diagnosis for proteinuria by a reagent strip, will contribute to maintain child health. The application of recombinant antigens may improve the sensitivity and specificity of urine ELISA.

In conclusion, the urine ELISA can be a useful tool for the surveillance of schistosomiasis.

ACKNOWLEDGEMENTS

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EFFECTS OF CpG OLIGONUCLEOTIDES ON *SCHISTOSOMA JAPONICUM* INFECTION IN MICE

TAKASHI KUMAGAI, MOHAMED EL-MALKY, HARUHIKO MARUYAMA, AND NOBUO OHTA
Department of Molecular Parasitology, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuhocho, Mizuhoku, Nagoya 467-8601, Japan
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SUMMARY

Effects of CpG oligonucleotides on murine experimental *Schistosoma japonicum* infections were examined. Intramuscular injection of CpG oligonucleotides at the dose of 100 mg/kg biased host immune responses toward Th 1-dominance. When mice were treated with CpG oligonucleotides at the infection with *S. japonicum*, the egg production by female adult worms was significantly suppressed, suggesteing that some unknown stimulation related to Th 1 responses had inhibitory effects on sexual maturation of *S. japonicum* female worms. However, there was no difference in the number of recovered worms between CpG-treated and non-CpG treated groups. The mean size of hepatic circum egg granulomas in CpG-treated mice was significantly smaller than that in non-CpG treated mice. Our present study suggests that CpG oligonucleotides might be applicable to morbidity control of schistosomiasis japonica as adjuvants in conjunction with classical vaccines.

Key Words: *Schistosoma japonicum*, helper T cell, CpG oligonucleotide, granuloma

熊谷　貴，モハメド・エルマルキー，丸山治彦，太田伸生
Address for Correspondence: Nobuo Ohta, M.D., Department of Molecular Parasitology, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuhocho, Mizuhoku, Nagoya 467-8601, Japan
Phone: +81-52-853-8186, FAX: +81-52-842-0149, e-mail: nohta@med.nagoya-cu.ac.jp
Abbreviations used: ABTS, (2, 2'-azino-di [3-ethyl-benzthiazoline sulfonate]); IFN, interferone; IL, interleukin; PBS, phosphate-buffered saline; SEA, soluble egg antigen; Th, T helper

INTRODUCTION

In the last two decades, it has been increasingly recognized that there are two major types of immune responses of humans as well as animals, based on antigen-specific T cells involved (1). Type 1 responses are characterized by the induction of T helper 1 (Th 1) cells that secrete interferon (IFN) -γ and interleukin (IL) -2 in response to antigen stimulation. Type 2 responses are dominated by T helper 2 (Th 2) cells that produce cytokines such as IL-4, IL-5, IL-9, and IL-13. In general, type 1 responses are protective against intracellular parasites and tumor cells, because IFN-γ and IL-12, which are produced during type 1 responses, stimulate effector cells including macrophages and NK cells. However, inappropriate stimulation of Th 1 cells is known to cause certain kinds of autoimmune diseases (2, 3). On the other hand, type 2 responses induce eosinophilia, mastocytosis, and elevated immunoglobulin production, especially IgE, because of the biological activities of secreted cytokines. Roles of Th 2 cells in allergy, atopic diseases, and helminth infections have been studied extensively as eosinophilia, mastocytosis, and elevated IgE are hallmarks of these diseases.

Infections with *Schistosoma mansoni* and *S. japonicum* are among the most powerful inducers of type 2 immune responses (4). These parasites inhabit the portal vein of host animals, and eggs deposited by adult female worms cause granuloma formation in the intestine and the liver. During granuloma formation, intense type 2 responses elicited by egg antigens take place in the infected hosts, which have profound effects on co-existing infections and other disease conditions (5, 6). For example, schistosome-infected mice become resistant to intestinal nematode infections (7-9), but in the same time, become vulnerable to protozoal infections (10, 11) and to malignant tumors (12). Because Th 1 and Th 2 cells are cross-inhibitory each other (13, 14), type 2 responses elicited by schistosome infections inhibit type 1 responses which are necessary for the elimination of viruses and protozoan parasites. Moreover, non-obese diabetic (NOD) mice, which spontaneously develop autoimmunity against pancreas islet cells (2, 3), are protected from the disease when mice are infected with schistosomes (15).

Immune responses of the host have various effects on schistosome infections themselves. It has been demonstrated that strong type 2 responses against egg antigens are beneficial for the worms, because egg excretion is significantly decreased in immunodeficient mice, in which no antigen-specific T cells are induced (16, 17). It is also known in mice that protective immunity against schistosomulae, migrating larvae of schistosomes, are of type 1 responses (18,

19). To take all the known findings into consideration, the onset and progression of schistosomiasis might be entirely changed, if the host mounts type 1 immune responses to schistosome infections. In the present study, we modulated immune responses of mice with CpG oligonucleotides and challenged them with *S. japonicum*. CpG oligonucleotides are strong inducers of type 1 responses (20-22). We found that CpG oligonucleotides significantly suppressed the production of eggs by adult worms, and granuloma formation in the liver. Our study suggests that vaccination of the host with CpG oligonucleotides and a certain antigen preparation might be able to induce anti-disease immunity, even if sterile immunity is difficult to achieve.

