A role for granulomatous inflammation in the transmission of infectious disease: schistosomiasis and tuberculosis

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SUMMARY

The relationship between cell-mediated granulomatous inflammation and transmission of disease in schistosomiasis and tuberculosis has been explored. In 2 experiments involving *Schistosoma mansoni*-infected normal and T cell-deprived mice, and infected deprived mice that had been variously reconstituted with immune or normal lymphocytes or immune serum, there was a significant positive numerical correlation between mean liver granuloma diameters and faecal egg counts in individual animals. Lymphocytes from donors with recently patent infections were more active than cells from chronically infected or uninfected donors in reconstituting egg excretion rates in deprived recipients, and mesenteric lymph node (MLN) cells were more active than spleen cells. Modulation of granulomatous activity with increasing chronicity of infection in the donors, resulting in a decrease in granuloma size around freshly produced tissue-bound eggs, was paralleled by a waning of the capacity of transferred lymph node cells to reconstitute egg excretion in the recipients. Serum taken from chronically infected donor mice over the same period and transferred to infected deprived recipients became more active in enhancing egg excretion in the recipients as the cell-mediated activity declined. A recent study in Kenya has found that *S. mansoni*-infected patients with concurrent human immunodefficiency virus (HIV) infection excrete fewer eggs than patients exposed to the same levels of schistosome infection, but who are not HIV-infected, thus indicating that schistosome egg excretion in humans is also immune-dependent. Attention is drawn to an apparently parallel situation in human tuberculosis, another pathogen which induces a cell-mediated granulomatous immune response. Several studies have shown that patients with tuberculosis who are also HIV-seropositive tend to have fewer tubercle bacilli detectable in their saliva than those with tuberculosis, but who are HIV-negative. This discrepancy, associated with differences in lung pathology in HIV-positive patients, suggests that in tuberculosis immune cell-mediated granulomatous inflammation causes the destruction of host tissue in a manner which facilitates onward transmission of the bacterial pathogen.

Key words: Schistosomiasis, tuberculosis, *Schistosoma mansoni*, *Mycobacterium tuberculosis*, egg granuloma, granulomatous inflammation, immunopathology, disease transmission.

INTRODUCTION

Continuity of the life-cycles of disease-causing organisms requires that the respective causative agents have evolved mechanisms which allow them to be effectively transmitted to new hosts. Schistosomes, in common with many other helminths, depend on their eggs leaving the definitive host in excreted stools or urine, while tubercle bacilli are released from diseased lung tissue and expectorated in aerosol droplets. A feature common to both schistosomiasis and tuberculosis is the extensive granulomatous inflammation induced in diseased tissue, and it has previously been indicated that in schistosome-infected animals this immunopathology is important in facilitating the egg excretion process (Doenhoff, Hassounah & Lucas, 1985, 1986; Damian, 1987). An objective of this review is to draw attention to the possibility that the immunological reactivity induced by tubercle bacilli is likewise important in the release of the infectious agent from the body.

With respect to schistosomes, in contrast to many other species of helminth, the adult parasites reside inside blood vessels of the host (the hepatic portal system in the case of *Schistosoma mansoni* and *S. japonicum*, the vesicle plexus and other vessels of the urino-genital tract for *S. haematobium*), and many of the eggs produced by female worms are not in fact excreted. Instead they embolize in capillary beds of host tissues, particularly those downstream of the sites of oviposition. Once entrapped in a capillary the egg induces an inflammatory reaction or granuloma which occludes blood flow, resulting in lesions to which many of the disease symptoms of schistosomiasis are attributable (Warren, 1973).

While it is clear that the symptoms of schistosomiasis have an immunopathological aetiology (Warren, 1975), the function of the granuloma in an adaptive or evolutionary context has been less well defined. It has been suggested that the granuloma protects host tissues against toxic schistosome egg products (von Lichtenberg, 1964), and indeed, 'nude' mice (Byram & von Lichtenberg, 1977) or T-cell depleted mice (Buchanan, Fine & Colley, 1973; Byram *et al.* 1979; Doenhoff *et al.* 1981) which cannot form granulomas around schistosome eggs, have been found to suffer from hepatotoxicity putatively induced by *S. mansoni* eggs. However, reconstitution of *S. mansoni*-infected T-deprived

Parasitology (1997) **115**, S113–S125. Printed in the United Kingdom
https://doi.org/10.1017/S0031182097001972 Published online by Cambridge University Press

mice with immune lymphocytes, which augmented the host's capacity for granuloma formation, did not protect against egg-induced hepatocyte damage as well as passive immunization with immune serum. Furthermore, serum from infected mice acquired hepatoprotective activity early after patency when granulomatous activity was still maximal (Doenhoff *et al*. 1986; Hassounah & Doenhoff, 1993).

Experiments with *S. bovis* and *S. haematobium* also are not entirely consistent with the notion that the sole role of the granuloma is host protection, since livers of immunosuppressed mice heavily infected with these two schistosome species showed no evidence of a toxic reaction despite the absence of granulomatous inflammation (Murare *et al*. 1987; Agnew, Lucas & Doenhoff, 1988).

