognosis of post-schistosomal liver fibrosis.

Keywords: *Schistosoma japonicum*; Cytokine; HLA; Single nucleotide polymorphism; Microsatellite; Liver fibrosis

1. Introduction

One of the important subjects in the biomedical research field is the analysis of human reactivity to exogenous stress. Exogenous stress in the infectious diseases is usually one species of microorganism that is called pathogen. The reactivity of the human host is so variable that wide range of clinical spectrum spanning from asymptomatic to grave is shown in almost all infectious diseases as shown in SARS. The reason why such a wide range occurred is that many factors are involved in the pathogenesis during the infection including host and parasite genetic factors (multifactorial diseases). Usually this multifactorial disease is very difficult to analyse, because to identify several responsible genes to develop the disease, statistical approach is necessary using linkage study or population based case–control study which needs a number of subjects. After you get an appropriate quality and quantity of subjects or family members, two different genetic approaches are generally undertaken to identify the responsible genes: targeted gene analysis and genome-wide survey. We adopted the former method, focusing especially on immunity-related genes such as those for HLA, cytokines and adhesion molecules.

HLA genes are highly polymorphic and their alleles are well characterized at the DNA sequence level. For example, the HLA-DRB1 gene has over 100 alleles in the human population. In particular,

*Tel.: +81-95-849-7820; fax: +81-95-849-7821.
E-mail address: hiraken@net.nagasaki-u.ac.jp (K. Hirayama).

1383-5769/04/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.parint.2004.01.008

HLA-DR, -DQ, -DP, -A, -B, and -C are believed to function as immune-response genes that are known to control host immune reactivity to specific antigen of T cells and NK (T) cells. Cytokines are also believed to play important roles in controlling the intensity and duration of the immune response. Recently, single nucleotide polymorphisms (SNPs) have been observed very commonly in the promoter regions of some cytokines, including tumour necrosis factor (TNF), interleukin (IL)-4, IL-13, and interferon-γ. Several studies have demonstrated that these polymorphisms directly affect promoter activity. Therefore, we focused on those supposed to be very strong candidate genes for the search to pathogenic or resistant genes to post-schistosomal liver fibrosis in humans.

2. HLA class II genes and the IL-13 promoter alleles genetically interacted to enhance the development of post-schistosomal liver fibrosis

Schistosomiasis japonica is a chronic helminthic infectious disease that affected at least 860 000 individuals in China in 1995. Morbidity and mortality are dependent on its chronic sequelae, post-schistosomal hepatosplenic disease, which is characterized by liver fibrosis, portal hypertension, ascites accumulation, oesophageal varices, and eventual death. The liver fibrosis seen in these patients is induced by a granulomatous immune response against the eggs that are deposited in the periportal area [1]. Schistosomal egg antigen-specific $CD4^+$ T cells play a major role in the formation of granuloma through Th2-type cytokine production in experimental schistosomiasis mansoni [2,3]. However, in humans, little is known about the immunological response during the chronic phase of hepatosplenic disease [4]. Because only 5–10% of patients with chronic schistosomiasis japonica develop hepatosplenic disease, and because the granulomatous response is initiated by $CD4^+$ T cells reactive to schistosomal antigen, polymorphisms of the HLA class II antigens, which control the reactivity of the $CD4^+$ T cells, may be associated with a susceptibility to hepatosplenic disease. Indeed, associations between schistosomal hepatosplenic disease and HLA alleles have been reported for schistosomiasis mansoni [5,6] and for schistosomiasis japonica [7,8]. Recently, more objective diagnostic methods using ultrasonography have become popular and have been standardized to measure changes in liver morphology [9]. Therefore, we used this method to categorize the patients into a 'fibrotic' group and a 'non-fibrotic' group, and examined their genetic characteristics by analysing the polymorphisms of candidate genes encoding HLA class II and class I antigens, TNF-α and cytokines.

A total of 230 current or former patients with chronic schistosomiasis japonica were examined for clinical changes. All patients were from the agricultural village of Beishan, in Yushan County, China, and had their first-episode of infection and treatment at least 10 years before the initiation of this study in 1994. The mean age of the subjects was 52.6 ± 10.5 years and the mean duration between 1994 and their initial treatment year was 27.4 ± 8.8 years. Ultrasonographic diagnosis was carried out according to the WHO standard for the diagnosis of liver fibrosis due to schistosomiasis japonica [9–11]. Ultrasonographic diagnosis determined that there were 44 persons with grade 0 fibrosis, 81 with grade I fibrosis, 99 with grade II fibrosis, and six with grade III fibrosis. The presence of hepatitis B virus (HBV) was not assessed in these patients, but the prevalence of HBV is approximately 15% in Jiangxi Province [12]. Most of the men in the village smoke tobacco and drink alcoholic beverages, but the women generally do not. The patients had all been treated after each positive faecal examination throughout their lives, but it was not possible to estimate the precise total worm burden of each patient during the clinical course of the disease. Therefore, we tentatively defined an appropriately exposed person as a repeatedly treated for schistosomiasis japonica over a 10-year period [13].

