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Immune mechanisms to *Ascaris suum* in inbred guinea-pigs

I. PASSIVE TRANSFER OF IMMUNITY BY CELLS OR SERUM

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Summary. Syngeneic Strain 2 guinea-pigs which received seven s.c. injections of infective eggs of *Ascaris suum* were shown to harbour less larvae in their lungs than control animals after mesenteric vein challenge with 10,000 infective larvae of *A. suum*. Serum and cell preparations harvested from these animals were able to transfer protective immunity to normal recipients to varying degrees. Significant protection with serum preparations was obtained with immune IgG2, IgE+IgG1 and whole immune serum. Best protection with cell preparations was obtained with a pool of cells from the mesenteric, hepatic (retropancreatic), and mediastinal nodes of immune animals. Cells from the hepatic nodes of immune animals were more effective than cells from the mediastinal and mesenteric nodes. Immune spleen cells enhanced, rather than reduced, the degree of the infection. Cells or serum preparations from normal animals were not capable of transferring protective immunity to normal recipients.

INTRODUCTION

Ascaris suum, a parasite of the pig, inhabits the small intestine and infection is passed from one animal to another by eggs which, if swallowed at an infective stage, hatch in the intestine releasing larvae. These penetrate the intestinal wall and reach the liver via the hepatic portal system. In the liver, they moult and then resume their migration to the lung and then back to the intestine where they undergo further moults to reach maturity. The major part of the immune response to *A. suum* is thought to be induced by the migrating stages and involves both humoral (Taffs 1964a, 1964b; Crandall & Crandall 1967, 1971; Dobson, Morseth & Soulsby 1971) and cellular (Soulsby & Muncey 1970; Soulsby 1972; Khoury 1973; Khoury & Soulsby 1976a, 1976b; Soulsby & Khoury 1975) elements. However, attempts to transfer protective immunity with serum or cells in experimental ascariasis have yielded variable and inconclusive results. Crandall (1965) was not able to transfer protection to challenge infections with a mixture of spleen cells and peritoneal exudate cells or with serum from immune mice to syngeneic normal recipients. However, parabiosis did lead to transfer of some protection. Using the definitive host of the parasite, Kelley & Nayak (1965a) demonstrated significant degree of protection to infection in specific pathogen-free pigs given subcutaneously either immune serum or globulin. In further experiments, Kelley & Nayak (1965b) demonstrated that immunity to *A. suum* could be transferred via the colostrum.

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In the present study, transfer of significant protective immunity by whole serum, IgG2, and IgE+IgG1 immunoglobulin preparations or lymphocytes harvested from the hepatic (retropancreatic) and mediastinal lymph nodes of immune guinea-pigs was demonstrated. Minimal protection was obtained with IgM and IgA preparations and spleen lymphocytes enhanced the degree of the infection in challenged normal recipient animals.

MATERIALS AND METHODS

Animals

Syngeneic Strain 2 guinea-pigs with an initial weight of 450–550 g and an age of 5 weeks were used in these experiments. These guinea-pigs were from a closed breeding colony at the Laboratory of Parasitology of the University of Pennsylvania. Their syngeneic status was assessed periodically by skin grafts and mixed lymphocyte culture.

Immunization of guinea-pigs

Guinea-pigs were immunized by seven s.c. injections of infective eggs of *A. suum* at bi-weekly intervals according to the method of Soulsby (1957) as modified by Khoury & Soulsby (1976b).

Preparation of serum

Two weeks after the last immunizing injection, the animals were exsanguinated by cardiac puncture and the serum was collected, pooled and centrifuged at 5600 *g* for 90 min and the resulting lipid layer was removed. The serum was then stored at $-20°$ until use. Serum from normal guinea-pigs was harvested similarly.

Preparation of immunoglobulins (Igs)

IgM, IgA and IgG2 were sequentially isolated from immune guinea-pig serum by Sephadex G-200 gel chromatography and DEAE Sephadex A-25 ion exchange chromatography as described by Khoury & Soulsby (1976a). The purity of these Igs was assessed by immunoelectrophoresis, gel diffusion, and inhibition of indirect haemagglutination systems using rabbit antisera specific for heavy chains of guinea-pig IgM, IgA, and IgG2 (Khoury 1973; Khoury & Soulsby 1976a).

