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Depressed In Vitro and In Vivo Antibody Response and Adoptive Transfer of Delayed Hypersensitivity to Myoglobin with Spleen Cells of Mice Chronically Infected with *Schistosoma japonicum* and Injected with Myoglobin

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Spleen cells from C57BL/6J mice infected for 21 weeks with *Schistosoma japonicum* did not show an in vitro secondary antibody response upon challenge with sperm whale myoglobin. Mice infected for 13 weeks showed almost no in vivo secondary antibody response to this antigen. Spleen cells from mice infected for 12 to 21 weeks did not adoptively transfer delayed hypersensitivity to this antigen.

We examined a number of antigen- and mitogen-induced responses of C57BL/6J mice in the course of infection with *Schistosama japonicum* (4). The syntheses of antibody to schistosomal egg antigens (SEA) and of nonantibody immunoglobulin and the production of lymphokine by hepatic granulomas peaked at 7 to 8 weeks of infection and then declined precipitously. Immunoglobulin production by spleen cells (SC) peaked at 6 weeks and then declined rapidly, whereas synthesis of antibody to SEA by SC peaked between 6 and 12 weeks and then declined moderately by 22 weeks. In vitro stimulation by SEA of SC immunoglobulin synthesis and [^{3}H]thymidine uptake was maximal at 5 weeks and declined by 12 weeks. The in vitro uptake of [^{3}H]thymidine by SC stimulated with concanavalin A and lipopolysaccharide was deeply depressed between 7 and 12 weeks. These kinetics were reminiscent of the spontaneous regression in size of hepatic granulomas and in level of portal hypertension in the course of this murine disease.

The profound depression in these antigen- and mitogen-induced cellular responses in the course of this infection raised the question whether immune responses of these infected mice to antigens not cross-reactive with SEA were also depressed. Therefore, the in vitro and in vivo secondary antibody responses to sperm whale myoglobin (Mb) by SC from *S. japonicum*-infected mice and the adoptive cellular transfer of delayed-type hypersensitivity (DTH) to Mb with SC from infected mice were compared with the responses of SC from normal mice.

C57BL/6J mice were employed because of their genetic disposition to marked granulomatous inflammatory responses (1). Female mice (Jackson Laboratories, Bar Harbor, Maine) were infected at Lowell University, Lowell, Mass., with 25 cercariae of a Philippine strain of *S. japonicum*. Rabbit antiserum to sperm whale Mb (Sigma Chemical Co., St. Louis, Mo.) for testing cross-reactivity of Mb and SEA and for radioimmunoassay of antibody to Mb was prepared as described (13). SEA was prepared from the livers of mice infected for 20 to 30 weeks, as described (2). Neither by the interfacial precipitation test (6) nor by double diffusion (4) did SEA and Mb cross-react.

Normal mice or age-matched infected (21 weeks) mice were injected intraperitoneally (i.p.) with 100 μg of Mb in complete Freund adjuvant and were reinjected 3 weeks later with 100 μg of Mb in incomplete Freund adjuvant. Seven days later, their SC (10^7) were cultured in 1 ml of Eagle minimal essential medium (minus leucine) supplemented as described (4). The Mb was washed out after 24 h, and the SC were placed in fresh medium. After 3 days of culture, 0.5 μCi of L-[^{14}C]leucine (254 μCi/mmol; Amersham Corp., Arlington Heights, Ill.) was added to each tube for 48 h. The cells were then removed by centrifugation and the radioactive antibody in the medium was assayed by a solid-phase method (10), to yield counts per minute in antibody per 10^7 cells. Twelve cultures were prepared from the SC of each mouse: three each in the absence of antigen and with 1, 10, or 100 μg of Mb. In a separate experiment, normal mice or age-matched infected (13 weeks) mice were immunized i.p. with 200 μg of alum-Mb on days 0 and 21 and were bled on day 28. The SC were cultured as before, but in addition the mice were bled before sacrifice, and the serum was titrated for hemagglutinating antibody by using sheep erythrocytes sensitized with 2 mg of Mb per ml of 2.5% (vol/vol) erythrocytes by the tannic acid method (11). The assay of DTH involved the immunization of normal or infected (12 or 21 weeks) mice with 100 μg of Mb-complete Freund adjuvant i.p. Seven days later, SC suspensions were prepared, and 0.025 ml of SC (1.2×10^7) containing 100 μg of Mb was injected into the left hind footpad of a normal mouse; as a control, the identical number of cells plus bovine albumin was injected into the right footpad of the same mouse. The data are expressed as net differences in thickness between the two feet at a number of times.

