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amounts without FREUND's adjuvant. Unembryonated eggs do not induce granulomas, and if embryonated eggs are depleted of soluble antigens by *in vitro* tissue culture they do not elicit granulomas. Granuloma formation around eggs is a sensitization phenomenon which is highly specific and is transferred with lymph node cells but not serum. Its onset is concomitant with delayed footpad swelling in mice and delayed skin reactivity, lymphocyte transformation, and migration inhibitory factor production in guinea pigs. Granuloma formation occurs in the absence of detectable γ_2 antibodies in the mouse and γ_1 and γ_2 antibodies in guinea pigs. Granulomas isolated from the livers of infected mice and cultivated *in vitro* release migration inhibitory factor in the presence of soluble egg antigens. Granulomas are inhibited by immunosuppressive agents which are active against cell-mediated reactions and are unaffected by inhibitors of antibody-mediated reactions.

D 8 (2)

CELL-MEDIATED IMMUNITY IN EXPERIMENTAL FILARIASIS

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Our knowledge of infection with *Filaria* and its importance in community health is mainly based on the epidemiology of the disease. Although many attempts have been made to elucidate certain aspects of the disease, there are difficulties in interpreting some of the epidemiological features. The cotton rat experimental model has been known for a long time, but we are quite ignorant of the detailed mechanism of the pathogenesis of the disease and the host-parasite relationship. Our preliminary findings on immunopathology of filariasis in the cotton rat will be presented and the implications of the results will be discussed.

D 8 (3)

HETEROLOGOUS IMMUNITY IN MICE INFECTED WITH TRICHINELLA SPIRALIS

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Primary infection of CFLP mice with *Trichinella spiralis* results in expulsion of the nematodes from the intestine about day 14 post-infection. *Trichuris muris* in a primary infection is expelled day 21 to 22 post-infection. Preliminary recent reports indicated the possibility of a host immunodepressive activity by *T. spiralis,* and therefore the interaction of the two species was examined. Repeated infections of either species of nematode renders mice specifically immune, but not immune to the other species. However, in mice in which *T. spiralis* is established a few days before or simultaneously with *T. muris, T. muris* is expelled at the same time as *T. spiralis,* i. e. a full seven days before they would be expelled in a solo primary infection. This phenomenon is evident in double primary infections where the interval between infections is as great as 18 days. Indomethacine (Boots, Ltd), a non-steroid, anti-inflammatory drug, delays expulsion of *T. spiralis* but seems to have no effect in preventing expulsion of *T. muris.* In double infections, indomethacine treatment resulted in delayed expulsion of *T. spiralis* and a delayed expulsion of *T. muris.* This may suggest that an inflammatory response is a primary factor in the species interaction.

Thymectomy which is known to delay expulsion of *T. spiralis* and *T. muris* in solo infections also had this effect in double simultaneous infections in NIH mice. However, in these mice there was a significant loss of *T. spiralis* from the small intestine by day 15 post-infection indicating that expulsion had not been entirely prevented. Thus the precise mechanisms of expulsion of either species and the interactions between them is not so far simply explained by either a non-specific inflammatory response or specific immune mechanisms.

D 8 (4)

THE EFFECT OF CONCURRENT MALARIA AND TRYPANOSOME INFECTIONS ON IMMUNITY TO TRICHINELLA SPIRALIS IN MICE

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Plasmodium berghei berghei (KSP 11 strain) in adult CFLP mice produces an acute infection with a patent period of 18 to 21 days. There is some mortality and in the experiments referred to below sulphadiazine (May and Baker) was used to assist the mice through the parasitaemic crisis. *Trypanosoma brucei* (TREV 792 strain) in CFLP mice follows a typical repeatedly relapsing course eventually culminating in the death of the host 6 to 12 weeks later. In this study Berenil (Hoechst Pharmaceuticals) was used to suppress acute trypanosome parasitaemias.

Experiments were carried out to examine the effect of concurrent *T. brucei* and *P. berghei* infections on the ability of mice to develop an acquired immunity to *Trichinella spiralis* and also the ability of immune mice to resist a challenge infection. A depression of the immune response of the mice to *T. spiralis* was indicated by the longer survival of adult worms in the small intestine and an increase in the number of larvae encapsulated in the muscle 5 to 6 weeks after *T. spiralis* infection. It was found that in mice in which *T. spiralis* and *T. brucei* infections were initiated on the same day, the expulsion of a large proportion of the adult worms (including larval producing females) was delayed for at least 4 to 5 days and the muscle larvae count at 6 weeks was three times that in control animals. There were similar but less significant findings where mice were infected with *T. spiralis* and *P. berghei* on the same day.

