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IMMUNOLOGY OF *ANGIOSTRONGYLUS* INFECTION

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Angiostrongylosis

Angiostrongylosis is a parasitic disease of mammals caused by nematodes in the genus *Angiostrongylus*[1]. Although there are several species of nematode in this genus that can potentially cause the disease, *Angiostrongylus cantonensis* is the only species known to be associated with human infection[1,2]. Because *A. cantonensis* and *A. malaysiensis* live side by side in nature and utilize the same intermediate host, it is difficult to rule out the potential danger of the latter[2].

Angiostrongylosis has been reported in a number of Pacific islands and Southeast Asian countries including Thailand[1,3]. However, sporadic cases of eosinophilic meningitis with clinical manifestations similar to those of the epidemic eosinophilic meningoencephalitis have also been reported in Europe and America[1]. In the endemic areas, the occurrence of wild rodents, molluscs and paratenic hosts, together with local cultural habits, favor the spread of the disease.

A. cantonensis is a parasite in the rat, but can readily spread to man following the ingestion of raw or inadequately cooked snails that harbor parasites[1]. In the human host, this rat lungworm has an extensive tissue migration and finally ends up in the central nervous system where it develops to fourth-stage larva or young adult[1,2]. Such a cerebral migration, together with the involvement of a host reaction to the parasite, elicits an intense inflammatory response typical of eosinophilic meningoencephalitis[1]. Although the signs and symptoms of meningeal involvement caused by infection with this nematode are non-specific, certain distinctive features should be noted, i.e., eosinophilic pleocytosis and eosinophilia[1,3]. In addition to the more common cerebral angiostrongylosis, a few cases of ocular angiostrongylosis have been reported[1,3]. In these cases, there are no meningeal symptoms but parasites are found in the eyes of the patients[1,3]. In addition to angiostrongylosis, there are a

few other conditions, both infectious and non-infectious in nature, that are commonly associated with eosinophilic pleocytosis[1]. Those that are caused by helminths include cysticercosis, echinococcosis and paragonimiasis[1]. Mereover, in Thailand there is yet another form of eosinophilic meningitis, commonly referred to as eosinophilic myeloencephalitis, that is difficult to differentiate from that caused by *A. cantonensis*[4]. The disease is caused by another nematode, i.e., *Gnathostoma spinigerum*, but it is generally more severe and has higher mortality rate than the one caused by *A. cantonensis*.

It was not until 1960 that *A. cantonensis* was universally accepted as an etiologic agent for eosinophilic meningoencephalitis in man[1]. This conclusion is supported by the following field observations and laboratory investigations:

1. Recovery of *A. cantonensis* from cerebrospinal fluid and brain of patients with signs and symptoms of eosinophilic meningoencephalitis[1-3].

2. Involvement of a central nervous system typical of human eosinophilic meningoencephalitis in experimentally infected rats and rhesus monkeys[5-7].

3. High incidence of disease in those ingesting snails from the endemic areas, e.g., Pacific Islands and Southeast Asia[1].

4. Demonstration of *A. cantonensis* in rats and molluscs from the areas where the disease has been reported[1].

The main clinical finding in human angiostrongylosis is meningitis and it is most likely that this coincides with the time of larval migration to the central nervous system. This pattern of migration, together with the local reactions elicited by the host in response to the presence of parasites in this locale, can evoke an intense inflammatory response within a few days after ingesting infective third-stage larvae. In contrast the main pathological finding in rat is observed during the migration of young adults from the subarachnoidal space to the lung[1]. In both situations, as well as in other experimental hosts, the severity of the disease appears to depend on the number of larvae ingested or injected[1,8]. The number of larvae required to elicit disease in man is not known as this number depends on many variable factors including the previous history of infection and the strain and virulence of larvae ingested. The median lethal dose (LD_{50}) for rodents infected by either oral or parenteral (subcutaneous) route has been determined in our laboratory and are presented in Table I. The results shown in the table suggest that subcutaneous injection, although not a natural route of infection by this nematode, is more efficient than the oral route. This observation is not unexpected as infection by the oral route requires an additional step before the larvae can reach the circulation, i.e., penetration of the mucosal barrier. In addition, we noted that infecting the animals by a subcutaneous route is associated with a higher worm recovery and a shorter survival period. These data suggest that on a weight basis, the rat, a natural definitive host for this nematode, is more susceptible to infection by *A. cantonensis* than the mouse which in this case acts as a wrong host for the parasite.

While the main pathology of human angiostrongylosis is attributable to the migration of the parasites to the central nervous system and to the host response to these parasites, the initial vomiting following ingestion of a large number of larvae

TABLE I: LD_{50} OF *A. CANTONENSIS* INFECTION IN RODENTS

Animals infected	Route of Infection	
	Oral	Subcutaneous
Rat (Natural definitive host)	180	85
Mouse (Wrong host)	115	63

Figures indicate numbers of infective third-stage larvae

is more likely to be related to the toxic effect of the larvae on the gastric mucosa[1]. However, a direct irritation of the vagal nerve endings by these infective larvae cannot be excluded. It should be kept in mind that the cerebral lesion could also be related to the toxic effect of the metabolic products secreted by developing larvae. However, such a toxic effect on the cerebral tissue has never been systematically investigated.