To obtain guinea-pig IgE+IgG1, immune serum was chromatographed on three in series Sephadex G-200 columns (Pharmacia, Sweden) and the fractions obtained were tested for homologous passive cutaneous anaphylaxis (PCA) activity (Dobson *et al.* 1971). Those fractions which eluted from Sephadex G-200 in the ascending arm of the 7S peak and which gave positive PCA reactions with both 6-h and 6-day latent periods, were pooled and concentrated and were then chromatographed on DEAE Sephadex A-25 (Pharmacia, Sweden) (Khoury & Soulsby 1976a). The resulting fractions obtained from the main protein peak and which gave positive 6-h and 6-day PCA reactions were pooled.

All samples were concentrated to their respective original whole serum volume equivalent and the protein content was determined by the Biuret technique. They were filtered through a 0·22 μm Millipore filter immediately prior to i.v. injection.

Preparation of cells

Immune guinea-pigs were killed 2 weeks after they had received their last immunizing injection. The mesenteric, hepatic (retropancreatic), and mediastinal lymph nodes and the spleen were excised and the lymphoid cells were separated as described previously (Khoury & Soulsby 1976a). Macrophages were removed from the lymphoid cell populations using carbonyl iron according to the method of Coombs, Gurner, Janeway, Wilson, Gell & Kelus (1970). The viability of the remaining cells was assessed by trypan blue exclusion and cells were suspended in 5 ml of RPMI 1640 (Associated Biomedic Systems, Incorporated, Buffalo, New York) plus 1 per cent normal guinea-pig serum before i.v. injection. Cells from normal animals were processed similarly.

Assessment of protective immunity

Two days after cell transfer and 8–12 h after the transfer of serum, recipient animals were challenged by injection of 10,000 artificially hatched infective larvae (Williams & Soulsby 1970) of *A. suum*, into the mesenteric vein, after laparotomy under 'Halothane' (Fluothane, Ayerst Laboratories) anaesthesia. Six days later the animals were killed and larvae were recovered from the lungs using the Baermann Technique. Results were recorded by calculating the mean and the standard error of the mean of the larval counts within each animal group. The difference between the groups was analyzed using the Student's *t*-test. A probability (*P*) value of 0·05 or lower was considered to constitute a

statistically significant difference between the groups. Protection was calculated as follows:

$$\text{Per cent protection} = \left(1 - \frac{\text{no. of larvae from lungs of animals that received serum or cell preparations}}{\text{no. of larvae from lungs of animals that received no treatment}}\right) \times 100.$$

RESULTS

Sequential larval recovery in experimentally immunized animals

To assess the efficacy of the active immunization procedure, thirty-two normal and thirty-two s.c. immunized animals were challenged each via the mesenteric vein with 10,000 infective larvae of *A. suum*. Four animals from each group were killed at day 0 and days 1, 2, 5, 6, 7, 9, and 12 after challenge and larvae were recovered from the lungs. Fig. 1 shows that the number of larvae recovered from the lungs of infected normal animals was significantly ($P < 0{\cdot}05$) higher than that recovered from the lungs of challenged immune animals at all times throughout the period of the study.

To determine whether the pattern of larval recovery in passively immunized animals was similar to that depicted in actively immunized animals (Fig. 1), two groups of thirty-two guinea-pigs were used. One group received immune serum (5 ml per recipient animal) and the other received a pool of cells from the mesenteric, hepatic, and mediastinal nodes and spleen ($2{\cdot}5 \times 10^7$ lymphocytes from each lymphoid centre per recipient animal) of immune animals. Recipient animals were killed sequentially after challenge as described above and larval counts were performed. The results showed that the pattern of larval recovery in these animals was similar to that of actively immunized animals and peaked at day 6 with $375{\cdot}6 \pm 31{\cdot}2$ larvae and $1014{\cdot}7 \pm 51{\cdot}6$ larvae harvested after passive transfer of immune serum and immune cells, respectively. Therefore, in all the following experiments, larval recovery was assessed on day 6 after challenge.