In preliminary experiments it has been found that *T. brucei* infections at least delay the expulsion of *T. spiralis* from the small intestine of mice immune to this nematode.

These results follow those of other workers who have variously reported that rats with an established *T. brucei* infection were not able to expel *Nippostrongylus brasiliensis*, and *T. brucei* and *P. berghei* infections impair both the development and the expression of acquired immunity to *Trichuris muris* in mice.

D 8 (5)

CELL MEDIATED IMMUNITY IN RODENTS INFECTED WITH ASCARIDOID NEMATODES

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In vitro lymphocyte transformations were monitored by measuring the incorporation of ^{3}H-thymidine in whole-blood cultures by liquid scintillation spectrometry. Blood was taken by cardiac puncture into heparinized sterile syringes and diluted 1 : 40 with RPMI 1640 culture medium (CSL Melbourne). Total and differential white cell counts were made on the blood and the ^{3}H incorporation calculated as dmp's/10^{6} lymphocytes. Dose response curves were plotted for antigen and phytohaemagglutinin (PHA) using lymphocytes from immune hosts. Antigen was prepared from adult *Toxocara canis* and *Ascaris suum* by disintegrating washed whole worms in saline centrifuging at 16,000 rpm for 30 min. and sterilizing the supernatant by passing it through 0.45 µ millipore membranes. Optimum responses were obtained with 50 µg PHA and 40 µg antigen per 2 ml culture after three and five days culture, respectively. Lymphocyte transformations were first observed after five days and thereafter reach a peak by the 10th day after infection. These responses will be discussed in relation to the PHA response and the dissemmination of the larvae into the various organs of the body.

D 8 (6)

CELLULAR RESPONSES OF GUINEA PIGS INFECTED WITH SCHISTOSOMA MANSONI: BLASTOGENESIS, MIF AND CHEMOTACTIC FACTOR PRODUCTION IN RESPONSE TO CERCARIAL, ADULT AND EGG ANTIGENS

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A large group of guinea pigs was infected once with 1000 *Schistosoma mansoni* cercariae each. Some of these animals were also immunized with H37RA. At intervals of 2, 4, 6, 7, 8, 11, 16 and 24 weeks after infection, peripheral lymphocytes and oil-induced peritoneal mononuclear exudates were collected from 5 infected animals, including 2 immunized with H37RA, and 2 uninfected non-immunized (control) animals. These cells were tested for *in vitro* responsiveness to four antigens – adult *S. mansoni* freezethaw antigen, soluble egg antigen, cercarial saline extract, and purified protein derivative of tuberculin (PPD). Lymphoid cell responsiveness was tested by three *in vitro* assays – blastogenesis, production of macrophage migration inhibition factor, and production of macrophage chemotactic factor.

Strong responses were recorded in infected animals to all schistosome antigens. The earliest response to develop was to cercarial antigen, the next to adult antigen, and the last to egg antigen. All three of these responses reached a peak between 4 to 8 weeks after infection, however, and then rapidly dropped to zero, where they stayed for the remainder of the study. In contrast, the responses to PPD gradually increased to a plateau at the 3rd week post-immunization and did not vary significantly thereafter.

It is concluded that the lymphoid cell responses to the three schistosome antigens tested were unusual in that the early strong responses disappeared so rapidly and completely. The loss of response appears to be antigen specific since cells from infected animals retained a high level of responsiveness to PPD. Experiments are currently being carried out to further characterize these responses and in particular to determine the cause of their transient nature.

The results of this study will be compared with findings in other host systems.

D 8 (7)

PROGRESSION OF LYMPHOID CELL RESPONSIVENESS IN EXPERIMENTAL ASCARIASIS

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Experimental ascariasis *(Ascaris suum)* infection in the guinea pig is associated with a spectrum of immune responses, ranging from those responsible for some of the *in vitro* correlates of cell mediated immunity to the production of antibodies, including reaginic and other homocytotropic antibodies. The relationship of the early responses, characterizable as cell mediated, to the later responses in which antibody production occurs must await studies in which populations of cells with defined surface determinants (e. g. theta antigen, immunoglobulin receptors, etc.) are transferred to challenge recipients.