As well as suffering from an egg-induced hepatotoxicity reaction, *S. mansoni*-infected immunosuppressed mice are incapable of excreting parasite eggs in normal numbers, a defect which could in part be rectified by injections of serum from normal infected donor animals (Doenhoff *et al*. 1978, 1981; Dunne *et al*. 1983). In subsequent experiments involving adoptive transfer of immune cells or serum there was a positive relationship between the numerical values for the mean size of granulomas in the liver and the number of eggs being excreted in individual recipient animals (Doenhoff *et al*. 1985, 1986). While it was understood that liver-bound eggs were terminally incapacitated with respect to further transmission of the parasite, it was suggested that the immune effector mechanisms responsible for granuloma formation may also be involved in the processes of egg excretion.

From his observations on non-human primates Damian (1987) arrived at a similar conclusion concerning promotion of schistosome egg excretion by the egg granuloma. The experimental observations, first made some 20 years ago, are now supported by the finding that *S. mansoni* egg excretion rates are impaired in subjects with concomitant human immunodefficiency virus (HIV) infection in Kenya (Karanja *et al*. 1997). Insight about the molecular mediators involved in the schistosome egg excretion process has also recently been gained with the observation that tumour necrosis factor alpha (TNF-α) promotes both *S. mansoni* egg granuloma formation and egg excretion (Amiri *et al*. 1992; McKerrow, this volume).

The experimental results reported here are from studies in which the respective roles of cellular and humoral immune responses in the processes of *S. mansoni* egg excretion in mice have been further investigated. It is shown that as schistosome infections become increasingly chronic, egg-induced granuloma formation and the capacity of immune cells to mediate in the processes of egg excretion are modulated downwards in parallel, while the capacity of immune serum to promote egg excretion increases. The activity in serum reaches a lower, but constantly maintained level of potency than that expressed by immune cells during acute infection.

In the discussion of these results below attention is drawn to the possibility that the cell-mediated immune responses generated by *Mycobacterium tuberculosis* may have a similar role to the schistosome egg granuloma; namely, to promote transmission of the infectious agent.

MATERIALS AND METHODS

Parasite and host

A Puerto Rican strain of *Schistosoma mansoni* was maintained in laboratory life-cycle in random-bred TO strain mice and albino *Biomphalaria glabrata* snails. Inbred CBA-H/*T6T6* strain mice were used for experimentation.

T cell-deprived mice were prepared as described previously by a combination of adult thymectomy and injections of rabbit anti-mouse thymocyte serum (Doenhoff *et al*. 1978, 1979). The mice were rested for approximately 30 days before use, and then infected percutaneously with *S. mansoni* cercariae (Smithers & Terry, 1965). Adult-thymectomized, anti-thymocyte serum-treated mice have approximately 10% of the normal level of circulating T cells (Doenhoff & Leuchars, 1977). Donors of immune lymphocytes and serum were infected with 25 cercariae, and recipients of the cells or serum and the appropriate control groups were given 200 cercariae.

Cell and serum transfers

Mesenteric lymph node and spleen cells and serum were obtained from donors that had been infected between 1 and 20 weeks previously. Cell suspensions for injection into recipient mice were prepared as described (Doenhoff *et al*. 1986; Hassounah & Doenhoff, 1993). Immune serum was obtained from infected immune-intact donors between 2 and 16 weeks after infection with 25 cercariae.

Recipient T cell-deprived mice and the appropriate control groups were infected with 200 cercariae. Approximately 40 days after infection lymphocyte recipients were given intravenous injections of cells in medium: if more than one injection was given, each injection was separated by 24 h. The number of cells transferred is specified in the text relating to respective experiments. Recipients of immune serum were injected intraperitoneally once daily from day 40 after infection until the day before perfusion. Experiments were terminated approximately 47 days after infection of the recipients.

Perfusions, tissue egg counts and faecal egg counts

On the day of termination of the experiment a 40–50 mg faecal pellet was obtained from each mouse

https://doi.org/10.1017/S0031182097001972 Published online by Cambridge University Press

Table 1. Granuloma diameters and faecal egg counts in mice given serum, spleen or mesenteric lymph node cells from normal and infected donors

A group of normal mice and the T cell-deprived mice which were to act as cell or serum recipients or unreconstituted controls were infected with 200 *S. mansoni* cercariae on day 0. Recipients were injected with 38×10^6 cells on each of days 38 and 39 after infection. Spleen cells (SpC) or mesenteric lymph node cells (LNC) were taken from uninfected mice (N) or from mice infected with 25 cercariae 8 weeks (AI), or 16 weeks (CI) previously. Recipients of serum (IS) were given daily intraperitoneal injections of serum (0·5 ml/mouse, the serum having been obtained from mice with patent 16 week-old infections) from day 40 to 46, and the experiment was terminated on day 47. Figures in parentheses to the right of values of faecal egg counts and granuloma diameters indicate position in numerical rank order with (1) the highest and (9) the lowest.

