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THE PRESENT UNDERSTANDING OF THE MECHANISM OF IMMUNITY TO *TRICHINELLA SPIRALIS**

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About twenty years ago, the decision was made to undertake a long-term study of immunity to *Trichinella spiralis* with the hope that ultimately the mechanism for expulsion of adult worms from the small intestine might be elucidated. Although considerable work on immunity to *T. spiralis* in a wide variety of animals had been completed before this decision was made, the results, even from use of the same experimental host, were difficult to compare both in that insufficient attention was given to the experimental designs and also the experimental methods varied greatly. For the latter reason, it was decided first to perfect and standardize techniques that could be used without major modifications throughout the period of study.[1] Moreover, the white mouse was selected as the experimental host, since it was anticipated that large numbers of animals would be needed to fulfill the requirements of complicated experimental designs. This host was known to develop immunity to this parasite,[2] but, based on the fact that only a few reports were available, it was not a popular host for such studies.

This long-term research project conducted by the author, staff collaborators, and many graduate students has provided considerable information on various aspects of immunity to this parasite.[3] It is the purpose of the present paper 1) to review briefly the results of certain of these studies that opened the way for the formulation of a working hypothesis to explain the mechanism of this immunity, and 2) to present recent proof that delayed (cellular) hypersensitivity plays the prominent role. Although most of the references to be cited concern work in our laboratory, other selected references are included. To avoid as much as possible the variable of host differences, most of the latter deal with studies in mice.

To understand the mechanism that brings about an expulsion of significant numbers of adult worms from the small intestine, it is necessary to relate the host responses after an initial infection, and after a challenging infection given to test the immunity developed in response to prior infections. Therefore, the most important of these will be presented before giving separate consideration to the role of humoral and cellular factors in this immunity.

HOST RESPONSES IN NONIMMUNIZED AND IMMUNIZED MICE AFTER A CHALLENGING INFECTION WITH *T. SPIRALIS*

Important Host Responses after an Initial Infection

Studies in "old" mice (more than 3 months old) have provided a good understanding of these responses. For 11 days after infection, the majority of adult worms is located in the anterior half of the small intestine.[4] Despite mechanical damage that may be extensive soon after infection, a tissue response in this region is not noted for about 4 days.[5] At this time, there is only a mild inflammation. Soon afterwards (between 5 to 7 days) and presumably due to the inflammation, the mice begin to consume less feed per day,[6] and as a result loss in body weight is noted between 6 to 9 days.[7] In the meantime, the intestinal inflammation has developed into an acute phase, and at the zenith (about 8 days), it is moderately severe. The dominant infiltrating cell during the acute phase is the polymorphonuclear leukocyte. The reductions in feed consumption and body weights continue until they reach their lowest levels about 9 to 13 days after infection.[7, 8] The inflammation begins to diminish after 8 days, and at about 10 days it develops into a characteristic subacute (or chronic) phase that lasts for more than a week. This phase is characterized by a mixture of infiltrating mononuclear cells (lymphocytes, plasma cells, and

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macrophages). During the early phase of the subacute response (11 to 14 days after infection), there is a significant loss of adult worms from the anterior half of the small intestine,[4] and soon thereafter the mice begin to consume more feed, and gain in body weight is noted.[7, 8] It should be mentioned that in such old mice a significant loss of worms from the posterior half of the small intestine does not occur until after 14 days. Nevertheless, the numbers lost from the anterior half are great enough to produce the significant reduction noted earlier in the entire small intestine.[4]

In addition to these events, it is of interest to add that by 7 days differential counts show a considerable reduction in lymphocytes so that neutrophils are about equal in numbers.[9] By 14 days, there is still not an increase in total numbers of circulating leukocytes,[7] but by this time a reversal in the lymphocyte/neutrophil ratio has occurred.[9] Also, the size of the spleen, especially in length, is much smaller at 14 days than at 7 days.[9] Finally, circulating precipitins in low titer (1:2) can be detected by 7 days after infection.[10]

It is evident that there is a close association between intestinal inflammation, reductions in feed consumption and body weights, and loss of adult worms from the anterior half of the small intestine. Moreover, it is of interest that neutrophilia develops at a time when polymorphonuclear cells are known to be the dominant ones in the acute inflammatory response in the upper portion of the small intestine. Assuming that these responses are not merely coincidental but associated with the initiation of acquired immunity, one would expect, on the basis of an anamnestic response, a more rapid occurrence of them in mice infected before being challenged. This is, indeed, the case.

