Experimental Models of *Schistosoma mansoni* Infection

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Experimental models of *Schistosoma mansoni* infections in mammals have contributed greatly to our understanding of the pathology and pathogenesis of infection. We consider here hepatic and extrahepatic disease in models of acute and chronic infection. Experimental schistosome infections have also contributed more broadly to our understanding of granulomatous inflammation and our understanding of Th1 versus Th2 related inflammation and particularly to Th2-mediated fibrosis of the liver.

Key words: *Schistosoma mansoni* - experimental models - schistosomiasis
has been employed less frequently. Some investigators have found naive mice nonresponsive to eggs injected into the portal vein (Leptak & McKerrow 1997) and that portal (Cuison et al. 1995) or enteric (Weinstock et al. 1985) injection of eggs induced tolerance to eggs subsequently injected. Others have not noted this effect and have used portal injection as they would the lung model (Edungbola & Schiller 1979, Raso et al. 1983, Eltoum et al. 1995, Jacobs et al. 1997a, 1999).

Granulomas in the mouse lung induce much less fibrosis than granulomas in the liver and although it is possible to study fibrosis in the lung model (Boros et al. 1983, Metzger & Peterson 1988), the high background levels of matrix and the low levels of fibrosis induced (Cheever et al. unpublished) complicate this use of the lung model. Examination of collagen mRNA levels in the lung (Warmington et al. 1999) will doubtless be increasingly used as will determination of mRNA for proteases.

GENERAL CONSIDERATIONS IN THE INTERPRETATION OF MODELS OF SCHISTOSOME INFECTIONS

A. The intensity of experimental schistosome infections is generally extremely high. A single S. mansoni worm pair in a mouse may be equivalent to more than 1,000 pairs in an infected person (Cheever 1969, Gryseels & de Vlas 1996).

B. Most schistosome infections in humans are acquired gradually over years while most experimental infections are given as a single exposure. When a mouse is given multiple inocula, the cumulative intensity of infection becomes progressively less realistic.

C. Most humans exposed to schistosomiasis are born to mothers who are or have been infected. There are ample reasons to think that in utero exposure to schistosome antigens or to idiotypic or anti-idiotypic antibodies may modify the response to subsequent infection in humans (Eloi-Santos et al. 1989, Novato-Silva et al. 1992) and in mice (Montesano et al. 1997, 1999a, b).

D. The chronicity of schistosome infections in humans is obviously not reproducible in most animal models. Although one may predict that in some respects a week or two in the life of a mouse may be equivalent to a year in humans, the calculation of equivalent times is uncertain.

E. While different strains (isolates) of S. mansoni clearly behave differently in laboratory hosts it is unclear if these patterns are relevant to human infections. Strains from patients with acute schistosomiasis or hepatosplenic disease did not differ in the pathology they produced in mice (Costa & Katz 1982, Costa et al. 1984) but Thiongo et al. (1997) found differences in egg production and egg passage in the feces of mice infected with different Kenyan strains of S. mansoni and felt these might be related to clinical differences in infected humans.

However, a given isolate may produce one pattern of infection (e.g. a higher proportion of eggs in the liver) in mice (Anderson & Cheever 1972) and a different pattern in monkeys (Powers & Cheever 1972). Rapid changes in isoenzyme patterns occur during successive generations of newly isolated S. mansoni strains in mice so that it is clear that genetic selection may occur rapidly in the laboratory (LoVerde et al. 1985, Bremond et al. 1993). Pinto et al. (1997) recently documented the greater genetic variability in worms from field isolates compared to the LE strain long maintained in the laboratory. Passage in the molluscan host may also result in genetic selection of the worms (Richards & Shade 1987).

### TABLE I

<table>
<thead>
<tr>
<th>Species and References</th>
<th>Development Early/Late</th>
<th>Fecundity Early/Late</th>
<th>Fecal egg excretion Early/Late</th>
<th>Hepatic fibrosis General/Pipesistem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimpanzee1</td>
<td>N / N</td>
<td>N / N</td>
<td>N / N</td>
<td>+++ / +++, or +/+/+++13</td>
</tr>
<tr>
<td>Baboon2a and</td>
<td>N / N</td>
<td>N / ↓</td>
<td>N / ↓</td>
<td>+-- / 0</td>
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<td>Cercopithecus monkey2b</td>
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<td>N / N?</td>
<td>N / N?</td>
<td>+-- / 0</td>
</tr>
<tr>
<td>Capuchin3</td>
<td>N / N</td>
<td>N / N?</td>
<td>N / N?</td>
<td>+-- / 0</td>
</tr>
<tr>
<td>Rhesus4</td>
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<td>N / ↓↓</td>
<td>N / ↓↓</td>
<td>+-- / 0</td>
</tr>
<tr>
<td>Aotus5</td>
<td>N / N</td>
<td>N / N</td>
<td>N / N</td>
<td>+ / 0</td>
</tr>
<tr>
<td>Stump tail macaque6</td>
<td>N / N?</td>
<td>N / N?</td>
<td>N / N?</td>
<td>+ / 0</td>
</tr>
<tr>
<td>Cynomolgus7</td>
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<td>N / N?</td>
<td>N / ↓?</td>
<td>+ / 0</td>
</tr>
<tr>
<td>Squirrel monkey8</td>
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<td>↓ / ↓</td>
<td>↓ / ↓</td>
<td>+ / 0</td>
</tr>
<tr>
<td>Marmoset9</td>
<td>↓ / ?</td>
<td>↓ / ↓</td>
<td>0 / 0</td>
<td>+ / 0</td>
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<tr>
<td>Tree shrew10</td>
<td>↓ / ↓↓</td>
<td>↓ / ↓</td>
<td>0 / 0</td>
<td>+ / 0</td>
</tr>
<tr>
<td>Tamarin11</td>
<td>N / N</td>
<td>N / N</td>
<td>N / ↓</td>
<td>+ / 0</td>
</tr>
</tbody>
</table>

N: normal, i.e. similar to a permissive host such as the mouse; ↑ ↓: increased or decreased; ?: unknown; 0: absent.

TABLE II

<table>
<thead>
<tr>
<th>Species and References</th>
<th>Development Early/Late</th>
<th>Fecundity Early/Late</th>
<th>Hepatic fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit(^1)</td>
<td>↓↓↓ / ↓↓↓</td>
<td>↓↓↓ / ↓↓↓</td>
<td>+</td>
</tr>
<tr>
<td>Opossum(^2)</td>
<td>↓ / ↓</td>
<td>N / ?</td>
<td>+</td>
</tr>
<tr>
<td>Armadillo(^3)</td>
<td>↓ / ↓</td>
<td>↓ / ↓</td>
<td>±</td>
</tr>
<tr>
<td>Raccoon(^4)</td>
<td>↓↓ / ?</td>
<td>↓↓ / ?</td>
<td>±</td>
</tr>
<tr>
<td>Skunk(^5)</td>
<td>↓↓ / ?</td>
<td>↓↓ / ?</td>
<td>0</td>
</tr>
<tr>
<td>Red fox(^6)</td>
<td>R</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

R: resistant to infection; NA: not applicable

1: Sylvilagus floridanus (Lichtenberg et al. 1962, Andrade et al. 1988); 2: Didelphis marsupialis (Lichtenberg et al. 1962); 3: Dasypus novemcinctus (Smith et al. 1988); 4: Procyon lotor (Lichtenberg et al. 1962); 5: Mephitis nigra (Lichtenberg et al. 1962); 6: Vulpes fulva (Lichtenberg et al. 1962).

Numerous other species have been exposed but little or no description of the pathology was noted; e.g. Loos (1964) exposed Erinaceus europaeus, Sorex araneus, Sciurus vulgaris, Glis glis, Chlethrionomys glareolus, Microtus arvalis, Ondatra zibethica, Microcyon minutus, Apodemus flavicollis, Rattus rattus, Rattus norvegicus and Apodemus sylvaticus. Stirewalt et al. (1951) exposed cats, dogs, three varieties of rabbits, cotton rats and albino rats (Sigmodon hispidus hispidus and Mus norvegicus albinus) in addition to mice and hamsters. Kunz and Malakatis (1955) exposed Mus musculus pratettus, Rattus rattus, Arvicantus niloticus, Acomys cahirinus, Gerbillus pyramiculus, Jaculul jaculus, Meriones s. shawi, Psammomys o. obesus, Nesokia indica suilla, Hemiechinus auritus aegypticus, Mustela nivalis subpalmata, Hesperustes i. icheunon and Vulpes v. aegyptiaca. Torrealba et al. (1958) exposed Didelphis marsupialis, Callomurus trinitatis venezuelae, Cebus nigritus and Pecari tajacu torvus.