MATERIALS AND METHODS

Parasites and animals

Schistosoma japonicum (Yamanashi strain) has been maintained at the Department of Molecular Parasitology, Nagoya City University Graduate School of Medical Sciences, by standard laboratory procedure in female BALB/c mice (SLC, Hamamatsu, Japan) and the snail host, *Oncomelania hupensis nosophora*. Mice were infected with 40 cercariae of *S. japonicum*.

Oligodeoxynucleotides

Phosphorothioates-modified oligonucleotides were purchased from Hokkaido System Science (Hokkaido, Japan). Oligonucleotides used in the present study were CpG 1826 (20), which contain two CpG motifs (underlined 5'-TCCATGACGTTCCTGACGTT-3'). CpG 1826 has been well characterized for its adjuvant activity with protein antigens. For nonstimulatory negative control, non-CpG 1745 (5'-TCCAATGAGCTTCCTGAGTCT-3') was used.

Infection

Mice, either injected with CpG oligonucleotides, non-CpG oligonucleotides, or untreated (n=6-7), were infected percutaneously with 40 cercariae of *S. japonicum*. For percutaneous infection, mice were anesthesized with pentobarbital, and abdominal region was shaved off. Then, a piece of cover glass was applied to the abdomen in such a way that cercaria suspension was placed between the cover glass and the abdominal surface. Mice were left untouched for 15 min to allow cercariae to penetrate the abdominal skin. Under these conditions, moer than 95% of cercariae successfully penetrate the skin. One hundred μg of oligonucleotides were

given intramuscularly for 3 times; 2 days before infection, 3 and 8 days post-infection. Eight weeks after challenge infection, the portal vein of infected animals was perfused with phosphate-buffered saline (PBS) to count the number of adult worms in each of mice. A small portion of the liver was removed and fixed with 10% buffered formalin for histopathological examination. The rest of the liver and the whole intestine were digested with 4 % KOH overnight at 37℃, and the number of eggs was counted under a microscope. The number of eggs / the number of female worms was calculated for each mouse (fecundity).

Enzyme-linked immunnosorbent assay (ELISA)

Antibody responses to *S. japonicum* soluble egg antigen (SEA) were measured in ELISA. For the preparation of SEA, freshly recoverd eggs were sonicated in PBS containing proteinase inhibitor cocktail (Sigama-Aldrich, St. Louis, USA), and incubated at 4℃ overnight. SEA preparations were then recovered after centrifuge at 15000 rpm for 10 min, and stored at -80℃ until used. Wells of microtiter plates (Nunc, Roskilde, Denmark) were coated with 10 μg/ml of SEA, and blocked with Tris-buffered saline containing 1% casein. Wells were then incubated with serum samples diluted at 1 to 1, 000. After washing, anti-mouse IgG 1 or IgG 2 a antibodies (Southern Biotechnology associates, Birmingham, USA) were added. ABTS (2, 2'-azino-di [3-ethyl-benzthiazoline sulfonate]) was used as a substrate, and optical densities were read in a microplate reader (Bio-Rad, Hercules, USA).

Histopathological examination

Paraffin-embedded sections of the liver were stained with hematoxylin and eosin (H. E.). The size of granulomas was measured for more than 10 granulomas in each mouse by using a VM-30 videomicrometer (Olympus, Tokyo, Japan) and NIH image software (national Institute of health, Bethesda, USA). Average granuloma area (μm^2) was calculated for each mouse.

Cytokine

Serum samples were collected after intramuscular oligonucleotide injection, and IL-12 concentration was measured in ELISA (PharMingen, San Diego, USA). IFN-γ and IL-4 production by spleen cells were tested at the time of perfusion. 1×10^6 spleen cells were stimulated with SEA at 10 μg/ml in RPMI 1640 (Sigma-Aldrich) containing 10% fetal bovine serum (GIBCO-BRL, Grand Islands, USA). After incubating at 37℃ for 48 h, culture superna-

tants were recovered and the concentrations of cytokines were determined in ELISA (PharMingen).

Statistical analysis

Statistical significance was evaluated in two-sided Student's *t* test.

RESULTS

CpG oligonucleotides elicits Th 1 responses

CpG and non-CpG oligonucleotides were administered to mice intramuscularly, and serum IL-12 was measured to monitor Th 1 activities. After injection with CpG oligonucleotides, serum IL-12 elevated quickly, whereas non-CpG oligonucleotides had no such effects (Fig. 1).