The SC from uninfected mice injected with Mb synthesized increased amounts of antibody upon challenge with 1 and 10 μg of antigen and a smaller amount upon challenge with 100 μg of Mb (Table 1). Although the SC from infected mice injected with Mb showed about the same amount of antibody synthesis as controls when Mb was not added, these cells did not produce additional antibody upon challenge with 1 or 10 μg of antigen; indeed, they usually showed a significantly reduced response relative to 0 Mb when 100 μg of Mb was added.

Table 2 shows the results of the hemagglutination titrations of the serum from the normal and 13-week-infected mice. It is clear that there was virtually no serum antibody produced in the infected mice compared with the normal

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TABLE 1. Effect of chronic infection with *S. japonicum* on in vitro secondary antibody response of murine spleen cells to Mb

Mouse no.	cpm ($\times 10^{-3}$) incorporated into antibody when spleen cells cultured with Mb[a]:			
	0 μg	1 μg	10 μg	100 μg
Normal mouse cells				
185	1.59	1.045 (0.7)	3.00 (1.9)	2.79 (1.8)
186	0.597	1.923 (3.2)	1.25 (2.0)	0.96 (1.6)
187	2.093	6.188 (3.0)	5.42 (2.6)	2.84 (1.4)
188	2.953	5.28 (1.8)	4.83 (1.6)	4.74 (1.6)
189	2.01	1.87 (0.9)	3.55 (1.8)	2.81 (1.4)
21-wk-infected mouse cells				
180	2.6	2.97 (1.2)	2.53 (1.0)	1.21 (0.47)
181	2.94	3.38 (1.1)	3.37 (1.1)	1.55 (0.5)
182	2.58	2.32 (0.9)	2.70 (1.05)	0.75 (0.3)
183	2.44	2.62 (1.1)	1.9 (0.8)	2.46 (1.0)

[a] SC (10^7) were cultured with 0, 1, 10, or 100 μg of myoglobin. Stimulation index is shown in parentheses. Mean values of duplicate determinations. Standard error of the mean was usually 10 to 20% of mean.

controls. The SC cultures showed similar impairment of the in vitro secondary antibody response to what was seen in the previous experiment (Table 1).

The adoptive transfer to normal mice of SC from Mb-injected normal mice together with Mb resulted in specific DTH (Table 3). However, the transfer of equal members of SC from infected (12 or 21 weeks) and Mb-inoculated mice did not result in any specific DTH.

In addition to the aforementioned impairments of in vitro and in vivo humoral and cellular responses to antigen and mitogens, *S. japonicum*-infected mice show impaired in vitro and in vivo humoral and in vivo cellular responses to a serologically non-cross-reactive antigen, Mb. Thus, murine *S. japonicum* infection is characterized by immunodepression, as are a number of other microbial, parasitic, and protozoan infections, including malaria (9), trypanosomiasis (18), lepromatous leprosy (17), tuberculosis (3), toxoplasmosis (14), and piroplasmosis (8). The mechanisms of immunodepression are not well understood in any of these infections. There is evidence for the involvement of an immunoglobulin G1 fraction of serum from mice chronically

TABLE 2. Reduced secondary antibody response to sperm whale Mb in mice chronically infected with *S. japonicum*[a]

Mouse no.	Hemagglutination titer
Normal mouse cells	
223	1/80
224	1/10
225	1/20
226	1/40
13-wk-infected mouse cells	
227	1/640
228	1/640
229	1/640
230	1/640
231	1/640

[a] Normal C57BL/6 mice and age-matched mice infected for 13 weeks with *S.* japonicum were injected i.p. with 200 μg of alum Mb on days 0 and 21. They were bled on day 28 to obtain serum for hemagglutination titrations.