The frequencies of several HLA class II alleles [14] were significantly increased or decreased in the fibrotic groups. When we compared the frequencies of alleles between grade 0 and grades I, II, and III, we found that HLA-DRB1*1101 ($P < 0.001$), DQA1*0501 ($P < 0.02$), and DQB1*0301 ($P < 0.03$), which are closely linked, were significantly elevated in the grade 0 group, and that

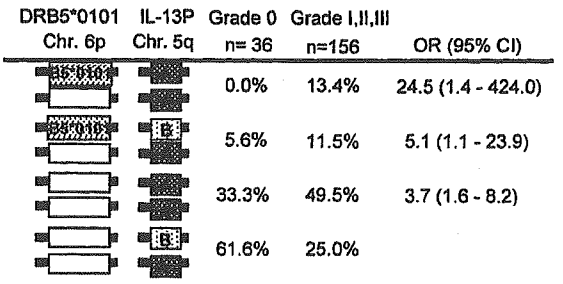


Fig. 1. Synergistic effect of the two susceptibility markers, HLA-DRB5*0101 and IL-13P- A/A. OR and 95% CI were calculated relative to individuals negative for both DRB5*0101 and IL-13P- A/A.

HLA-DRB5*0101 was significantly elevated in grade I, II, and III fibrotic patients ($P<0.03$). This suggests that the HLA-DRB1*1101–DQB1*0301–DQA1*0501 haplotype ($P<0.02$) decreases susceptibility to grades I, II, and III fibrosis, whereas the HLA-DRB1*1501–DRB5*0101 haplotype ($P<0.02$) increases this susceptibility. If we assume that these genetic associations arise from the functions of the HLA molecules themselves, then the critical question is: how do these molecules present antigens to $CD4^+$ T cells to initiate the immunological processes leading to fibrosis? We have not yet identified any pathogenic or protective T cells via such HLA molecules in the exposed donors.

We further analysed the polymorphisms of the Th2 cytokine genes in the same subjects. There was a significant association between IL-13 gene promoter polymorphism and the liver fibrosis group. Because the IL-13 gene is localized on the long arm of chromosome 5, the IL-13P allele must be inherited independently of the HLA class II allele that occurs on the short arm of chromosome 6. Therefore, the next question is whether there is any interaction between these two genetic markers, HLA and IL-13. As shown in Fig. 1, both HLA-DRB5*0101- and IL-13P- A/A-positive subjects had much higher odds ratios (OD=24.5) than subjects positive for only one of these polymorphisms (OD=5.1 for HLA, OD=3.7 for IL-13P-A/A), indicating these two genetic markers synergistically enhance the development of fibrosis after infection. This synergy made us propose this hypothetical story that the pathogenic IL-13- high producer $CD4^+$ T cells are preferentially stimulated by the antigen-presenting cells expressing HLA-DRB5*0101. Actually the in vitro experiment using many healthy volunteers showed that IL-13 mRNA levels of the PHA stimulated T cells from IL-13P A/A genotype donors showed significantly higher than those from IL-13P A/B donors. This strongly supports our hypothesis that IL-13 producing CD4 T cells were pathogenic.

Acknowledgments

Mihoko Kikuchi (Nagasaki University), Chen Honggen, Dong Yin, Xiaonan Gu Shaoji Zhang (Jiangxi Provincial Institute of Parasitic Diseases) and Jianxiang Liu, Hong-Chang Yuan (Shanghai Medical University) contributed to the study. This work was supported, in part, by Grants-in-Aid for Scientific Research from the Ministry of Education, Sports, Science and Culture, Japan, (08281104, 08044316, 09470071, 10044317).

References

[1] Cheever AW. Schistosomiasis. Infection vs. disease and hypersensitivity vs. immunity. Am J Pathol 1993;142:699–702.
[2] Wynn TA, Cheever AW, Jankovic D, et al. An IL-12-based vaccination method for preventing fibrosis induced by schistosome infection. Nature 1995;376:594–6.
[3] Kaplan MH, Whitfield JR, Boros DL, Grusby MJ. Th2 cells are required for the *Schistosoma mansoni* egg-induced granulomatous response. J Immunol 1998;160:1850–6.
[4] Wiest PM, Wu G, Zhang S, et al. Schistosomiasis japonica on Jishan Island, Jiangxi Province. People's Republic of China: persistence of hepatic fibrosis after reduction of the prevalence of infection with age. Trans RI Soc Trop Med Hyg 1993;87:290–4.
[5] Salam EA, Ishaac S, Mahmoud AA. Histocompatibility-linked susceptibility for hepatosplenomegaly in human schistosomiasis mansoni. J Immunol 1979;123:1829–31.
[6] Secor WE, del Corral H, dos Reis MG, Ramos EAG, Zimon AE, Matos EP, et al. Association of hepatosplenic schistosomiasis with HLA-DQB1*0201. J Infect Dis 1996;174:1131–5.
[7] Ohta N, Hayashi M, Tormis LC, Blas BL, Nosenas JS, Sasazuki T. Immunogenetic factors involved in the

pathogenesis of distinct clinical manifestations of schistosomiasis japonica in the Philippine population. Trans RI Soc Trop Med Hyg 1987;81:292–6.