Lower fecundity of adult worms in CpG-administered mice

Having confirmed the immunomodulating effects of CpG oligonucleotides, we infected mice with *S. japonicum*, to examine how biased immune responses affect the course of *S. japonicum* infection. After infection, both of CpG administered and non-CpG administered mice excreted *S. japonicum* eggs in the stool, indicating that CpG alone could not prevent infection. Seven weeks after infection, adult worms in the portal vein and eggs in the liver and

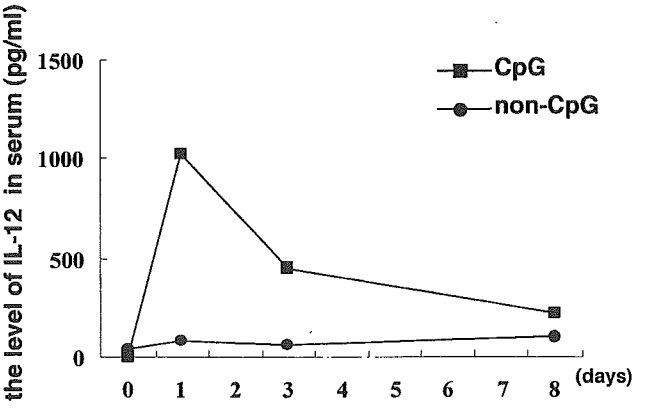

FIG. 1. Serum IL-12 responses after intramuscular administration of CpG and non-CpG oligonucleotides. After oligonucleotide injection, serum samples were collected at day 0, day 1, day 3 and day 8. Only CpG oligonucleotides elicited IL-12 production.

the intestine were recovered and counted. We found that the number of eggs recovered from CpG administered mice was significantly reduced as compared to that from non-CpG administered and untreated mice, although there was no significant difference in the number of adult worms (Fig. 2). Our findings indicated that the fecundity of female adult worms was suppressed in CpG administered mice.

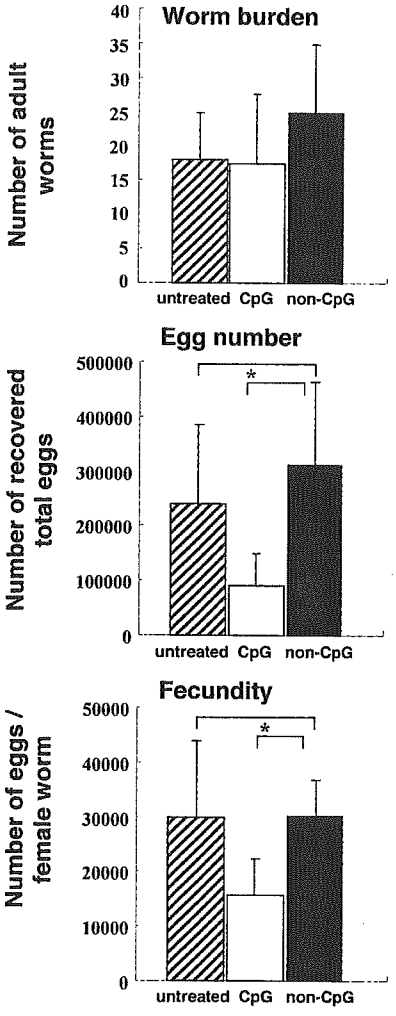

FIG. 2. The numbers of adult worms and eggs recovered from mice, either treated with CpG, non-CpG oligonucleotides, or untreated. Although there is no significant difference in worm burden, the total number of deposited eggs and the fecundity of female worms were significantly decreased in CpG-treated group. (*$p < 0.05$).

Antibody responses to soluble egg antigen (SEA)

We measured IgG 1 and IgG 2 a antibodies specific to *S. japonicum* soluble egg antigen (SEA), because IgG 1 and IgG 2 a antibody classes depend on Th 2 and Th 1 responses, respectively. In CpG administered mice, the production of IgG 1 antibodies to SEA was significantly low as compared to that in non-CpG administered and untreated mice (Fig. 3). However, IgG 2 a antibodies to SEA were not detected in all groups of mice.

Hepatic granuloma formation

It has been demonstrated that Th 2 resopnses are important for the formation of liver granulomas in schistosomiasis (4). Histological examination of the liver revealed that the size of granulomas around deposited eggs in CpG administered mice was significantly smaller than that in non-CpG administered and untreated mice (Fig. 4 A). Interestingly, some hepatic tissues around the eggs in CpG treated mice went necrotic (Fig. 4 B), which was also observed in immunosuppressed mice (4, 16). The present findings indicated that CpG oligonucleotides administered in the early phase of infection had effects on the histopathological changes in the later phase of infection, in spite that serum IL-12 dropped rapidly (Fig. 1).

Cytokine production

Finally, we measured IL-4 and IFN-γ production by spleen cells of infected mice. As shown in Figure 5, there

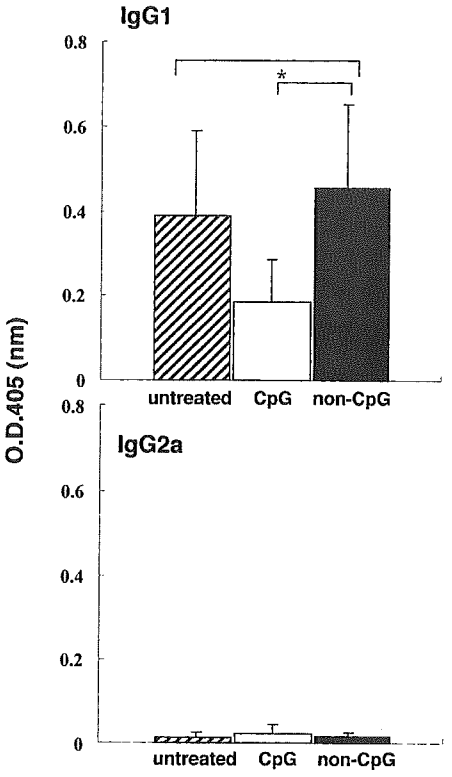

FIG. 3. IgG 1 and IgG 2 a antibody responses against *S. japonicum* SEA. IgG 1 response was suppressed in CpG -treated mice (*$p<0.05$). IgG 2 a antibodies against SEA were under detection limit in all three groups.

was no significant defference in the production of IL-4 and IFN-γ between CpG treated and non-CpG treated mice.