TABLE 3. Adoptive transfer of DTH to Mb with spleen cells of normal versus *S. japonicum*-infected mice[a]

Time (h)	Net increase in footpad thickness (mm) in recipient when donor mice were[b]:		
	Normal	12-wk infected	21-wk infected
0	0.02 ± 0.02	0	0.02 ± 0.02
1	0.04 ± 0.04	0.06 ± 0.04	0.06 ± 0.04
3	0.04 ± 0.06	0.07 ± 0.04	0.07 ± 0.06
24	0.32 ± 0.03	0.05 ± 0.05	0.06 ± 0.04
48	0.18 ± 0.11	0.05 ± 0.05	0.01 ± 0.05

[a] Groups of five mice were immunized with 100 μg of Mb in complete Freund adjuvant i.p. in 0.2 ml. Seven days later, the mice were sacrificed, and splenic cell suspensions were prepared and transferred to recipient mice. Cells 1.2×10^7 in 0.025 ml containing either 100 μg Mb or bovine serum albumin (control) were transferred to the feet of normal syngeneic recipients.

[b] Footpad thickness was measured with calipers at various times after transfer of the cells. Data are expressed as net increases in the thickness of the foot which received cells plus Mb over that which received cells plus bovine serum albumin. The donor mice which were immunized with Mb were of three types: normal C57BL/c, or infected at 12 or 21 weeks with 25 cercariae of *S. japonicum*. Means of duplicate determinations ± standard errors of the mean are shown.

infected with *S. japonicum* in the regulation of the in vitro SEA-induced [^{3}H]thymidine incorporation (5) as well as in modulation of the granuloma and portal hypertension in vivo (7). It is not known whether immunoglobulin G1 is at all responsible for modulation of the humoral or cellular responses to Mb. A number of other mechanisms are being considered. The data in Table 1 show that the SC from infected mice produce as much antibody as the controls in the absence of added Mb. There is evidence that this background antibody synthesis is due to the interaction of persistent antigen on accessory cells with T and B memory cells (12, 15). Thus, it appears that the processing of antigen by accessory cells and T- and B-cell memory are intact in the spleens of infected mice but that the addition of Mb to these cells—in contrast to SC from normal mice—does not induce additional antibody synthesis. There is also clear evidence for a markedly impaired secondary antibody response in vivo (Table 2). Whether this failure to induce antibody production is due to the generation of specific or nonspecific suppressor cells or other mechanisms must be determined. The negative adoptive transfer experiments can be explained by an absence of T-cell population(s) required for DTH from the SC of infected mice, the presence of suppressor cells or their precursors in the transferred populations, or both. However, the failure of the DTH to occur within 24 h of transfer seems more plausibly explained by the former mechanism than by the latter. There is now evidence for the presence in the spleens of mice chronically infected with *S. japonicum* of T suppressor cells which inhibit the in vitro proliferative response to schistosomal egg antigens of spleen cells from acutely infected mice. (G. R. Olds, A. B. Stavitsky, and L. B. Peterson, unpublished observations). However, it has not yet been determined whether these supressor cells suppress the antibody response to Mb either in vitro or in vivo or the proliferative response to egg antigens in vitro. These mechanisms as well as others are under investigation to explain the impaired responses to Mb as well as the diminished responses to SEA both in vitro and in vivo in the course of this parasitic infection.

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