Nevertheless a sequence in responsiveness is evident in lymphoid organs draining parasitized tissues. Initially draining lymph nodes of parasitized tissues show increased blastogenesis; this is followed by cells capable of undergoing antigen induced blastogenesis in culture. Two days later a proportion of such cells express surface immunoglobulin determinants detectable by rosette techniques. At this stage *in vitro* correlates of cell mediated immunity, such as the inhibition of macrophage migration are present. A proportion of cells capable of rosette formation progress to the stage of antibody secretion and eventually a population of cells is found which secretes antibody but is not responsive to antigen induced blastogenesis in rosette formation.

Circumstantial evidence suggests that this progression of responsiveness represents the sequence by which lymphoid centers respond to the infection. If so then the early stages of the response, which possess the characteristics of cell mediated immunity can be regarded as a means of facilitating the antibody producing part of the response.

D 8 (8)

MACROPHAGE MIGRATION INHIBITION ASSAY IN STRONGYLOIDIASIS OF RABBITS

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Protective immunity against *Strongyloides papillosus* has been obtained after repeated experimentally infections with 3rd-stage filariform larvae in sheep (TURNER, 1956, 1957, 1959; STANKIEWICZ, 1969, 1971; STANKIEWICZ and BROZOZOWSKA, 1972), goats (TURNER, 1956, 1957, 1959), calfs (VEGORS, 1954; DAVIS et al., 1960) and rabbits (JARON, 1964; GEYER, 1965). Single infection with X-irradiated larvae induced sufficient protection against reinfection in rabbits (GEYER, 1969); some degree of immunity after single infection could be demonstrated in sheep (BEZUBIK, 1970). Repeated injections of somatic antigen preparations from 3rd-stage larvae elicited protective immune response in rabbits (GEYER, 1965).

The question of the basic immune mechanism in *S. papillosus* infection has not been studied. Strongyloidiasis of sheep, goats and rabbits is accompanied with serological response as experimentally demonstrated in IFT, CFT and PT, respectively, using 3rd-stage larvae antigen (DU PLESSIS, 1970; STANKIEWICZ, 1970; GEYER, 1965; FUNK and GEYER, unpubl.).

In this study the macrophage migration inhibition assay (capillary tube method) with peritoneal exudate cells was used to detect sensitive lymphocytes in experimentally immunized male Alaska rabbits after single and repeated infections in the presence of antigen prepared from 3rd-stage larvae (AL) and adult parasitic female worms (AA). Antigen from intestinal/fecal bacteria from rabbits (AFB) was also tested. Antigen concentrations used were 0.4; 2.0; 10.0; 50.0; 100.0 and 200 µg protein/ml. Macrophages from control rabbits showed maximum migration in the presence of *Strongyloides* AL- and AA-antigen and saline control medium, but were significant inhibited as is to be expected at high concentrations of AFB-antigen. On the other

hand inhibition indices of macrophages from immunized animals were significant in the presence of both AFB- and *Strongyloides* AA-antigen at high concentrations. No or extremely weak inhibition was observed when using *Strongyloides* AL-antigen. Our study strongly indicates that in *S. papillosus* infection adult nematodes play an important role in the development of immunity.

D 8 (9)

LOCAL IMMUNE RESPONSE IN EXPERIMENTAL ASCARIASIS

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The possible local nature of the immune response to *Ascaris suum* infections in the guinea pig has been suggested by the work of DOBSON et al. (1971) and SOULSBY (1972). This possibility was investigated further using antigen-induced blastogenesis and rosette formation techniques.

Three experimental groups of guinea pigs were studied. Each group consisted of 14 normal and 14 immune animals. Group I animals were infected orally with 10,000 infective eggs of *A. suum;* Group II were infected by injection of 10,000 artificially hatched second stage larvae of *A. suum* via the mesenteric vein; and Group III were infected by injection of 1500 third stage larvae of *A. suum* via the saphenous vein. Two animals from each group were sacrificed at day 0 and at days 1, 2, 5, 7, 9 and 12 after infection. Lymphoid cells from the mesenteric, hepatic and mediastinal lymph nodes, from PEYER's patches and the spleen were assessed by blastogenesis and rosette formation. For the former, lymphoid cells were cultured for four days with adult *A. suum* antigen (WWAg), pulsed with tritiated thymidine and processed for liquid scintillation spectrometry. For the latter, lymphoid cells were centrifuged at 4° C with WWAg coupled to sheep red blood cells with glutaraldehyde.