Group	No. of mice	No. of worms	Total gut eggs ($\times 10^{-3}$)	No. of eggs/ 100 mg faeces	Granuloma diameter
Normal	7	70·4 ± 17·1	59·7 ± 20·1	216·8 ± 103·7 (1)	354 ± 79 (1)
Deprived	8	91·3 ± 19·3	43·8 ± 17·4	5·0 ± 6·5 (9)	142 ± 44 (9)
Dep. + IS	8	88·6 ± 14·5	32·3 ± 13·8	16·0 ± 11·3 (6)	196 ± 50 (7)
Dep. + AI-LNC	8	84·9 ± 14·5	38·3 ± 9·1	93·9 ± 62·0 (2)	282 ± 65 (2)
Dep. + AI-SpC	8	73·5 ± 9·5	31·6 ± 8·0	36·7 ± 26·0 (3)	232 ± 73 (3)
Dep. + CI-LNC	8	75·7 ± 10·4	31·0 ± 16·1	26·3 ± 13·3 (4)	228 ± 65 (4)
Dep. + CI-SpC	8	83·3 ± 14·9	40·0 ± 13·9	12·6 ± 10·0 (8)	196 ± 57 (8)
Dep. + N-LNC	8	76·3 ± 11·9	34·9 ± 6·6	20·5 ± 25·4 (5)	220 ± 56 (5)
Dep. + N-SpC	8	90·5 ± 10·2	39·7 ± 12·7	13·8 ± 11·0 (7)	212 ± 56 (6)

and processed to display ninhydrin-stained eggs as previously described (Doenhoff *et al.* 1978). Adult worms were retrieved from infected mice by perfusion via the hepatic portal vein (Smithers & Terry, 1965) and they were counted on the same day. The intestine from the duodenum to the rectum was removed and kept at −20 °C for later digestion in 5 % KOH for tissue egg counting (Cheever, 1968).

Measurement of granuloma diameters

After perfusion of the mouse the two ventral median lobes of the liver were removed, fixed in formal saline, embedded in wax, and three 5 μm-thick sections cut at intervals of 200 μm. Following haematoxylin and eosin staining and mounting the sections were scanned and the diameters of granulomas around single separate eggs measured with an ocular micrometer as described by von Lichtenberg (1962). The mean liver granuloma diameter for each mouse was estimated after a minimum of 20 lesions had been scored, and the group mean value subsequently calculated from the individual mouse means.

Statistics

The Student *t*-test was used to determine the probability of the difference between group mean values being significant, with values of $P < 0{\cdot}05$ being considered significant.

RESULTS

Results from preliminary unpublished experiments indicated that the degree to which lymphoid cells from *S. mansoni*-infected mice enhanced the rate of egg excretion in infected immunosuppressed recipients varied with the source organ of the transferred cells (spleen or mesenteric lymph nodes) and with the age of the schistosome infections in the donors. Table 1 gives results from an experiment examining the effect of these two parameters.

The results in Table 1 are in agreement with earlier observations in that: (1) heavily infected T cell-deprived mice have liver egg granuloma diameters which are smaller than those in the normal animals, and the mean number of eggs excreted by the deprived mice is reduced by 98 % compared with only 27 % reduction in the intestinal egg count in the deprived animals; and (2) the egg excretion rate and granuloma size were increased marginally in the group of deprived mice that received immune serum injections. The results in the other groups in Table 1 indicate that with respect to enhancement of egg excretion rate and liver egg granuloma size: (i) cells from infected donors were more effective than cells from non-infected normal donors; (ii) transferred mesenteric lymph node cells were more effective than spleen cells; and (iii) cells from donors infected for 8 weeks (AI) were more effective than cells from donors infected for 16 weeks (CI).

The group mean results for faecal egg count and liver egg granuloma diameter in Table 1 showed, with one exception, the same numerical rank order, and regression analysis of the results from individual animals gave a significant positive linear correlation between the two numerical values ($P < 0{\cdot}001$, result not illustrated).

The effect of chronicity of infection on the ability of donor mouse lymphoid cells to facilitate egg excretion was investigated in more detail. Mesenteric lymph node cells were taken from groups of donor

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Table 2. Experiment 1. Granuloma diameters and faecal egg counts in mice reconstituted with lymph node cells from groups of donors infected for different times. Experimental design as in Table 1. Mesenteric lymph node cells were collected from groups of mice infected with 25 cercariae 1, 4, 6, 8, 10, 15 or 20 weeks previously. Each recipient received one injection of 44×10^6 lymphocytes on day 39 after infection with 200 cercariae. Perfusion etc. was on day 47. Experiment 2. Egg excretion rates in deprived mice reconstituted with immune serum. Groups of deprived mice infected with 200 cercariae were given serum from donor mice that had been infected with 25 cercariae between 2 and 16 weeks previously. Each recipient was injected with 0·5 ml serum/day between days 40 and 45 after infection, and the mice were perfused on day 46.

Exp.	Duration of infection in cell/serum donors	No. of mice	No. of worms	Total gut eggs ($\times 10^{-3}$)	No. of eggs/100 mg faeces	Granuloma diameter in recipients (μm)	Granuloma diameter in donors (μm)
1	—	7	112·7 ± 11·0	55·0 ± 15·9	7·5 ± 6·9	160 ± 69	—
	1	6	109·0 ± 22·7	43·0 ± 12·9	37·9 ± 38·2	208 ± 77	—
	4	7	111·8 ± 13·4	50·9 ± 9·2	81·8 ± 56·6	196 ± 90	—
	6	7	117·0 ± 16·2	58·2 ± 9·0	115·9 ± 101·3	192 ± 95	—
	8	6	116·2 ± 15·9	54·1 ± 7·0	291·0 ± 198·6	218 ± 94	305 ± 79
	10	7	87·4 ± 26·1	45·6 ± 8·4	97·6 ± 61·4	211 ± 130	357 ± 36
	15	6	103·2 ± 17·7	46·7 ± 13·2	79·3 ± 105·6	192 ± 81	318 ± 32
	20	7	95·0 ± 29·7	41·7 ± 5·3	37·9 ± 28·7	180 ± 86	258 ± 31
2	—	6	56·5 ± 11·8	31·5 ± 9·8	8·2 ± 8·5	ND*	
	2	6	52·5 ± 9·6	24·1 ± 10·9	4·4 ± 5·3	ND	
	4	6	55·5 ± 10·1	36·2 ± 9·3	13·2 ± 10·5	ND	
	6	6	52·8 ± 16·4	31·5 ± 13·4	10·7 ± 13·2	ND	
	8	6	65·3 ± 13·7	37·7 ± 6·5	47·4 ± 23·1	ND	
	10	6	55·8 ± 6·2	34·1 ± 5·6	92·9 ± 50·9	ND	
	13	6	61·8 ± 11·1	37·5 ± 10·3	74·6 ± 60·0	ND	
	16	6	44·0 ± 10·0	25·9 ± 5·5	64·9 ± 34·8	ND	

