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Attempts to Transfer Immunity against *Clonorchis sinensis* in Nude and DS Mice

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Abstract: The effects of peritoneal exudate cells(PEC) and sera of athymic nude and DS mice infected with *Clonorchis sinensis* metacercariae or sensitized by injection of metabolic products into footpad on transfer of immunity against the fluke to the syngeneic mice were studied.

There was no significant difference in eggs per gram pattern between the sensitized and control groups, and between nude and DS mice. However, the worm burdens were slightly greater in nude mice than in DS mice. Also, a few plaque forming cells were found in only DS mice given PEC and serum from Group II DS mice.

In the light of these results, it is likely that PEC and sera of nude or DS mice which are deficient, at least partially, in the cellular immune system are unable to transfer immunity against *C. sinensis* to syngeneic recipients.

Key words: *Clonorchis sinensis*, nude mouse, DS mouse, immunity transfer, peritoneal exudate cells, serum

INTRODUCTION

In a series of experiments on transfer of immunity against *Clonorchis sinensis*, somewhat different results were revealed according to the choice of laboratory animals as well as the methods of sensitizing donors. In general, the golden hamster, *Mesocricetus auratus*, was considered as more suitable experimental animal than the mouse (Choi *et al.*, 1987a). However, there seemed to be interstrain difference in the ability to transfer immunity against *C. sinensis* (Choi and Lim, 1986; Kwon *et al.*, 1987; Choi and Park, 1989; Choi *et al.*, 1990a).

Choi and Lim(1986) reported that spleen cells from ICR mice sensitized by intraperitoneal injection(IPI) of the excysted metacercariae failed to transfer the immunity against *C. sinensis* to recipient mice.

On the other hand, Kwon *et al.* (1987) reported that a significant reduction in the worm burden, compared with the controls, was found in inbred BALB/c mice given peritoneal exudate cells(PEC) from syngeneic donor mice sensitized by IPI of excysted *C. sinensis* metacercariae.

Choi and Park (1989) revealed that DDY mice given PEC from donors sensitized by injection of the metabolic products of adult *C. sinensis*, emulsified with Freund's incomplete adjuvant, harbored fewer flukes than the control mice. Choi *et al.* (1990a) reported similar results using BALB/c mice, although the transfer was not successful in athymic nude mice.

The purpose of the present study is to attempt to transfer immunity against *C. sinensis* to recipient nude and DS mice with the immune lymphoid cells and serum from the donor mice infected with the metacercaria, or sensitized with two injections of metabolic products of

the adult worm into footpads, and to compare the results from the two strains to each other.

MATERIALS AND METHODS

Animals: Female mice of inbred strains of DS and nude mice were used for immunization and passive transfer experiments.

Parasites: The metacercariae of *C. sinensis* were collected from the Southern top-mouthed minnow, *Pseudorasbora parva*, collected in the river Chongdo, Chongdo County, Kyungpook Province, Korea. The recovered metacercariae were given orally to outbred rabbits. Three months after oral infection the rabbits were bled to death under deep anesthesia and the adult *C. sinensis* were recovered. The flukes recovered were washed in 3 changes of physiologic saline for 2 hours and the injured or dead flukes were removed with pipettes.

Preparation of Medium-199 (M-199): Powdered Medium-199 (Gibco, Grand Island, New York) was dissolved in 500 ml of distilled water and the pH of the solution was adjusted to be alkaline by addition of 7% sodium bicarbonate.

Preparation of metabolic products of adult *C. sinensis*: As described by Sun (1969), each adult *C. sinensis* was cultured in a tube containing 0.5 ml of M-199 under the condition of 100% relative humidity and 5% CO_2 concentration for 5 days.

Sensitization of donor mice: The donor nude and DS mice were divided into two groups. Group I was infected orally with twenty metacercariae of *C. sinensis* and Group II was sensitized by injecting 0.1 ml of the admixture of the metabolic products of adult *C. sinensis* and Freund's incomplete adjuvant into footpads twice at two weeks interval.

Collection of peritoneal exudate cells (PEC) and serum: Four weeks after oral infection or 2 weeks after the last sensitization, the PEC and serum were collected from donor mice under deep anesthesia. Firstly, each mouse was bled to death from orbital venous plexus (Cate, 1969) and the blood was left to clot at room temperature. Then the mouse was completely soaked in a beaker containing 70% ethanol. The mouse was placed on paper towel and arranged left side up. Pulling the abdominal wall upwards with forceps, 4.5 ml of M-199 in a plastic syringe was injected into the peritoneum. After massaging the abdomen with fingers as the syringe inserted, the PEC were collected with the syringe. From the clotted blood, the clot was detached and clot-free liquid was poured into a centrifuge tube and centrifuged for 30 min at 3,000rpm at 4°C.