[8] Ohta N, Nishimura YK, Iuchi M, Sasazuki T. Immunogenetic analysis of patients with post-schistosomal liver cirrhosis in man. Clin Exp Immunol 1982;49:493–9.

[9] The Cairo Working Group. The use of diagnostic ultrasound in schistosomiasis: attempts at standardization of methodology. Acta Trop 1992;51:45–63.

[10] Hirayama K, Chen H, Kikuchi M, et al. Glycine–valine dimorphism at the 86th amino acid of HLA-DRB1 influences the prognosis of post-schistosomal hepatic fibrosis. J Infect Dis 1998;177:1682–6.

[11] Hatz C, Murakami H, Jenkins JM. A review of the literature on the use of ultrasonography in schistosomiasis with special reference to its use in field studies. 3. *Schistosoma japonicum*. Acta Trop 1992;51:29–36.

[12] Li Y, Yu DB, Li YS, Ross AG, McManus DP. Infections with hepatitis B virus in three villages endemic for schistosomiasis japonica in the Dongting Lake region of China. Ann Trop Med Parasitol 1997;91:323–7.

[13] Hirayama K, Chen H, Kikuchi M, et al. HLA-DR-DQ alleles and HLA-DP alleles are independently associated with susceptibility to different stages of post-schistosomal hepatic fibrosis in the Chinese population. Tissue Antigens 1999;53:269–74.

[14] Kimura A, Sasazuki T. Eleventh International Histocompatibility Workshop reference protocol for the HLA DNA-typing technique. In: Tsuji K, Aizawa M, Sasazuki T, editors. HLA 1991 Volume 1. Proceedings of the Eleventh International Histocompatibility Workshop and Conference, New York, USA: Oxford University Press, 1992:397–419.

住血吸虫感染と体質

平山謙二

はじめに

体質の本質は遺伝子である。体質改善という言葉があるが，もし最初の前提を正しいとすれば体質改善を一世代でなしうることはありえないことになる。しかし遺伝子の妙は遺伝子の表現型が環境によって左右されるところにある。遺伝子を変えるのは難しいが，その表現型を変化させることは可能なのである。この可能性を期待して世の親たちは必死で子供たちに勉強をさせ，遺伝子に最大限の表現型を発揮させるべく努力しているのである。

疾病に対する抵抗性や感受性も同様で，遺伝子であらかじめ決められた運命は環境によりその表現が変化していく。ある疾患の原因遺伝子を明らかにしようとする時，問題となるのは数多くのヒトの疾病が遺伝子も環境要因もほとんど不明なことである。このような宿主と環境という二つの変数間の相互作用を解析することは今の研究体制では不可能である。そこで著者らは，環境要因がほぼ明確な感染症において，その疾患感受性を決定する遺伝子の同定する努力を続けている。

感染症の外的なストレスは，普通単一の病原微生物でありこれに対する反応パターンが，いわゆる臨床的な感染症として現れるが，この反応パターンには個体差が存在し，まったく症状のない不顕性感染から致死性の重篤な感染症に至る幅広いスペクトラムが観察されている。このような現象が観られるのは反応性を規定する遺伝因子が複数存在し，それらが複雑に関与していることによると推測されている。これらの因子を1つ1つ明らかにしていくことにより，感染症に対するヒトの反応性を理解することが可能となる。住血吸虫感染は，セルカリアの経皮感染により引き起こされるが，約2ヵ月で成虫となり血管内に寄生する。寄生の成立から産卵の維持までには寄生虫と宿主免疫とのせめぎ合いが行われ，抵抗性の個体では寄生数の減少が起こるとされている。一旦寄生が成立すればいよいよ数年にわたる産卵活動に入り虫卵の数は1日に数千に及ぶ。この虫卵の慢性的な刺激効果により，流行地の患者の約10%で重症の肝線維症あるいは肝硬変を発症し，肝不全や食道静脈破裂などにより死亡する。ここでも，感受性に個人差が観察されるのである。これらの個体差を規定している遺伝子はいかなるものであろうか。

ある疾患遺伝子を考える場合，一般的にとられる方法は，その病態生理から最も強く病因として疑われる生理活性物質をコードする遺伝子座に着目する方法である。例えば神経疾患ではニューロンの機能と関係する物質などが挙げられるだろう。感染症では免疫関連遺伝子に着目した解析が広く行われている。そのうち最も盛んに行われたのは，HLA 遺伝子領域である。最近では免疫学の進歩と相まって，サイトカインや接着因子などをコードする遺伝子領域の解析も進んでいる。住血吸虫症に関わる宿主遺伝要因について候補遺伝子領域からの解析を行った我々の結果を中心に考察したい。