Important Host Responses after a Challenging Infection Given to Previously Infected Mice

Old mice given three stimulating infections and then challenged show evidence of intestinal inflammation within 12 hours after challenge,[5] and their feed consumption and body weights drop drastically soon after challenge.[7, 8] Both of the latter reach the low point at about 4 days. Between 3 to 9 days, the numbers of circulating leukocytes reach their highest level,[7] and by 7 days there is already a reversal in the lymphocyte/neutrophil ratio.[9] Moreover, at the latter period the size of the spleen is much smaller, especially in length (2 mm shorter), than that of mice given only the challenging infection.[9] The precipitin titers at 7 days average 1:1,024.[11] The intestinal inflammation is the same as that noted above in mice given an initial infection, except that in the present case it is initiated sooner, runs a more rapid course, and is more severe.[5] As stated above, it is already evident at 12 hours after infection. It is minimal in degree at 24 hours, after which the acute phase develops rapidly and reaches a peak at about 4 days when it is much more severe than at the peak (8 days) in mice given only one infection.

In view of these various observations, it is obvious that immunized mice respond immediately to a challenging infection, *i. e.*, there is no lag phase. As a result, most of the various host responses (inflammation, reduction in feed consumption, loss in body weight, etc.) are clearly evident about 1 week earlier than in mice given only one infection. As would be expected if these responses are associated with acquired immunity, a significant loss of worms from the small intestine also occurs about one week earlier (between 5 to 7 days vs. 11 to 14 days).[4] It is worth noting again that this loss in such old mice is only from the anterior half of the small intestine. The rate of loss from the posterior half is no greater than that in nonimmunized mice.[4]

These observations show that old mice given several stimulating infections acquire the ability to eliminate adult worms from a challenging infection at a significantly accelerated rate. Inasmuch as both humoral and cellular factors are present, the important question arises as to the role of each in producing the demonstrated effect on expulsion of worms. This leads to a consideration of these two separate host responses and certain evidence in support of each.

THE ROLE OF HUMORAL FACTORS

In view of the complexity of *T. spiralis*, it is not surprising that a mosaic of antigens has been demonstrated in its metabolic products (ES antigens), its somatic portion, and most recently in its cuticle.[12-16] These antigens have been of primary interest to serologists seeking those most suitable for serologic tests. However, certain ones have been shown to produce immunity as, for example, the acid-soluble protein fraction of

Melcher,[17] metabolic antigens,[18, 19] and cuticular antigens.[20] Unfortunately such studies fail to shed light directly on the relative importance of humoral and cellular factors in accounting for the immunity demonstrated.

The most convincing evidence to date for direct action of an antibody against *T. spiralis* has been provided by studies of precipitating antibodies.[14, 21-23] When larvae or adult worms are incubated in antiserum, protein precipitates occur around certain body openings. In view of the principles involved, there is no reason to doubt that these *in vitro* effects are duplicated *in vivo*. Direct evidence for this is not available as in the instance of *Nippostrongylus brasiliensis*, in which precipitates have been shown within the digestive tract of worms *in situ*.[24] However, it is known that precipitin titers in mice increase progressively (1:4 to 1:64 to 1:128 to 1:1,024) after each of four stimulating infections with 200 larvae given at 21-day intervals.[11] Also, there is indirect evidence to support the view that precipitating antibodies play a role in inhibiting the normal development and reproductive potential of the worms during infection. Interference with metabolic activities is the most logical explanation for the stunting of worms in immunized hosts and reduced numbers of muscle larvae that cannot be accounted for by a loss of adult worms from the small intestine.[18, 19, 25, 26] It is probable that soon after reinfection, these antibodies combine with antigens in areas of high concentration, especially at the oral opening. This is supported by the fluorescent-antibody studies of Jackson.[14] The source of "primary effective antigens" (excretions and secretions) appears to be the cells of the digestive tract, whereas "secondary effective antigens" are probably secretions from the reproductive organs, which in females are excreted at the vulval orifice. There are, of course, potentially numerous other antigenic sites, such as those in other internal organs shown by staining with certain samples of fluorescent antisera from rabbits.