Passive transfer of immunity to *A. suum* with serum or IgM, IgA, IgG2, or IgE+IgG1 from immune animals

Seven groups of four guinea-pigs were used to assess the protective effect of transferred normal serum, immune serum or Igs. The numbers of larvae harvested following challenge are presented in Table 1. Significant protection ($P < 0{\cdot}05$) was obtained with immune serum or Igs but not with normal serum. Protection obtained with IgA (15·9 per cent) or IgM (17·5 per cent) was minimal whereas a high degree of protection was obtained with whole immune serum (72·4 per cent), immune

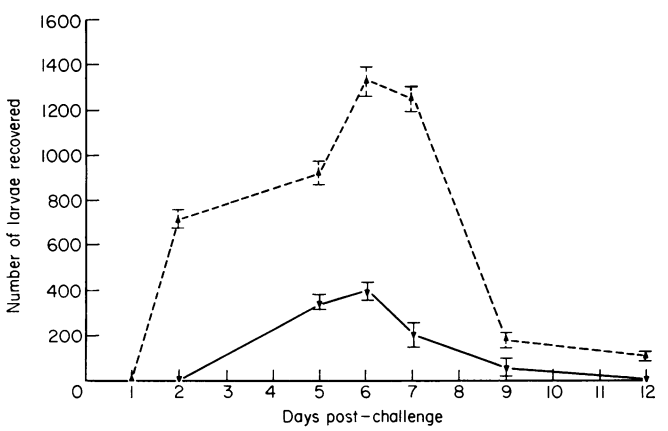

Figure 1. Number (mean ± s.e. mean) of *A. suum* larvae recovered from the lungs of infected normal and immune guinea-pigs. Thirty-two normal animals (- - - -) and thirty-two immune animals that had been previously immunized at bi-weekly intervals with seven s.c. injections of infective eggs of *A. suum* (——), were infected via the mesenteric vein with 10,000 artificially hatched infective larvae of *A. suum*. Four animals from each group were killed at day 0 and at days 1, 2, 5, 6, 7, 9, and 12 after challenge and larvae were recovered from the lungs using the Baermann technique.

Table 1. Passive transfer of immunity to *A. suum* with normal serum, immune serum or IgM, IgA, IgG2 or IgE+IgG1 from immune guinea-pigs*

Animal§ group	Treatment	Larvae harvested from lungs mean±s.e.	Per cent protection†	*P* Value‡
1	None	1369·3±49·0	—	—
2	Normal Serum (5 ml)	1269·7±50·4	7·3	n.s.
3	Immune Serum (5 ml)	377·3±29·4	72·4	<0·0004
4	Immune IgM (8·9 mg protein)	1129·7±54·6	17·5	<0·009
5	Immune IgA (7·6 mg protein)	1151·6±58·7	15·9	<0·006
6	Immune IgE+IgG1 (7·75 mg protein)	299·7±22·6	78·1	<0·0005
7	Immune IgG2 (6·1 mg protein)	153·0±20·4	88·8	<0·0006

n.s. = Not significant.

* Seven groups of normal animals each comprising four animals were used. Five ml of each serum preparation were injected i.v. into normal recipients 8–12 h prior to a challenge infection with 10,000 infective larvae of *A. suum* via the mesenteric vein. Six days later, the animals were killed, and larvae were recovered from the lungs by the Baermann technique.

$$\dagger \text{ Per cent protection} = \left(1 - \frac{\text{(no. of larvae from lungs of animals that received serum preparations)}}{\text{(no. of larvae from lungs of animals that received no treatment)}}\right) \times 100.$$

‡ $P<0{\cdot}05$ = statistically significant difference between animals of group 2 and those of groups 3–7.

§ Comparison between groups: group 1 *vs* 2 (n.s.), group 4 *vs* 5 (n.s.), group 3 *vs* 6 ($P<0{\cdot}003$), group 3 *vs* 7 ($P<0{\cdot}009$), group 6 *vs* 7 ($P<0{\cdot}004$).