1. 感染抵抗性を決定する遺伝子

経皮感染の際の感染抵抗性については，ある程度の獲得免疫が作用していると考えられている。とりわけ，マンソン住血吸虫症やビルハルツ住血吸虫の高度浸淫地においては，年齢により感染率や感染強度の違いが認められ，加齢と共に感染強度が低下することから，加齢に伴う感染抵抗性が存在することが示唆されている[1,2]。彼らの報告では血清中の成虫虫体に対する特異的 IgG4 抗体価が低年齢層で高く加齢とともに低下し，反対に特異的 IgE 抗体価が加齢とともに上昇することから，特異的 IgE 抗体価が年令依存性の防御免疫に関わっているとされている[3,4,5]。そうであるならば，この防御的な IgE 抗体の産生量に個体差があればそれがそのまま何らかの遺伝子の相違によりもたらされているかもしれない。IgE に関する個体差については臨床的にはアレルギー疾患に関連して世界的に多くの研究が進行しており，すでにいくつかの遺伝子領域がマッピングされている。これらの遺伝子とアフリカの感染抵抗性遺伝子の関連が注目される。しかしながら，日本住血吸虫症においては，同様の解析は十分になされていない。

我々が行った中国江西省の Poyang 湖周辺での日本住血吸虫の集団治療後の再感染抵抗性に関する研究によれば，感受性と考えられる再感染群と抵抗性の非再感染群について調べても血清中の住血吸虫特異的抗体価との関連は認められなかった。またアフリカの研究で観察されたような加齢による再感染率の低下も認められず，それどころか逆に 30 歳以上の高年齢群のほうが高い感染率を示した。同じ江西省の Poyang 湖に浮かぶ南山島で Zhosong らが行った同様の調査でもわれわれの観察とほぼ同じ結果であったことが報告されており[6]，日本住血吸虫では加齢に伴う感染防御能の増強は顕著ではない。しかし年齢による階層化を行ったあとに個人間の感染強度を比較すると，高い感染強度を示す者，あるいは低い感染強度を示す者がランダムに存在し，加齢以外のなんらかの個体差が感染防御に関係していることが推測された。しかし，ここでも抗体価との関連は明らかではなかった。一般には日本住血吸虫の感染成立が危険な水との接触の度合いと相関関係にあるとされている。上記の研究では個人の水接触について職業などを指標に感染危険度を解析に加味してはいるが，遺伝的な個体差を解析するにはもう少し客観的な水接触指数の計測法や信頼できる定量性を持った Kato-Katz 法あるいは感染量の測定法の確立が必要であると思われた。

住血吸虫症に多かれ少なかれ免疫応答性が関与するのはおそらく間違いないと考えられるが，個々人の抵抗性を定量的に判定し，家系や集団を用いて遺伝要因を解析することは必ずしも容易ではない。Dessein らは 1991 年ブラジルのマンソン住血吸虫症流行地で，各人の感染抵抗性を虫卵排出数で定量化し，20 家系 269 名の家系調査により，共優性の感染性 / 抵抗性遺伝子座 SM-1 の存在を示唆した[7]。その後，1996 年に同じ対象を用いたゲノムワイド解析により，SM-1 が 5q31-q33 の CSF1R 付近にマップされることをつきとめた[8]。ゲノムワイド解析の結果は，確かに驚くべきことで何らかの遺伝子多型が感受性を決定していることを強く示唆するものである。この近傍には，IL-13, 4, 5 などの遺伝子座があることから，その本体が注目されているが，まだ明らかになっていない。

以上のように，感染抵抗性に関する遺伝子の関与はいまだ明らかではないが，しかし確実に存在するように思われる。組織移行するヒトぜん虫感染症における免疫機構を解明するためにより一層の努力が必要である。

2. 慢性の肝脾疾患の感受性遺伝子

日本住血吸虫と HLA に関しては，笹月らによる先駆的な研究により，山梨甲府盆地の流行地の住民の中に，肝硬変に感受性あるいは抵抗性と相関を示す HLA のハプロタイプが存在することが示された[9]。その後我々はこの研究をさらに発展させ，中国の慢性の肝線維症と HLA-クラス II 遺伝子アレルとの強い相関を報告している[10,11,12,13]。特に最近見出された HLA-クラス II と IL-13 プロモータ遺伝子多型の重症化への相乗効果は驚くべきもので，HLA 多型が確かに慢性疾患の病型に直結することを状況証拠とはいえ明らかにしたものと考えられる。これについて以下に少し詳しく記述したい。

日本住血吸虫症の流行地として設定したのは，中国江西省の山間の村で，人口約 2 千人のうち 30 代以上では，ほとんどの人が数回の感染を経験していた。1995 年にここで 10 年以上の感染歴のある成人 230 名を対象に，肝病変の進行度を超音検査により正常の Grade 0 から肝硬変の Grade 3 までの 4 段階に診断し(図 1)，各人の血液から DNA を抽出し，HLA やサイトカイン遺伝子多型と重症度との相関を解析した。その結果 HLA-DRB1*1101 が進行と共に頻度が下がり，逆に HLA-DRB5*0101 と HLA-DPB1*0301 が重症度で増加する傾向があった[12]。HLA 分子は T リンパ球への抗原提示分子として働くことから，アレルにより抗原提示能に差が生じ，その結果として肝硬変