* ND = not determined.

mice that had been infected with *S. mansoni* for periods of between 1 and 20 weeks. The cells were transferred to a series of 7 groups of infected deprived mice, with inclusion of an eighth infected, but unreconstituted deprived group.

The results (Table 2, Exp. 1) indicate that the capacity of a given number of cells maximally to enhance egg excretion in the recipients coincided temporally with their ability to generate inflammation around eggs in the recipients' livers, and that the peak response was obtained from 8-week-infected donors. Maximum liver granuloma size in the donor animals was, however, found in mice with 10-week-old infections (last column, Table 2, Exp. 1). To facilitate visualization of these results the group mean values in Table 2 have been plotted in Fig. 1*a* (liver granuloma diameters in cell donors), 1*b* (liver granuloma diameters in recipients) and 1*c* (egg excretion rates in recipients).

As in the preceding experiment, there was a significant positive correlation between the pooled individual mouse values for faecal egg count and mean liver egg granuloma diameter, but at a lower probability level ($P < 0{\cdot}025$, result not illustrated).

To determine at what time after infection serum acquired the capacity to promote egg excretion, blood from a group of normal donor mice was taken at intervals between 2 and 16 weeks after infection with 25 cercariae. The results of transferring 7 pools of serum are given in Table 2 (Exp. 2) and the mean egg excretion rates in the recipient groups have been included in Fig. 1*c*. The cell-dependent effector mechanisms which facilitate egg excretion thus show maximum activity at an earlier time than humoral effector mechanisms, and the serological activity plateaus out at a lower, though subsequently constantly maintained level than the cellular activity.

DISCUSSION

Induction and regulation of schistosome egg-associated immunopathology

Granulomas induced by *S. mansoni* eggs in mice have many of the characteristics of a thymus- or T-dependent cell-mediated delayed-type hypersensitivity reaction (Warren, Domingo & Cowan, 1964). Earlier evidence suggested that naive animals could be sensitized for granuloma formation only with egg antigens (Warren & Domingo, 1970), but recent reports indicate that enhanced responses to injected eggs occur also in mice with unisexual worm infections (Cheever, Lewis & Wynn, 1997; Leptak & McKerrow, 1997). Granulomas induced by eggs of *S. haematobium* and the related species *S. bovis* share with *S. mansoni* granulomas characteristic of T

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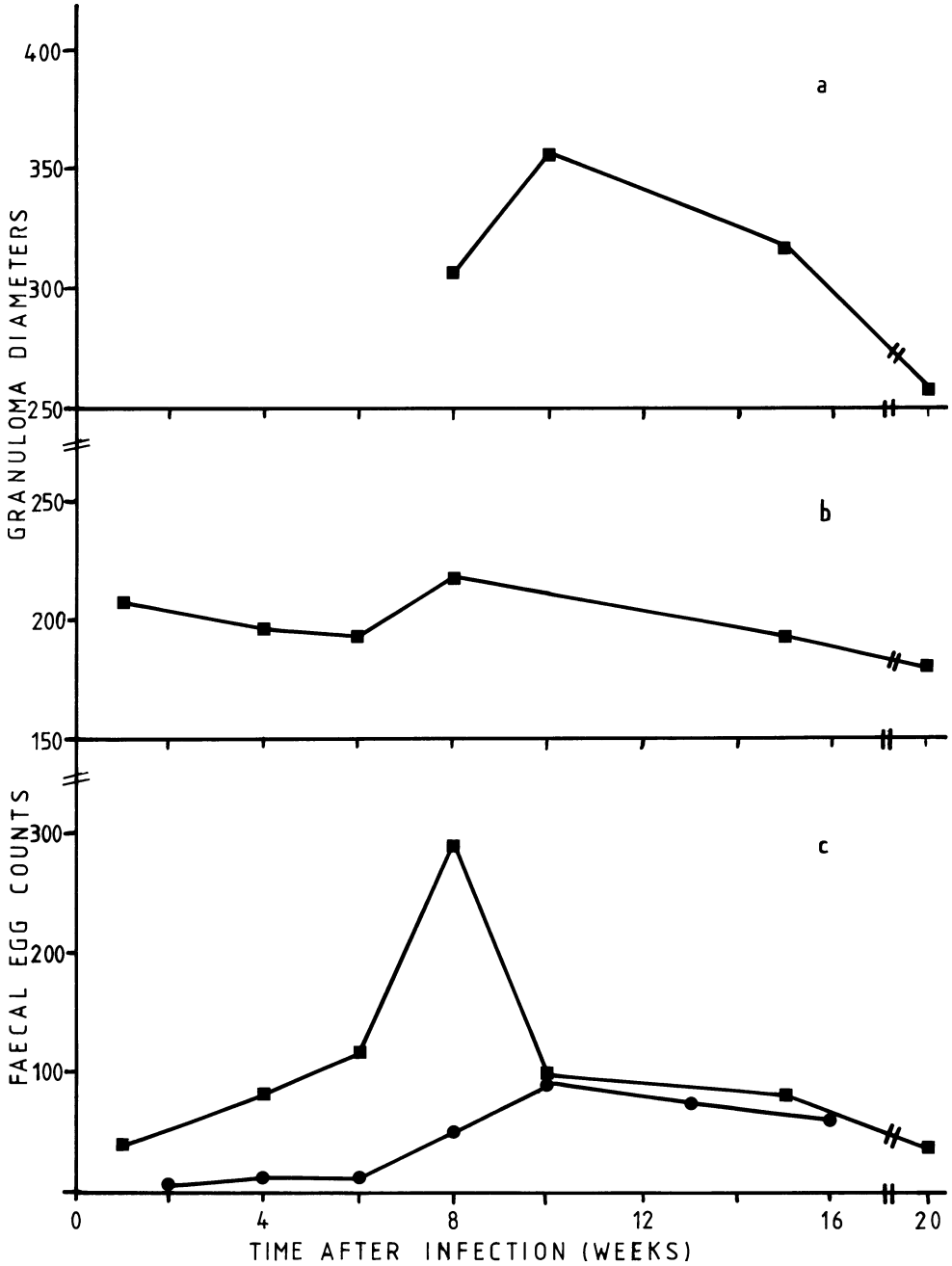