The pathological findings of the brain from a few fatal cases of eosinophilic meningoencephalitis in man and in other animals experimentally infected with *A. cantonensis* larvae have many features in common[1,5]. For instance, a tract created by the migration of the larvae could be demonstrated and is characterized by a heavy infiltration of eosinophils[1,5]. Eosinophil granulomas of various sizes have also been noted, particularly around dead and disintegrated parasites[1,5]. Both the inflammatory and granulomatous responses are rarely observed around actively migrating larvae because the latter can easily relocate themselves. This does not mean however that the living parasites are not harmful as the metabolic products left behind by these parasites may also stimulate these responses.

It is believed that the signs and symptoms of meningitis appear at the time of larval migration from the brain to subarachnoidal space[1]. The inflammatory response in the meninges may damage or kill the parasites. These damaging or dead parasites may release soluble toxic substance(s) which could further aggravate the condition, making it even less favorable for the remaining parasites to survive or driving some of them back into the brain. The inflammatory response may be more intense during a recurrence of the disease[1]. There is also a report of a severe headache following a skin testing in patients suspected of having *A. cantonensis* infection, particularly in those with a previous history of eosinophilic meningitis[1]. The possible involvement of the inflammatory reaction in the pathogenesis of the disease is also consistent with the observation that an anti-inflammatory agent is sometimes helpful in alleviating the condition[9].

From the above discussion on the pathology and pathogenesis of the disease, it would be unwise therefore to use a method of treatment that will kill the parasites in mass because the soluble substance(s) released from dead or damaged para-

also a few isolated reports on the recovery of young adults in the pulmonary vessels of patients heavily infected with *A. cantonensis*[2].

Under suitable conditions, the first-stage larvae excreted with the feces of definitive host infect a susceptible molluscan intermediate host, i.e., different species of aquatic and terrestrial snails and terrestrial slugs, either by ingestion or by active penetration of the body surface[1,11]. In the intermediate host, these larvae differentiate, molt twice and develop into infective third-stage larvae within 2-3 weeks. Although these third-stage larvae may escape from the intermediate host spontaneously and directly infect susceptible mammalian host, most of them remain within the tissues throughout the life of the molluscs[1]. Mammalian hosts become infected mainly by ingesting the molluscan tissues harboring these infective larvae[1,11]. On the other hand, the parasites may also be ingested by paratenic hosts which are in turn eaten by the mammalian hosts[1,11]. Paratenic hosts for *A. cantonensis* include a variety of predaceous land planarians, freshwater prawns, and land crabs[1].

Antigenic mosaic

Like other metazoan parasites, *Angiostrongylus* has a large variety of antigens capable of eliciting immune responses with diverse specificity in mammalian hosts. Moreover, this nematode is known to have an extensive tissue migration while it continues to develop from infective third-stage larva to sexually mature adult living in the pulmonary vessels of its definitive hosts. Such a situation allows antigens from these different developmental stages to have a close contact with the host immune machinery. Furthermore, in a chronic infection, both eggs and first-stage larvae also have ample opportunity to stimulate the immune response as they are found in close contact with the lung tissue during the development from one-celled egg into first stage larva. It is not surprising therefore that several groups of investigators have found the immune response developing after a natural infection as well as after an immunization with appropriate *A. cantonensis* antigen preparations have a rather diverse specificity[7,12]. Many of these *A. cantonensis* antigens have been reported to cross-react with those of *Ascaris*, *Caenorhabditis*, *Panagrellus* and *Dirofilaria* species[12]. Sera of animals infected with *Anisakis* and *Toxocara* also cross react with *A. cantonensis* extract[7]. Despite the presence of these cross-reactive antigens, there is evidence for the existence of species-specific and strain-specific antigens[7,12,13]. An attempt to prepare antigens specific for *A. cantonensis* by the immunoadsorbent technique has been reported[7].

Both humoral and cell-mediated immune responses have been demonstrated in animals naturally or experimentally infected with *A. cantonensis*[1,8,14-19]. Although in these animals, antibodies specific for most of the antigens presented to them are formed, not all of these antibodies are protective in nature. Nonetheless, the presence of these non-protective antibodies may be useful in the diagnosis of the disease[2,10,20-22]. Both protective and non-protective antibodies and the cell-mediated response may play a role in the pathogenesis of the disease[1,5].

For simplicity, in this review the antigenic composition of *A. cantonensis* will be discussed as two separate headings, namely adult worm antigens and larval antigens.

