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Accessory Cell Function of Liver Granuloma Macrophages of *Schistosoma mansoni*-Infected Mice

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In murine schistosomiasis mansoni, the inflammatory macrophage comprises 30% of the granuloma which forms around parasite eggs in the tissue. These granuloma macrophages (GR-MØ) displayed dense Fc and C3 receptors, and about 50% expressed *H-2I* region-encoded determinants (Ia antigens). These GR-MØ were able to effectively reconstitute the burro erythrocyte-specific immunoglobulin M and G antibody response of primed macrophage-depleted spleen cells. However, in contrast to splenic macrophages, GR-MØ gave only minimal reconstitution of the primary immunoglobulin M response. The reconstitution of the T-cell proliferative response to L-glutamic^{60}-L-alanine^{30}-L-tyrosine^{10}, an antigen under *Ir* gene control, was also observed when GR-MØ were added to purified lymph node T-cells. The addition of a monoclonal antibody recognizing a determinant on the Ia complex effectively blocked L-glutamic^{60}-L-alanine^{30}-L-tyrosine^{10} presentation by GR-MØ. These studies demonstrated that inflammatory GR-MØ could function as antigen-presenting cells and that this accessory function was mediated by *H-2I* region gene products.

Schistosomiasis mansoni is a helminthic disease of the tropics characterized by the formation of T-cell-mediated granulomas around parasite eggs in the tissues (3, 27). The presence of macrophage (MØ)-rich granulomas and fibrous repair of the inflamed foci in the liver and intestines are major factors in the pathology of the disease (3). In previous studies, MØ of the schistosome egg-induced granulomas were shown to be activated inflammatory cells. The majority of cells were esterase positive, displayed dense membrane receptors, and exhibited receptor-mediated phagocytosis (2, 29) and nonspecific tumoricidal activity (12). The finding that about half of the granuloma macrophages (GR-MØ) also carried Ia membrane antigens (29, 31) indicated that these cells may also serve in an immune accessory function.

In the past, a variety of MØ obtained from the peritoneum (8), spleen (7), alveoli (11), liver (20), and bone marrow (21), as well as dendritic cells of lymph nodes (25) and Langerhans cells of skin (26), were shown to function as antigen-presenting cells. A common requirement for the accessory role of these cells was the capacity to present antigen in the context of immune response (*Ir*) gene products, the *I* subregion membrane-associated antigens.

A recent study showed that the immune response-associated (Ia) antigen-bearing MØ of the schistosome granuloma also presented soluble antigens to sensitized T cells (23). The present study confirms and extends this observation, showing presentation of both particulate and soluble antigen by the Ia$^+$ GR-MØ, demonstrating an accessory role in both humoral and cell-mediated immune responses.

MATERIALS AND METHODS

Animals and infection. Female 7- to 8-week-old CBA/J mice (Jackson Laboratory, Bar Harbor, Maine) were infected by subcutaneous injection of 25 cercariae of the Puerto Rico strain of *Schistosoma mansoni* (19, 23).

Preparation of GR-MØ. Granulomas from the livers of mice infected 8 weeks, (28, 30) earlier were obtained by low-speed homogenization in a Waring blender for 30 s and sedimented at 1 × *g*. The granulomas were collected on wire screens, rinsed, and incubated for 50 min at 37°C in 2% collagenase (type 1; Sigma Chemical Co., St. Louis, Mo.) in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 0.1% gentamycin, and 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid) buffer. Single cell suspensions were obtained by gently pressing the collagenase-treated granulomas through a wire

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screen. The cells were then washed three times in RPMI 1640 medium. Viability was assessed by eosin dye exclusion and was consistently between 80 and 90%.

Monoclonal antibodies and antisera. The following hybridoma cell lines and their respective antibody specificities were used in the experiments: 10-2.16 (Ia.17) was obtained from the Salk Institute, and MK-D6 ($I\text{-}A^d$) was generously provided by P. Marrack, Denver, Colo. Rabbit anti-mouse immunoglobulin serum was prepared by injecting rabbits with rabbit erythrocytes coated with mouse anti-rabbit erythrocyte hyperimmune antibodies. Complement source was serum obtained by cardiac puncture from 2- to 4-week-old New Zealand White rabbits. The complement was screened against thymocytes and lymph node cells for natural cytotoxicity. Only those batches with less than 5% of natural cytotoxicity were used.

Immunizations. Experimental animals were immunized with L-glutamic^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) (Miles-Yeda, Rehovot, Israel) emulsified in complete Freund adjuvant (Difco Laboratories, Detroit, Mich.) that contained *Mycobacterium butyricum* (500 μg/ml). The animals received 0.2 ml divided between injection sites in both footpads and the base of the tail by the method described by Corradin et al. (6).