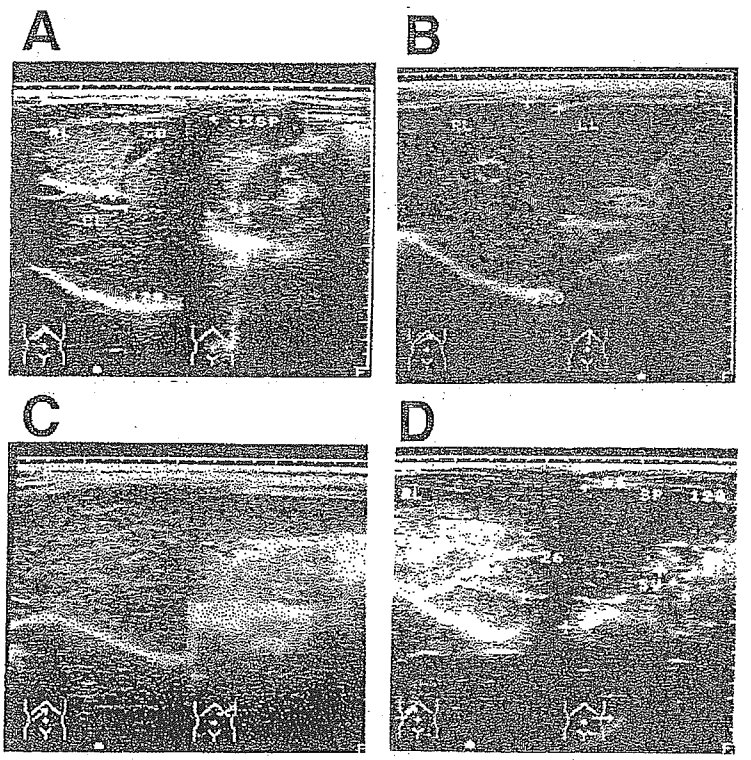


図 1　超音波診断法による住血吸虫性肝線維化症診断像 A; Grade 0. B; Grade 1. C; Grade 2. D; Grade 3 典型的な亀甲模様を呈する

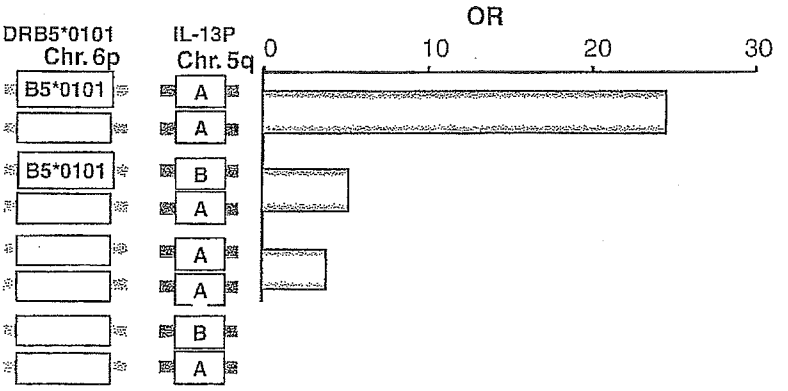


図 2　二つの感受性マーカーである HLA-DRB5*0101 と IL-13P*A/A の相乗効果

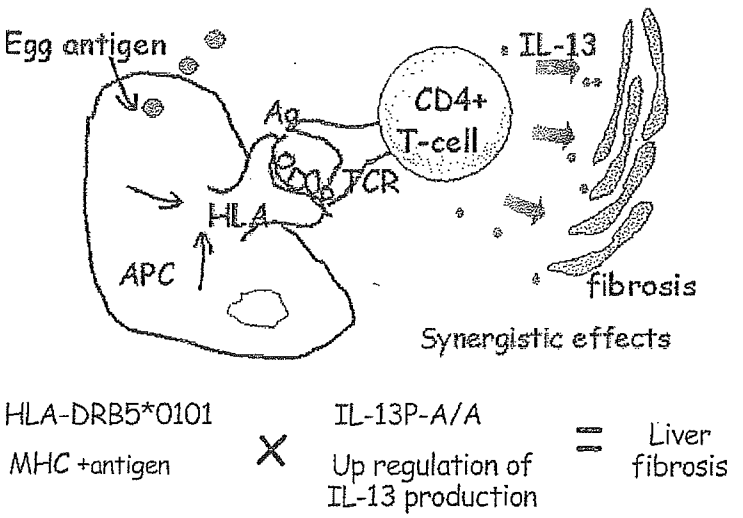


図 3　T ヘルパー 2 モデルによる相乗効果の一元的な説明

が現れたと考えられる。一般的にここからは遺伝子の性格付けから，その機能解析へと展開することになるが，今のところ，実際にどの抗原分子によってこのような HLA アレル間の反応性の違いが引き起こされるのか明らかではない。