Table 2. Passive transfer of immunity to *A. suum* with normal cells or with immune cells harvested from the mesenteric, hepatic and mediastinal lymph nodes and from the spleen of donor animals*

Animal‡‡ group	Treatment†	Larvae harvested from lungs mean±s.e.	Per cent protection‡	*P* value††
8	Normal cells§	1290·3±68·8	5·8	n.s.
9	Immune cells¶	1017·3±48·8	25·7	<0·006
10	Immune hepatic node cells	672·0±41·8	50·9	<0·0004
11	Immune mediastinal node cells	866·3±30·7	36·7	<0·0006
12	Immune mesenteric node cells	1246·0±45·0	9·0	n.s.
13	Immune spleen cells	1705·0±37·0	−24·5**	<0·004
14	Immune mesenteric+hepatic+mediastinal node cells	389·0±42·5	71·6	<0·0005
15	Immune mesenteric+hepatic node cells	656·0±41·8	52·1	<0·0003
16	Immune hepatic+mediastinal node cells	420·3±71·0	69·3	<0·0004
17	Immune mesenteric+mediastinal node cells	820·3±79·2	40·1	<0·004

n.s. = Not significant.

* Ten groups of normal animals each comprising 4 animals were used. Cell preparations were injected i.v. into normal recipients 2 days prior to a challenge infection with 10,000 infective larvae of *A. suum* via the mesenteric vein. Six days later animals were killed and larvae were recovered from lungs.

† Contribution of cells from each lymphoid centre in each instance was always $2{\cdot}5 \times 10^7$ lymphocytes.

$$\ddagger \text{ Per cent protection} = \left(1 - \frac{\text{(no. of larvae from lungs of animals that received cell preparations)}}{\text{(no. of larvae from lungs of animals that received no treatment)}}\right) \times 100.$$

§ A pool of cells from the mesenteric, hepatic and mediastinal nodes and spleen of normal animals.

¶ A pool of cells from the mesenteric, hepatic and mediastinal nodes and spleen of immune animals.

** The negative sign denotes that more larvae were recovered from the lungs of group 13 than from those of groups 1 or 8.

†† $P<0{\cdot}05$ = statistical significance between animals of group 8 and those of groups 9–17.

‡‡ Comparison between groups: group 1 *vs* 8 (n.s), group 14 *vs* 16 (n.s.), group 10 *vs* 15 (n.s.), group 11 *vs* 17 (n.s.), group 10 *vs* 16 ($P<0{\cdot}03$), group 11 *vs* 16 ($P<0{\cdot}03$).

IgE+IgG1 (78·1 per cent), and immune IgG2 (88·8 per cent).

Passive transfer of immunity to *A. suum* with lymphocytes from the mesenteric, hepatic, and mediastinal lymph nodes and from the spleen of immune animals

Ten groups of four guinea-pigs were used as recipients of normal or immune cells. The contribution of cells from each lymphoid centre in each instance was always $2{\cdot}5 \times 10^7$ lymphocytes. The numbers of larvae harvested from the various groups are presented in Table 2. There was no significant protection ($P > 0{\cdot}05$) with a pool of cells from the mesenteric, hepatic (retropancreatic), and mediastinal nodes and the spleen of normal animals (group 8). However, significant protection though of a low order (25·7 per cent) was obtained with a pool of cells from the same lymphoid organs of immune animals (group 9).

Cells from the hepatic nodes of immune animals were more effective (50·9 per cent protection) than mediastinal node cells (36·7 per cent protection) and mesenteric node cells (9·0 per cent protection: not significant). More larvae (24·5 per cent increase) were recovered from the lungs of normal animals that received immune spleen cells (group 13) than from those that received no treatment (group 1).

Maximum protection (71·6 per cent) was obtained when immune cells from the mesenteric, hepatic, and mediastinal nodes were transferred to normal recipients in the absence of immune spleen cells (Group 14). Such protection was not statistically different from that obtained when a mixture of immune hepatic and mediastinal node cells was transferred in the absence of immune mesenteric node cells (69·3 per cent protection; group 16). There was no significant difference between the protective capacity of immune hepatic or immune mediastinal node cells when transferred separately and when transferred in combination with immune mesenteric node cells (groups 15 and 17). However, there was a significant difference between the protection obtained with immune hepatic or immune mediastinal node cells when transferred separately and when both of these cell populations were combined (group 16).