Fig. 1. Liver egg granuloma diameters and egg excretion rates of mice in Table 2. (*a*) Mean granuloma diameters of cell donor mice (last column, Table 2). (*b*) Granuloma diameters of cell recipient mice (6th column, Table 2). (*c*) Group mean egg excretion rates of mice reconstituted with cells (■: Table 2, Exp. 1) or serum, (●: Table 2, Exp. 2).

cell dependence (Murare *et al*. 1987; Agnew *et al*. 1988, 1989), but considerably less work has been done on the former species. Because the aetiology of *S. japonicum* egg granulomas is still not very well defined, this species will be ignored in this review. Space has also precluded exhaustive citation of the literature on *S. mansoni* immunopathology, but see recent reviews by Boros (1994), Lukacs *et al*. (1994), Wynn & Cheever (1995) and Pearce *et al*. (1996*a*.)

Schistosome egg granulomas are dynamic, both in terms of their growth and decay around individual eggs (von Lichtenberg, 1962), and because the immune effector mechanisms mediating granuloma formation undergo modulation as the infection progresses (see below). In contrast to the protoypical granulomatous inflammatory lesion induced by tubercle bacilli, which consist almost entirely of macrophage-like cells (see below), many different cell types are found in schistosome egg granulomas, including eosinophils, macrophages, mast cells, neutrophils, T and B lymphocytes, plasma cells and fibroblasts (Stenger, Warren & Johnson, 1967; von Lichtenberg, Erickson & Sadun, 1973; Moore, Grove & Warren, 1977; Smith, 1977; Epstein *et al*.

https://doi.org/10.1017/S0031182097001972 Published online by Cambridge University Press

1979). The proportions of each cell type present in an egg granuloma depend on the tissue in which the granuloma is located (Weinstock & Boros, 1983), and the time after infection (Chensue & Boros, 1979).

Leptak & McKerrow (1997) have shown that tumour necrosis factor-α (TNF-α), the production of which can be induced in liver cells by infecting with worms, is involved in priming the host for egg granuloma development. Granuloma formation is also dependent on $CD4^+$ T helper cells, and there is a consensus that the activities of Th2 cells, and the cytokines they produce (especially IL-4), are involved in granuloma formation during the early stages of infection (Lukacs & Boros, 1993; Wynn & Cheever, 1995; Jankovic & Sher, 1996). However, the results from IL-4 gene-knockout mice, which give diminished Th2 responses and enhanced Th1 responses, are inconsistent, with the intensity of hepatic pathology having been found to be either unaffected (Pearce *et al*. 1996*b*) or reduced (Metwali *et al*. 1996). Furthermore, SEA-specific Th0 and Th1 clones, as well as Th2 clones, have granuloma formation capability (Chikunguwo *et al*. 1991; Zhu, Lukacs & Boros, 1994). Th1 cell activity may thus not be completely inconsequential in granuloma formation during the early stages of patent infection, but its IgE-dependence (King *et al*. 1997) and the presence in these complex lesions of numerous eosinophils that are IL-5 dependent (Sher *et al*. 1990) argue strongly for the activity being dominated by Th2 cells. Hepatic fibrosis also appears to be particularly dependent on Th2 cell activity (Czaja *et al*. 1989; Cheever *et al*. 1994; Wynn *et al*. 1995*a*), and is perhaps a consequence of a failure to down-regulate hepatic TNF-α production (Adewusi *et al*. 1996) and the lack of anti-egg antibody of appropriate idiotype (Montesano *et al*. 1997).