Determination of cell viability and viable cell count: The number and percentage of viable PEC and spleen cells was determined by trypan blue exclusion method (Kruse and Patterson, 1973). On the day of the count, 4 parts of 0.2% trypan blue soluted were mixed with 1 part of 5-fold concentrated saline. A small amount of cell suspension was added to an equal volume of trypan blue saline solution (1:2 dilution). The mixture was loaded into a hemocytometer (American Optical) and the number of unstained (viable) and stained (dead) cells were counted separately. More than a combined total of 200 cells were counted for greater accuracy. The number of viable cells per ml was determined by the following formula:

viable cells/ml
= average number of viable cells in large square $\times 10^4$/ml $\times 2$

Primary sensitization of recipient mice: Recipient mice were divided into six groups. The nude mice of Group 1 were injected intraperitoneally (IP) with 5×10^5 PEC and 0.1 ml of serum from Group I (infected orally with 20 metacercariae) donor nude mice, and those of Group 2 were injected IP with 5×10^5 PEC and 0.1 ml of serum from Group II (sensitized by two injection of the admixture of metabolites and adjuvant) donor nude mice. The DS mice of Group 3 were injected IP with 5×10^5 PEC and 0.1 ml of serum from Group I donor DS mice, and those of Group 4 were injected IP with 5×10^5 PEC and 0.1 ml of serum from Group II donor DS mice. The control nude and DS mice

of Group 5 and 6 were injected IP with 5×10^5 PEC and 0.1 ml of serum from nonsensitized donors.

Challenging infection of recipient mice: Seven days after the primary sensitization, the individual recipient mouse was challenged orally with 20 metacercariae of *C. sinensis*.

Egg and worm count: Twenty days after challenge infection, stool examination was performed by formalin-ether sedimentation technique (Ritchie, 1948) to demonstrate the eggs of *C. sinensis*. Once the eggs were demonstrated by the sedimentation technique, Stoll's egg counting method(1923) was employed to determine the number of eggs per gram of feces (EpG) at three days' intervals to the 50th day of infection. The recipient mice were killed 50 days after challenge, and the worm burdens were determined by pressing the livers, gall bladder and bile ducts between two large slide glasses (9×12 cm).

Agar solution for indirect Jerne plaque assay: Bactoagar (Difco, Detroit, Michigan) was dissolved in M-199 solution with the concentration to be 0.7%. In order to eliminate the anti-complementary effect, 0.1 ml of 10% DEAE dextran (Pharmacia Fine Chemicals, Sweden) was added in every 20 ml of the agar solution.

Conjugation of sheep red blood cells (SRBC) with trinitrophenol(TNP): One milliliter of packed SRBC were added a drop at a time into a beaker containing 7.0 ml of cacodylate buffer supplemented with 20 mg of TNP for 10 minutes with a magnetic bar stirring slowly. The beaker was wrapped in aluminum foil to prevent photodecomposition of the mixture during the reaction. The suspension was transferred to a 50 ml centrifuge tube and modified barbital buffer was added. The suspension was centrifuged at 2,000rpm for 10 minutes and the supernatant was discarded. The precipitated SRBC were washed in two changes of 35 ml of MBB containing 7.3 mg glycyl-glycin at 2,000 rpm for 20 minutes. The resulting precipitate was diluted 15-fold with M-199.

Preparation of rabbit anti-mouse IgG antibody: Forty days after oral infection with every 20 metacercariae of *C. sinensis*, the nude and DS mice were bled by the retroorbital bleeding technique as described by Cate(1969) and the serum was separated. The serum separated was added drop by drop into a centrifuge tube containing an equal volume of saturated ammonium sulfate with the tube shaking and left to stand for 30 minutes in the refrigerator. The mixture was centrifuged at 3,000rpm for 3 minutes and the precipitate was recovered. The precipitate was dissolved in distilled water and dialysed overnight against phosphate buffered saline (PBS, pH 7.2) at 4°C. The solution dialysed was mixed with an equal volume of Freund's incomplete adjuvant to form an emulsion. The emulsion was injected subcutaneously to one side of the back of a rabbit and boosted to the other side 3 weeks after the primary injection. One week after boost injection, blood was collected from ear vein before every use.