DISCUSSION

Schistosomiasis is a serious health problem in tropical and subtropical countries. The countermeasure currently taken for the disease control is chemotherapy, mass or selected, of infected individuals, together with strengthening social infrastructures such as safe water supply (23). However, eradication of schistosomiasis is extremely difficult in endemic foci in Asia, because Asian schistosomiasis, mainly caused by *Schistosoma japonicum*, is zoonotic in its nature. Therefore, vaccine development has been a major issue in the control of schistosomiasis in Asia.

In the present study, we demonstrated that CpG oligonucleotide treatment reduced the fecundity of *S. japonicum* adult worms, and granuloma formation around the eggs in the liver. These findings suggest that CpG oligonucleotides might be able to suppress liver pathology in chronic schistosomiasis in humans. In general, vaccines against helminthic infections fall into two categories. Infection control vaccines, which reduce disease incidence, and disease control vaccines, which reduce morbidity. In helminthic diseases, infected people often suffer from chronic disabilities caused by pathological changes, in spite that the disease itself is not directly life-threatening. In this sense, CpG oligonucleotides are expected to have such 'disease control' effects.

It is unclear how CpG oligonucleotides induced antifecundity effects in mice. In severe combined immunodeficiency (SCID) mice, schistosomes fail to mature, and do not start oviproduction (24). Such impaired sexual maturation can be restored by certain kinds of T cell cytokines, suggesting that egg production by female schistosomes is dependent on the host immune responses. In the case of CpG treatment, suppression of immune responses, as evidenced by reduced granuloma size, seemed to be associated with the inhibition of the sexual

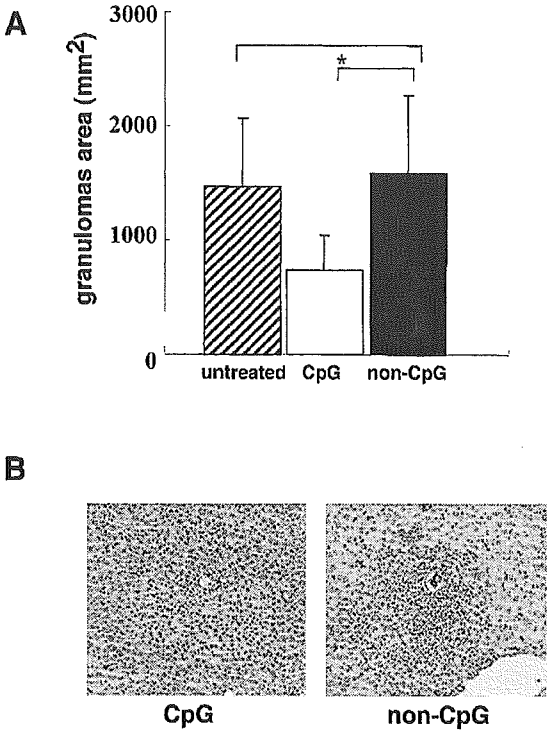

FIG. 4. Granulomas of the liver. (A) The granuloma size of CpG-treated mice was significantly smaller as compared to non-CpG and untreated mice (*$p<0.05$). (B) H.E. staining of a representative liver granuloma of CpG-treated mice and of non-CpG-treated mice (x 400). Note that periovular hepatic tissues in CpG oligonucleotide -treated mice show signs of necrosis.

maturation of worms. Alternatively, Th 1 cytokines might have suppressed the sexual maturation directly. It has been reported that Th 1-dominant responses are induced in early phase of *S. mansoni* infection in mice (25, 26). CpG-treatment might have further enhanced type 1 responses in the early phase of infection, when parasite growth seemed sensitive.

The pathological lesion in the liver was significantly milder in CpG-treated mice, which agreed with a study on *S. mansoni* infection (27). However, there was little evidence that T cell responses against egg antigens were biased to Th 1, in spite of the reduced granuloma size in the liver (Fig. 5). This might be due to the short half-life of the effects of CpG oligonucleotides in vivo (Fig. 1), or the sugar structure of soluble egg antigens, that stimulates Th 2 responses (28). In *S. japonicum* infection, immunological mechanisms are not fully understood as to how granulomas are formed, and it is still controversial whether host immune response