**ACUTE TOXEMIC SCHISTOSOMIASIS**

Humans infected for the first time with *S. mansoni* often develop an acute disease characterized by fever, malaise, diarrhea, intense eosinophilia and occasionally allergic manifestations such as asthma or angioedema (hives). Symptoms may appear before the onset of oviposition and are accentuated after oviposition. Acute disease is virtually unknown in residents of endemic areas but is frequent in outsiders exposed for the first time. Acute toxemic schistosomiasis is associated with high levels of immune complexes and with a vigorous cellular response to schistosome antigens (Hiatt et al. 1980). Symptoms, signs and immune reactivity decrease over a period of months while the infection continues unabated. An acute toxemic phase is obvious in many animal models judging from the appearance of the animals and from an initial vigorous cellular immunity and high eosinophilia. As the infection becomes “chronic”, at 10-20 wks, the appearance of the animals improves and the cellular response to antigen is downregulated while egg laying by the parasite continues unchanged (Tawfik et al. 1986, Damian et al. 1992). Particular efforts to study the toxemic phase have been made in baboons, which exhibit fever as well as the other features noted in mice and other animal models (Damian et al. 1992, 1996). It is unclear, however, how to relate the experimental acute disease to that in humans. The cytokine patterns which may be related to the fever, malaise and other symptoms and signs of the acute phase in baboons included TNF-α, IL-1 and IL-6 (Damian et al. 1996), findings remarkably similar to those later reported in humans (Jesus et al. 2002).

Decreased levels of corticotropin-releasing hormone, adrenocorticotropic hormone and dehydroepiandrosterone were reported by Morales-Montor et al. (2001) in acutely infected baboons and mice but not in rechallenged chronically infected baboons. In baboons, but not mice, the lower hormone levels correlated with unmodulated granulomas.

**THE FORMATION OF CIRCUMOVAL GRANULOMAS AND MODULATION OF GRANULOMA SIZE**

Studies in infected mice often give different results than those obtained from intravenous injection of eggs. A general overview will be presented here and differences between the lung model and the use of infected mice will be detailed later.

It has long been clear that T helper cells (CD4+ T cells) are instrumental for the formation of granulomas around *S. mansoni* eggs (Mathew & Boros 1986) and that CD8+ T cells are instrumental for the formation of granulomas around *S. mansoni* eggs (Mathew & Boros 1986). As the infection becomes "chronic", at 10-20 wks, the appearance of the animals improves and the cellular response to antigen is downregulated while egg laying by the parasite continues unchanged (Tawfik et al. 1986, Damian et al. 1992). Particular efforts to study the toxemic phase have been made in baboons, which exhibit fever as well as the other features noted in mice and other animal models (Damian et al. 1992, 1996). It is unclear, however, how to relate the experimental acute disease to that in humans. The cytokine patterns which may be related to the fever, malaise and other symptoms and signs of the acute phase in baboons included TNF-α, IL-1 and IL-6 (Damian et al. 1996), findings remarkably similar to those later reported in humans (Jesus et al. 2002).

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**TABLE III**

| Species                | Development Early/Late | Fecundity Early/Late | Hepatic fibrosis (mice=+++)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Woodchuck(^1)</td>
<td>N / ↓↓↓</td>
<td>N / ↓↓↓</td>
<td>+</td>
</tr>
<tr>
<td>Calomys(^2)</td>
<td>N / ?</td>
<td>N / N</td>
<td>±</td>
</tr>
<tr>
<td>Mastomys(^3)</td>
<td>N / N</td>
<td>N / N</td>
<td>+</td>
</tr>
<tr>
<td>Rat-lat(^4)</td>
<td>1 / sc</td>
<td>↓↓↓ / sc</td>
<td>±</td>
</tr>
<tr>
<td>Rattus rattus(^5)</td>
<td>&gt;&gt; lab rat</td>
<td>&gt;&gt; lab rat</td>
<td>?</td>
</tr>
<tr>
<td>Hamster(^6)</td>
<td>N / N</td>
<td>N / N</td>
<td>±</td>
</tr>
<tr>
<td>Gerbill(^7)</td>
<td>N / N</td>
<td>N / N</td>
<td>±</td>
</tr>
<tr>
<td>Gray squirrel(^8)</td>
<td>N / ?</td>
<td>N / ?</td>
<td>+</td>
</tr>
<tr>
<td>Chipmunk(^9)</td>
<td>↓↓ / ?</td>
<td>↓↓ / ?</td>
<td>±</td>
</tr>
<tr>
<td>Nutria(^10)</td>
<td>↓ / ?</td>
<td>↓↓ / ?</td>
<td>±</td>
</tr>
<tr>
<td>Meadow vole(^11)</td>
<td>↓ / ?</td>
<td>↓↓ / ?</td>
<td>±</td>
</tr>
<tr>
<td>Muskrat(^12)</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Guinea pig(^13)</td>
<td>↓ / ?</td>
<td>↓ / ?</td>
<td>? / ? +</td>
</tr>
<tr>
<td>Agouti(^14)</td>
<td>N / ?</td>
<td>N / ?</td>
<td>+</td>
</tr>
</tbody>
</table>

R: resistant to infection; ↑ ↓ increased or decreased; ±: slight change; N: normal; ?: insufficient data; sc: self cure, i.e. adult worms are killed.

1: Marmota monax (Anderson et al. 1991, Andrade et al. 2001); 2: Calomys callosus (Lenzi et al. 1995); 3: Mastomys coucha (Cheever 1965a, b); 4: (Phillips et al 1987); 5: (Denoya et al 1987, Cêtre et al. 1999); 6: Mesocricetus auratus (Cheever 1965a, b); 7: Pachyomys duprasi natronensis (Cheever 1965a, b); 8: Sciurus carolinensis (Lichtenberg et al. 1962); 9: Tamias striatus (Lichtenberg et al. 1962); 10: Myocaster coypus (Lichtenberg et al. 1962); 11: Microustus pennsylvanicus (Lichtenberg et al. 1962); 12: Ondatra zibethicus (Lichtenberg et al. 1962); 13: (Hsu et al. 1973, Pearce & McLaren 1983); 14: Dasyprocta aguti (Price 1953).
T cells appear to be important for downregulation of granuloma size in chronic infections (Chensue & Boros 1979, Henderson et al. 1992), but antibody is necessary in addition to downregulated T cells (Jankovic et al. 1998).

In acute infections (8 wk) regulation of granuloma size seems to differ fundamentally from downregulation in chronic infection. Thus in acute infection granuloma size seems controlled principally by T cells and volume is decreased by IL-10 (Wynn et al. 1998) and increased by administration of cyclophosphamide or cymetidine (Weinstock & Boros 1981, Weinstock et al. 1983). Cyclophosphamide also increased granuloma size in chronic infections and one needs to postulate that the effect on T cells can partially overcome the effects of immunoglobulins (Colley et al. 1979, Weinstock et al. 1983). Antibody has a significant but modest effect in acute infections (Jankovic et al. 1998).

Delayed type hypersensitivity (DTH) is commonly taken to be indicative of a Th1-type cellular reaction, although there are several instances in which contact hypersensitivity etc. have been shown to be predominantly a Th2-type response (Assherson et al. 1996). The reaction to schistosome eggs is a cell-mediated hypersensitivity reaction which has usually been considered to be a DTH reaction. We think that the evidence indicates that the reaction to schistosome eggs is predominantly Th2 and propose that the schistosome granuloma be considered as a type-2 DTH, although it is clear that the granuloma can begin as a Th1-type response and can also later be manipulated toward Th1. Rakasz et al (1998) found that granulomas contained numerous activated Th1 and Th0 cells but that these were under tight control.

Lenzi et al. (1998) have given a detailed morphogenic and biomechanical description of granuloma development and involution and have detailed the spatial deployment of collagen fibers within the granulomas (Lenzi et al. 1999).

The Role of Cytokines in Granuloma Formation and Downregulation (See Tables IV-VI)

Several comprehensive reviews dealing with this subject have been published recently (Lukacs & Boros 1993, Wynn & Cheever 1995, Cheever & Yap 1997, Cheever et al. 1998, Fallon 2000) and the discussion here will be oriented toward the models described. Adhesion molecules such as ICAM-1 are presumably important for circulating cells to reach the site of the granuloma (Langley & Boros 1995, Jacobs et al. 1997b) and adhesins are upregulated in acute and chronic murine infections.