Preparation of spleen cell suspension: Recipient nude and DS mice were killed by cervical dislocation and placed in the beaker containing 70% ethanol to be thoroughly wet. Then the mice were placed on paper towels soaked with 70% ethanol and arranged left sides face up. In each mouse, a cut through the loose skin in the inguinal region was made and the cut skin was pulled toward the head and tail of the mouse with fingers on either side of the cut until the peritoneal wall was widely exposed. The peritoneal wall was flooded with 70% ethanol to remove any loose hair. The peritoneal wall over the spleen was lifted with the forceps and a large U-shaped cut was made around the spleen. The spleen was lifted with the forceps and separated from the vessels and connecting tissue with the scissors. The spleen removed was placed on stainless steel screen(100 mesh) in plastic petri dish(13×100 mm) containing 5 ml of cold M-199 and teased gently with the rubber policeperson (Difco, Detroit, Michigan). The cell suspension was diluted 4-fold with the M-

199 before assay.

Slides for indirect Jerne plaque assay: The microscopic slides with one end frost (American Scientific Products) were used. The slides precoated with 0.1% agarose were placed on slide warmer (Precision Scientific) adjusted at 42~45°C.

Complement: Commercial guinea pig complement preabsorbed were used to prevent nonspecific lysis of SRBC. Lyophilized guinea pig complement (Gibco, Grand Island, N.Y.) was dissolved and diluted 10-fold in M-199.

Indirect Jerne plaque assay: The assay (Jerne and Nordin, 1963; Zaleski, 1981) modified by Choi and Eun(1985) was employed.

Seven per cent agar solution in M-199 and culture tubes were placed in the 45°C water bath to allow them to warm. To the prewarmed tube, 0.3 ml of agar solution, 0.05 ml of conjugated SRBC suspension and 0.05 ml of spleen cell suspension were added and mixed by rolling the tube between both palms. The mixture was poured on the prewarmed slide and distributed evenly with 90° angled capillary tube. The slide was left to stand at room temperature until the agar jellified. Duplicated slides were prepared per immune cell suspension. After jellification, the slides were placed agar side down on the specially-made complement tray and the space between the bottom of the tray and slides was filled with M-199. The slides were incubated at 5% CO_2 concentration and 100% relative humidity for 60 minutes. After the initial incubation, in place of M-199, the medium containing 10 folds diluted guinea pig complement and 300 times diluted rabbit antimouse IgG antisera was injected in the space and the slides were incubated for an additional 60 minutes under the same conditions. The slides, then, were dried for 20 minutes, fixed in 95% ethanol for 15 minutes, washed in distilled water and dried again as described by Fuji *et al.*(1971). The plaques formed on the slides were counted.

Statistical analysis: The paired t-test was employed to analyse the statistical significance of the difference between mean worm burdens from sensitized and nonsensitized group. P values of <0.05 were considered significant.

RESULTS

The numbers and proportions of viable cells per ml of peritoneal exudate cells from donor nude and DS mice by trypan blue exclusion is presented in Table 2. The mean number of viable cells was the greatest, 2.10×10^5, in control nude mice and the least, 1.78×10^5, in group II nude mice, with no significant difference. The proportion was also the highest, 94.6%, in control nude mice and the lowest, 89.0%, in group II DS mice.

Table 3 presents the effect of sensitized peritoneal exudate cells and sera on the fecundity of *C. sinensis* in recipient nude and DS mice after challenge infection by counting the number of eggs per gram of feces(EpG). The eggs were found for the first time on the 23rd day of infection from both sensitized and control recipients. The fecundity increased stepwise and showed the highest on the 33rd or 36th day without regard to the method of sensitization. After this, EpG was found to decrease gradually to the day of necropsy.

Table 4 shows the effect of the peritoneal exudate cells and sera on worm burdens of *C. sinensis* in recipient mice. The mean number of flukes recovered from the control unde mice was 1.8 per mouse, whereas those from group 1 and group 2 nude mice were 1.8 and 1.6, respectively. The number was 1.4 from the control DS, whereas those from group 3 and group 4 DS mice were 1.6 and 1.4, respectively.

Table 5 presents viability of spleen cells used in indirect Jerne plaque assay and number of plaque forming cells per spleen in recipient mice on which necropsy was performed 50 days after challenge infection. The viability was the highest, 91.3% in group 1 nude and Group 4 DS mice and the lowest, 88.2% in group 3 DS mice. A few plaque forming cells were found in only DS mice given PEC and serum from Group II DS mice.