HLA 以外にも TNF，インターフェロン-γ や IL-4，IL-13 など Th1，Th2 系のサイトカインの遺伝子領域についても解析したが，唯一，IL-13 のプロモーター領域の SNP ハプロタイプに弱い相関が観られた。以上のように住血吸虫感染後 HLA-DR アレルおよび IL-13 プロモーター SNP ハプロタイプとの相関が認められたが，これらは，各々第 6，第 5 染色体上に存在するため，これらのマーカーの相互作用について調べたのが図 2 である。感染性マーカーの HLA-DRB5*0101 と IL-13P*A/A は同時に存在すると，OR 値が単独の OR 値の和よりはるかに大きく，これらのマーカーが相乗的に作用していることがわかった。それに対して，抵抗性マーカーである HLA-DRB1*1101 と IL-13P*B については，同時に存在するときの OR 値は各単独 OR 値の和に等しく互いの相互作用は認められなかった。上記の 2 アレルの解析から図 3 のような肝線維化感受性のメ

カニズムが推測されている。ただし，最初に紹介したゲノムワイド解析の SM-1（感染抵抗性感受性遺伝子）が IL-13 遺伝子座を含む 5q31-q33 にマップされていたことから，可能性として我々が見出した肝線維化に対する HLA と IL-13 の相乗効果が実は，感染感受性が増した結果，大量の虫体，虫卵に曝露されたために起こったということも考えられる。

感染抵抗性に関するブラジルでの遺伝解析を行ったグループは，アフリカのスーダンで慢性のマンソン住血吸虫性肝線維症の重症群について同様の家系調査を行い，6q22-q23 の IFN-γRI 遺伝子の近傍に肝線維症の感受性遺伝子 SM-2 をマップした[14]。しかし，その後の研究の進展は報告されておらず，真の責任遺伝子の同定には至っていない。

3. 今後の展望

感染症の遺伝などというのは，まだ微生物という概念のない時代の家族集積などをさしていたという歴史的な経緯があり，また簡単に結果が得られる感染モデルの動物実験と比して，一般に好まれない傾向にある。しかし，現在のようにヒト及び病原体のゲノムの情報が集積し，感染症の環境要因も明らかになりつつある状況下では，遺伝解析を通してゲノムと微生物との相互作用を解明することは十分可能であり，このような解析の結果これまで見えなかった色々な相互作用が明らかになることが期待される。住血吸虫ワクチンや慢性疾患の予防薬などの開発につながっていけば本望である。

参考文献

1) Butterworth AE, Dunne DW, Fulford AJ, Ouma JH, Sturrock RF. Immunity and morbidity in *Schistosoma mansoni* infection: quantitative aspects. Am J Trop Med Hyg 1996, 55 (5 Suppl): 109–15
2) Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. Nature 1991, 17; 349 (6306): 243–5
3) Dunne DW, Butterworth AE, Fulford AJ, Kariuki HC, Langley JG, Ouma JH, Capron A, Pierce RJ, Sturrock RF. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to reinfection. Eur J Immunol 1992, 22 (6): 1483–94
4) Grogan JL, Kremsner PG, van Dam GJ, Deelder AM, Yazdanbakhsh M. Anti-schistosome IgG4 and IgE at 2 years after chemotherapy: infected versus uninfected individuals. J Infect 1995, 173: 1242–7
5) Grogan JL, Kremsner PG, van Dam GJ, Metzger W, Mordmuller B, Deelder AM, Yazdanbakhsh M. Antischistosome IgG4 and IgE responses are affected differentially by chemotherapy in children versus adults. J Infect 1997, 176 (5): 1344–50
6) Zhang Z, Wu H, Chen S, Hu L, Xie Z, Qiu Y, Su C, Cao JP, Wu Y, Zhang S, Wu G. Association between IgE antibody against soluble eggantigen and resistance to reinfection with *Schistosoma japonicum*. Trans R Soc Trop Med Hyg 1997, 91 (5): 606–8
7) L. Abel, F. Demenais, A. Prata, A.E. Souza, A. Dessein, Evidence for the segregation of a major gene in human susceptibility/resistance to infection by Schistosoma mansoni. Am J Hum Genet 1991, 48: 959–970
8) Marquet S, Abel L, Hillaire D, Dessein A. Full results of the genome-wide scan which localizes a locus controlling the intensity of infection by Schistosoma mansoni on chromosome 5q31–q33. Eur J Hum Genet 1999, 7: 88–97
9) Hirayama K, Matsushita S, Kikuchi I, Iuchi M, Ohta N, Sasazuki T. HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans. Nature 1987, 4–10; 327 (6121): 426–30

10) Secor WE, del Corral H, G. dos Reis M, Ramos EAG, Zimon AE, Matos EP, Reis EAG, do Carmo TMA, Hirayama K, David RA, David JR and Harn Jr. DA. Association of Hepatosplenic Schistosomiasis with HLA-DQB1*0201. J Infect Dis 1996, 174, 1131-1135