In considering humoral factors, one cannot overlook the possibility that those causing immediate hypersensitivity may have a bearing on the immunity demonstrated. Among these types, anaphylaxis has been given attention recently by those working with *T. spiralis*. In anaphylactic hypersensitivity, it appears that the reaction results from absorption of humoral antibody or antigen-antibody complexes onto susceptible mast cells in the presence of complement, but other mechanisms for the reaction have been proposed.[27] In any event, the cells are injured, and they release amines, such as histamine and 5-hydroxytryptamine (serotonin), which are pharmacologically active agents. The latter probably is the foremost elicitor of symptoms in the mouse. These agents can initiate a variety of tissue responses, including alterations of tissue permeability. It follows that anaphylactic reactions are susceptible to prevention and treatment with drugs that interfere with the activities of these harmful substances.

Morphologic evidence for the release of mast-cell contents in mice after exposure to antigens of *T. spiralis* has been presented by Briggs.[28] In mice actively sensitized to *T. spiralis*, either by infection with living larvae or by injection of somatic antigens, a marked local reaction occurred after subcutaneous inoculation either of metabolic or somatic antigens, or both. Mast cells disrupted and released granules into the supporting ground substance of the connective tissue; these granules showed a striking metachromasia. This sensitivity was noted about 2 weeks after an initial infection with 50 larvae and persisted for at least 15 months. Much less striking reactions were noted in infected mice tested with heterologous somatic antigens from *Strongyloides ratti* and *N. brasiliensis*, and, especially, in mice passively sensitized by transfer of antibodies from mice or rabbits. When metabolic or somatic antigens were injected intravenously into mice infected with 50 larvae, the generalized allergic host responses to the exogenous antigens were dose dependent and characterized by alterations in behavior within 1 hour after injection.[29] Those that died or were killed generally showed visceral congestion, especially of the small intestine. The degree of host sensitivity was dependent on the number and the duration of infections. Slight sensitivity to somatic antigen was evident 5 to 7 days after an initial infection; it increased during the 2nd week (the period of significant loss of adult worms), reached the maximum level 3 to 4 weeks after infection, and persisted for many weeks. This sensitivity was increased by a challenging infection given 8 to 10 weeks after the initial infection, and in this case was demonstrated within 3 to 5 days. Therefore, there was an accelerated response related to a more rapid elimination of adult worms. Cortisone by various injection schedules protected the

mice against the effects of the homologous somatic antigen. It likewise protected infected mice against the effects of intravenously injected serotonin, which in sensitized mice produces effects similar to those of specific antigen.[30]

In view of the above, it is of interest that an antihistamine drug (chlorpheniramine maleate, a pyridine derivative), an antiserotonin drug (B.A.S., a tryptamine derivative), and a drug with both properties (cyproheptadine, a piperidine derivative), prolonged the intestinal phase of *T. spiralis* in mice.[31]

As will be discussed below, the expulsion of adult worms from the small intestine is due to factors associated with the characteristic intestinal inflammation described above. Therefore, it may be that the release of amines after a specific anaphylactic reaction in the mucosa causes sufficient tissue damage to initiate the inflammation. The fact that antihistamines have an anti-inflammatory property[31] and serotonin, in the mouse, has a histaminelike action[32] support this. Also, the presence and degree of sensitivity to exogenous somatic antigen was associated with loss of adult worms, suggesting that this loss may be due at least in part to allergic responses to endogenous antigens released during infection.[29] However, the comparatively low-grade passive sensitivity of mice to *Trichinella* antigens,[33] the failure to show an accelerated elimination of worms in mice treated with pertussis vaccine to potentiate anaphylactic sensitivity,[33] and, especially, the failure to produce significant loss of worms in mice by high-titered antiserum (see below) make this unlikely. In any event, more work is needed before conclusions can be drawn as to the role of anaphylaxis or other types of immediate hypersensitivity in the expulsion of intestinal worms.