The earliest hepatic granulomas form in a Th1 environment with downregulation of granuloma size and hepatic fibrosis (Czaja et al. 1989a) while chronically infected (immunologically downregulated) mice treated with IL-4 make larger granulomas than do untreated animals (Yamashita & Boros 1992). IL-4 and IL-13 are largely compensate for each other for formation of hepatic granulomas in infected mice. Thus only minute granulomas are formed when both are suppressed, as in IL-4 receptor ko mice (Jankovic et al. 1999) or IL-4 ko mice in which IL-13 action is suppressed (Chiramonte et al. 1999b). Kaplan et al. (1998) found that Stat6 knockout mice mounted a minimal Th2 response and formed small granulomas. Stat4 deficient mice showed a minimal Th1 response but normal hepatic granulomas. Wynn et al. (1995a) found that immunization with SEA and IL-12 produced immune deviation toward Th-1 type reactions with reduction of granuloma size. All of these effects are consistent with the concept of the granuloma as a Th2 dominant reaction, but under some conditions Th1 granulomatous responses may be predominant and damaging (Stadecker & Hernandez 1998, Chen & Boros 1999, 2001, Hoffmann et al. 2000, Rutitzkky et al. 2001). cDNA microarrays are a powerful tool for examining arrays of activated genes in schistosome infected-mice with Th1 and Th2 type mediated reactions and led to recognition of the importance of neutrophils in hepatic lesions of wild type and immune deviated mice (Hoffmann et al. 2001).

<table>
<thead>
<tr>
<th>Type of mouse and References</th>
<th>Hepatic granuloma size Early/Late</th>
<th>Hepatic fibrosis</th>
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</thead>
<tbody>
<tr>
<td>Germfree 1a</td>
<td>↓?/↓?</td>
<td>↓</td>
</tr>
<tr>
<td>Inbred 2</td>
<td>V↓/↓</td>
<td>+ to +++</td>
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<tr>
<td>Biozzi 2a</td>
<td>HIII &gt; LIII</td>
<td>HIII = LIII</td>
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<tr>
<td>Immuno-deficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nude 3a</td>
<td>↓↓↓</td>
<td>±</td>
</tr>
<tr>
<td>SCID 3b</td>
<td>↓↓↓</td>
<td>±</td>
</tr>
<tr>
<td>W/Wy 3c</td>
<td>=?/ =</td>
<td>=</td>
</tr>
<tr>
<td>bg/bg 3d</td>
<td>=/?/ =</td>
<td>=</td>
</tr>
<tr>
<td>XID 3e</td>
<td>↓= /</td>
<td>=</td>
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<tr>
<td>Transgenic IL-7</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>IL-99</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>IL-9pataneous 9</td>
<td>=/?</td>
<td>↑</td>
</tr>
<tr>
<td>TGF-β1 4a</td>
<td>=/?</td>
<td>↑</td>
</tr>
<tr>
<td>Knockout CD8 5a</td>
<td>= /</td>
<td>=</td>
</tr>
<tr>
<td>β-2mic 5b</td>
<td>= /</td>
<td>=</td>
</tr>
<tr>
<td>TAP 1 5c</td>
<td>= /</td>
<td>=</td>
</tr>
<tr>
<td>TGF-β1 +/- 5t</td>
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<td>=</td>
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<tr>
<td>ClassII MHC 5v</td>
<td>↓↓↓↓↓↓/?</td>
<td>↓↓↓↓↓↓</td>
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<td>B cell 5f</td>
<td>↑ / ↑↑↑</td>
<td>↑↑↑</td>
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<td>Fce-RI 5g</td>
<td>↑? /</td>
<td>↑</td>
</tr>
<tr>
<td>FcY-R 3h</td>
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<td>↑↑</td>
</tr>
<tr>
<td>IL-4 129xB6 5i</td>
<td>=/?</td>
<td>=</td>
</tr>
<tr>
<td>IL-4 B65</td>
<td>↓/?</td>
<td>=</td>
</tr>
<tr>
<td>IL-4 B65k</td>
<td>=/?</td>
<td>?</td>
</tr>
<tr>
<td>IL-4R 5i</td>
<td>↓↓↓↓↓↓/?</td>
<td>↓↓↓↓↓↓</td>
</tr>
<tr>
<td>IL-5 5i</td>
<td>=/?</td>
<td>=</td>
</tr>
<tr>
<td>IL-6 5z</td>
<td>=/?</td>
<td>?</td>
</tr>
<tr>
<td>IL-10 5m</td>
<td>=/?</td>
<td>=</td>
</tr>
<tr>
<td>IL-10 &amp; IL-4 5n</td>
<td>=/?</td>
<td>↓</td>
</tr>
<tr>
<td>IL-13 8</td>
<td>=</td>
<td>↓↓↓↓↓↓</td>
</tr>
<tr>
<td>IL-13 &amp; IL-4 8</td>
<td>=</td>
<td>↓↓↓↓↓↓</td>
</tr>
</tbody>
</table>

TABLE IV  Murine models of Schistosoma mansoni infection
In chronic infections (20 wk) Th2 responses are blunted (Grych et al. 1991, Henderson et al. 1992, Chensue et al. 1992) and Borozzi (1992) regards the chronic phase of murine schistosomiasis as predominantly Th1 mediated, largely on the basis of increasing ratio of IgG2a to IgG1 and decreasing eosinophil and IgE levels in chronically infected mice. IFN-γ, IL-4 and IL-10 exert cross-regulatory effects on the Th1-Th2 balance as IL-4 drives the reaction toward Th2, IFN-γ toward Th1 and IL-10 may inhibit either trend depending on the circumstances (Chensue et al. 1994a, b, Jankovic & Sher 1996, Wynn et al. 1997, 1998, Boros & Whitfield 1998). IL-10-knockout mice also formed very large granulomas 8 wk after infection but subsequently downregulated granuloma size (Wynn et al. 1998).

IL-5 does not seem to be directly involved in mediating granuloma size or fibrosis. Anti-IL-5 treated mice lacked eosinophils but granuloma size and hepatic fibrosis were virtually unaffected in both acute (Sher et al. 1990) and chronic (Cheever et al. 1992c) infections and the pathology was not greatly altered in IL-5 ko mice (Rosa Brunet et al. 1999a). Rumbley et al. (1999), however, note that eosinophils form the majority of cytokine producing cells in the granuloma and are the dominant source of IL-4.

### TABLE V

<table>
<thead>
<tr>
<th>Cytokine or antibody</th>
<th>Hepatic granuloma size</th>
<th>Hepatic fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>↓ or ↑</td>
<td>↓</td>
</tr>
<tr>
<td>anti-IFN-γ</td>
<td>= or =</td>
<td>=</td>
</tr>
<tr>
<td>IL-12</td>
<td>↓± or ↑</td>
<td>↓± or ↑</td>
</tr>
<tr>
<td>anti-IL-4</td>
<td>↑↑ or ↑</td>
<td>↑↑ or ↑</td>
</tr>
<tr>
<td>anti-IL-10</td>
<td>↑↑ or ↑</td>
<td>↑↑ or ↑</td>
</tr>
<tr>
<td>IL-2</td>
<td>↑↑ or ↑</td>
<td>↑↑ or ↑</td>
</tr>
<tr>
<td>anti-IL-5</td>
<td>↓ or ↓</td>
<td>↓</td>
</tr>
<tr>
<td>anti-IL-6</td>
<td>= or =</td>
<td>=</td>
</tr>
<tr>
<td>anti-IL-10</td>
<td>= or =</td>
<td>=</td>
</tr>
<tr>
<td>anti-IL-10/Fc</td>
<td>= or =</td>
<td>=</td>
</tr>
<tr>
<td>PGE-14</td>
<td>↑↑ or ↑</td>
<td>↑↑ or ↑</td>
</tr>
<tr>
<td>PGF-2β</td>
<td>↓ or ↑</td>
<td>↓</td>
</tr>
<tr>
<td>anti-NK1.1.19</td>
<td>↑↑ or ↑</td>
<td>↑↑ or ↑</td>
</tr>
<tr>
<td>SOM analogue</td>
<td>↑↑ or ↑</td>
<td>↑↑ or ↑</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

†, ↓: increase, decrease or no change; ?: unknown; ±: slight change.
The use of T cell lines and clones would seem to be a definitive way of resolving the relative importance of Th1 and Th2 cells. However granulomas are induced by transfer of Th0, Th1 and Th2 cells specific for SEA (Chickunguwo et al. 1991, Zhu et al. 1994, Jankovic et al. unpublished).

The downregulation of granuloma size has often been regarded as an effect of CD8+ suppressor cells (Chensue & Boros 1979, Green & Colley 1981) which one might expect to be mediated at least in part by IFN-γ. Normal downregulation was found in ko mice unable to make IFN-γ, in CD8 ko mice, in β-2 microglobulin ko mice (also virtually unable to produce CD8+ cells) and in TAP1 ko mice which are unable to process antigen in the context of class I (Hernandez et al. 1997b, Yap et al. 1997). It is unclear how these findings can be reconciled with the studies implicating CD8+ cells in downregulation of granuloma size. Rumbley et al. (1998, 2001) hypothesize that selective apoptosis of sensitized lymphocytes within the granulomas contributes to immunoregulation (Rumbley et al. 1998, 2001, Lundy et al. 2001, Lundy & Boros 2002).

