REGULATION BY THE *H-2* GENE COMPLEX OF MACROPHAGE-LYMPHOID CELL INTERACTIONS IN SECONDARY ANTIBODY RESPONSES IN VITRO*

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The development of optimal antibody responses to T-cell-dependent antigens requires the participation of and interactions among at least three distinct types of cells in the immune system: macrophages (Mϕ)[1]; thymus-derived cells (T cells); and precursors of antibody-producing cells (B cells) (1). Mϕ have the critical function of presenting antigen in a highly immunogenic form to T and B cells to initiate the immune response (1, 2). Efficient physiologic interactions among antigen-specific murine T and B cells in the development of secondary IgG antibody responses appear to require that these cells share specificities encoded by the *I* region of the *H-2* complex (3, 4). Similarly, the generation of carrier-specific helper T cells in vitro has been reported to require that Mϕ and T cells also share specificities encoded by the *I* region of the *H-2* complex (5). In another experimental system, combinations of immune guinea pig lymphocytes and Mϕ must be syngeneic for successful development of DNA synthetic responses to the immunizing antigen in vitro (6, 7). By contrast, genetic restrictions have not been observed in interactions among murine Mϕ and lymphoid cells (T cells and B cell) necessary for the development of primary plaque-forming cell (PFC) responses in vitro; lymphoid cells develop comparable primary PFC responses when incubated with antigen and syngeneic or allogeneic Mϕ (references 1, 2, and 8-14; and footnote 2).

In the course of the experiments investigating the lack of genetic restrictions in primary PFC responses, it became obvious that one significant difference between our experiments and those demonstrating genetic restrictions in Mϕ-lymphocyte interactions was that the latter used immune lymphocytes. In this communication, we report experiments investigating the ability of syngeneic and allogeneic antigen-bearing Mϕ to stimulate secondary PFC responses by immune lymphoid cells in vitro. The results demonstrate that immune lymph-

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[1] Abbreviations used in this paper: GAT, terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; HBSS, Hanks' balanced salt solution lacking sodium bicarbonate; IgG, refers to IgG_1 and IgG_2 in aggregate; Mϕ, macrophage(s); PFC, plaque-forming cell(s).

[2] Pierce, C. W., and J. A. Kapp. Lack of genetic restrictions regulating macrophage-lymphoid cell interactions in primary antibody responses *in vitro*. Manuscript in preparation.

oid cells develop secondary PFC responses preferentially when stimulated by antigen-bearing Mϕ syngeneic to the Mϕ used to immunize the lymphoid cells and that these genetic restrictions are controlled by the *H-2* complex.

Materials and Methods

Mice. Male C57BL/6 (*H-2^b*), C57BL/10 (*H-2^b*), P/J (*H-2^p*), DBA/1 (*H-2^q*), B10.G (*H-2^q*), D1.LP (*H-2^b*), B10.A (*H-2^a*), A/J (*H-2^a*), and (C57BL/6 × DBA/1)F_1 (*H-2$^{b/q}$*) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine, or the Department of Pathology Animal Facility at Harvard Medical School, Boston, Mass. The mice were maintained on laboratory chow and acidified-chlorinated water and used when 2-6-mo old.

Antigens. Sheep erythrocytes (SRBC) (Grand Island Biological Co., Grand Island, N. Y.) were washed three times with Hanks' balanced salt solution (HBSS) before use as indicator cells in the PFC assay or as antigen in culture (15). The synthetic linear random terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) (Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill.), mol wt approximately 45,000, was prepared for use as antigen in culture (16), for preparing GAT-Mϕ (13, 17), and for coupling to SRBC for use as indicator cells in the PFC assay (16), as described previously.

Culture System and Hemolytic Plaque Assay. Spleen cells or splenic lymphoid cells, depleted of Mϕ by adherence techniques (18), at 10^7 cells/ml in completely supplemented Eagle's minimal essential medium (MEM) containing 10% fetal calf serum (lot M26302; Reheis Chemical Co., Kankakee, Ill.), were incubated with graded numbers of GAT-Mϕ, or Mϕ and 10^7 SRBC, or 5 μg GAT for 5 days under modified Mishell-Dutton conditions (15). Details of specific experiments are in the text. IgM and IgG PFC responses to SRBC and IgG GAT-specific PFC responses were assayed on SRBC and GAT-SRBC indicator cells, respectively, using the slide modification of the Jerne hemolytic plaque assay (15, 16). Preparation of GAT-SRBC and specificity controls for the GAT PFC assay have been described (16).