Adult worm antigens. A systematic analysis of the antigenic composition of *A. cantonensis* has been carried out by Bouthemy and associates[12]. In this report aqueous crude extracts of adult worms and fourth-stage larvae were used for the production of antisera to *A. cantonensis* antigens. These hyperimmune sera, together with those obtained from humans and rats infected with *A. cantonensis*, were allowed to react with these extracts in immunodiffusion and immunoelectrophoresis. Under these conditions, as many as 25 different antigens could be demonstrated in the crude somatic extract of adult worms. Some of these somatic antigens are related to those of other helminths. In addition to these cross-reacting antigens, some of the antigens detected may have also originated from the host, because following an appropriate absorption of the antisera with rat tissues as many as 4 precipitating bands disappeared from the immunoelectrophoretic pattern of adult worm extract[12]. Using an immunoadsorbent column specific for rat serum proteins, Suzuki and his associates[7] were able to eliminate these host component(s) from the crude extract. In conjunction with this observation, we have also noted that one of the antigens in adult female extract reacted with rabbit antiserum to normal rat serum and this was later identified to be rat IgG with monospecific antiserum (unpublished observation). The contamination of host antigen(s) in the extract of adult worms could have occurred because the adult worms live in blood vessels and acquire nutrients from the blood of the host. However, this possibility is unlikely because in our experiment we were unable to eliminate this antigen from our preparation which was prepared from adult worms that had been thoroughly washed and maintained in an *in vitro* condition for as long as 10 days before extraction. On the other hand, the IgG found in such an extract may also represent a specific antibody that has reacted with the worms *in vivo*. In addition to specifically or non-specifically acquired host antigen, the synthesis of host-like antigens by the parasites themselves is another possibility and this has been reported for many other parasites[23].

Several approaches have been made in an attempt to identify some of these somatic antigens but due to the complexity of the antigenic system, very little progress has been achieved. By an immunofluorescent technique, Ishii and Kamiya[24] were able to demonstrate a positive reaction in different organs, particulary in the muscular, digestive and reproductive systems. Under the same conditions, the cuticle was found to be negative, indicating that it is not immunogenic in these animals. The female worms appear to be more immunogenic than the male counterpart, as the antibody could be detected earlier in normal rats receiving a female worm transplant than in those receiving a male worm transplant[26]. Some of these adult worm antigens were also found to be sex specific. We as well as Kamiya and his group[25], have found that the immunoelectrophoretic patterns of male and female adult worm extracts are different from one another. Using the number of precipitating bands in immunoelectrophoresis as a criterion, Kamiya and his group[25] found the male to be antigenically more complex than the female, as there were as many as 25 different components detected in the male extract compared with only 12 in the female.

Using polyacrylamide gel electrophoresis, we were able to demonstrate differences between male and female extracts (to be published). Under appropriate conditions, we could detect as many as 25 to 26 protein components in these somatic extracts. Some of these components were however found to be glycoprotein in nature. Our estimation of the molecular weight of these components suggest most of them to be between 23,000 to 70,000 daltons, a conclusion that fails to agree with that of Kamiya *et al.*[25]. The elution profile of the crude extract from a Sephadex column obtained by these investigators is consistent with a molecular size greater than 200,000 daltons. The discrepancy of the results from the two groups is still obscure and needs to be clarified.

In addition to the somatic antigens and the host or host-like antigens mentioned earlier, evidence is also available indicating that the metabolic products that are present in such a crude extract are also immunogenic. Bouthemy *el al.*[12] were able to detect enzymatic activities in the adult worm antigen preparation that they used. Particularly notable were those of the oxidoreductase system. Indirect evidence suggesting the presence of these excretions and secretions (ES antigens) in the crude antigen preparation from adult worms was presented by Kamiya *et al.*[27] and by Bouthemy *et al.*[12]. These investigators found that infected rat serum gave a band of identity between an extract of infected rat lungs and adult worms. We have a more direct evidence for this occurrence as we could demonstrate that the antiserum to ES antigens cross reacted with adult worm extract and *vice versa* (unpublished observation).

To characterize the ES antigens from adult worms, a large quantity of antigens, preferably without contamination by somatic antigens, is needed. We have found two culture media (NCTC 109 and BME) to be suitable for the maintenance of adult worms metabolically active for several days without any serum supplement[8,28]. Polyacrylamide gel electrophoretic analysis of the concentrated culture fluid showed female ES antigens to consist of one or two major protein components and at least 9 other minor components. Male ES antigens, analyzed at a lower protein concentration, had only one component but the presence of other minor components has not been ruled out.

Larval antigens. Antigenically, the larvae are more complicated than would have been expected from their size. The extract of fourth-stage larvae collected from brain and subarachnoidal space ("cerebral" larvae) of infected rats was found to react with infected rat serum[8,12]. We have found that when larval antigens were used, the hemagglutinating activity could be demonstrated in the serum of infected rats as early as one week after infection[8]. In contrast, such activity could not be detected until the fifth week of infection when either somatic or ES antigens from adult worms were used. Bouthemy *et al.*[12] were able to detect as many as 11 antigenic components in the extract of fourth-stage larvae. We found that the early fourth-stage larvae, obtained from the brain of rats 7-10 days after infection, cross reacted weakly with the antiserum to adult worm extract (unpublished observation). Different lines of indirect evidence to be described in the latter section are also consistent with the conclusion that the antigens of adult worms and other developmental stages are immunologically related.