Infected mice rendered B cell deficient by treatment with anti-µ antiserum (Cheever et al. 1985) or by genetic manipulation (Jankovic et al. 1998, Ferru et al. 1998) (µMT ko mice) did not modulate granuloma size or hepatic fibrosis and showed increased granuloma size and fibrosis in acute (8 wk) and chronic infections. T cell responses to antigen, however, were downregulated normally in chronically infected µMT ko mice. Another type of B cell ko mice (JHD B-less) showed no difference in granuloma size from controls 8 wk after infection (Hernandez et al. 1997a) but chronic infections were not examined. Mice lacking the Fc receptor also do not modulate granuloma size normally, indicating that the effect of antibody in modulation is directed through the Fc receptor (Jankovic et al. 1998). This result is consistent with the reports of downregulation of in vitro granuloma size by immune complexes (Goes et al. 1991, 1994, Rezende et al. 1997b, c).

SCID mice show almost no reaction to S. mansoni eggs but after injection with recombinant TNF-α formed granulomas around S. mansoni eggs (Amiri et al. 1992) and infected, immunologically intact mice treated with anti-TNF-α formed granulomas reduced in size (Joseph & Boros 1993). Administration of recombinant murine TNF-α to mice with chronic S. mansoni infection restored granuloma size to that seen in acutely infected animals (Joseph & Boros 1993). Nevertheless mice lacking both the p55 and p 75 chains of the TNF-α receptor formed normal granulomas in 8 wk infections (Yap et al. unpublished). Cheever et al. (1999) were unable to affect granulomas in infected SCID mice by injection of TNF-α.

Flores Villanueva et al. (1994) consider the granuloma to be a Th1-type response and attribute downregulation to anergy. Treatment of acutely infected animals with IL-10Fc fusion protein decreased the size of granulomas in acute infection while diminishing levels of Th1 cytokines (IFN-γ and IL-2) and augmenting levels of Th2 cytokines (IL-4 and IL-10) (Flores Villanueva et al. 1996). Chen and Boros (1998, 1999) produced “Th1” or “Th2” granulomas to egg antigen epitopes p38 or P4 depending on the adjuvant used in mice of the H2k haplotype.

IL-4 knockout mice exhibited increased Th1-like responses and markedly diminished Th2 responses but granuloma size and hepatic fibrosis during infection were equivalent to those in intact controls (Pearce et al. 1996, Rosa Brunet et al. 1997). However, T cell responses were more generally affected in IL-4 ko mice (Pedras-Vasconcelos et al. 2001). Metwali et al. (1996) found granuloma size to be diminished in IL-4 ko mice but gave a different interpretation to cytokine patterns similar to those reported by Pearce et al. (1996) and Chiramonte et al. (1999b) found enlarged granulomas but decreased fibrosis in IL-4 ko mice. Other experiments in various strains of IL-4 ko mice have given discrepant results which we cannot yet interpret (Cheever et al. 1998, Jankovic et al. unpublished).

Weinstock and his colleagues (1992, 1998) have extensively investigated the role of angiotsin converting enzyme and neuropeptides in the formation and modulation of schistosome granulomas (Tables IV-VI). Chemokine expression has been examined broadly by Park et al. (2001) and Qiu et al. (2001).

**TABLE VI**

Use of cytokine or anti-cytokine treatments and knockout (ko) mice using the lung model in experimental murine schistosomiasis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on granuloma size</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>↓</td>
</tr>
<tr>
<td>anti-IFN-γ</td>
<td>↑</td>
</tr>
<tr>
<td>IFN ko 1</td>
<td>↑</td>
</tr>
<tr>
<td>IFN ko 1d</td>
<td>↑</td>
</tr>
<tr>
<td>TNF-α2a</td>
<td>↑</td>
</tr>
<tr>
<td>anti-TNF-α2b</td>
<td>± ↓</td>
</tr>
<tr>
<td>anti-B7-12c</td>
<td>=</td>
</tr>
<tr>
<td>anti-B7-22c</td>
<td>↓</td>
</tr>
<tr>
<td>anti-IL-21b</td>
<td>↓</td>
</tr>
<tr>
<td>IL-4 ko 5b</td>
<td>↓</td>
</tr>
<tr>
<td>anti-IL-45c</td>
<td>↓</td>
</tr>
<tr>
<td>anti-IL-65b</td>
<td>↓</td>
</tr>
<tr>
<td>IL-10v7b</td>
<td>=</td>
</tr>
<tr>
<td>anti-IL-10v7b</td>
<td>↓</td>
</tr>
<tr>
<td>IL-4 &amp; IL-10ko 7d</td>
<td>↓</td>
</tr>
<tr>
<td>IL-12v8a</td>
<td>↓</td>
</tr>
<tr>
<td>anti-IL-12v8b</td>
<td>↑</td>
</tr>
<tr>
<td>IL-4 + IL-13 ko 3</td>
<td>↓</td>
</tr>
<tr>
<td>IL-13R α2Fc 21</td>
<td>↓</td>
</tr>
<tr>
<td>IL-13 transgenic mouse 24</td>
<td>↓</td>
</tr>
<tr>
<td>Stat4 ko 18</td>
<td>↓</td>
</tr>
<tr>
<td>Stat6 ko 18</td>
<td>↓</td>
</tr>
<tr>
<td>β2m ko 9</td>
<td>=</td>
</tr>
<tr>
<td>Anti-ICAM-1 10</td>
<td>↓</td>
</tr>
<tr>
<td>Anti-MCP1 11</td>
<td>↓</td>
</tr>
<tr>
<td>Anti-MIP-1 12a</td>
<td>↓</td>
</tr>
<tr>
<td>CCRI-MIP-1 10R ko 12b</td>
<td>↓</td>
</tr>
<tr>
<td>CCRII-MCP-1R ko 12c</td>
<td>↓</td>
</tr>
<tr>
<td>INOS ko 17</td>
<td>↓</td>
</tr>
<tr>
<td>B cell ko 18</td>
<td>=</td>
</tr>
<tr>
<td>TGFβ1 1 ko 13a</td>
<td>± ↓</td>
</tr>
<tr>
<td>TGFβ1 1 g 13b</td>
<td>± ↑</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on granuloma size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-NK1.1 or anti-AsialoGM1</td>
<td>↑</td>
</tr>
<tr>
<td>PGE1</td>
<td>↓</td>
</tr>
<tr>
<td>PGF2α</td>
<td>↓</td>
</tr>
<tr>
<td>p47phox−/−</td>
<td>=</td>
</tr>
<tr>
<td>NK-1RA</td>
<td>↓</td>
</tr>
<tr>
<td>Osteopontin ko−/−</td>
<td>↓</td>
</tr>
</tbody>
</table>

↑, ↓: increased, decreased or unchanged; ±: slight change; [N: naive mouse; S: sensitized mouse; I: infected mouse; E: eggs injected; B: antigen co-authorized beads injected].

<table>
<thead>
<tr>
<th>Reference</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jones et al. (1983)</td>
<td>Used H-2b and H-2k mice on both the C57BL/10 and BALB/c backgrounds and found that both the strain background and the H-2 haplotype influenced antibody response and passage of eggs in the feces. The effects of crosses between mouse strains were complex (Jones &amp; Kussell 1985). Kee et al. (1986) tested fractionated antigens in mice with a variety of haplotypes and found that a 14kDa fraction was recognized only by H-2k mice while an 8kDa fraction was not recognized by any of 4 H-2k strains of mice. Responses to the 86 kDa fraction were further examined by Schweitzer and Taylor (1991) and Schweitzer (1992). Trzyna and Cordingly (1993) found H-2k mice unable to produce IgG to a major egg antigen, p-40 and Hernandez et al. (1997c, 1998). Stadecker and Hernandez (1998) and Hernandez and Stadecker (1999) found that H-2k mice, including C3H and B10.10Br, made a T-cell response to a recombinant fraction of p40 while C57BL/6 mice (H-2b) did not. Granuloma size and other pathologic changes did not, however, follow H-2 haplotypes in a large number of mouse strains examined nor in congenic mice (Fanning et al. 1981, Colley et al. 1983, Fanning &amp; Kazura 1985, Cheever et al. 1987).</td>
</tr>
</tbody>
</table>