11) Hirayama K, Chen H, Kikuchi M, Yin T, Itoh M, Gu X, Zhang S, Yuan H. Glycine-valine dimorphism at the 86th amino acid of HLA-DRB1 influenced the prognosis of postschistosomal hepatic fibrosis. J Infect Dis 1998, 177 (6): 1682-6

12) Hirayama K, Chen H, Kikuchi M, Dong Y, Gu X, Liu J, Zhang S, and Yuan H. HLA-DR-DQ alleles and HLA-DP alleles are independently associated with susceptibility to different stages of post-schistosomal hepatic fibrosis in the Chinese population Tissue antigens 1999, 53: 269-274

13) MacManus DP, Ross AGP, Williams GM, Sleigh AC, Wiest P, Erlich H, Trachtenberg E, Guanling W, McCarvey WST, Li YS, Waine GJ. HLA class II antigens positively and negatively associated with hepatosplenic schistosomiasis in a Chinese population. Int J Parasitol 2001, 31: 674-680

14) Dessein AJ, Hillaire D, N.E.M.A. Elwali et al. Severe Hepatic fibrosis in Schistosoma mansoni infection is controlled by a major locus that is closely linked to the interferon-gamma receptor gene. Am J Hum Genet 1999, 65: 709-721

Schistosomiasis in Asia

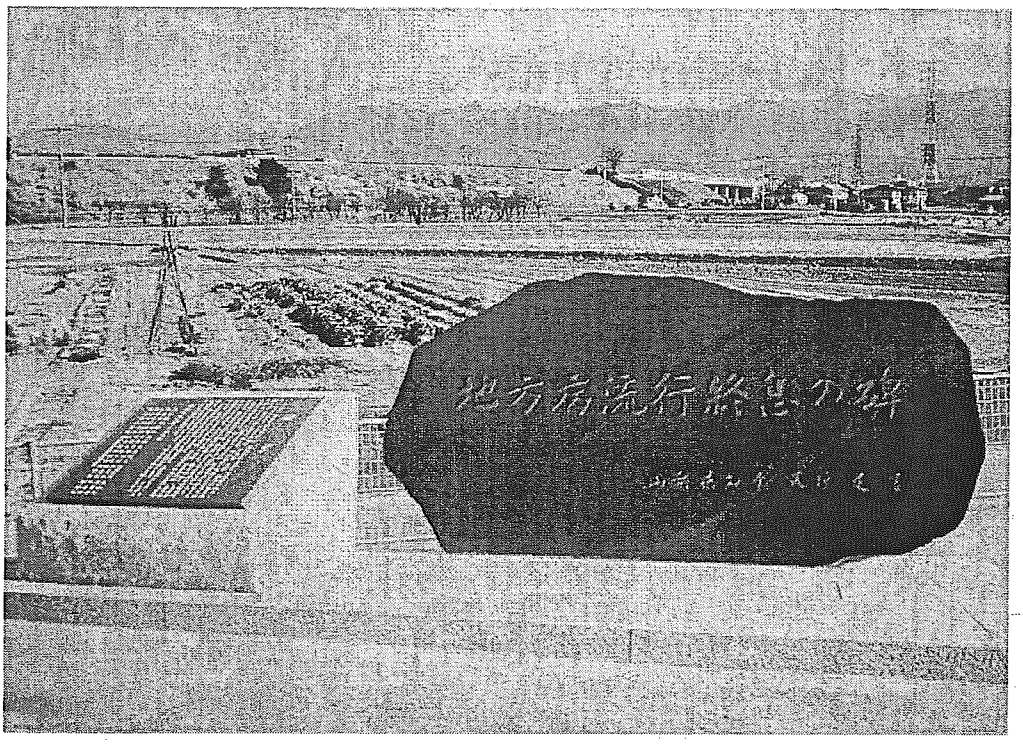
Ming-gang Chen
Xiao-nong Zhou
Institute of Parasitic Diseases,
Shanghai, P.R.China

and

Kenji Hirayama
Institute of Tropical Medicine
Nagasaki University
Nagasaki, Japan

Asian unique strategy for Asian people
being suffered from parasitic diseases by Asian parasitologists
(AAA)

The monument of the completion of eradication of 'regional disease' or Chihou-byou.



Built on December 26, 2002, Oshigome, Showamachi, Nakakoma-gun, Yamanashi, Japan. Calligraphy by Mr. Ken Amano, Governor of Yamanashi Prefecture.

Description

Regional disease (Schistosomiasis) had been endemic for more than 400 years. The area was spanned over 20,000 ha along three rivers, Kamanashi River, Arakawa, and Huehuki River. The people living there had been suffered from this fatal disease that was characterized by swollen abdomen or abdominal ascites.

Our regional eradication program was initiated at the time when the regional leader of a village, Kasugai, Higashi-Yamanashi gun, processed a requirement paper for the control of this special disease characterized by swollen abdomen to the Governor Shiro Fujimura. Since then the people have been struggling with this disease for 115 years and finally we declared that this disease ended on February 19, 1996.