Specific immune reactivity to the parasite

Different lines of evidence indicate that following a natural or an experimental infection of either definitive hosts or wrong hosts by *A. cantonensis*, both humoral and cell-mediated immune responses develop. Some of the more important lines of evidence supporting this a conclusion are discussed below:

1. Low worm recovery in wild rats, suggesting the presence of acquired immunity following continuous light infections in nature[1,5,11].

2. Wild rats appear to be more resistant than laboratory rats to experimental infection[29].

3. Cellular infiltration and granuloma consisting mainly of eosinophils are frequently found in the brain of experimentally infected animals[1,5].

4. Rising of antibody titers in the serum of animals experimentally infected with *A. cantonensis*[8,14,16].

5. Demonstration of cell-mediated immune response with increasing magnitude in animals experimentally infected with *A. cantonensis*[17-19].

6. Demonstration of antibodies to *A. cantonensis* antigens in the serum and cerebrospinal fluid of patients with eosinophilic meningoencephalitis[10,12,20-22].

7. Development of a positive skin reaction in some of the patients suspected of having been infected with *A. cantonesis*[1].

8. Increased resistance to *A. cantonensis* infection in animals previously immunized with appropriate worm antigens[28,30], or infected with irradiated larvae[31] or a sublethal dose of unirradiated infective third-stage larvae[32].

Antibody response

The antibody response to infection by *A. cantonensis* third-stage larvae has been much studied in several laboratories including ours in an attempt to find specific and sensitive serological method(s) for the diagnosis of the disease caused by this nematode. Sera obtained from infected rats from the fifth week onward were found to give positive hemagglutination when tested with red blood cells sensitized with adult worm antigens[8,14-16]. The results were similar regardless of whether the somatic antigens from male or female worms were used[8]. However, when the cells were sensitized with larval antigens, hemagglutinating activity could be detected as early as 1-2 weeks after infection[8]. This interval coincides with the presence and the migration of larvae within the central nervous system[1]. The hemagglutinating antibody that developed in these animals seems to be associated with female ES antigens, as the hemaggulutinating pattern obtained with the latter was indistinguishable from those obtained with the female somatic antigens[8]. Other evidence demonstrating a correlation of hemagglutinating activity in the serum of infected animals with the maturation and fecundity of the adult females have also been presented [28,33]. Both Jacobs *et al.*[33] and Uahkowithchai[8] demonstrated that only the culture medium from

mature adult female, but not adult male, was able to sensitize sheep red blood cells in the hemagglutination test. These investigators also failed to demonstrate hemagglutinating antibody against female ES antigens in the serum of experimental hosts in which *A. cantonensis* did not develop to maturity.

When antibody activity was analyzed by immunoelectrophoresis[10] or by passive cutaneous anaphylaxis[17] using crude antigen extracted from adult worms, a positive reaction could also be demonstrated 1 and 2 weeks after injection respectively. The skin-sensitizing antibody was found to be heat labile and sensitive to reduction by mercaptoethanol[17], suggesting an IgE or IgE-like nature. Although in humans, IgE antibody to *A. cantonensis* has never been convincingly demonstrated, the report of a positive skin reaction in many patients with eosinophilic meningitis is consistent with this notion[1]. High titers of skin-sensitizing antibody with properties similar to human IgE are frequently detected in other helminthic infections[34]. The detection of precipitating antibody in the absence of hemagglutinating antibody in the serum of infected animals and humans[10] is surprising in view of the fact that in general both immunodiffusion and immunoelectrophoresis are considerably less sensitive than the hemagglutination test. It is however possible that these antibodies are directed against different worm antigens.

Kamiya and his group[10,15] demonstrated that all hemagglutinating activity in the serum of infected rats is associated with the 7S fraction eluted from a Sephadex column. No 19S antibody could be demonstrated, even in the specimens obtained as early as 2 weeks after infection. It is possible however that had the specimens been taken earlier, the 19S antibody might have been demonstrated. The association of antibody with the 7S fraction may explain why precipitating antibody is more readily detected early during the course of infection as precipitating antibody is most commonly associated with 7S IgG antibody. Similarly, we have demonstrated previously that antibody activity in the serum of patients with eosinophilic meningitis is IgG[21,35]. However, in some patients the antibody activity is also associated with other immunoglobulin classes. The elevated serum IgG level in these patients[21,35] may result from the preferential stimulation of IgG synthesis by *A. cantonensis*.