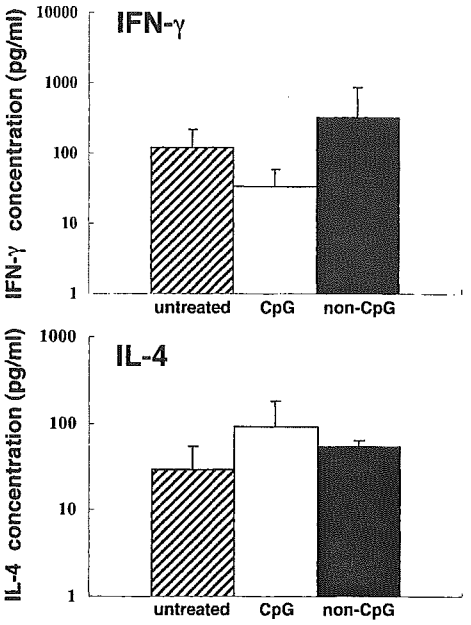

FIG. 5. IFN-γ and IL-4 production by spleen cells stimulated with *S. japonicum* soluble egg antigen (SEA). Spleen cells were cultured with 10 μg/ml of SEA for 48 hours, and culture supernatants were tested for cytokines in ELISA. There was no significant difference among all groups.

and immunopathogenesis are same between *S. japonicum* and *S. mansoni* infections (29). Roles of Th 2 responses in the development of pathological changes in *S. japonicum* infection remain to be clarified.

Based on the present study, we believe that CpG oligonucleotides are candidate adjuvants for human schistosomiasis japonica, in which morbidity control is an urgent issue. It has turned out that some vaccine preparations are effective only under Th 1-dominant circumstances (30). Since known Th 1 inducing adjuvants, such as complete Freund adjuvant, cannot be used in humans, CpG oligonucleotides deserve consideration for human use. Undoubtedly, more intensive studies are needed for the practical use of CpG oligonucleotides against human schistosomiasis. For example, simple suppression of granuloma formation could be harmful to the hepatocytes because of the toxic effects of schistosome egg antigens (31). Choice of antigens, optimization of dose and administration schedules of CpG oligonucleotides should be considered carefully.

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Review

Historical view of schistosomiasis japonica in Japan: implementation and evaluation of disease-control strategies in Yamanashi Prefecture

Masaru Minai[a], Yukio Hosaka[b,*], Nobuo Ohta[c]

[a]*Yamanashi Institute for Public Health and Hygiene, Kofu 400-0027, Japan*
[b]*Biomedical Science Association, 1-28-801, Takadanobaba, Shinjuku-ku, Tokyo 167-0075, Japan*
[c]*Department of Molecular Parasitology, Nagoya City University Graduate School of Medical Sciences, Nagoya 467-8601, Japan*

Abstract

We summarized historical aspects of disease control activities targeting schistosomiasis japonica in Kofu basin, Yamanashi Prefecture, Japan. Kofu Basin was one of the biggest endemic foci of schistosomiasis japonica in Japan, and the last place where transmission of *Schistosoma japonicum* was confirmed in Japan. Because of the most severe endemic situations in Yamanashi, intensive control measures had been implemented by the central as well as the local government. The last human case in Japan was in 1977, which is just before praziquantel being available. Therefore, the main efforts were focused on snail control. Mass examination and mass chemotherapy were implemented, however, the compliance was not so good because of the severe side effects due to the available therapeutics, Stibnal. Along with socioeconomic development after World War II, big changes in land use, life style, and farming led drastic reduction in the disease prevalence in Kofu Basin in the 1960s. A large amount of budget was also used for disease control. Cementing water canals covered more than 95% of paddy fields in Kofu Basin, and this resulted in ecological changes. After elimination of schistosomiasis, environmental repair is the urgent subject in Kofu Basin. Our experiences in Yamanashi contain both good influences and also a lot of reflection. It is important to evaluate each activity in our history before we give intensive cooperation with countries where endemic foci is still present.

Keywords: Schistosomiasis japonica; Countermeasure; *Oncomelania nosophora*; Molluscicide; Yamanashi

1. Introduction

There were several endemic foci of schistosomiasis japonica in Japan. Among them, Kofu Basin, Yamanashi Prefecture, was the most severe focus not only in patient number, but also in the width of snail bleeding areas [1]. Katayama note, written in 1847, was the first medical record of schistosomiasis japonica, however, the earliest political appeal to the local Government to control the unknown disease was raised by residents of Kofu Basin in 1881 [2]. Local people noticed the presence of disease endemicity of unknown origin, however, high transmission situation had continued until the 1960s in Kofu Basin even after cause of

*Corresponding author. Tel.: +81-3-3200-6752; fax: +81-3-3200-5206.

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Table 1
History of control activities against schistosomiasis japonica before World War II in Yamanashi

Year	
1881	Chief of Kasugai village, Higashi-Yamanashi County, Yamanashi Prefecture sent a proposal to the prefectural governor to investigate the endemic disease
1888	Eggs of unknown parasites were observed in the liver of autopsized case in Yamanashi.
1898	Ms. Naka Sugiyama, a farmer suffered from the 'unknown' disease donated her corpse to autopsy to investigate the cause of her disease.
1900	Dr. Sabro Mikami found parasite eggs in patient's stool.
1901	Dr. Mikami hypothesized that the new parasite seemed to be a trematode.
1904	Dr. Fujiro Katsurada found an 'unknown' parasite from Dr. Mikami's cat, and reported to be a new species, *Schistosomum japonicum.*
1914	The discovery of intermediate host snails enabled to implement prophylactic measures by killing the snails. Lime was the first molluscicide to be used.
1917	A method of buying snails was tested
1918	Local low for buying snails was issued; 0.5 Yen for 180 ml of snails, and 0.1 Yen for another 180 ml.
1925	A committee for controlling schistosomiasis was organized in Yamanashi Prefecture
1929	Recommendation to use 'improved toilet'
1930	Stibnal was generally used for deworming in Yamanashi
1933	Yamanashi Prefecture appointed 'Endemic areas' and registered patients
1938	Calcium cyanamide was used as a molluscicide. Construction of cementing water canals was planned

the disease being clarified. In 1996, Yamanashi Prefecture finally declared elimination of schistosomiasis japonica after countermeasures continued for more than 100 years. Japan is, then, the first country to get a success in complete elimination of schistosomiasis in the world. In this review, we introduce countermeasures implemented in Kofu Basin, evaluate our activities, and we would like to discuss future prospect of control of schistosomiasis in the world.