THE ROLE OF CELLULAR FACTORS

Indirect Evidence for the Prominent Role of Cellular Factors

The characteristic intestinal inflammation described above varies in intensity as to the degree of immunity demonstrated. The response is strongest in mice given repeated stimulating infections with untreated larvae before being challenged,[5] it is intermediate in degree when the larvae used for stimulating infections are irradiated to prevent sexual maturity of the developing adults,[34] and it is least in mice stimulated with larvae irradiated to prevent development beyond the preadult stage.[35] Likewise, the immunity and inflammation are less striking in young mice than in old mice.[36] In all cases, worms in significant numbers were eliminated a few days after the peak of the acute inflammatory response, *i.e.*, during the early stage of the subacute (or chronic) phase of inflammation. These cellular effects are not direct, as against certain other parasites, since the reaction is a panmucosal one and thus is about the same throughout large areas of the mucosa and submucosa as it is in areas adjacent to the worms. The suggestion that the elimination of worms is due to the creation of an unfavorable environment is supported by at least two different observations. One, many of the worms are not killed, since after leaving the small intestine they live for a time in the large intestine.[4] Two, intestinal inflammation produced by *Ancylostoma caninum*[37, 38] or *Salmonella typhimurium*[39] can drive out *T. spiralis* adults in significant numbers even when the specific immunity is feeble. It seems logical that the altered biochemical environment may be harmful to resident parasites. Among other things, in acute inflammation there is an increased concentration of CO_2, a decreased tension of oxygen, and the accumulation of organic acids, especially lactic acid.[40]

Other indirect evidence that factors associated with the intestinal inflammation are of major importance in the mechanism of the elimination of adult worms was provided by studies with cortisone and whole-body X-irradiation.

Cortisone

Old mice were selected for study after they had been strongly immunized by repeated stimulating infections to rule out any effect cortisone might have on the development of this immunity.[9] After daily cortisone injections these mice failed to eliminate adult worms for long periods after a challenging infection and on this basis had been rendered essentially nonimmune. Associated with this loss of immunity, there was prompt and striking reduction in spleen size (an average of 3.1 mm shorter in length and 1.0 mm shorter in width than in untreated immunized mice), and a pronounced reduction in circulating lymphocytes. As a result of the latter, a reversal of the lymphocyte/neutrophil ratio to a marked degree was noted at 7 days after challenge. The most striking effect was noted in histopathologic

studies of the small intestine.[41] The characteristic inflammation was prevented almost entirely for at least 9 days after challenge. Although the possible immunosuppressive effects of cortisone on humoral factors (preformed antibodies and those stimulated by antigens from the challenging infection) cannot be ignored, the most logical explanation for the retention of worms is interference with the characteristic intestinal inflammation. Although this host response has been shown in various studies to be associated with the elimination of adult worms, there is no evidence that significant reduction in worms occurs in its absence.

The effect of cortisone on this immunity should not be dismissed without mention of the effect of adrenalectomy.[10] In view of the wide range of physiological effects of adrenalectomy that may influence immune responses directly or indirectly or the maintenance of the parasite, or both, it was surprising that the operation performed in immunized mice 4 days before a challenging infection increased rather than decreased the acquired immunity, based on adult worm counts of adult worms 7 days after challenge. It is worth noting that this treatment failed to affect preformed precipitin titers. In retrospect, there would seem to be a close association between these results and those with cortisone. In the latter case, there is an association of an excess of cortisone, a decrease in lymphoid reserves, and decreased acquired immunity, whereas in the former there is a decrease in cortisone (implied in adrenalectomy), an increase over normal in lymphoid reserves[42], and an increased acquired immunity. Therefore, it is tempting to speculate that adrenalectomy, by eliminating cortical steroids shown by the later cortisone studies to prevent the intestinal inflammation, allowed the inflammation to proceed without interference, thereby bringing about the expulsion of unusually large numbers of adult worms. The opposite has been suggested by Davis and Reed[43] to explain the retention of worms in male wild mice subjected to socio-psychological stress by daily crowding that leads to vicious fighting. Such effects of crowding are a well known cause of adrenal hypertrophy with increased secretion of cortical steroids and a chain of physiological consequences. Among these, one can readily visualize inhibition of intestinal inflammation and retention of worms as suggested by the authors.