Antibody to *A. cantonensis* is present not only in the serum but also in the cerebrospinal fluid of patients with angiostrongylosis[21,22,35]. While Kamiya[22] was able to detect hemagglutinating activity in the cerebrospinal fluid of most patients, a smaller percentage of positive cerebrospinal fluid specimens was reported by Tungkanak *et al.*[21]. The discrepancy between these two studies cannot be readily explained but may be due, at least in part, to the use of patients with different clinical manifestations. In addition, the latter investigators also reported that in contrast to serum, the antibody activity in cerebrospinal fluid was not always associated with IgG. This observation, together with the lack of a significant correlation of the antibody titers in serum and cerebrospinal fluid, suggest a local synthesis of antibody in the central nervous system in response to a larval migration. This postulation is consistent with the recent report by Yoshimura and associates[17,18] demonstrating that early during the course of infection a cellular response was only limited to the cells taken from the cervical lymph nodes.

One interesting observation regarding the antibody response in patients with eosinophilic meningoencephalitis is that the patient sera consistently give positive reactions with somatic antigens prepared from adult worms[2,10,12,20-22]. This suggests that there is immunological cross reactivity between larval and adult worm antigens, if one assumes that the parasite does not develop into the adult stage in the human host. Such a proposal is consistent with our recent unpublished observation of a positive reaction between antibody to adult worm and fourth-stage larval antigens. These observations are also consistent with the report by Kamiya[6] showing that the serum taken early during the course of infection, i.e., before the larvae reached adult stage, could also react with adult worm antigens. However, one must still be cautious in making such a conclusion because on a few occasions young adults have been found in the brain and lung of patients who died of *A. cantonensis* infection[1,2]. Further investigation on the development of the parasite in different hosts is required before a definite conclusion on the immunological cross reactivity among antigens from different developmental stages can be obtained.

Cell-mediated immune response

In contrast to the information on antibody response, only limited information is currently available on the cell-mediated immune response in *A. cantonensis* infection[17-19]. Intradermal skin tests with adult worm antigens in patients suspected of having been infected with this nematode have given variable results[1]. However, the recent report by Yoshimura *et al.*[18] using guinea pigs infected with *A. cantonensis* is rather promising. These investigators showed that in contrast to rats and humans, infected guinea pigs exhibited a positive skin reaction that correlated well with the *in vitro* assay for lymphocyte response[18].

In their first report, Yoshimura and Soulsby[17] determined the responsiveness of the lymphoid cells from spleen, peripheral blood, and cervical, mediastinal and mesenteric lymph nodes of rats experimentally infected with *A. cantonensis.* Using an increase of the *in vitro* uptake of tritiated thymidine as a criterion for cellular responsiveness, only the cervical lymph node cells were found to give a significant response to stimulation by adult worm antigens and such a response was demonstrable only during the first four weeks of infection. A similar conclusion is reached in a subsequent report[18] using a macrophage migration inhibition test. The pattern of cellular reaction suggests a local rather than a systemic response and it is during this period that the parasites were found in the central nervous system of infected animals. Evidence for the possible presence of a local immune response has also been presented previously by Tungkanak *et al*[21].

In addition to the adult worm antigens used by other investigators[17,18], we have also used female ES antigens to examine the status of the cell-mediated immune response in rats infected with *A. cantonensis*[19]. Using a macrophage migration inhibition test, it was possible to show a positive cellular response to both of these antigens when peritoneal exudative cells were tested. Under the conditions employed, the peak response was observed earlier with somatic antigens than with ES antigens (6 wk vs

12 wk). The cellular response returned to normal when tested again from the 20th week onward. With both antigens however, no significant response could be demonstrated with the specimens taken during the 4-5 week period of infection. Like Yoshimura and associates[18], we found the delayed skin reaction to both of these antigens to be rather variable. However, it was possible to demonstrate in some animals a mononuclear cell infiltration at the site of antigen injection 48 hours later. Although the overall results presented in these studies establish the occurrence of a cell-mediated immune response in animals infected with *A. cantonensis*, its role in acquired resistance or in pathogenesis of the disease remains to be investigated.

Eosinophil response

Although strictly speaking eosinophil response by itself should not be regarded as part of the host immune response to antigenic stimulation, its involvement in several immune phenomena deserves a brief discussion in this section. This is particularly relevant in view of the fact that eosinophil pleocytosis and eosinophilia are commonly observed in angiostrongylosis[1]. Both eosinophilia and enhanced IgE synthesis are common features of helminthic infections[34, 36-38]. However, their functions with regard to host defense have been more clearly defined only recently[34, 37-41]. Nowadays the eosinophil can no longer be regarded as an innocent bystander in the inflammatory response.