Purification of T cells. Popliteal, inguinal, and periaortic lymph nodes were obtained from mice 14 to 21 days after immunization with GAT. The nodes were minced into a single cell suspension, and cell aggregates were removed by gravity sedimentation for 5 min. The cells were washed twice at 350 × *g* and suspended in HEPES-buffered Hanks minimal essential medium containing essential and nonessential amino acids, glutamine, and vitamins and supplemented with 3% human AB+ serum. One milliliter of the cell suspension (50×10^6 cells) was added to nylon wool (0.6 g) columns by the method of Julius et al. (10). The nylon wool-nonadherent cells were collected in the first 15 ml of void volume, and carbonyl iron was added to the nylon wool-purified cells to remove any contaminating MØ (16, 17). After removal of the carbonyl iron, the cell sample was incubated with rabbit anti-mouse immunoglobulin serum at 37°C for 30 min, followed by 30 min at 37°C with rabbit complement. The cells were washed twice, suspended in culture medium containing 1% fresh-frozen autologous serum at a concentration of 1.5×10^6/ml, and incubated overnight in 16-mm culture plates (2 ml per well) to allow adherence of any residual MØ. After 16 to 18 h, the nonadherent T-cell population was removed and used for the antigen-induced proliferation arrays. This cell population was greater than 95% Thy-1^+ and did not respond to antigen.

Culture conditions for reconstitution of a T-cell proliferative response. The GR-MØ were seeded in flat-bottomed microassay plates and allowed to adhere for 2 h in culture medium containing 10% fetal calf serum. The nonadherent cells were removed by washing, and the adherent cells were antigen pulsed for 2 h. Excess antigen (GAT) or purified protein derivative (PPD) was decanted, and the GR-MØ were treated with mitomycin C (4 μg/10^6 cells) for 30 min. All treatments were performed at 37°C in 5% in CO_2. The purified lymph node T cells (4×10^5 per well) were added, and in some experiments, antigen was also added at this time. After the initial 24-h incubation, the culture medium was supplemented with human AB^+ serum to a final concentration of 3%. The cultures were harvested after 96 h of incubation and pulsed with 1 μCi of [^{3}H]thymidine for 18 h before harvesting.

Culture conditions for in vitro antibody response. Dispersed spleen cell cultures were prepared by the method of Mishell and Dutton (14, 17). The cultures were established in 35-mm plastic dishes, with each dish receiving a daily addition of 90 μl of enriched culture medium. Culture were stimulated with 10^7 burro erythrocytes (BRBC) and maintained for 5 days at 37°C in an atmosphere of 10% CO_2–7% O_2–83% N_2 while rocking at 7 cycles/min.

MØ-depleted T-B cells were prepared as previously described (16, 17, 22). MØ-depleted T-B cells obtained from normal mice or primed mice were added to each MØ monolayer (10^7 cells per ml per dish), and the BRBC were added. The in vitro antibody response was assayed at the end of a 5-day culture period by determining the number of plaque-forming cells (PFC) using a modified Jerne plaque assay (9).

RESULTS

GR-MØ as antigen-presenting cells in an in vitro antibody response. Granulomas after 8 weeks of infection were used as the source of inflammatory MØ. Adherence for 2 h resulted in a population which comprised approximately 85% MØ by morphology. This number of adherent cells represented 30% of the total unseparated granuloma cell population. Fifty percent of the adherent cells were Ia antigen positive, and 95% displayed Fc receptors. Other characteristics of these cells have been described by us previously (29, 31).

Since precise numbers of GR-MØ after adherence could not be obtained, the determination of their antigen-presenting capacity was done by using a dose-dependent addition of GR-MØ to MØ-depleted spleen or lymph node cells. The indicated number of GR-MØ added per culture represents the estimated number of adherent cells after the 2-h incubation period (e.g., the number of cells added per culture was twice the stated value, assuming 50% of the cells are adherent). Initial attempts to induce a primary immunoglobulin M (IgM)-PFC response of MØ-depleted normal spleen cells to BRBC using GR-MØ were unsuccessful, as shown in Table 1.

When increasing numbers of GR-MØ (5×10^4 to 2×10^6) were allowed to adhere onto culture dishes and 10^7 primed MØ-depleted spleen cells were added, an optimal IgM- and IgG-PFC/culture response occurred with 5×10^4 and 1×10^5 GR-MØ. Higher GR-MØ numbers only resulted in suppression of the response. We currently are determining whether the secretion of PGE by MØ can specifically inhibit the primary antibody response of unprimed T-B cells.