In all groups, the peak blastogenic and rosette formation responses occurred in the draining lymph nodes at the time when the parasite was migrating through the respective parasitized tissues. Peak blastogenic and rosette formation responses occurred in Group I ("complete" infection) in the mesenteric, hepatic and mediastinal lymph nodes; in Group II ("abbreviated" infection: intestinal phase bypassed) in the hepatic and mediastinal lymph nodes; and in Group III ("abbreviated" infection: intestinal and hepatic phases bypassed) in the mediastinal lymph nodes only. The draining lymph nodes of infected immune animals in each group studied showed similar but more marked responses than those of infected normal animals.

The results indicate that the immune response to the migratory larval stages of *A. suum* is of a local nature at least for the first 10 to 12 days of infection.

D 8 (10)

THE ROLE OF CELLS IN IMMUNITY TO DEVELOPING LARVAE AND STATIC ADULT WORM POPULATIONS OF NIPPOSTRONGYLUS BRASILIENSIS

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The immunological control of both the developing larvae and the adult stages of *Nippostrongylus brasiliensis* in mature rats and mice requires the collaborative action of antibodies and cells obtained from the mesenteric lymph node of syngeneic immune donors. It has been suggested by DINEEN and KELLY that the rejection of adult worms also requires the participation of cells from the bone marrow.

We are comparing the immunological control of developing larvae and adult worms in neonatally infected and lactating rats. In these animals, infections are prolonged and although the action of antibodies on the worms appears to be essentially unaffected, the cellular

step is impaired. 2.5 x 10^8 viable syngeneic immune lymph node cells were injected intravenously into neonatally infected animals at various times during an infection, either simultaneously with larvae on day 0, or at intervals after the worms had become mature (on days 7, 14, 21 or 28). When cells were given on day 0, the infection was rejected from the young animals. When cells were given on day 7, there was partial expulsion of the worms but the injection of cells on days 14 to 28 had no effect even though at the time cells were injected the worms were damaged by antibodies. These experiments have been confirmed in animals infected with antibody-damaged worms transferred surgically into their intestine from mature donors. The injection of bone marrow cells as well as lymph node cells also failed to cause rejection of adult worms from neonates infected 18 days previously. Preliminary experiments in lactating rats have given similar results.

We think these results show that (a) even though both require antibodies and lymphocytes, there is a qualitative difference between the immune mechanism which controls developing larvae and adult worms and (b) that the immunological defect in neonates and lactating animals is not explained simply by their failure to produce sensitized effector lymphocytes.

D 8 (11)

GRANULOMA FORMATION TO CAPILLARIA HEPATICA EGGS: CELLULAR AND HUMORAL ASPECTS

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Experimental granuloma formation to *Capillaria hepatica* eggs in mice has an immunological basis and the cellular composition of the granuloma suggests a cell-mediated component is involved as part of the specific response. Following primary intravenous mesenteric vein inoculation of eggs into the liver, distinct granulomatous lesions

developed, characterized by macrophages and lymphocytes. Prior intraperitoneal sensitization led to an earlier and an enhanced reaction to an intravenous (secondary) egg challenge. Specificity of the cellular response was suggested by the lack of an enhanced reaction to presensitization with eggs of a closely related species, *Trichuris muris*.

The uptake of tritiated thymidine during primary and secondary granuloma formation in mice indicated early responses in the regional lymph nodes draining the liver and high dpm/mg of liver tissue in presensitized-challenged hosts.

Peripheral immunological responses have been assessed in mice during primary and secondary granuloma formation. Hemagglutinating (IHA) and homocytotropic antibodies as well as delayed dermal reactivity but not precipitating antibodies were detected in animals with primary and secondary granulomas.

Persistent IgM and IgG class antibodies occur throughout primary granuloma formation, although sensitized-challenged granulomatous mice had no demonstrable IgM during the latter stages of secondary granuloma formation.

Homologous homocytotropic antibody activity, assessed in mice, was present in sera from primary and secondary granulomatous mice. Two hour PCA activity was heat stable, whereas 2 day activity was heat labile at 56° C for 2 hours.

The demonstration of circulating antibody during the course of granuloma formation indicates a possible role for antibody in the response.

D 8 (12)

THE DEVELOPMENT OF IMMUNITY AGAINST DICTYOCAULOSIS IN CALVES WITH TRANSPLANTATED LYMPHATIC TISSUE TAKEN FROM CALVES IMMUNE AGAINST DICTYOCAULOSIS

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