Eosinophil chemotactic factors are released when the the IgE coated mast cells react with appropriate antigen or when the complement system is activated[34, 37]. There is also some evidence suggesting that lymphocytes can release factor(s) that are also chemotactic for eosinophils[37]. These factors may also stimulate selective eosinophil production by the bone marrow. Eosinophils can release factors that counteract the biological activities of pharmacological mediators released from mast cells or basophils, thereby serving as a modulator of inflammatory responses, particularly those involving IgE mediated response. Beside this damping effect, the eosinophil is also known to interfere with degranulation and replenishment of mediator-containing granules from mast cells or basophils[37, 38]. On the other hand, eosinophils are less effective than polymorphonuclear leukocytes in phagocytosis but have preference for the phagocytosis of IgE antibody-antigen complex[37-39]. In addition to these functions, the eosinophil has been shown recently to participate in acquired resistance in some parasitic infections, possibly by acting as effector cell in antibody-dependent cell mediated cytolytic reaction[37-40]. Immunoglobulin and complement receptors have also been demonstrated on its membrane[42].

In *A. cantonensis* infection, there is always a selective increase of eosinophils in cerebrospinal fluid suggesting that they are attracted by the migration of larvae into the central nervous system[1]. Eosinophil infiltration into the brain tissue and spinal cord is also commonly observed, particularly around dead parasites and along the old tract left by migrating larvae[1, 5]. In addition, granuloma formation consisting of eosinophils as the main cell type is not uncommonly seen[1, 5]. The latter is similar to what have been observed in schistosomiasis[43]. In addition to the central

nervous system, we have also noted eosinophil infiltration in the lung of rats experimentally infected with *A. cantonensis*.

Besides the localized accumulation of eosinophils in the tissues, eosinophilia is also a common feature of *A. cantonensis* infection both in human and in experimental animals[1,5]. In experimental animals, eosinophilia may occur in two or three phases[12,35,44,45]. The first phase reaches the peak around the end of the first week of infection and coincides with the cerebral phase of the life cycle. The appearance of the first phase and its peak depend in part on the dose of infection[45]. The second phase is generally larger and peaks between 6-8 weeks after infection and coincides with the pulmonary phase of the life cycle and the appearance of first-stage larvae in the feces. On occasions, there may be another phase of eosinophil response that follows the main second phase. Such a cyclic response has also been observed with other parasitic infections, e.g., schistosomiasis[43]. It should be noted at this point that although the data are not as clearcut as those of the eosinophil response, the lymphocyte response, may also have a biphasic appearance[17-19]. Whether or not these two cellular events are interrelated remains to be investigated.

We have attempted to analyze the first phase of eosinophil response in more detail. In these experiments rats and mice were exposed to different numbers of infective (unirradiated), irradiated, or dead third-stage larvae. A more pronounced eosinophilia was noticed in mice and the magnitude of such a response depends in part on the infective dose. We found that with a larger infective dose, the latent period was shorter and the peak was reached sooner and higher (unpublished observation). A similar number of dead larvae injected subcutaneously were unable to evoke an eosinophil response. Irradiated larvae were effective only when the dose was fairly large. Even in this latter situation, eosinophilia was of a low magnitude only. The difference between different forms of third-stage larvae cannot be attributable to the association of larvae with the enteral phase of its life cycle because in these experiments all larvae were given by a subcutaneous route. These observations suggest that the occurrence of eosinophilia is associated with actively metabolizing larvae, possibly in response to stimulation by the metabolic products secreted by these developing larvae and their active migration in the tissue. The occurrence of eosinophilia is reported to be delayed somewhat when the animals is given anti-inflammatory agent[45], suggesting, among other possibilities, the involvement of the immune phemomenon in this process.

Specific acquired immunity in angiostrongylosis

One of the earlier clues suggesting the development of acquired resistance following infection by *A. cantonensis* in mammalian hosts is the frequent recovery of a low number of parasites from wild rats in endemic areas[1,5,11]. Immunity to a subsequent challenge is attributable to continuous light infections throughout the life of these animals[1,5,11]. On the other hand, immunity in humans is less complete as the disease is known to recur within a few months after the previous attack[1]. How-

ever, due to the lack of a reliable diagnostic method for *A. cantonensis* infection, the possibility of misdiagnosing the cases cannot be definitely ruled out.

Infection by *A. cantonensis* seems to be an interesting model for the study of acquired immunity for several reasons. Firstly, the parasite has an interesting life cycle in that it has enteral, cerebral and pulmonary phases of development in its definitive host but only the enteral and cerebral phase in the wrong hosts, e.g., human, monkey and mouse. As mentioned earlier, the enteral phase of development is not essential for its development as it can be bypassed by using parenteral route of infection. Secondly, mature adult worms in the pulmonary vessels of definitive host continue to survive and remain fertile in the presence of both humoral and cell-mediated immune responses that they themselves evoke. And thirdly, there is a possibility of inducing immunity with non-living adult worm antigens, as it has been shown previously that an immunological cross-reactivity exists among antigens from different developmental stages.