2. Days in discovery of *Schistosoma japonicum* and control measures in Yamanashi

Biomedical research projects were started in years of the early 1880s both in Yamanashi Prefecture and Katayama district. In Yamanashi Prefecture, protest from residents motivated the local government. Professor Fujiro Katsurada discovered adult worms of *Schistosoma japonicum* in Kofu Basin in 1904 [3], and its intermediate snail hosts, *Oncomelania nosophora* was discovered by Drs. Miyairi and Suzuki in the Chikugo river area in 1913 [4]. Based on those scientific discoveries on the parasite life cycle, the local government organized control strategies in each endemic focus in Japan. Just after the discovery of the intermediate snail hosts, snail control was the only available control measure until discovery of therapeutics (Tables 1 and 2). We will summarize the tactics of schistosomiasis control in Yamanashi Prefecture as follows.

2.1. Snail control activities

Biological and non-biological approaches were tested in Yamanashi. In very early days, snails were buried, or the local government purchased snails from the people in endemic areas at 0.5 Yen for 180 ml of snails. As biological control measures, molluscicides had been used. Lime was the first one to be tested around 1914, however, it was not so effective. In 1938, calcium cyanamide became a choice of molluscicide in Japan, and it was much more effective than lime. After the last world war, the US military agent joined our snail control activities. They introduced sodium pentachlorophenate (Na-PCP), and Yamanashi Prefecture had used it from 1953 until 1967. Although molluscicidal effects of Na-PCP were high enough, the local government stopped using it because of environmental toxicity. The next molluscicide

Table 2
History of control activities against schistosomiasis japonica after WWII in Yamanashi

Year	
1947	The US occupation forces took initiative of schistosomiasis control project.
1953	Na-PCP was introduced as a new molluscicide
1953	Committee for promoting schistosomiasis control was settled
1954	Construction of cementing water canals was started
1958	Flamethrowers were tested for killing snails
1959	Intradermal response (skin test) was employed for mass examination
1966	Anbihar was tested as oral medicine for schistosomiasis
1968	Yurimin was tested as a new molluscicide
1975	B-2 was introduced as a new molluscicide
1978	Parasite egg 'Zero' in stool examination in endemic areas
1984	ELISA replaced with skin test in mass examination
1984	Praziquantel was tested for chronic patients in Yamanashi
1996	Declaration of elimination of schistosomiasis japonica in Yamanashi Prefecture
1996	Committee for promoting schistosomiasis control was changed to be Committee for watching schistosomiasis
2001	Committee for watching schistosomiasis was finally dispersed

introduced was Na-PCP, and it replaced Yurimin (3,5-dibromo-4-hydroxy-4′-nitroazobenzene) in 1968 [5]. It was again forced to stop using because of mutagenicity. Research group at National Institute of Health, Japan (currently, National Institute of Infectious Diseases), reported high efficacy of B-2 (Sodium 2,5-dichloro-4-bromophenol) without detectable environmental toxicity [6]. After detailed field-testing, Yamanashi Prefecture replaced Yurimin with B-2 in 1975, and had continued to use B-2 until 1996, when Yamanashi Prefecture declared the disease elimination.

Non-biological measures included physical snail control, as well as environmental modification in Yamanashi. In 1916, hot water was used for killing snails in the field, however, it cost too much. Flamethrowers came in use in 1958 as supplemental measures of chemical molluscicides. In case of a small endemic focus, flamethrowers were so effective that it killed all snails. As an approach of environmental modification, cementing water canals were constructed in agricultural fields. Although the original idea was raised in 1938, it was in 1954 when the budget of the central government allowed implementation of this project. The cost for the cementing canal construction was the biggest in control measures implemented in Yamanashi Prefecture. More than 95% of water canals in endemic areas in Kofu basin became cementing ditches. Changes of land use were also important factors, however, details will be mentioned later. Another approach of snail control included an ecological way. For example, geese or larvae of firefly were released to paddy fields to eat snails, but no successful results were obtained.