### Comparison of Granuloma Formation and Regulation in Infected Animals Versus the Response to Injected Eggs

The lung model allows study of granuloma formation and cytokine production in vivo in naive animals at defined periods after egg injection and the model also allows sensitization with defined antigens or antigenic fractions as well as the testing of defined antigens as targets, i.e. the injection of antigens on beads which are roughly the size of schistosome eggs. Although the lung model generally seems to reflect the sequence of events, and regulation, present during the course of infection induced by cercariae, important exceptions exist. Administration of IL-13R fusion protein, inhibiting IL-13 function, dramatically reduced granuloma size around eggs injected into the lung (Chiramonte et al. 1999a) but did not affect the size of hepatic granulomas in infected mice (Chiramonte 1999b). Additionally, although granulomas around injected eggs were downregulated by the injection of anti-IL-4 granulomas around eggs laid by worms in infected animals, in the lung or in the liver, were not (Cheever et al. 1994c). The differences were attributed to the inferior antigenic quality of eggs extracted from the tissues as neither the site of granuloma formation nor the state of sensitization of the host were important (Elton et al. 1995). Granulomas around eggs injected into the lungs downregulate in a fashion similar to hepatic granulomas while granulomas around pulmonary eggs shunted from the portal system in infected mice are not downregulated (Sousa Vidal et al. 1993). In infected IL-10 ko mice, liver granulomas were larger than in control mice while granulomas in the lung of sensitized mice were smaller in IL-10 ko mice than in controls (Wynn et al. 1997, 1998). Although Elton et al. (1995) did not find the site (lung vs liver) of the granulomas to be important, there are obvious differences in the liver and lung, e.g. the hepatic and pulmonary microenvironments are different and the miracidia in eggs injected via the tail vein are killed much more rapidly than those in eggs injected into the portal vein (Feldman et al. 1990) and all remnants of the eggs are

### Role of Murine Host Genotype on Immune Reactivity, Granuloma Size and Hepatic Fibrosis

Claas and Deelder (1979) found C3H/Sn (H-2k) mice had lower mortality, antibody titers and DTH (ear swelling) than did C3H.B10 (H-2b) mice but worm numbers and cell proliferation in vitro did not differ. The differences extended to the I-region of the H-2 complex and B10.A(2R) showed lower mortality but higher antibody titers than B10.A(4R) mice (Class & Deelder 1980). Mendlovic et al. (1989a, b) further explored the effects of I-A and I-E on the responses of congeneric mice to crude and purified antigens. Jones et al. (1983) used H-2b and H-2k mice on both the C57BL/10 and BALB/c backgrounds and found that
removed from the lungs more quickly (Cheever & Anderson 1971, Almeida & Andrade 1983). Kupffer cells in the liver of infected mice contribute to the type 2 reaction (Hayashi et al. 1999).

ICAM-1 was the only adhesion molecule identified in the liver of infected wild type (control) mice (Ritter & McKerrow 1996) while VCAM-1 was the predominant adhesion protein identified in the vicinity of lung granulomas (Ritter & McKerrow person. commun.). VCAM-1 was seen in the livers of infected ICAM-1 ko mice, affirming a striking illustration of the ability of ko mice to adapt to their deficiencies (Ritter & McKerrow 1996). However, VCAM-1 was found to be rapidly upregulated in the livers of infected-wild type mice by Rathore et al. (1996), a result in apparent conflict with the above. Leptak and McKerrow (1997) found little IL-4 in livers of mice given injected portal eggs and postulate that TNF-α plays a prominent role in the formation of hepatic granulomas while IL-4 is important for initiation of pulmonary granulomas, but Eltoum et al. (1995) found similar levels of IL-4 mRNA after injection of eggs at either site.

The regulation of granuloma size in the mouse gut also differs from that in the liver (Weinstock & Boros 1981, Jacobs et al. 1998a).

HEPATIC FIBROSIS

General

Hepatic fibrosis is related to the immune response to the egg and is virtually absent in infected nude and SCID mice (Cheever et al. 1994a, Cheever et al. 1999) or in mice infected with only male or female parasites.

Female C3H/HeNN mice infected with 25 male worms for 20 wk had only 2.21 μmoles of hydroxyproline per liver as compared to 1.53 in uninfected mice and 30 in mice infected with 2-3 worm pairs. However mice unisexually infected for 8 wk and then given bisexual infections modulated fibrosis (but not granuloma size) and had 21% less fibrosis (p < 0.05 in each of two experiments) than control mice given the same infection (Cheever et al. unpublished).

The regulation of fibrosis is often independent of the regulation of granuloma size, i.e. larger granulomas are not always associated with greater hepatic fibrosis (Olds et al. 1989, Cheever et al. 1994c, Phillips et al. 1996, Cheever 1997, Chiramonte et al. 1999b, Fallon et al. 2000b). Hepatic fibrosis in schistosome infected mice is clearly linked to IL-13 and the Th2 response, although fibrosis also clearly sometimes occurs around granulomas formed in the Th1 milieu (Chen & Boros 1999, Hernandez et al. 1999, Hoffmann et al. 2000, Hesse et al. 2000).

Animals treated with anti-IL-4 showed a decrease in Th2 response, an increased Th1 response and a reduction in hepatic fibrosis (Cheever et al. 1994c). IL-13 is even more important for fibrosis and treatment of infected mice with sIL-13Rα2Fc fusion protein led to a marked decrease in fibrosis with little effect on granuloma size (Chiramonte et al. 1999b). IL-4 ko mice treated with sIL-13Rα2 had tiny granulomas but fibrosis equivalent to that in sIL-13Rα2 treated wild type (WT) mice, although the cytokine pattern was Th2-like in WT mice and Th1-like in IL-4 ko mice (Chiramonte et al. 1999b) and in IL-4R ko mice, in which neither IL-4 or IL-13 can signal in the absence of the receptor (Jankovic et al. 1999). Mice treated with anti-IL-4 might be expected to have low IL-13 levels. IL-13 ko mice had granulomas the same volume as wild-type mice but showed minimal fibrosis (Fallon et al. 2000b). Arginase-1 activity is important in influencing both granuloma size and hepatic fibrosis, probably through increasing hepatic proline levels (Hesse et al. 2001).

Mice vaccinated with eggs + IL-12 before infection developed an increased Th1-like cytokine response, a moderate decrease in granuloma size and a marked decrease in hepatic fibrosis (Wynn et al. 1995). This effect is dependent on IFN-γ, IL-12 and TNF-α (Hoffmann et al. 1999) and mediated through nitric oxide synthase-2 (Hesse et al. 2000). Treatment with IFN-γ resulted in a marked decrease in hepatic fibrosis (Czaja et al. 1989a) but anti-IFN-γ had no effect on granuloma size or hepatic fibrosis in infected mice (Sher et al. 1990). SEA given without adjuvant intravenously or intraperitoneally before infection also affected granuloma size (Botros et al. 1997) and fibrosis (uncorrected for egg number, Pancré et al. 1999).

Hassanein et al. (2001) noted amelioration of pathology in mice immunized intravenously with either SEA or recombinant S. mansoni glutathione S-transferase.

Wolowczuk et al. (1997) reported that a single subcutaneous dose of IL-7 given at the site of exposure to S. mansoni increased worm recovery and hepatic collagen (μg collagen/mg protein) but their analysis of fibrosis did not allow for the increased intensity of infection in treated mice. IL-7 ko mice had small granulomas and little fibrosis (Wolowczuk et al. 1999) and IL-7 Tg mice showed increased fibrosis (Roye et al. 2001). A somatostatin inhibitor, octreotide, diminishes hepatic fibrosis in infected mice (Mansy et al. 1998).

Hepatic fibrosis in infected mice is related to egg numbers, but not in a linear fashion, i.e. mice with heavier infection have more total hepatic fibrosis but less fibrosis per egg (Cheever 1986). The rate of hepatic fibrosis, as indicated by collagen mRNA (Wynn et al. 1995a, b) or collagen synthetic rates (Olds et al. 1989), was decreased in chronically infected mice compared to those with acute (6-8 wk) infections. However Takahashi and Simpser (1981) felt that decreasing levels of collagenase in chronic infections were more important than decreased synthetic rates and in acute infections mice receiving a single injection of concanavalin A showed increased collagenase activity and decreased hepatic collagen (Takahashi & Koyayashi 1982). We are unaware of attempts to adjust the rate measurements of hepatic fibrosis for infection intensity. Collagen synthetic rates were proportional to liver collagen per egg in ICR mice with high fibrosis compared to C57BL/6 mice with low fibrosis (Cheever et al. 1983).