Most of the studies on acquired immunity in *A. cantonensis* infection have been conducted in rats infected sublethally with different numbers of third-stage larvae and the results suggest that immunity is associated with the continuous presence of living parasite in these animals[21,29-32]. This conclusion is not unexpected as in general helminthic infections are most frequently associated with concomitant immunity[23]. The criteria widely used in the study of immunity to *A. cantonensis* are mortality rate and worm recovery[13,29-32]. Using these criteria, it was found that a low-grade chronic infection conferred an almost complete protection against a subsequent challenge with a lethal dose of infective larvae. However, immunity to reinfection appears to be highly strain specific. Lim and Heyneman[13] for instance found no cross protection between Malayan, Thai, and Hawaiian strains, suggesting the presence of strain-specific protective antigens.

Acquired immunity induced by a sublethal infection of the host with infective larvae could be associated with the response to any of the antigens of different larval stages or of adult worms because all these antigens are present in the definitive host. In an attempt to define protective (functional) antigen(s) for this parasite, Lee[29] exposed rats to irradiated third-stage larvae and found that they had increased resistance against a subsequent challenge with unirradiated larvae. A complete protection was observed in some animals as evident from the worm recovery determined 42 days after challenge. He suggested that the resistance was associated with larval antigens as practically all irradiated larvae failed to develop into mature adults. These antigens are more likely associated with their metabolic products, as we have previously demonstrated that killed larvae failed to protect the animals (Table II). This conclusion does not necessarily exclude the role of adult worm antigens as it has been discussion previously that common antigens exist between these developmental stages and adult worm and these antigens can effectively induce immune response in the rodents[8].

We have been interested in using *A. cantonensis* infection in rodents as a model for the study on the mechanism of acquired immunity in tissue nematode infections. Although the data obtained cannot be directly applied to other helminthic infections,

TABLE II: THE EFFICIENCY OF VARIOUS ANTIGENIC PREPARATIONS OF *A. CANTONENSIS* IN STIMULATING IMMUNITY IN RATS AGAINST CHALLENGE INFECTION WITH INFECTIVE THIRD-STAGE LARVAE.

Antigen used for immunization	No. rats	Challenging dose (no. larvae)	Mortality rate (%)	Survival period (days)	Gross lesions		% *A. cantonensis* recovered		
					Brain	Lung	Brain	Lung	Total
Living third-stage larvae	4	0	0	>60	ND	ND	ND	ND	ND
	6	500	0	>60	−	+	0	8±0.2[b]	8±0.2
	6	1000	16.6	>60(5)[a]	−	+	0	19±0.2	19±0.2
Dead first-stage larvae	6	500	100	28±1.5	+	+	39±0.1	3±0.1	42±0.1
	6	1000	100	25±0.7	+	+	40±0.5	4±1.4	44±1.1
Dead third-stage larvar	6	500	100	25±0.3	+	−	42±0.3	3±0.1	45±0.3
	6	1000	100	24±0.8	+	+	45±0.2	1±0.1	46±0.2
Dead cerebral larvae (extract)	12	500	100	28±0.4	+	−	42±0.4	3±0.3	45±0.5
Dead adult worms (extract)	6	500	100	45±0.7	−	+	0	16±0.3	16±0.3
	6	1000	100	35±0.3	+	+	0	27±0.1	27±0.1
Female ES	5	500	100	38±2.7	−	+	0	14±1.6	14±1.6
Male ES	5	500	100	20±0.4	+	+	31±3.7	1±0.3	32±3.8
NCTC 109 and/or saline controls	12	500	100	23±0.4	+	+	47±2.4	8±1.6	56±1.5
	6	1000	100	23±0.3	+	−	43±0.2	0	43±0.2

a. Number in parenthesis indicates the number of animals that survived longer than 60 days.
b. Mean ± 1 standard error of the mean.
ND. Not done.

they can nevertheless serve as a background information which may be useful in the investigation of other nematode infections. In one of our earlier experiments Lungdhara[30] demonstrated that neither dead parasites nor crude extracts from different stages could induce any significant protective immunity in rats (Table II). However, among the antigens used, somatic antigens from adult worms might have some protective value judging from worm recovery and survival period. On the contrary, under the same experimental conditions a sublethal infection with living third-stage larvae conferred a solid immunity to a lethal challenge, similar to the previous observation reported by other investigators[13,31,32]. Subsequent to this, we immunized the animals with the *in vitro* culture fluid containing ES antigens from either adult male or female worms, and found that the female ES antigens could provide a certain degree of immunity to a lethal infection by third-stage larvae[8,28]. Although in our first set of experiments with ES antigens, we failed to reduce the mortality rate in immunized rats (Table II), subsequent experiments[28] using more ES antigens and smaller challenging dose showed that the mortality rate too could be reduced. As many as 75% of the immunized

rats were found to survive a lethal challenge and the worms recovered from these animals were found to be reduced in number as well as in their size. Moreover, their reproductive potential was also affected, judging from the number of first-stage larvae recovered in the feces and the number of eggs found in the culture fluid during the *in vitro* cultivation (to be published). Evidence was also obtained suggesting the possible interference with the migration to and the development of third-stage larvae in the central nervous system. The inability of male ES antigens to induce immunity in these animals is obscure and need to be investigated further. These observations indicate that effective protection against infection by *A. cantonensis* can be induced with non-living antigens, particularly with those associated with the maturation and fecundity of the female worms.