DISCUSSION

In the present study, guinea-pigs were immunized by seven s.c. injections of infective eggs of *A. suum* and were assessed for their ability to resist a challenge infection of 10,000 infective larvae of *A. suum* administered via the mesenteric vein. In addition, serum Igs or lymphocytes from the mesenteric, hepatic (retropancreatic), and mediastinal lymph nodes and the spleen of immune animals were used in attempts to transfer immunity to normal recipients.

Immunized animals were significantly protected against challenge infection as measured by the number of larvae recovered from the lungs of these animals at intervals throughout the period of the infection. These results supported the studies reported by Soulsby (1957) and indicated that the majority of the larvae were held up and destroyed prior to the lungs. The liver of an immune animal is considered to be the site of such a reaction (Soulsby, 1961; Taffs 1964a; 1964b) although some larvae might have been killed in the lungs as well.

The studies on the transfer of immunity with serum preparations showed that whole serum, IgE+IgG1 or IgG2 from immune animals transferred a reasonable level of protective immunity to normal recipients. Immune IgA or IgM were poorly protective whereas immune IgE+IgG1 or IgG2 were more effective than whole immune serum.

The mechanism by which immune IgE+IgG1 or IgG2 protected normal animals against challenge infection is not known. Homocytotropic antibodies have been shown to play a protective role in certain helminthic infections, e.g., *Nippostrongylus brasiliensis* (Ogilvie & Jones, 1971). These antibodies may mediate inflammatory reactions at the local site of infection and may act against the parasite either directly by damaging it or indirectly by rendering its local environment unfavourable for its survival. In the present study, significant protection was obtained with immune IgE+IgG1 both of which have been shown to be homocytotropic in the guinea-pig (Dobson *et al.*, 1971).

Serum fractions rich in IgG antibodies have been shown by Leventhal & Soulsby (1976) to promote *in vitro* significantly greater adhesion and degranulation of polymorphonuclear leucocytes on the surface of early stage larvae of *A. suum* than fractions rich in IgM. However, whether IgG or its subclasses (IgG1 or IgG2) mediate the destruction of these larvae at the local site of the infection has to be determined.

Transfer studies with cells from the lymphoid

organs of immune animals showed that maximum protection (71·6 per cent) was transferred with a pool of cells from the mesenteric, hepatic, and mediastinal nodes. The presence of immune cells from the mesenteric nodes in the latter pool did not contribute to its protective capacity since a similar degree of protection was obtained with a mixture of immune hepatic plus mediastinal node cells. When immune mesenteric node cells were transferred separately, they were not able to confer significant protection to normal recipients. However, significant protection was obtained with immune hepatic or immune mediastinal node cells. These results might be due to the presence of significantly higher numbers of antigen-reactive lymphocytes and antigen-specific effector cells (as assessed by rosette formation and plaque-formation techniques, respectively) in the hepatic and mediastinal nodes than in the mesenteric nodes of challenged immune animals (Khoury & Soulsby, 1976b). However, the effect of these cells on the migrating stages of the parasite is still to be determined.

Immune cells from the hepatic nodes (draining the hepatic phase of the infection) were significantly more effective in conferring protective immunity upon normal recipients than immune mediastinal node (draining the pulmonary phase of the infection) cells. This would suggest that the major protective immune mechanisms to *A. suum* take place at the hepatic level and might be due to the immunogens that are produced in the liver by the moulting larvae before they migrate to the lung where moulting does not occur.

Immune spleen cells were exceptions in two respects. Firstly, when they were used in combination with immune mesenteric, hepatic, and mediastinal lymph node cells, the protective capacity of the cells of the various nodes dropped from 71·6 per cent to 25·7 per cent. This result suggested that immune spleen cells contained lymphocytes that either had an inhibitory action against the lymph node cells or were suppressor cells, and as such, would counteract the ability of immune lymph node cells to confer protection upon normal recipient animals. Secondly, when immune spleen cells were used separately in the transfer experiments, they enhanced, rather than reduced, the infection by 24·5 per cent. This would again suggest the presence of suppressor cells amongst the lymphocyte population of the immune spleen. Such cells could either be T or B lymphocytes (Sinclair & Singhal, 1976), and work is underway to separate the various subpopulations of spleen lymphocytes. These will be assessed for their capacity to reduce or enhance challenge infections in normal recipients.

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