Table 1. Experimental groups to study the effects of peritoneal exudate cells and sera on transfer of immunity against *Clonorchis sinensis* in nude and DS mice

Donor mice			Recipient mice			Challenging
Group	No. & strain of mice	Sensitization	Group	No. & strain of mice	Primary sensitization	infection
I	5 nude	20 metacercariae injected orally	1	5 nude	5×10^5PEC* and 0.1 ml of serum from Group I nude mice injected IP**	20 metacercariae injected orally
	5 DS	20 metacercariae injected orally	2	5 nude	5×10^5PEC and 0.1 ml of serum from Group II nude mice injected IP	〃
II	5 nude	0.1 ml of metabolic products of adult *C. sinensis* and adjuvant admixtures injected into footpads twice at two-weeks interval	3	5 DS	5×10^5PEC and 0.1 ml of serum from Group I DS mice injected IP	〃
			4	5 DS	5×10^5PEC and 0.1 ml of serum from Group II DS mice injected IP	〃
	5 DS	0.1 ml of metabolic products of adult *C. sinensis* and adjuvant admixtures injected into footpads twice at two-weeks interval	5	5 nude	5×10^5PEC and 0.1 ml of serum from nonsensitized nude mice injected IP	〃
			6	5 DS	5×10^5PEC and 0.1 ml of serum from nonsensitized DS mice injected IP	〃

* PEC means peritoneal exudate cells.
** IP mean intraperitoneally.

Table 2. Proportion of viable cells per ml of peritoneal exudate cells in donor nude and DS mice by trypan blue exclusion

Group	No. & strain of mice	Peritoneal exudate cells in primary sensitization		
		Total No. of cells($\times10^5$)	Mean No. of viable cells($\times10^5$)	Viable cells proportion(%)
I	5 nude	2.00	1.86	93.0
	5 DS	2.14	1.94	90.7
II	5 nude	1.98	1.78	89.9
	5 DS	2.18	1.94	89.0
Controls	5 nude	2.22	2.10	94.6
	5 DS	2.06	1.92	93.2

Table 3. Effect of sensitized peritoneal exudate cells and sera on Eggs per Gram of feces in recipient nude and DS mice after challenging infection

Group	No. & strain of mice	No. of cysts challenged(ea)	Mean EpG# from 23 to 50 days after challenge($\times100$)											
			22	23	25	27	30	33	36	38	41	44	47	50
1	5 nude	20	—*	+**	1	2	4	8	9	8	2	4	1	1
2	5 nude	20	—	+	2	2	6	9	7	5	1	6	3	1
3	5 DS	20	—	+	2	1	4	9	6	2	6	3	4	2
4	5 DS	20	—	+	1	6	5	8	8	4	3	4	3	3
5	5 nude	20	—	+	2	2	5	10	11	6	3	2	4	2
6	5 DS	20	—	+	2	6	3	11	8	7	5	3	4	4

\# EPG means Eggs per Gram of feces.
* — means negative by formalin-ether sedimentation technique.
** + means positive by formalin-ether sedimentation technique.

Table 4. Effect of peritoneal exudate cells and sera sensitized by oral infection or footpad injection of metabolic products of adult *C. sinensis* on worm burdens of *C. sinensis* in recipient nude and DS mice

Group	No. & strain of mice	No. of cysts challenged (ea)	Interval between challenge and necropsy (days)	No. adult worms recovered						Worm recovery rate (%)
				Mouse						
				1	2	3	4	5	Mean	
1	5 nude	20	50	1	2	2	2	2	1.8	9.0
2	5 nude	20	50	1	1	2	2	2	1.6	8.0
3	5 DS	20	50	1	1	2	2	3	1.8	9.0
4	5 DS	20	50	0	1	1	2	3	1.4	7.0
5	5 nude	20	50	1	2	3	3	3	2.4	12.0
6	5 DS	20	50	1	2	2	3	3	2.2	11.0

Table 5. Viable spleen cells and plaque forming cells in recipient mice after challenging infection

Group	No. & strain of mice	Spleen cells per ml of cell suspension			Plaque forming cells per spleen (ea)
		Total No. of cells ($\times 10^5$)	Mean No. of viable cells ($\times 10^5$)	% viable cells	
1	5 nude	1.214	1.108	91.3	0
2	5 nude	1.142	1.026	89.8	0
3	5 DS	1.120	0.988	88.2	0
4	5 DS	1.236	1.128	91.3	600
5	5 nude	1.104	0.984	89.1	0
6	5 DS	1.122	1.006	89.7	0