Widely different rates of resorption of hepatic collagen have been reported following chemotherapeutic cure of infection (Morcos et al. 1983, Andrade & Grimaud 1986, Cheever et al. 1992a), but in general collagen resorption is relatively rapid (collagen half-life of wk or months) in mice treated during the first few weeks of egg deposition and much more gradual when chronically infected mice were treated (Warren & Klein 1969). The morphologic pattern of collagen resorption in mice resembles that in humans (Andrade & Grimaud 1988, Andrade et al. 1992). Colchi-
Symmers' Clay Pipestem Fibrosis

Symmers' fibrosis is present in virtually all cases of portal hypertension attributable to schistosome infection in humans and nearly all cases with Symmers' fibrosis at autopsy also manifest signs of portal hypertension. Symmers' fibrosis in the chimpanzee was morphologically indistinguishable from that in man but did not produce portal hypertension (Sadun et al. 1970), probably because of the extensive portal-systemic collaterals formed. Njenga et al. (1998) and Nyindo and Farah (1999) describe, but do not convincingly illustrate, Symmers'-like perportal fibrosis in *S. mansoni*-infected baboons and Farah et al. (2000) relate portal fibrosis in baboons to reinfection and TGF-β and IL-4 production.

The massive infections present in mice produce a portal hypertension related to granuloma number and size which is probably not relevant to the mechanism of portal hypertension in humans (Cheever 1965a). However, mice also share the obstructive portal-venous lesions apparently responsible for portal hypertension in humans. Portal fibrosis resembling Symmers' fibrosis in humans was first described in mice by Warren (1966). Andrade (1987) and Andrade et al. (1997) described similar fibrosis in infected mice in which low intensity infections allowed gradual obstruction of peripheral portal venules with subsequent preferential shunting of eggs into the portal spaces surrounding larger veins. In infected persons it was also noted that eggs did not concentrate in the large portal spaces until pipestem fibrosis had already begun (Cheever 1969). Splenectomized mice developed pipestem fibrosis but less frequently than intact mice (Andrade et al. 1998).

Henderson et al. (1993) not only described a more marked and uniform Symmers'-like fibrosis in infected CBA male mice but also related this fibrosis to the absence of regulatory idiotypic antibodies in these mice, a finding strikingly similar to their findings in humans with Symmers' fibrosis (Montesano et al. 1990a, b). Exposure of neonatal mice to cross-reactive idiotypes (CRI) of mice without portal fibrosis, but not to CRI of mice with portal fibrosis, resulted in decreased granuloma size and decreased hepatic fibrosis in mice subsequently infected (Montesano et al. 1999b). Thus both mechanistic and immunological mechanisms in the murine model of pipestem fibrosis resemble those in humans. These CBA mice also had elevated serum levels of TNF-α and TNF-α mRNA in their livers (Adewusi et al. 1996) and decreased levels of IL-10 (Bosshardt et al. 1997). However idiotypes from mice without portal fibrosis stimulated IFN-γ formation (Montesano et al. 1997). Clinical hepatosplenic disease in humans was associated with a Th1-type immunological response (Mwantha et al. 1998) but Montenegro et al. (1999) saw IFN-γ production in such patients only after neutralization of IL-10. Mice with and without pipestem hepatic fibrosis did not show differences in the production of anti-idiotypic antibodies against *S. mansoni* (Andrade et al. 1998).

Gross examination of the livers of mice (and other rodent hosts) can be misleading. Macroscopic white thickening of the portal areas is frequent in chronic infections, but microscopically this usually reflects marked proliferation of bile ducts rather than fibrosis. Bile duct proliferation is often associated with eosinophilic crystals in the duct lumen. The crystals are apparently derived from epithelial secretions and not eosinophils. Similar changes are seen in the lungs of chronically infected mice and are presumably IL-4 and IL-13 dependent as similar crystals are seen in the lungs of IL-4 and IL-13 transgenic mice.

Extrahepatic Pathology

**Lung** - The key pulmonary pathology in infected humans is granulomatous schistosomal pulmonary arteritis resulting in pulmonary hypertension and cor pulmonale. Granulomatous pulmonary arteritis is seen routinely in mice injected with schistosome eggs, but the attempts to model human pulmonary schistosomiasis have generally involved the shunting of eggs in infected animals to the lungs through partial ligation of the portal vein which created a shunt from the portal system to the lungs (Warren 1964) producing granulomatous pulmonary endarteritis similar to that in infected humans (Andrade & Andrade 1964), lesions only partially reversed by chemotherapy (Almeida & Andrade 1983). Granulomas in the lungs of shunted mice did not undergo the downmodulation in size seen in the livers of the same animals after chronic infection (Souza Vidal et al. 1993).

Pulmonary granulomas in mice with portacaval shunts were resistant to downregulation by anti-IL-4 while granulomas around intravenously injected eggs were downregulated, a difference attributed to the lower antigenic potency of the eggs injected compared to those laid in situ by the worms (Eltourn et al. 1995).

**Intestines** - Granulomas in the colon and small intestine of mice are smaller than those in the liver, are not always subject to the same downregulation (Weinstock & Boros 1981, Jacobs et al. 1998a) and are associated with less fibrosis than are hepatic granulomas (Dunn & Kelley 1979, Santos et al. 1992). Inflammatory colonic polyps were seen in *S. mansoni*-infected chimpanzees (Sadun et al. 1970) but these did not ulcerate and were not associated with diarrhea, a hallmark of schistosomal colonic polyposis in man. Heavily infected animals of several species may develop colonic ulcerations and bloody diarrhea but it is unclear whether these bear any relation to lesions or symptoms and signs in humans. Infected woodchucks have only slight hepatic fibrosis but marked fibrosis of the intestine (Andrade et al. 2001).

Alterations in the vasculature and innervation of the intestines of infected mice have been reported (Kloetzl 1971, Block 1980, Varilek et al. 1991). Death of intestinal neurons is uncommon (Nassauw et al. 2001). In spite of the predominance of small intestinal over colonic pathology in infected mice and the evident gross and microscopic lesions, the functional changes seem slight. Domingo and Warren (1966) did not find functional changes in the guts of infected mice while Vengesa and Lesse (1979) and Sadek et al. (1986) noted changes in the absorption of glucose and fluid transport and of disaccharidase activity. Moreels et al. (2001) found that at 12 but not 8 wk after infection that increased muscular con-
tractility was present in the inflamed mouse gut and that transit through the GI tract was decreased. Immuno-deficient mice may have severe intestinal disease, as noted below under "Causes of Death".

Kidneys - The glomerulonephritis seen in schistosome-infected humans has not been described in S. mansoni-infected animals although both chimpanzees and rabbits seem susceptible to this lesion when infected with S. japonicum. S. mansoni-infected rabbits show similar changes, albeit of a lesser degree (Andrade et al. 1988). Mild glomerular lesions in infected capuchin monkeys were described by de Brito et al. (1971) and in baboons by Houba et al. (1977). Houba (1979) reviewed experimental schistosomal glomerulopathy.

Glomerular deposits of immune complexes and ultrastructural glomerular lesions and Ig deposits have been described in infected mice, but the kidneys were often normal by light microscopy (Andrade & Susin 1974, Rousse & Romeiro 1974, Natali & Cioli 1976, Carneiro & Lopes 1986, Water et al. 1988). Antigen and immune complex deposition were augmented by portocaval shunting (van Marck et al. 1977) but were present even in unisexual infections (Lopes et al. 1981). El-Sherif and Befus (1988) found IgA to be the predominant Ig isotype in the glomeruli of infected mice. Severe glomerulonephritis developed in female BXSB mice exposed to 10^5 cercariae but most mice did not have granulomas in the liver and unisexual infections were not ruled out (Fugiwara et al. 1988). Hematuria was documented in a substantial proportion of infected mice by Valadares and Pereira (1983). S. mansoni-infected hamsters developed severe amyloidosis of the kidneys which resulted in marked ascites and amyloidosis of the liver and spleen, which complicates interpretation of the lesions in these organs (Cheever 1965b).

Central Nervous System - No proper model has been described for the focal mass lesions most frequently reported in humans, but Aloe et al. (1996) have described granulomas in the brain and altered nerve growth factor levels in chronically infected mice. Behavioral changes have been noted in mice and are perhaps attributable to effects of cytokines on the central nervous system (Fiore et al. 1996). Focal egg deposition and granulomatosus encephalitis associated with convulsions were seen in an infected cercopithecus monkey (Cheever & Duvall 1974). Recently, Silva et al. (2002) have called attention to the inadequacy of the murine model for studies concerning neuroschistosomiasis.

Causes of Death

Causes of death in patients - Humans rarely die from acute S. mansoni infections. Nearly all persons with lethal chronic infections have Symmers' fibrosis and most of these die from bleeding esophageal varices. Others with Symmers' fibrosis die with chronic hepatic cellular failure and a smaller number from complications of pulmonary hypertension or glomerulonephritis (Cheever & Andrade 1967) but death from schistosomiasis in patients without Symmers' fibrosis is very uncommon.