We built this monument to give our sincere appreciation to all the persons who had contributed to this historical achievement and to memorize it forever.

A good day of December, 2002

Yamanashi Prefecture Government, Kofu, Yamanashi, Nirasaki, Kasugai, Isawa, Misaka, Ichinomiya, Yashiro, Nakamichi, Sakaigawa, Toyotomi, Mitama, Masuho, Nakatomi, Ryuoh, Shikishima, Tamaho, Shouwa, Tatomi, Hatta, Shirane, Wakakusa, Kushigata, Kousai, Futaba, Association for the eradication of schistosomiasis (Regional Disease) in Yamanashi Prefecture

Preface

Kimio Saitoh
President
Association for the eradication of schistosomiasis
(Regional Disease)
in Yamanashi Prefecture

The history of our eradication program began at the time the pathogen was found in 1904. The program was enhanced by the finding that Oncomelania snail was the intermediate host in 1913. Regional community based control programs had been aggressively processed for more than 100 years and finally our Governor declared the end of this disease in Yamanashi Prefecture in February 1996.
Schistosomiasis Japonica had been endemic in several different regions in Japan, such as Hiroshima, Okayama, Fukuoka, Saga and Yamanashi. Our Association was established in 1950 and has been working as a facilitating organization for the control collaborated with other Prefectures' associations. When we established this association, the national and the prefectural financial situation showed very difficult due to the defeat of the World War II. However our association strongly pushed the Japanese government to make a time limited law to support our eradication program mainly composed of Snail control. I appreciate the people in the endemic areas for their voluntary effort that was essential and critical for this final success. Of course the great advancement of the medical and pharmaceutical treatments contributed to this achievement.
In 1947, the expected years of life of male and female were 46.06 and 49.6 years respectively, but now those are 77.10 for male and 83.99 years for female in Japan. I am sure our effort against schistosomiasis contributed a lot to improve our prefectural health condition. On this historical occasion of the declaration of eradication of schistosomiasis, we decided to publish this booklet titled 'The war against the regional disease, History of the eradication of schitosomiasis in Japan to give all the precious records that should be kept as our common heritage. I hope it will be the first level historical record in future.
I would like to give my sincere gratitude to all the persons who have been supporting our association and also who had contributed to this publication by donating the manuscripts and or materials.

Notification

Association for the eradication of schistosomiasis (Regional Disease) in Yamanashi Prefecture
March, 2003

For a long period, the farmers living in the Kofu Basin area had been suffered from the regional disease (Schistosomiasis japonica). Therefore, the eradication of this disease was a kind of dream for them over generations and generations.

Our eradiation project started after the findings of the pathogen in 1904 and of the intermediate host Oncomelania snail in 1913. The inhabitants, and many doctors and researchers who were pursuing the new methods for prevention and treatment, were performing the projects under the strong supports by regional and prefectural offices. Those activities with additional public health improvements gradually decreased the morbidity and mortality, but until 1955, there were still many active patients in any places in the region. After that there were sporadic cases until 1985. Finally Governor declared that the disease ended in February 1996.

Our Association was established in 1950 and has been working as a facilitating organization for the control of schistosomiasis collaborated with other Prefectures' associations. We have already published a series of books that describe the disease situation and the history, 'The War against the regional Disease, 1977', 'The War against the regional Disease, memories of the soldiers, 1979', and 'The War against the regional Disease, Medical aspects,1981'. Since 1992, we have been collecting available materials that related to the eradication program to prepare this final memorial booklet which we hope will be informative for all the persons who are still suffering schistosomiasis japonica in South East Asia.

The contents of this volume are as follows,
The first chapter, 'History of the control program by the prefectural government' described the outline of the disease and the history of the control program at a glance and put the table that summarize the complicated activities year by year. The second chapter, 'The collected materials that tell the myth' shows you the pictures and the records that will help you to understand the situations more vividly. The third chapter, 'Records during the war' describes each regional history by city, town, or village level by using their records, articles in the news paper, and books. Our Association's history is also put here as 'Footprints of Association for the eradication of the regional disease'. The fourth chapter, 'Renewed materials' includes two historical booklets, 'I am a doctor of regional disease!' published in 1916, and 'Manuals for the diagnosis of schistosomiasis japonica' published in 1996 by the society of the researchers of the regional disease. The fifth chapter, 'Statistics tables regarding the regional disease', shows the epidemiological records since Meiji Era (1985-) based on the official reports, public health statistics and the materials stored in the Yamanashi prefectural institute of health.

Even now we all have a memory of 'the 100 years' war against the regional disease' deep in mind. I hope this booklet will tell our children that we had a glorious history to eradicate a disease that had been there for hundreds of years.

Contents

Chapter 3. Records during the war
(No English translation of this chapter)

Chapter 4. Renewed materials

Chapter 5. Statistics tables regarding the regional disease

Closing