After 10 weeks of infection in mice mean liver granuloma size begins to diminish (Andrade & Warren, 1964; Cheever, 1965), a process termed 'endogenous desensitization' (Domingo & Warren, 1968) or 'spontaneous modulation' (Boros, Pelley & Warren, 1975). Modulated lesions have a reduced ratio of $CD4^+$ helper T cells (Ragheb & Boros, 1989) and a higher percentage of B cells (Chensue & Boros, 1979), as well as an intrinsic capacity to synthesize immunoglobulins and specific antibody (Boros, Amsden & Hood, 1982). Infected mice with modulated granulomas show decreased activity in tests of cell-mediated immunity such as antigen-stimulated lymphocyte blastogenesis, delayed hypersensitivity responses to egg antigens and MIF production, and increased evidence of humoral immunity (Boros, Warren & Pelley, 1973; Boros *et al*. 1975; Colley, 1975).

Cytokines associated with Th1 cell differentiation and activity, particularly IL-12 and interferon-γ, suppress Th2 cell-mediated granuloma formation (Chensue *et al*. 1992, 1994; Wynn *et al*. 1994, 1995*b*), implicating Th1 cells in down modulation of granulomatous activity. $CD8^+$ T cells have also been implicated (Chensue & Boros, 1979; Chensue *et al*. 1993), and although *S. mansoni* egg granuloma formation cannot be suppressed by antibody with specificity for egg antigens (Colley, 1976), anti-idiotypic antibodies appear to exert a regulatory influence in infections of both humans (Montesano *et al*. 1990) and mice (Bosshardt, Nix & Colley, 1996).

The biological role of schistosome egg-induced immunopathology : host protection or parasite survival?

The pathological consequences of schistosome egg-induced inflammation have been well documented (Warren, 1973), and as the above brief review demonstrates, considerable progress has been made in defining the cellular and molecular factors which induce and regulate granuloma formation. Less consideration has been given to defining the adaptive advantages of granuloma formation for the host and/or the parasite?

The intuitive assumption is that the egg granuloma is an immune response which, like most others, is induced to defend the host (von Lichtenberg, 1964) and that the pathological sequelae are but a consequence of immunological over-reactivity. There is, however, no consistency between schistosome species with regard to the apparent relative toxicity of their eggs. Thus, while evidence of hepatotoxic potential has been found for *S. mansoni* eggs in immunosuppressed mice (Buchanan *et al*. 1973; Byram & von Lichtenberg, 1977; Byram *et al*. 1979; Lucas *et al*. 1980), a factor which may in turn contribute to the infected immunosuppressed animals dying earlier than comparably infected, immunologically intact controls (Doenhoff *et al*. 1979; Lucas *et al*. 1980), the same does not hold for *S. bovis* or *S. haematobium* eggs. Granuloma formation around eggs of the latter two species is as T cell-dependent as for *S. mansoni* eggs, but no evidence of toxicity was seen around *S. bovis* and *S. haematobium* eggs in livers of immunosuppressed mice, even when the eggs were present in relatively large numbers (Murare *et al*. 1987; Agnew *et al*. 1988). T cell-deprived mice infected with *S. bovis* in fact survived longer than comparably infected intact controls (Murare *et al*. 1987), a result which is commensurate with the assertion first made by Ken Warren, that schistosomiasis is an 'immunologic disease' (Warren, 1975). Furthermore, *S. mansoni* egg-induced hepatotoxicity is more effectively neutralized by transfer of immune serum from homologously-infected intact donors than by transfer of immune lymphocyte populations, even though the serum acts without concomitant restoration of granuloma formation (Byram *et al*. 1979; Doenhoff

et al. 1981; Hassounah & Doenhoff, 1993). The hepatoprotective activity in infected donor serum has been attributed to antibodies which react with ω-1, a 31 kDa *S. mansoni* egg glycoprotein (Dunne *et al*. 1981; Dunne, Jones & Doenhoff, 1991) that is stage- and species-specific (Dunne *et al*. 1984; Murare *et al*. 1992). The activity is transferable with serum generated as early as 8 weeks after infection of the serum donors (Doenhoff *et al*. 1981) i.e. at the time when, in terms of lesion size, granulomatous activity around eggs is still at a maximum level in the mouse experimental system.

In contrast to the discrepancy between schistosome species with respect to the hepatotoxic potential of their eggs, rates of egg excretion of both *S. mansoni* (Doenhoff *et al*. 1978) and *S. bovis* (Murare & Doenhoff, 1987) are severely impaired in immunosuppressed mice. *S. mansoni* egg excretion rates can be partly restored in infected T cell-deprived mice by transfer of serum or lymphocyte populations from infected normal donor mice (Doenhoff *et al*. 1978, 1985, 1986). In these earlier experiments, and in the experiments reported above, there was a significant correlation between the respective numerical values for the number of schistosome eggs found in the faeces and the mean liver granuloma diameter in individual animals.

The results in Table 2 and Figs. 1*b* and 1*c* confirm the efficacy of immune cells in mediating *S. mansoni* egg excretion in homologously infected immunosuppressed recipients, with the group of mice that received cells from 8-week-infected donors showing both the highest rate of egg excretion and greatest mean granuloma size in the liver. However, maximum granuloma size occurred at a later time in the donor mice than in the recipients. This is probably due to the lymphocytes which mediate granuloma formation having become sensitized in lymphoid organs in the infected donor mice some time before they were removed and induced to participate in formation of the lesions around liver-bound eggs in the recipients.