Variability between laboratories - It is clear that in some laboratories lethal infections in mice are associated with lower numbers of infecting worms than in other laboratories. It is unclear whether this should be attributed to the parasite, the host, to concomitant bacterial or viral infections or to other variables associated with animal husbandry.

Acute hemorrhage into the gastrointestinal tract causes the great majority of deaths in S. mansoni-infected mice in our experience. AJ mice incur this complication early in infection and at low infection intensities (Dean et al. 1981, Colley & Freeman 1983) while C57BL/6 and BALB/c mice are unusually resistant to death after infection. The mechanism of intestinal hemorrhage in mice is unrelated to gastrointestinal hemorrhage in humans with schistosomiasis. Esophageal varices in the mouse are on the serosal surface of the esophagus and are not the source of bleeding, which is presumably from minute lesions in the gut. This does not seem to be a cause of death in other models, and perhaps is lethal for the mouse because it cannot afford to lose the 1-2ml of blood involved.

Cytokine shock in mice - Marked malaise and early death without hemorrhage into the gut lumen were reported in IL-4 ko mice on the C57BL/6 background, but not in IL-4 ko mice which were F1 129XJxB6. The lethal effect was related to IFN-γ and NO (La Flamme et al. 2001, Patton et al. 2002) but was independent of IL-12 (Patton et al. 2001). Survival was improved by treating the mice with anti-TNF-α or with recombinant IL-4 (Rosa Brunet et al. 1997a, b, 1999). Mice deficient in IL-4 and IL-13 are especially susceptible and Fallon et al. (2000b) give an excellent detailed description of the pathogenesis of cytokine shock in these mice, including the shift to a type 1 type cytokine response and the effects of bacterial lipopolysaccharides leaking into the circulation from the intestine. Fallon (2000d) provides an excellent review. Hoffmann et al. (2000) found that either extreme Th1 or Th2 polarization resulted in the early death of S. mansoni-infected mice but probably not from cytokine shock in Th2-polarized mice.

Other immunocompromised mice die in the first wk of egg laying without evident cytokine shock. Fallon et al. (2000) found that mice transgenic for IL-9 developed a fatal enteropathy in a Th2 milieu and MacDonald et al. (2002) noted wasting and early death of CD154 ko mice which had impaired Th2 responses.

T cell deficient mice, including SCID and nude mice, infected with S. mansoni, but not S. japonicum or S. haematobium, frequently die 7-9 wk after infection, apparently from hepatotoxicity [and presumably cytokine shock] (Byram et al. 1979, Cheever et al. 1999). Mice deficient in Class II MHC (Angyalosi et al. 1998), CD-4 depleted mice (Fallon et al. 2000a) or made tolerant to S. mansoni egg antigens seem to die a similar death (Fallon & Dunne 1999). Gharib et al. (1999) have described increased eosinophil peroxidase activity and increased oxygen radical production (Abdallahi et al. 1999) in the livers of S. mansoni-infected mice with a concomitant decrease in hepatic antioxidant defenses, apparently predisposing infected mice to hepatotoxicity. It is not clear that the entities we have attributed to "cytokine shock" are all similar nor has this toxicity in mice been shown to be relevant to death or other illness in humans (Cheever et
al. 2000). If the syndrome is related to acute schistosomiasis then it is not clear why immunodeficient mice do not show cytokine shock when infected with schistosome species other than \textit{S. mansoni} (Cheever et al. 2000).

**WORM DEVELOPMENT, FECUNDITY AND FECAL EGG EXCRETION**

Fewer worms develop in male rodent hosts than in females and castration and exogenous hormones have the expected effects (Berg 1957, Nakazawa et al. 1997). Fulford et al. (1998) propose that in mice and in humans that this might be related to dehydroepiandrosterone levels. Worm development and egg production were delayed in mice deficient in the 5th component of complement at 7 wk of infection (Ruppel et al. 1982) but no difference was found in another laboratory at 12 wk (Cheever et al. 1987). Biozzi high responder mice developed higher numbers of parasites and larger worms, and significantly more eggs/worm pair were found in the liver at 10 but not at 15 wk (Blum & Cioli 1978). IL-7 deficient mice had fewer worms and fewer eggs/female 7-8 wk after infection (Wolowczuk et al. 1999).

Decreased numbers of eggs/worm pair were found in the livers of severely malnourished mice and hepatic granulomas in these mice were smaller than in well nourished mice (Knauff & Warren 1969, Akpm & Warren 1975, Coutinho et al. 1997) and pipistem fibrosis was absent and total hepatic fibrosis diminished in malnourished mice (Coutinho et al. 1997).

Fecundity is defined as the number of eggs laid per day per worm pair. Delayed inception of egg laying is more common than decreased fecundity so that measurement of eggs/worm pair at a single point in acute infections is of limited value. Strictly speaking one should know the total number of eggs in the tissues and eggs passed in the feces to calculate fecundity. Generally, only the number of eggs in the liver and intestines is known, and this comprises almost all eggs in the tissues. Eggs destroyed in the tissues also constitute a portion of the eggs laid and in rhesus monkeys the rate of egg destruction was extremely rapid [half life of 8 days] (Cheever & Powers 1971) and the same has been assumed to be true for cercopithecus monkeys and baboons (Cheever & Duvall 1974, Damian et al. 1986). In mice egg destruction is much slower and its effects can often be ignored (Cheever et al. 1992a). Quantitative estimates of worm fecundity for many of these species have been presented previously (Cheever et al. 1994b). Farah et al. (1997) described higher fecundity in multiply infected baboons than after single infection.

In mice infected with a single pair of worms, fecundity varied greatly between individual mice but did not vary with duration of infection (Cheever et al. 1994a). Fecundity did decrease in time in more heavily infected mice (Cheever et al. 1994b). Worm fecundity in vitro also varied greatly among worm pairs and with the host used and the duration of infection (El Ridi et al. 1997). The fecundity of worms in infected rhesus monkeys decreased with increasing duration of infection. This decrease was much more rapid in heavily infected animals than in those lightly infected and the decrease in fecundity presumably had an immune basis as worms were also much more rapidly destroyed in the heavily infected monkeys (Cheever & Powers 1969).

The fecundity of the worms was generally reflected by the number of eggs passed in the feces at various stages of infection in mice (Cheever et al. 1994b) and in monkeys (Cheever & Powers 1969). The percentage of eggs laid passed in the feces can vary greatly in different species and even in different strains of mice (Jones et al. 1989). Nude mice (Cheever et al. 1993) and T-cell depleted mice (Dunne et al. 1983, Doenhoff et al. 1978, 1985) passed less eggs in the feces than did intact mice and SCID mice passed almost no eggs in the feces in the first weeks of oviposition (Amiri et al. 1992, Cheever et al. 1999). These findings anticipated those in patients with AIDS who may pass many fewer eggs in the feces than comparably infected patients without AIDS (Karanja et al. 1997). The number of eggs per worm pair in the tissues of nude and SCID mice was low early in infection (Amiri et al. 1992, Cheever et al. 1993, 1999) but the rate of egg accumulation approached that in intact mice by the 10th wk of infection (Cheever et al. 1993, 1999). Davies et al. (2001) correlate worm development with the presence of a subset of CD4$^+$ lymphocytes in the liver.

Lenzi et al. (1987) found that eosinophils favored the passage of \textit{S. mansoni} eggs in the feces, although mice treated with anti-IL-5 and lacking eosinophils passed normal numbers of eggs in the feces (Sher et al. 1990). Worms in mice deficient in IL-4 or IL-4 and IL-13 showed normal to slightly increased fecundity as judged from tissue eggs but almost no eggs were passed in the feces (Fallon et al. 2000b).

More selective immune depletion, i.e. treatment with anti-IgE, has been reported to inhibit the development of \textit{S. mansoni} suggesting that the worms need this immunoglobulin for normal development (Amiri et al. 1994). This is difficult to reconcile with the increased numbers of worms developing in IgE ko mice (King et al. 1997) and the normal development of worms in FcεRI ko mice, in B cell ko mice and in SJA/9 mice unable to produce IgE (Jankovic et al. 1997, 1998, El Ridi et al. 1998).

**INTERACTION OF SCHISTOSOMIASIS WITH OTHER DISEASES**

Murine viral hepatitis was more severe in schistosome infected mice (Warren et al. 1969) and the clearance of vaccinia virus was delayed for a period during the acute phase of schistosome infection (Actor et al. 1994). Woodchuck hepatitis B infection was apparently not affected by \textit{S. mansoni} infection (Andrade et al. 2001). Surprisingly hepatitis B replication in HBV transgenic mice was inhibited by \textit{S. mansoni} infection (McCrary et al. 2000).