DISCUSSION

The studies on acquired immunity to parasitic helminths have advanced slowly because of the difficulties in culturing them *in vitro* and partly because of their complex and long-standing life cycles. On the other hand, since most of the parasitic helminths do not replicate within the host, they offer an unique advantage in studying immunity, that is, the possibilities of recovering the worms after challenge and of statistical analysis to determine the significance of difference in the numbers recovered from experimental and control groups.

Larsh *et al.* (1964) reported for the first time the successful transfer of immunity against the parasitic helminths by the use of lymphoid cells. Larsh *et al.* (1966) confirmed the transfer of immunity against *Trichinella spiralis* with peritoneal exudate cells from infected donors.

In infection with trematodes, Lang *et al.* (1967) showed that peritoneal exudate cells (PEC) from donor mice infected with *Fasciola hepatica* conferred immunity on the recipient. Since it was shown by Dodd and Nuallain (1969) that anti-rabbit lymphocyte serum raised in sheep and horses produced striking suppression of cellular responses in the rabbits infected with *F. hepatica*, lymphoid cells had been considered to be operable against trematodes. Moreover, it is suggested that cell-mediated immunity might play a major part in the immunopathogenesis of experimental schistosomiasis (Colley, 1971; Vernes *et al.*, 1972).

Recently, the attempts to transfer immunity against *C. sinensis* which is one of the most prevalent flukes in Korea with lymphoid cells and/or serum from donor animals sensitized with various antigenic materials or infected with the metacercariae (Choi and Lim, 1986; Kwon *et al.*, 1987; Cho *et al.*, 1987a & b; Choi and Park, 1987; Cho *et al.*, 1988; Lim *et al.*, 1988; Choi and Park, 1989; Choi *et al.*, 1990a; Choi

et al., 1990).

However, unlike *Schistosoma mansoni* and *F. hepatica*, *C. sinensis* is not such a tissue penetrating parasite that the oral infection with this fluke would not be potent enough to stimulate the experimental animals to be sensitized. Therefore, in an attempt to transfer immunity against *C. sinensis*, it is reasonable to sensitize the donor animals with parenteral administration of antigenic materials such as metabolic products of adult *C. sinensis* (Choi and Park, 1987; Choi and Park, 1989; Choi *et al.*, 1990a; Choi *et al.*, 1990) or juveniles (Cho *et al.*, 1988), somatic constituents of the adult worm (Choi and Park, 1987), and newly excysted metacercaria itself (Choi *et al.*, 1987a & b; Lim *et al.*, 1988). Recent reports have suggested that immunity against *C. sinensis* in recipient animals could be transferred with PEC from donors sensitized by intraperitoneal injection (IPI) of excysted metacercariae of *C. sinensis* (Choi *et al.*, 1987a; Kwon *et al.*, 1987), by IPI or footpad injection of the metabolic products of the adult fluke (Choi *et al.*, 1987b; Cho *et al.*, 1988; Choi and Park, 1989; Choi *et al.*, 1990a).

The other reports suggested that there were synergistic effects of the immune lymphod cells and sera on transfer of the immunity when recipients animals received both components (Choi and Park, 1987; Lim *et al.*, 1988; Kwon *et al.*, 1987).

In order to study the *in vivo* immune responses against helminthic infection, the treatments such as removal of some components from the immune system, and transfer of the other components from sensitized donor to naive recipient was usually applied. Before inbred strains of laboratory animals have been used, immune serum, in general, was transferred. Since the lymphoid cells transferred from allogeneic or heterogeneic donor were destroyed by the mechanism of graft rejection response, the transfer of immunity had been unsuccessful. *In vivo* studies on cellular immune response were performed by the comparison of results between experimental group neonatally thymectomyzed and intact controls. However, the establishment of the inbred strains of experimental animals has allowed cellular components to be transferred to syngeneic strain animals. Moreover, congenitally athymic nude mice have presented a powerful tool for research in cellular immunity.

The nude mouse was first reported by Flanagan (1966), and two years later, it was learned that the animal lacked the thymus (Pantelouris, 1968). The mouse thus represented the first known occurrence of natural thymectomy (thymic agenesis or dysgenesis) and spontaneous immune deficiency in a laboratory animal.