Granuloma size in recipients given cells from donors with 1-week-old infections was enhanced when compared with unreconstituted controls, but this was without concomitant induction of an ability to enhance egg excretion rates. This may be due to the recently incoming larvae bearing some antigens that are cross-reactive with egg antigens (Dunne & Bickle, 1987), but which sensitize for inflammatory activity around eggs that is irrelevant to egg excretion.

The results in Table 1 indicate that transferred syngeneic mesenteric lymph node cells are more effective than an equivalent number of spleen cells in mediating schistosome egg excretion. This may not be due solely to the presence of a proportionately greater number of T cells in lymph nodes than in spleen, as in a separate unpublished experiment groups of infected deprived mice given twice as many spleen as lymph node cells still excreted fewer eggs. The discrepancy between spleen and lymph node cells is noteworthy since the spleen has been used almost exclusively as the source of lymphoid cells in investigations into the immune aetiology of schistosome egg granulomas. Cells involved in the egg excretion process and which mediate in granulomatous activity may, however, be preferentially sensitized and/or retained in the mesenteric lymph nodes of infected mice.

Cell-mediated immunological activity induced by schistosome eggs appears therefore to have a pivotal role in the process of schistosome egg excretion, at least during early infection patency. Extensive histological observations on *S. mansoni*-infected baboons likewise led Damian (1987) to propose that 'the granuloma is the agent of translocation (of a schistosome egg) from the site of oviposition … to the intestinal lumen'. Evidently, eggs in the liver and the granulomas formed around them play no direct role in parasite transmission. The liver is, however, the site where schistosome eggs are first found in substantial numbers after infection, and antigens from these early eggs are likely to initiate sensitization of the host for granuloma formation.

There is still much to elucidate about the egg excretion process at the cell and molecular levels. The identity and properties of the antigens which sensitize for granuloma formation and egg excretion have to be determined, and the manner in which the sensitized lymphocytes and the subsequently generated specific antibodies mediate egg excretion also has to be worked out. Are the sensitizing antigens the same for both the cell- and antibody-mediated events? It also remains to be established whether the post-8-week down-turn in cell-mediated activity noted in Fig. 1*c*, and the concomitantly increased serum-borne activity, have their counterparts in human infection. While the time-spans of this infection in mice and humans bear no comparison, faecal eggs counts do routinely reduce from their maxima as patients age in endemic areas.

Host factors other than those responsible for adaptive immunity are also involved in schistosome egg excretion. Thus, schistosome eggs are potent aggregators of blood platelets, and excretion rates are impaired in platelet-depleted mice (Ngaiza & Doenhoff, 1990). Platelets may aggregate around eggs as soon as they emerge from the female into the bloodstream, but it is not known whether they just serve to anchor the eggs to the endothelial surface against blood flow, or actively help the eggs penetrate through. Schistosome eggs interact actively with vascular endothelial cells (Freedman & Ottesen, 1988; Ngaiza, Doenhoff & Jaffe, 1993; File, 1995), but again we do not yet know much about the nature of this interaction, nor how the integrity of the endothelium is breached. And how does the granu-

loma which forms around the egg as it traverses the intestine wall act a 'translocation' device (Damian, 1987)?

Justification for a continuation of the experimental research on immune-dependent schistosome egg excretion is provided by the novel results of Karanja *et al*. (1997) who showed that *S. mansoni*-infected people in Kenya that were concurrently sero-positive for HIV had significantly (approximately 3-fold) lower *S. mansoni* faecal egg counts than HIV sero-negative people exposed to similar levels of schistosome infection. Control for variation in schistosome infection intensity levels between the 2 patient groups was provided by analysis of concentrations of a parasite worm-derived circulating antigen (Barsoum *et al*. 1991). The pathogenic effects of HIV infection are complex and heterogeneous, and perhaps more difficult to control for. However, the HIV positive group had a significantly lower mean $CD4^+$ blood cell count and a significant positive correlation between faecal egg counts and the percent of $CD4^+$ cells in peripheral blood was found in this group.

A role for granulomatous inflammation in the transmission of tuberculosis

Schistosome egg granulomas were initially called 'pseudotubercles' in acknowledgement of their similarity with the lesions which occur in host tissue infested with *Mycobacterium tuberculosis* (MTB). In contrast to the mixed cell nature of schistosome egg granulomas, however, MTB granulomas are comprised of macrophages or macrophage-derived epithelioid cells alone.

Lung tissue is the commonest site for mycobacterial growth and pathogenesis in tuberculosis patients. Although growth of MTB and ensuing pathology can occur in tissues other than the lungs, extrapulmonary lesions do not contribute to onward transmission of infection (similarly to those schistosome eggs which become trapped in tissues other than those from which they can be excreted). Transmission of the infection depends on bacteria being released from lung tissue into the airways and subsequent expectoration from the body in aerosol droplets, particularly during bouts of coughing. Transmission from index cases to secondary contacts occurs mainly within households or between individuals living at close quarters (e.g. in hostels or hospital wards) (Murray, 1990), and it is estimated that a minimum density of approximately 5000 bacterial cells per mL of saliva is necessary for transmission to occur (Grange, 1996). In one study it was estimated that children living in a household with a tuberculosis sputum smear-positive index case had approximately 8 times more chance of becoming infected than children in a household with a smear-negative source case (Geuns, van Meijer & Styblo, 1975).