2.2. *Mass examination/mass chemotherapy*

Mass examination was implemented as a countermeasure in the activities by Yamanashi Prefecture. Stool examination had been the method of case-detection. In the 1960s, immunological methods were developed, and individuals positive for immunodiagnosis were further tested for stool examination as the secondary screening. Intradermal testing was the first method employed as an immunodiagnosis, and later, ELISA became a method for the first screening. From the base-line survey, cutoff of ELISA testing was set to be OD value of 0.2 at serum dilution of 1:100. Considering the zoonotic characteristics of schistosomiasis japonica, domestic animals such as cattle and dogs were subjects of mass examination. For treating patients, Stibnal, antimonyl potassium tartrate, had been only a drug of choice in Japan since the 1930s [7]. It was, however, pointed out that Stibnal injection caused severe side effects, and patients were injected every consecutive day for almost 3

weeks. In spite of such difficult treatment, no complete deworming was expected in many cases. Although anti-schistosome drugs of oral administration were developed in the mid-1960s [8], there was no occasion to use such newly developed medicine in Kofu Basin because schistosomiasis was almost controlled in the late 1960s in Japan [9]. Mass examination of domestic animals was also tried, and animals positive for *S. japonicum* were injected with the toxic antimonyl tartrate as in humans. In any way, snail control was the first choice in countermeasure because safe and effective therapeutics were not available at that time. Mass chemotherapy had been never of higher priority over snail control in Kofu Basin.

2.3. Changes in land use

Paddy fields were the main place of disease transmission in Japan. Many infected cases, if not all were farmers, and changes in land use such as from paddy fields to mulberry fields or to orchard fields had a big impact on the reduction of disease transmission in Yamanashi. This resulted in the increased fruit production in some areas, and increase in income of farmers in such areas was the by-product of the changes in land use. Urbanization in Kofu basin raised needs of housings, and paddy fields were also changed to be houses. Industrial use of land is also an important part of the changes in land use in endemic areas. Such a change in social structure also caused reduction in incidence of schistosomiasis. Educational sector in Yamanashi Prefecture constructed swimming pools in primary schools and public parks to reduce a chance of contact to contaminated natural water in school-age children. Some snail habitat areas were used for athletic fields or golf courses, and this also reduced the incidence.

2.4. Improved social hygiene

Stool containing schistosome eggs were the direct source of environmental contamination. It was important to control contaminated feces not to be spread in the field, however, it was not acceptable for farmers in Japan because night soil was commonly used as a cheap fertilizer. Methods to kill contaminated parasite eggs were developed. Since parasite eggs were killed after being preserved for a long period, the local government recommended using 'improved toilet' which contained 2 or 3 tanks to keep feces for a long period. It was possible to keep human feces, however, almost impossible to control feces of reservoir host animals. Water supply was also an important subject. Rivers and ponds were places of disease transmission; therefore, wells and service water were developed. Constructions of drainages in urban areas were good for fecal treatment.

2.5. Reservoir host animals

Schistosomiasis is a zoonosis, and almost all mammalians, wild or domestic, have susceptibility to *S. japonicum*. For countermeasures targeting the reservoir host animals, controlling wild mice and street dogs were tried, and replacing cattle with horses for farming were implemented. Cattle are highly sensitive to *S. japonicum*, and quantity of excreted eggs is relatively high. However, horses are resistant to schistosome infection, and egg output is low even in the cases of *S. japonicum* infection in horses. Those countermeasures depended on community participation, and both the local government and community people in Yamanashi owed the responsibility for the implementation.

2.6. Prophylactic efforts

Blocking cercarial invasion was also the target of countermeasures. Ointment reagents effective for this purpose were developed and tested, however, frequent water contact made the less effective, and no such ointment was in practical use as a prophylactics. To avoid contact with contaminated water, a swimming pool was constructed in every primary school in endemic areas in Kofu Basin. School teachers stressed to the pupils, not to swim in natural waters.

2.7. Health education

Concept of health education was different from place-to-place, and was different in various target

populations. The most effective targets were the farmers in Yamanashi. The local government instructed farmers how to find *Oncomelania* snails, and this enabled the community people collect the snails. It was not easy to change the traditional life style in rural areas, and farmers did not understand several critical points for disease control. For example, farmers did not understand importance of the period of keeping feces to kill the parasite eggs, and they used night soil containing live eggs in seasons of tight farming.

3. Critical factors lead to the disease elimination in Japan

Essential factors in the success of disease elimination in Kofu basin have been discussed. Although written record is officially not available, community leaders and the local government officers shared, to some extent, impression and reflection of their battle with schistosomiasis japonica during the past 50 years in Kofu Basin.

3.1. Countermeasures led by local government

This was the main component which led to the success of our disease elimination in Yamanashi Prefecture. Among them, snail control was the biggest part, and a huge amount of budget was spent for this. Between 1945 and 1985, 10 billion Japanese Yen was spent for constructing cementing water canals in Kofu Basin, and more than 95% of water canal was covered with cementation. Of course, there was no other choice in disease control in those days because safe and effective therapeutics, such as praziquantel, was not available. In spite of such a huge amount of budget, effects were doubtful from the view point of cost-effectiveness, and snail-habitat areas still present in Kofu Basin. Furthermore, community people feel that cementing canals caused environmental problems.