Reconstitution of the T-cell proliferative response to GAT by GR-MØ. The reconstitution of

TABLE 1. PFC response of MØ-depleted normal spleen cells to BRBC

T-B lymphocytes[b] (10^7)	No. of GR-MØ	PFC/culture ± SD[a]			
		Expt 1		Expt 2	
		IgM	IgG[c]	IgM	IgG[c]
NS		849 ± 153	0	176 ± 32	0
PS[d]		899 ± 126	699 ± 218	450 ± 94	575 ± 114
NS	2×10^6[e]	0		1 ± 2	
NS	1×10^6	16 ± 19		3 ± 4	
NS	5×10^5	13 ± 6		6 ± 11	
NS	1×10^5	43 ± 21		51 ± 24	
NS	5×10^4	19 ± 8		35 ± 13	
PS	2×10^6	0	4 ± 7	8 ± 8	10 ± 12
PS	1×10^6	26 ± 11	21 ± 9	38 ± 20	38 ± 20
PS	5×10^5	38 ± 14	53 ± 13	95 ± 9	75 ± 61
PS	1×10^5	541 ± 188	383 ± 68	325 ± 56	93 ± 20
PS	5×10^4	533 ± 258	253 ± 34	360 ± 91	113 ± 42

[a] Experiments 1 and 2 were stimulated with the BRBC antigen, and PFC represent BRBC-specific plaques on day 5 of culture ± standard deviation of quadruplicate dishes.

[b] NS, Normal spleen (unfractionated); PS, primed spleen (unfractionated).

[c] IgG-PFC were developed with anti-IgG (Cappel Laboratories) in the presence of anti-μ chain serum, which completely blocks IgM-PFC formation.

[d] Mice were primed with 10^8 BRBC intravenously 14 to 21 days before use.

[e] Estimated number of cells after adherence of granuloma cell preparation.

the T-cell proliferative response to GAT, an antigen under *Ir* gene control mapping to the *I-A* subregion, by GR-MØ was examined. Initial experiments with GAT (250 μg/ml) present during the entire culture period showed that optimal proliferation of T cells occurred ($P < 0.001$) when 10^5 GR-MØ were added to 5×10^5 purified lymph node T cells (Fig. 1). The PPD response (100 μg/ml), albeit much lower, was also optimal with 10^5 GR-MØ.

Although significant responses were observed, these cultures had high backgrounds (9,000 to 15,000 cpm) probably caused by contaminating MØ in our T-cell preparation which acted as antigen-presenting cells with the constant presence of antigen (GAT, 250 μg/ml) during the culture period. To overcome this problem, the GR-MØ were adhered for 2 h and pulsed with GAT (250 μg) before the addition of the T-cell preparations. As shown in Fig. 2, antigen-pulsed GR-MØ not only induced T-cell proliferation but also yielded a clear-cut dose response. The background values (T cells pulsed with GAT) were less than 5,000 cpm. Thus, GR-MØ effectively functioned as antigen-presenting cells after brief exposure to antigen. Maximal proliferation occurred with approximately 10^5 GR-MØ per well and 4×10^5 T cells. Higher numbers of GR-MØ resulted in suppression of the response.

Role of GR-MØ *H-2I* gene products in antigen presentation. As previously discussed, we had demonstrated that antigen presentation of GAT by BMDM, as with splenic MØ, was mediated through *H-2I* region gene products (21, 22). Figure 3 shows that antibodies recognizing a product(s) of the *I-A* subregion expressed on GR-MØ could inhibit T-cell responses. Experiments were performed with GR-MØ which had been pulsed either with GAT (250 μg) or PPD (100 μg), and maximum response was observed with 3×10^4 GR-MØ. The addition of 10-2.16 monoclonal anti-Ia antibody recognizing a product of the *I-A*^{k} subregion to the GR-MØ obtained

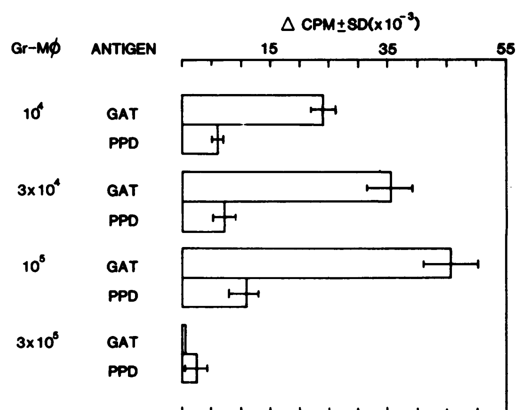

FIG. 1. Presentation of GAT or PPD antigens to immune T cells by adherent GR-MØ. The granuloma cell preparation was allowed to adhere for 2 h, washed, and mitomycin c treated. The number of granuloma cells added per well was twice that given for GR-MØ, since only ~50% of the cells had previously been shown to be adherent. Lymph node T cells (4×10^5 per well) and antigen (GAT, 250 μg/ml; PPD, 100 μg/ml) were then added. SD, Standard deviation.