The protective value of adult female ES antigens in rats is not entirely unexpected because in this definitive host the parasite develops into adult stage and therefore the immune components developed against these antigens would have ample opportunity to interact with the parasite. However, our recent observation that these ES antigens also have some protective value for mice, as judged from the prolonged survival period when compared with the unimmunized controls (unpublished observation), is rather surprising. This is because in mice the parasite dies prematurely and does not normally develop into adult stage. One possible explanation is based on the existence of an immunological cross reactivity between larval and adult worm antigens. The protection conferred in mice also raises the possibility that humans too may be protected against *A. cantonensis* infection if properly immunized. Although such a practice is not necessary nor practical for this parasitic infection, it encourages further investigation on the possibility of developing a safe and effective vaccine for other helminthic infections.

The next question is on the mechanism of acquired immunity induced by adult female ES antigens. We have previously demonstrated that immunized animals developed high titers of antibody to these antigens[8]. Although the development of cell-mediated immune response following an immunization with ES antigens have never been investigated, there is no reason to suspect why this should not occur because it is known to develop in animals sublethally infected with *A. cantonensis*[17-19]. Our limited observation on passive serum transfer suggests the possible involvement of serum antibody in this process[28]. It is not known exactly how antibody to ES antigens interferes with worm development and disease production. It is possible that these antibodies could interfere with either the feeding process or waste elimination or both, thus damaging their metabolic function in general. Kanjanabutara has demonstrated that the serum from infected rats could interfere with the oxygen uptake of adult *A. cantonensis*[46]. On the other hand, one should not disregard the possible involvement of lymphocytes, eosinophils, or other cellular elements which by themselves or in collaboration with antibodies could damage the parasite.

Evasion of the immune response

Many parasites are able to evade the host immune response, thus enabling them to live and survive in the immune host. The mechanisms for such a phenomenon

include antigenic variation, acquisition of host-like antigens, production of anticomplementary factors, reduced antigenicity and interference with the host immune response[47]. Like many other helminths, adult *A. cantonensis* are able to live and remain fertile in the presence of both humoral and cell-mediated immune responses. How these adult worms could tolerate these immune factors is still obscure. There are both direct and indirect evidences that suggest several different possibilities. For instance, Yoshimura and Soulsby[17] observed that the response of lymphoid cells from *A. cantonensis* infected rats to phytohemagglutinin gradually declined as the infection progressed, particularly during the period of 5 to 10 weeks after infection. This observation suggests that the parasite may have exerted some immunosuppressive effect on the host's immune system. The high level of circulating antibody to parasitic antigens may also interfere with either the induction or the expression of the cell-mediated immune response or both. Lastly, it is also possible that adult worms may have acquired the host or host-like antigens on their surface, thus preventing them from immune attack. We have recently noticed that adult female extracts gave a precipitin line with antiserum to rat IgG (unpublished observation). This was observed even when the worms had been thoroughly washed and maintained *in vitro* in the culture medium for more than one week, suggesting that if it is not incorporated as part of the worms during their development, it must adhere tightly to the worms. The possibility that this component is the worm antigen that cross reacts with anti-rat IgG has not yet been ruled out. More work is needed to distinguish between these possibilities.

Conclusion

Infection of mammalian hosts with *A. cantonensis* is associated with the development of both humoral and cell-mediated immune responses. These immune components are specifiic for both adult worm and larval antigens. Both somatic components and metabolic products (ES antigens) of these different stages are highly immunogenic. Depending on the antigens and on the techniques used, these immune responses could be demonstrated as early as one or two weeks following infection. The animals that survive the primary infection are highly resistant to reinfection. How the parasites that become established in the immune host survive has never been systematically investigated. There is some evidence suggesting the existence of a low degree of immunodepression in these infected animals.

The relative roles of humoral and cellular components of the immune response in providing an effective acquired immunity to reinfection remain to be determined. A preliminary observation from a passive serum transfer experiment suggests that antibody may participate in this process. Using *A. cantonensis* infection in rat as a model for the study of acquired immunity in nematode infection, it is demonstrated that the presence of living parasites is not essential for the development of an effective immunity in definitive host. Rats that have been immunized with female ES antigens are highly resistant to infection by infective third-stage larvae, as evident from mortality rate, survival period, worm development and recovery. A lower

degree of protective immunity could also be induced in a wrong host. This observation represents one of the few helminthic infections whereby acquired immunity can be demonstrated in the absence of living parasites, suggesting the feasibility of having a safe and effective vaccine for some of these helminthic infections. Immunoprophylaxis, either alone or in association with economical, biological and chemotherapeutic measures, can be a useful and significant contribution to the control and treatment of parasitic diseases.

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