3.2. Socioeconomic development

There is no doubt that socioeconomic development has been tightly related to improved endemic situations of various infectious diseases. In case of schistosomiasis japonica in Japan, high transmission had been observed in less developed socioeconomic situation. Disease prevalence reduced drastically in parallel with economical development in our country. It was in late the 1940s when a large scale project of schistosomiasis control was organized in Japan, and socioeconomic situation at that time was miserable in Japan. Furthermore, political confusion during and after World War II left schistosomiasis uncontrolled in Japan. Even in a difficult social background, the US occupational forces supported organizing control activities in endemic foci of schistosomiasis in Japan. People felt that it was almost interference by the US military government, but it resulted in accelerated success of disease elimination in Japan. Together with such political pressure for controlling schistosomiasis, socioeconomic development in the 1950s had deep influence on the disease elimination. Socioeconomic development changed agricultural structure. Night soil was replaced with cheep chemical fertilizers, and this reduced soil contamination with schistosome eggs. Use of cultivating machines reduced direct water contact of farmers in paddy fields.

3.3. Medical contribution

As mentioned in a booklet of Hashimoto Initiative [10], background information about biomedical aspects of *S. japonicum* required for disease control had been uncovered in the 1960s. Even before the appearance of praziquantel, disease transmission had been almost controlled in Japan. Together with this, community people felt that contribution from scientific approaches was not great enough in Yamanashi. Scientific contribution for disease elimination might have included operational research, which provided new tools and methodologies of disease control activities, or new molluscicides with less environmental toxicity. However, budget of health sectors and/or political commitment determined whether such scientific tools were used in the control activities in Yamanashi.

3.4. Cultural background

Life style and/or culturally determined human behavior were also a factor to be considered. There

have been few reports of sociological or cultural anthropological analysis in Yamanashi. Evaluation of this is, thus, not easy, but only limited contribution was supposed from this factor. Although health education is thought to be important in controlling schistosomiasis, it is difficult to distinguish between results of health education and changes in life style caused by socioeconomic development.

4. Conclusion

In this review, we summarized the countermeasures implemented in Yamanashi Prefecture. Almost 100 years were spent for controlling schistosomiasis japonica in Japan. During, and just after the last world war, people in endemic areas did not believe the disease elimination in Kofu basin. It is, therefore, admirable that Yamanashi Prefecture declared elimination of schistosomiasis in 1996. It was lucky that Japan eliminated schistosomiasis, however, we should reflect on many matters in our activities. Not all our previous control activities can be transferred to currently endemic countries. Strategy of cementing water canal should not be transferred because of the too much budget and deep effects on ecological situations. Schistosomiasis control should be on balanced approaches composed of community participation, political enthusiasm, biomedical science, and culturally determined human behavior. It depends on the background situations which component is important. Yamanashi might have had a unique situation, and other endemic foci in Japan had their own strategies. For the future controlling activities in many tropical countries, Japanese experiences are good examples. There are good experiences, and also bad ones. Through detailed discussion with personnel in endemic areas, we will a make contribution for controlling schistosomiasis in the world.

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Research on calpain of *Schistosoma japonicum* as a vaccine candidate

Nobuo Ohta[a,*], Takashi Kumagai[a,1], Haruhiko Maruyama[a], Ayako Yoshida[a,1], Yongkan He[b], Renli Zhang[a,c]

[a]*Department of Molecular Parasitology, Nagoya City University Graduate School of Medical Sciences, Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan*
[b]*Hunan Institute of Parasitic Diseases, Yueyang, PR China*
[c]*Department of Molecular Biology, Shenzhen Hygiene and Anti-epidemics Academy, PR China*

Abstract

Vaccine development by the use of calpain of *Schistosoma japonicum* has been tried in our laboratory. We cloned cDNA encoding the heavy chain of *S. japonicum* calpain, and prepared recombinant molecule of a possible vaccine region of the heavy chain. When BALB/c mice were immunized with our recombinant calpain of *S. japonicum* with Freund's complete adjuvant, we observed significant reduction in worm burden (41.2% reduction, $P<0.05$), and also significant anti-fecundity effects. In this sense, calpain of *S. japonicum* seems to have infection control as well as anti-disease effects. Mechanisms of vaccine effects of calpain remain to be clarified, however, several effecter mechanisms are suspected. In immunized mice, raised level of iNos expression was observed, while adhesion of peritoneal exudates cells were also observed in the presence of calpain-immunized sera, suggesting the possibilities of both cellular and humoral protective mechanisms. We examined tissue distribution of calpain in various developmental stages of *S. japonicum*. Strong signal was observed around excretory grand of cercariae, and they secreted calpain during their migratory movement tested in vitro. Together with the findings, calpain seems to induce larvicidal effects in the immunized mice. We observed time-course kinetics of antibody production against vaccine candidates in experimental *S. japonicum* infection in pigs. Although significant levels of antibody production were observed for paramyosin and GST, no significant antibody production was observed for calpain. This suggests that calpain is less immunogenic, and route of immunization and/or choice of adjuvant are important in future trials of calpain vaccine.

Keywords: *Schistosoma japonicum*; Calpain; Vaccine; Anti-fecundity; Immunogenicity

*Corresponding author. Tel.: +81-52-853-8184; fax: +81-52-842-0149.
E-mail address: nohta@med.nagoya-cu.ac.jp (N. Ohta).
[1] *Current address:* Department of Parasitology, Juntendo University School of Medicine, Tokyo 113-8421, Japan, and TK: Department of Tropical Medicine and Parasitology, University of Occupational and Environmental Health, Kitakyusyu 807-8555, Japan.

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