Irradiation

As was the case in the cortisone studies, old mice were strongly immunized before being exposed to whole-body X-irradiation.[7] When the challenging infection was given 4 days or 8 days after irradiation (450 r), the mice failed to show a significant loss of worms at 7 days after infection. They had been rendered essentially nonimmune, since their worm burden was similar to that of nonimmunized, untreated control mice given only the challenging infection. On the other hand, immunized, untreated control mice showed the significant reduction expected at this period. During the period when adult worms were being eliminated in significant numbers from immunized, untreated mice, the irradiated mice showed an 80 to 90% reduction in circulating leukocytes. This severe leukopenia, due largely to lymphopenia, was noted 1 day after irradiation and persisted for about 21 days before recovery. Conversely, the immunized, untreated mice showed a leukocytosis and a reversal of the lymphocyte/neutrophil ratio between 3 to 9 days after infection, which encompasses the period of significant loss of worms. The fact that a neutrophilia occurs during this particular time is of considerable interest in that polymorphonuclear cells are the dominant ones during the acute phase of the intestinal inflammation. Further evidence that this host response is associated closely with elimination of worms was provided by a histopathologic study.[34] At 7 days after infection (15 days after host irradiation with 450 r), the inflammatory response of the irradiated mice was less in degree than that noted in nonimmunized, untreated mice, while that of the immunized, untreated controls was about that expected for this period (*i.e.*, during the early subacute phase).

As was stated for the demonstrated effect of cortisone, one cannot rule out entirely the possible immunosuppressive effect of the irradiation on humoral antibodies. However, in this case, there is evidence that such treatment fails to affect precipitin titers[44] and hemagglutinin titers[45] produced prior to exposure. This is consistent with the view that X-irradiation does not alter the rate of preformed antibody destruction.[46] The ability to produce "new" antibody (*i.e.*, that produced in response to the challenging infection given after irradiation) may or may not be impaired, depending on various circum-

stances. In the present case, the only information is that provided by Yarinsky.[45] The hemagglutinin titers failed to change significantly in either the immunized, irradiated mice or the immunized, untreated mice for long periods after the challenging infection. Therefore, as for cortisone, the most plausible explanation for the retention of worms in the irradiated mice is the demonstrated effect on prevention of the intestinal inflammation.

Direct Evidence for the Prominent Role of Cellular Factors Provided by Studies on Delayed (Cellular) Hypersensitivity

When it became apparent from the indirect evidence presented above that intestinal inflammation is mainly responsible for the expulsion of adult worms, attention was directed to the mechanism of this host response. It seemed evident from the initial study of the intestinal histopathology of immunized and nonimmunized mice[5] that the inflammation was triggered by a specific antibody-antigen reaction. Moreover, there was reason to expect, on the basis of related studies, that a humoral antibody was involved. However, at a later date when the above-mentioned effects of cortisone on the inflammation and worm burdens were demonstrated, the mechanism did not appear to be mediated by a reaction of antigen with a serum-borne immunoglobulin. Rather, the striking lymphopenia of the mice raised a suspicion that delayed hypersensitivity might be involved.

This suspicion was strengthened considerably by certain observations made in the above studies of immunized, irradiated mice. The splenic measurements gave indirect evidence of severe irradiation damage to the lymphoid-macrophage system and, as expected, a striking, long-lasting lymphopenia resulted. Moreover, the evidence suggested no interference with antibody titers. Therefore, the fact that X-irradiation, if sufficient to destroy lymphoid tissue, does not affect an established immediate hypersensitivity, but strongly depresses tuberculin reactivity,[47] made delayed hypersensitivity attractive as a hypothesis to explain the expulsion of adult worms.

Despite the rather convincing evidence against the importance of humoral antibodies in the elimination of worms, it was decided to test the matter directly by passive transfer experiments (Larsh and Goulson, unpublished data). Numerous attempts to demonstrate passive immunity that would bring about a significant reduction of adult worms after a challenging infection were unsuccessful. Large volumes of high-titered antiserum from rabbits as well as from mice, and likewise large volumes of whole blood, were given by various schedules and routes without positive results being obtained in a single experiment. At this point, there was ample justification for the early suspicion that delayed hypersensitivity might be involved. While delayed-type skin reactions had been demonstrated many years before,[48] there were no reports on the successful transfer of this type of hypersensitivity produced in response to infection with an animal parasite. Therefore, this necessitated an acquaintance with modern techniques in the field of transplantation immunity and in others dealing with this type of hypersensitivity before the designing of experiments to test this hypothesis. Before a description of the highlights of these studies, a few comments about delayed hypersensitivity are in order.