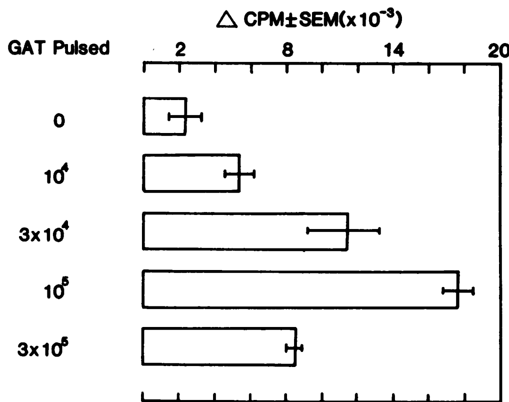

FIG. 2. Induction of T-cell proliferation by GAT-pulsed adherent GR-MØ. After adherence as described in the legend to Fig. 1, the GR-MØ were pulsed with GAT (250 μg) for 1 h. SEM, Standard error of the mean.

from CBA (I-A^k) mice resulted in inhibition of the GAT-specific T-cell response. Control cultures which received antibodies which recognize the *I-A* product of a different haplotype (I-A^d) had no effect. Similar results were obtained when the GR-MØ were pulsed with PPD before culturing with immune T cells and monoclonal anti-*I-A* antibodies (data not shown).

DISCUSSION

In the present experiments the accessory role of the GR-MØ in humoral and cell-mediated immune responses was examined. Whereas adherent GR-MØ were ineffective in the in vitro induction of the primary humoral response, they effectively reconstituted IgM and IgG responses of spleen lymphocytes primed to BRBC. Because the primary immune responses may need more prolonged cell-cell interaction, it is possible that GR-MØ, which lose their surface Ia antigens between 2 and 4 days (3, 14), failed to participate in antigen presentation during the primary response.

GR-MØ also effectively reconstituted the proliferative response of lymph node T lymphocytes primed to the synthetic terpolymer GAT. These experiments revealed two important points: (i) antigen presentation (GAT or PPD) was more efficient when MØ were briefly pulsed rather than coincubated with antigen over a period of several days; and (ii) higher MØ-lymphocyte ratios invariably suppressed not only the secondary proliferative responses of T cells but also T- and B-cell interaction during the anti-BRBC response. Recently, MØ-mediated suppression of lymphocyte proliferation responses has been the subject of numerous investigations. These studies showed that MØ activated by various means may suppress lymphocyte proliferation by secretion of prostaglandins (24), interferon (15), thymidine (18), or hydrogen peroxide (13). Because the schistosome GR-MØ are lymphokine activated (5) and demonstrate tumoricidal activity (12), it is likely that they exerted their suppressive effect by one or more of these secreted agents. This point is presently being investigated.

The effective abrogation of both GAT and PPD presentation by anti-*I-A* antiserum treatment of MØ clearly indicated that the GR-MØ presented these antigens in association with *H-2I* region gene products. These observations confirm a recent report that described the nonspecific accessory function of the Ia$^+$ schistosome GR-MØ in the T-cell proliferative assay (23) and extend it by showing that these cells handle both particulate and soluble antigens and are capable of interacting with T lymphocytes in the mediation of cell-mediated as well as humoral responses. Recently, the schistosome egg-induced liver granuloma has been shown to focally synthesize immunoglobulins and egg-specific antibodies (4). Thus, the intralesional MØ are presumed to interact with various subsets of granuloma lymphocytes in cell-mediated as well as humoral responses. Ongoing studies clearly indicate that these cells also reconstitute the egg antigen-specific proliferative response of lymph node lymphocytes from infected mice (Fed. Proc. **41**:962, 1982). The role of these Ia$^+$ GR-MØ in the induction and regulation of the granulomatous response is the subject of current studies.

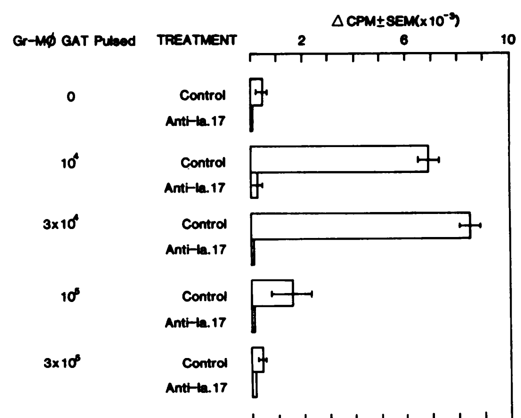

FIG. 3. Effects of anti-*I-A* serum treatment on the induction of T-cell proliferation by GAT-pulsed adherent GR-MØ. After adherence and antigen pulsing as described for Fig. 1 and 2, T cells were added. Control cultures received 50 μl of supernatant from MK-D6 hybridoma (anti-I-A^d), and experimental cultures received 50 μl of supernatants from hybridoma 10-2.16 (anti-*I-A*. Ia.17). SEM, Standard error of the mean.

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