Four to five distinguishable stages have been identified in the disease process of tuberculosis in a rabbit model system, namely: onset, symbiosis, initial caseous necrosis, liquefaction and cavity formation (Dannenberg, 1991; Dannenberg & Rook, 1994). In the first stage of infection the inhaled bacterium is ingested by a macrophage and the process may end there if, as is often the case, the micro-organism is destroyed. The 'symbiotic' second stage occurs if the bacterium survives and replicates, and a lesion consisting of pathogen-loaded macrophages develops. The third stage begins when logarithmic growth of the microbe is halted, assumedly as a result of control by induced cell-mediated immune (CMI) and delayed-type hypersensitivity (DTH) responses. Lesions may regress with destruction of bacteria or become necrotic, depending on the infection resistance/susceptibility status of the host. If the disease progresses to the final stages the lesions liquefy and host lung tissue cavitates, and these extremes may occur even if host CMI responses are well developed. In tuberculosis, as in schistosomiasis, the complicated interplay between the pathogen and cells of the host immune system, mediated to a large extent by host cell-derived cytokines (Dannenberg, 1994; Rook & Bloom, 1994) is still being intensively investigated.

Exit of mycobacteria from the body occurs particularly when the disease has progressed to give liquefaction and cavitation of lung tissue. Despite host immunity, the liquefied lung tissue occurring in the final stages of the disease process appears to provide an especially good environment for growth of the bacteria (Dannenberg, 1991) and following tissue necrosis and rupture (cavitation) the micro-organisms are discharged into the airways.

Two counterintuitive aspects of tuberculosis pathogenesis are noteworthy in the present context: (1) the frequency of liquefied caseous foci and cavitation tends to be less in immunosuppressed individuals (Dannenberg, 1991) and in those infected with HIV (Pitchenik & Rubinson, 1985; Pozniak *et al*. 1995; Haramati, JennyAvital & Alterman, 1997) than in tuberculous subjects with otherwise uncompromised immune responsiveness; and (2) rabbits selectively bred for enhanced resistance against infection with MTB suffered more severely from tissue liquefaction and cavitation than rabbits selected for increased susceptibility (Lurie & Dannenberg, 1965). Furthermore, delayed-type hypersensitivity to MTB antigens has been shown to be involved in the liquefaction reaction (Yamamura *et al*. 1974).

In view of the above two observations it is perhaps less surprising that several studies have shown that patients with tuberculosis and who were sero-positive for HIV infection were more likely to have sputum that was smear- or culture-negative for MTB than those with tuberculosis, but who were

HIV sero-negative (Klein *et al*. 1989; Elliott *et al*. 1990, 1993*a*, *c*; Long *et al*. 1991*a*; Pozniak *et al*. 1995). The concentration of bacteria in sputum of HIV +ve patients is also lower than in that of HIV −ves (Elliott *et al*. 1990, 1993*a*). In some other studies concurrent HIV infection had either no or only a marginal effect on MTB sputum smear positivity (Githui *et al*. 1992; Long *et al*. 1991*b*; Houston *et al*. 1994; Smith *et al*. 1994), but in these instances the HIV +ve and HIV −ve groups had been included in the study only if there was previous clinical evidence, including sputum smear- and culture-positivity, which indicated that they had tuberculosis.

Although the heterogeneity of HIV-induced pathology and $CD4^+$ cell counts were generally not controlled for in these studies, there seems to be little published work in which the prevalence of tuberculosis-positive sputum smears was found to be markedly higher in HIV +ve than in HIV −ve subjects, nor any evidence that the rate of growth of mycobacteria is inhibited in the lung tissue of the former patients to account for these results. Indeed, the increased mortality rates suffered by late-stage HIV patients with tuberculosis is assumed to be due to the opportunistic bacterial infection (de Cock *et al*. 1992).

A practically important consequence of the failure of mycobacteria to enter the airways as a result of immunosuppression is the delay in diagnosis of tuberculosis in HIV-infected patients which has been noted (Kramer *et al*. 1990), and attributed in part to a reduced sensitivity of sputum-smear examinations in these subjects.

At least 2 studies have shown that tuberculosis patients with AIDS or HIV infection were less infectious (with respect to transmission of the bacterial infection) than HIV −ve tuberculosis patients (Elliott *et al*. 1993*b*; Cauthen *et al*. 1996). In a third study investigating secondary transmission (Klausner *et al*. 1993) there was no evidence for such a difference, but in this instance again only sputum smear-positive individuals had been selected as index patients.

That cell-mediated immune responses play a vital role in controlling the growth of mycobacteria (Dannenberg, 1994) is not being questioned here. The studies summarized above do, however, suggest that, as with schistosomiasis, the tubercle bacillus gains advantage from host-defensive cell-mediated granulomatous inflammation in terms of facilitated transmission to new hosts.

ACKNOWLEDGEMENTS

Assistance from Dr O. A. Hassounah and Ms J. Bain and financial support from the Wellcome Trust, London, are gratefully acknowledged with respect to completion of the experimental schistosome work described in the results section above. Critical evaluations of the manuscript by Dr S. Gillespie (Royal Free Hospital, London) and Dr S. Lucas (UMDS, London) helped considerably to improve it. This paper is dedicated to the memory of the late Dr Kenneth S. Warren in appreciation of his considerable positive influence on the course of schistosomiasis research, first as an experimentalist and subsequently as administrator and scientometrist.

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