Since the athymic nude mouse would accept xenografts, it was possible to study neoplasms of human origin *in vivo* in a murine milieu. This mutant should also offer an important role in many areas of immunology.

Recently, both protozoal and helminthic infections have been studied in nude mice. Especially, for *Pneumocystis carinii*, an important cause of pneumonia in the immune compromized host, nude mice present a new animal model (Armstrong and Walzer, 1978; Tamura *et al.*, 1978; Ueda *et al.*, 1977; Walzer *et al.*, 1977; Walzer and Powell, 1982).

For the experimental infection with helminths, Jacobson and Reed (1974) reported that with increasing age, the athymic nude mice became more heavily infected with the pinworms, *Aspiculus tetraptera* and *Syphacia obvelata*, while the intensity of infection in both normal littermates and nude mice implanted with thymus glands or inoculated with thymus cells failed to show a significant change. Based on these results, they proposed the thymus dependency of resistance to pinworm infection in mice.

Isaak *et al.* (1975) also revealed that experimental infection with *Hymenolepis diminuta* persisted chronically in nude mice, and were expelled only after thymic transplantation or injection of thymus cells. Hsu *et al.* (1976) infected experimentally nude mice with *S. mansoni* and revealed that the mice exhibited impaired granuloma formation, eosinophil,

IgG, and IgE responses as compared with controls. Both infected and uninfected nude mice failed to develop mitogenic responses to *S. mansoni* antigen or phytohemagglutinin (a T cell mitogen), but responded well to lipopolysaccharide (a B cell mitogen).

On the other hand, DS strain has been used as host for a transplantable hormone-dependent mouse carcinoma (Kitamura *et al.*, 1978). The suitability of the strain as an experimental host of parasites has not yet been tested.

The present study was the first to use DS mice as experimental hosts of *C. sinensis* and to attempt transferring the immunity against the fluke in DS model. The results, however, were not different essentially from those in other strains, ICR (Choi and Lim, 1986) and GPC (Choi and Park, 1989). Although partial success in transfer of the immunity with PEC from BALB/c and DDY were reported (Kwon *et al.*, 1987; Choi and Park, 1989; Choi *et al.*, 1990a), the suitability of murine strains as experimental hosts could not be compared with that of hamsters (Choi *et al.*, 1987a & b; Choi and Park, 1987; Lim *et al.*, 1987; Cho *et al.*, 1988).

In the present study, nude mice were found not to be significantly more susceptible than DS mice to infection with the metacercariae of *C. sinensis*. This result coincides with the reports of Choi *et al.* (1990a) using *C. sinensis*, Rajasekariah *et al.* (1979a) using *F. hepatica*, and Phillips *et al.* (1977) using *S. mansoni* in which only a marginal increase in worm burden was observed in nude mice compared with intact mice.

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=국문초록=

近交系 nude 및 DS 마우스 腹腔滲出細胞와 血淸의 肝吸蟲 感染에 대한 免疫移入의 試圖

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肝吸蟲 被囊幼蟲의 經口感染 또는 肝吸蟲 成蟲의 代謝産物의 皮內注射로 感作한 近交系 nude 및 DS 마우스의 腹腔滲出細胞와 血淸이 肝吸蟲 感染에 대한 免疫을 同系(syngeneic) 마우스에 移入하는지 與否를 究明하기 위해 對照群 마우스에서의 EpG, 負荷蟲體數 및 脾臟當 plaque 形成細胞數를 基準으로 感作群에서의 그 成績과 比較하였다.

Challenge 感染後 EpG의 變動은 感作群과 非感作 對照群 마우스와 nude 및 DS 마우스와의 사이에 有意한 差를 認定할 수 없었다. 肝吸蟲 成蟲의 回收率에서는 感作群과 非感作 對照群 사이에 有意的 差를 認定할 수 없었으나 nude 마우스는 DS 마우스에 비하여 多少 높은 回收率을 나타내었다. 脾臟에서 plaque 形成細胞는 腹腔滲出細胞 5×10^5와 Group II DS 마우스의 血淸 0.1 ml를 腹腔에 感作한 第4群에서만 少數 檢出할 수 있었다.

以上의 成績으로 미루어 보아 細胞性 免疫系에 缺損이 있다고 알려진 近交系 nude 및 DS의 腹腔滲出細胞와 血淸에 의해서는 肝吸蟲에 대한 免疫이 移轉되지 않음을 확인하였다.

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