Schistosomiasis is intriguingly related to several bacterial infections, most notably with prolonged typhoid fever syndromes (Chieffi 1992) and numerous experimental studies have been published using a number of bacterial species (Rocha et al. 1980). The association of bacteria with the schistosome gut or tegument (Loverde et al. 1980) protects the bacteria from chemotherapy and presumably from immune attack as well.
S. mansoni infected mice also showed increased susceptibility to Entamoeba histolytica (Knight & Warren 1973), Trypanosoma cruzi (Kloetzel et al. 1973) and Toxoplasma gondii (Kloetzel et al. 1977). Marshall et al. (1999) found increased plasma TNF-α and increased hepatotoxicity in mice with combined S. mansoni and T. gondii infections and this effect was diminished in IL-12 ko mice (Araujo et al. 2001). The susceptibility to Plasmodium yoelli and P. berghei was little affected (Lwin et al. 1982).

There has long been controversy about the association of human hepatocellular carcinomas and schistosome infections, but S. mansoni infection is apparently not associated with hepatoma in man (Cheever & Andrade 1967). Hepatomas are infrequently seen in infected animals but infected mice given low doses of some carcinogens developed hepatomas much more frequently than did mice given the carcinogen alone (Domingo et al. 1967, Kakizoe 1985).

The vigorous Th2 response to schistosome infection may have a role in several of the above situations and in the response to immunizing antigens such as diphtheria toxoid (Haseeb & Craig 1997). Diabetes was ameliorated in NOD (non-obese diabetic) mice infected with S. mansoni or injected with eggs (Cook et al. 1999) and oral administration of SEA plus insulin led to a Th2 response in NOD mice (Maron et al. 1998). Second helminth infections may be influenced as well. Mice usually susceptible to Trichuris muris resolved their infections when infected with S. mansoni (Curry et al. 1995).

USE OF SCHISTOSOME-RELATED MODELS FOR THE STUDY OF OTHER DISEASES

Many of these studies are implicit in the discussion above. Schistosome infections have been widely used as an example of hypersensitivity granulomas (Boros 1983) and as model systems to examine the biochemistry and morphology of hepatic collagen formation (Silva et al. 1990 Prakash et al. 1995, Frizell et al. 1995) or resorption, as noted above. The interactions of the field of immunology and the study of the immunopathology of schistosome infections have been mutually beneficial. The importance of anti-idiotypic T cell responses in infectious disease was first noted in schistosome infected individuals (Montesano et al. 1990a, b). The study of T helper subsets has been extremely useful in analyzing infections with schistosomoses and with other helminths but investigations of these infections have also contributed substantially to our knowledge of Th1 and Th2 lymphocyte subsets (Jankovic & Sher 1996, Jankovic et al. 2000, MacDonald et al. 2002) and are relevant to understanding other Th2 dominated reactions such as asthma. Sabin et al. (1996) recently used schistosome eggs to demonstrate the importance of eosinophils as an early source of IL-4.

Coelom-associated lymphomyeloid tissue (CALT or milky spots) is greatly expanded in schistosome infected mice and examination of these animals has contributed to our knowledge of CALT (Lenzi et al. 1996). Many other studies have added to our understanding of eosinophils, macrophages, lymphocytes, non B, non T cells, endothelial cells etc.

SPECIAL CONSIDERATION OF THE RABBIT AND WOODCHUCK

Rabbits are a host of choice for the study of the pathology of schistosomiasis japonica. Large periovular granulomas are formed in the liver during acute infection and typical areas of periportal “pipestem” fibrosis appear. However, rabbits are not good hosts for S. mansoni since adult worms are eliminated within a few wk after cercarial exposure (Warren & Peters 1967, Andrade et al. 1988). Furthermore the eggs do not mature in the tissues and do not appear in the stools. Thus the main factor in the pathogenesis of schistosomiasis, the miracidium, is absent and therefore there is no disseminated liver fibrosis, nor obstructive portal vascular lesions and no portal hypertension or splenomegaly. Lesions in the liver are produced by the worms rather than the eggs. The basic lesion is composed of focal phlebosclerosis, vascular dilatation and polyloid endophlebitis. This curious and characteristic lesion is formed by projections of chronically inflamed subintimal connective tissue which are covered by hyperplastic endothelial cells. At first they do not affect the general liver architecture but in severe chronic infection some degree of portal and sepal fibrosis appear and may become prominent in prolonged infections. In extremely severe cases the combination of fibrosis and vascular proliferation may transform the liver into a huge angiomatous organ. Thus focal portal vascular changes caused directly by living or dying adult worms dominate the pathology of schistosomiasis mansoni in rabbits. Periovular granulomas are not a feature and the fibrotic changes do not resemble human “pipestem” fibrosis.

Woodchucks (Marmota monas and M. marmota) are worth considering as an experimental model for schistosomiasis because they are susceptible to both S. mansoni and to a hepatitis virus very similar to human hepatitis virus B (Woodchuck hepatitis virus - WHV). They are highly susceptible hosts with several peculiarities. Acute infection can run a severe and fatal course if the cercarial load is heavy (1,000 cercariae or more). Death in these cases is preceded by diarrhea due to intestinal lesions. The most severely damaged organ is the intestines rather than the liver. Large periovular granulomas are numerous in the submucosa and in the muscular coat, the mucosa being least involved. Granulomas in the liver are smaller than those in the intestines and are scattered. They cause some degree of portal fibrosis which is never disseminated or systematized. The infection runs an auto-cure course (Andrade et al. 2001).

SPECIAL CONSIDERATION OF CALOMYS COLLOSUS

The first report of S. mansoni infection in C. callosus was from Coelho et al. (1979). Borda (1972) showed experimentally that it is permissive to S. mansoni.

Our histopathological observations in C. callosus, infected by percutaneous exposure to 70 cercariae of S. mansoni, and killed at 42, 55, 80, 90 and 160 days after infection (six animals/point), have showed some peculiar aspects. In the liver, the granulomas were of two types: larger, in portal spaces, and smaller ones, in the parenchyma, which were often intravascular. The former were usually constituted by internal (periovular) and peripheral zones, while the intra-parenchymatous granulomas,
during all the studied period, were predominantly composed of large and mature macrophages, often with abundant schistosomal pigment, less eosinophils and neutrophils, and scarce lymphocytes, as described (Lenzi JA et al. 1995, 1998). There was no significant change in the granuloma morphology, size and extracellular matrix composition during the studied periods, suggesting that they are not modulated.

The granulomas exhibited fine texture of reticular fibers, mainly in the periphery and few or absent collagen fibers were visible by picrosirius (Junqueira et al. 1979) or phosphomolybdic acid-picrosirius stains for confocal laser scanning microscopy (Dolber & Sprach 1993) (Fig. 1). However, they were rich in carboxylated proteoglycans. By immunohistology, they showed deposits of fibronectin, laminin, collagen types I, Pro-III, IV, V, which remained

![Figures 1-6](image-url)
more or less constant during all the time of infection, always being less than seen in mice. Gelatinase was detected in large macrophages surrounding the eggs.

Focal parenchymal necrosis was found and portal inflammation and fibrosis were varied, depending on the time of infection. They were absent or minimal in more recent infection (42 days). At 90 days in some animals, there was an exacerbation of portal inflammation, and there appeared also portal perivenular and periductal fibrosis, causing sac-like dilatations in portal lymphatics, which filled with large macrophages.

Although the granulomas never exhibited peripheral metaplasia of hematopoietic cells, as observed in mice (Lenzi et al. 1995), from 55 days on, intrasinusoidal megakaryocyte, eosinophil and erythropoietic metaplasia was observed.

In *C. callosus* intestines, there were numerous eggs in all layers forming granulomas with collagen and reticular fibers detected only in the peripheral zone (Fig. 2). Numerous intestinal nodules, appearing from 55 ups to 160 days after infection, were localized at the interface between external muscular layer and intestinal serosa (Fig. 3) (Lenzi et al. 2002). From 55 days of infection on, an intense mast cell infiltration was detected, occupying all the intestinal layers. In the mucosa, the mast cells were mainly of the mucosal mast cell-type (MMC), while, in the muscular layer and in the subserosal nodules they were almost exclusively of the connective tissue type (CTMC) or transitional from MMC to CTMC.

Pancreatic involvement was frequent, and occasionally severe (Figs 4, 5). The omentum and mesentery were strongly activated during the infection, showing many milky spots and a diffuse infiltration of mast cells (Fig. 6). Milky spots were mainly of the lymphoplasmacytic type (Lenzi et al. 1996).

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