Delayed (cellular) hypersensitivity differs in certain important respects from immediate (humoral) hypersensitivity.[27] The terms *delayed* and *immediate* refer to the period of time required for elicitation of a reaction in a sensitized host, and not to the induction period for establishing the sensitivity. The latter is about the same (7 to 10 days) for both.[40] The fundamental difference between the two types of sensitivity, and thus the most certain means for distinguishing them, is the substance needed for passive transfer. Immediate hypersensitivities can be transferred with serum antibodies, and these can be detected, in most categories of this type of hypersensitivity, by common serologic techniques, such as the complement-fixation and precipitin tests. On the other hand, delayed hypersensitivity is transferable only with lymphoid cells or their derivatives, and to date no certain *in vitro* method has been devised to detect the antibodies involved. There are theories that such antibodies are serum-borne immunoglobulins ("cytophilic antibody" and another possessing very high "avidity" for the antigen[49]), but a recent study[50] yielded no evidence that a circulating high-affinity antibody played a role in this type of immunologic reaction. In any case, most workers hold the view that, for practical purposes, these antibodies may be considered to be intracellular (thus "endoantibody"

vs. "exoantibody" in immediate hypersensitivity[27]). There are distinctions, other than the substance needed for passive transfer, that can be made between the various types of immediate and delayed hypersensitivities, but they are more subtle and, therefore, would require more space for discussion than justified here. However, it is germane to add that the histopathology of a local delayed hypersensitivity reaction is characterized by cellular infiltration rather than by vascular and edematous changes noted in the immediate type of reaction.

In the first of a series of studies on delayed hypersensitivity[51], it became apparent that many variables must be taken into account in attempts to produce sensitivity in donor animals and to transfer it to recipients, viz., the number and size of stimulating infections given to donors and the period from final stimulation to cell collection, and for the recipients, the cell dosage transferred, number of transfers, period from final transfer to challenging infection, and size of the challenging infection. Significant reductions in adult worms were obtained in recipients in only one of 11 experiments that involved the use of lymph-node cells, but the results aided in designing a second series of experiments. In this case, the results with the use of peritoneal exudate cells (mostly lymphocytes) were encouraging both from the point of view of producing a significant reduction of adult worms in certain cases and in shedding light on important factors to be considered in further studies.[52] There was a trend whereby larger numbers of worms were eliminated as the number of transferred cells was increased, and with the same number of cells involved the effect on worm reduction was evident when a small (100 to 150 larvae) challenging infection was given, but not when a much larger (400) one was used. In other words, as would be expected in a hypersensitive state, there was a suggestion that the effect on the number of worms had a quantitative basis.

In a later study,[53] it was confirmed that the transfer of peritoneal exudate cells from infected donors to normal recipients causes a significant elimination of adult worms after an initial infection, and, in addition, other supporting data were obtained. The mice were shown by the ring skin-graft procedure to be compatible, hence fulfilling the requirement that an isologous strain be used in studies on delayed hypersensitivity. The transfer of peritoneal exudate cells from non-infected donors had no demonstrable effect on worm elimination, showing that this effect is dependent upon the use of cells from mice previously exposed to worm antigens. Moreover, serologic results with five different tests (indirect hemagglutination, bentonite flocculation with Melcher's and metabolic antigens, latex agglutination, and the indirect fluorescent-antibody test) precluded the possibility that the demonstrated effect of the transferred cells was due to adoptive immunity. Finally, strong supporting evidence was obtained in the histopathologic phase of the study. This deserves further comment.

The pattern of the intestinal inflammation, including the cellular components, was similar in the experimental and control mice to that described above from various earlier studies. However, the timing and severity were different, presumably due to the use of a much smaller challenging infection (100 vs. 2 to 500). Also, this was the probable reason for observing mononuclear cells in the early stages before they were masked by massive numbers of infiltrating polymorphonuclear leukocytes. In any case, the inflammatory response was initiated earlier, developed more rapidly, and was more severe in the mice given cells from infected donors before being challenged. The acute phase was noted by 4 days after infection, and by 10 days it was marked in degree as indicated by the numbers of polymorphonuclear leukocytes. Based on the earlier studies, the degree of inflammation at this time would explain the significant loss of adult worms noted the next day. In contrast, the initial appearance and the striking increase in these cells were delayed several days in the controls.

The above histopathologic observations in the recipients given cells from infected donors prove that the characteristic cellular response in the small intestine, which was demonstrated after infection in various earlier studies, is an allergic inflammation. It is, no doubt, triggered by the local injury produced after the specific delayed sensitivity reaction. Whether this injury is due entirely to union of the sensitized antibody-carrying cells and an antigen(s) of the invading parasite or, in addition, to direct parenchymal-cell injury by the adsorbed antigen(s) is not known. Also, the causative antigen(s) and the immunologically competent cells are unknown. Until recently, studies with the light microscope indi-

cated that these cells in skin sites were medium-large lymphocytes.[54] However, evidence obtained by studies with the electron microscope of skin-test sites in rats sensitized to tuberculin indicates that the predominant cell type is the blood monocyte.[55] These authors point out that lymphocytes and monocytes do not differ significantly in size and are difficult to differentiate by light microscopy, which may explain the prior difficulty in identifying the predominant cell. In any event, due to the fact that the mechanism of the immunity to adult *T. spiralis* depends upon it, we need to consider a bit further the relation between the delayed response and the resultant inflammation.

Inflammation is a nonspecific internal defense of the body, and as such may play an important role in natural immunity. One of its protective attributes is the rapid accumulation of phagocytic cells, especially polymorphonuclear leukocytes. This response is much more intense as the result of a local hypersensitivity reaction, whether of the immediate or delayed type, than occurs in normal tissues exposed to the same exciting agent.[40] It is important to emphasize that the immunologic reaction of the sensitivity is the primary event and that the inflammation is initiated later in response to the local tissue injury. In other words, whereas, as mentioned above, inflammation itself is not an acquired phenomenon, an accelerated and intensified reaction is always associated with acquired immunologic responses. In such a reaction, the rapid and pronounced infiltration of phagocytic cells often makes it difficult to delineate the features of a local hypersensitivity reaction. In fact, in early studies of delayed sensitivity, the appearance of massive numbers of polymorphonuclear leukocytes led some workers to conclude that these cells were responsible for the specific reaction. Later, it was shown that they are an important component of the secondary nonspecific response (allergic inflammation) and may mask entirely the presence of infiltrating mononuclear cells of the primary specific response, especially if tissue observations are delayed.

The important question arises whether the exaggerated response noted in allergic inflammation has a protective role. This possibility does not seem warranted in the case of various microorganisms, since the localizing effect of the inflammation is not more, and may be less, than that provoked by the same antigenic stimulus in normal tissues.[40] In this connection, the present results are most unusual in that the delayed sensitivity response is responsible for the events that cause the ultimate expulsion of adult worms from the small intestine. In view of this, it must be agreed that this specific response plays the prominent role in the total immunity to *T. spiralis*. While this phenomenon has been demonstrated only in mice, there is every reason to believe that it operates in other hosts, including man.

The present demonstration of the successful cellular transfer of immunity to a parasitic agent was the first to be reported. Later, success was reported after transfer of mesenteric lymph nodes from donor to recipient guinea pigs before infection with *Trichostrongylus colubriformis*.[56] Also, recipient mice given peritoneal exudate cells from isologous donors demonstrated immunity to *Fasciola hepatica*.[57] On the basis of chemical similarities of various parasite antigens, there is every reason to expect that delayed sensitivity, whether or not protective, is a consequence of many parasitic infections. Delayed skin-test reactions attest to this,[48] and in many reports the tissue histopathology is suggestive of a delayed response. Therefore, if recent interest continues, it may be predicted that this will become a fertile field for experimental parasitologists.

SUMMARY

Our present knowledge warrants the conclusion that the mechanism causing expulsion of adult *T. spiralis* is triggered by a specific delayed hypersensitivity reaction. The resultant tissue damage provides the stimuli for initiation of nonspecific allergic inflammation, which in turn directly effects expulsion of worms, probably due to the creation of an unsuitable biochemical environment. Further, the evidence indicates that humoral antibodies at best play only a minor role in the elimination of worms. Nevertheless, they are important in the total immunity in that they produce direct deleterious effects against the worms, such as interference with growth and reduction of the reproductive potential, and they are responsible for contributing to cellular destruction as a consequence of anaphylactic hypersensitivity reactions.

Considerable work remains to clarify various aspects of the delayed response to *T. spiralis*, such as demonstration of the responsible

antigen(s) and, by electron microscopy, the true identity of the immunologically competent cells. In the meantime, it is hoped that the present findings will encourage others to study this phenomenon and its relation to immunity demonstrated against other parasitic agents.

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