Preparation of GAT-Bearing Macrophages. Peritoneal exudate cells, as a source of Mϕ, were collected from mice injected intraperitoneally (i.p.) with 1 ml sterile 10% proteose peptone broth (Difco Laboratories, Detroit, Mich.) 3 days previously. This procedure routinely yielded 6-8 × 10^6 cells per mouse, approximately 85% of which were morphologically Mϕ. These cells were washed three times with HBSS, adjusted to 2 × 10^6 cells/ml in HBSS, and reacted with 100 μg/ml GAT (containing 1% [^{125}I]GAT) at 4°C for 45-60 min (13, 17). The cells were washed three times with 50 volumes of HBSS and adjusted to the desired density in HBSS. GAT bound to Mϕ was quantitated by counting a portion of the cells in a Packard Autogamma Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) and is expressed as nanograms GAT/10^5 or 10^6 cells in the tables. GAT-Mϕ were added in graded numbers to cultures of 10^7 spleen or splenic lymphoid cells immediately after preparation. In one experiment (Table I) peritoneal exudate Mϕ, not reacted with GAT, were added in graded numbers to cultures of 10^7 splenic lymphoid cells and 10^7 SRBC.

Immunization of Mice. Mice were immunized by i.p. injection of 3-4 × 10^6 GAT-Mϕ (bearing 20-30 ng GAT/10^6 cells) or 10 μg GAT in a mixture of magnesium-aluminum hydroxide gel (Maalox; Wm. H. Rorer, Inc., Fort Washington, Pa.) and pertussis vaccine (Eli Lilly & Co., Indianapolis, Ind.) (16). At various intervals after immunization, spleen cells from these mice were assayed for secondary PFC responses to GAT in vitro.

Results

PFC Responses of GAT-Primed Lymphoid Cells Stimulated by Syngeneic and Allogeneic Mϕ. The lack of genetic restrictions regulating efficient Mϕ-lymphoid cell interactions in primary PFC responses will be reported in detail separately.[2] However, to present the data on genetic restrictions in Mϕ-lymphoid cell interactions in secondary PFC responses in the proper context, the relevant data on the lack of such restrictions in the primary response should be summarized. First, syngeneic and allogeneic Mϕ supported development of comparable primary PFC responses to SRBC and GAT in vitro. Second, when

GAT-Mϕ were incubated for 24 h alone before addition to lymphoid cell cultures, approximately 90% of the GAT initially associated with Mϕ was released into the culture medium. Since the PFC responses stimulated by these aged syngeneic and allogeneic GAT-Mϕ were comparable and not significantly less than responses stimulated by freshly prepared GAT-Mϕ, the possibility that GAT is transferred from allogeneic Mϕ to syngeneic Mϕ contaminating the splenic lymphoid cells and that this GAT actually stimulates the PFC response is unlikely. Third, since allogeneic Mϕ may stimulate a mixed lymphocyte response with resultant release of factors which stimulate antibody responses, DNA synthetic responses have been measured in the same cultures used to assess PFC responses; 7×10^4 allogeneic Mϕ have never stimulated significant DNA synthetic responses in cultures of 10^7 C57BL/6 lymphoid or spleen cells ($E/C < 1.5$). Further, factors which stimulate or enhance PFC responses have not been demonstrated in the medium from such cultures.

In other experiments, we observed that splenic lymphoid cells from mice immunized with GAT developed secondary PFC responses to GAT preferentially when incubated with syngeneic GAT-Mϕ. To probe this phenomenon further, C57BL/6 mice were immunized with GAT in Maalox-pertussis and their splenic lymphoid cells were incubated with graded numbers of syngeneic or allogeneic (DBA/1 $H\text{-}2^q$) GAT-Mϕ or normal Mϕ and SRBC (Table I). As a control, primary responses to GAT by virgin C57BL/6 lymphoid cells stimulated by the same GAT-Mϕ were determined. Syngeneic and allogeneic GAT-Mϕ stimulated comparable primary PFC responses to GAT. By contrast, GAT-primed C57BL/6 lymphoid cells preferentially developed secondary PFC responses to GAT when stimulated by syngeneic GAT-Mϕ, the same Mϕ that were involved in the immunization process of the lymphoid cells in vivo. These same GAT-primed lymphoid cells, however, developed comparable primary PFC responses to SRBC when incubated with syngeneic or allogeneic Mϕ, indicating that the restrictions observed in secondary responses are antigen-specific and not a general result of immunization.

In further experiments, C57BL/6 mice were immunized by i.p. injection of syngeneic or allogeneic (DBA/1) GAT-Mϕ or normal Mϕ as indicated in Table II. 28 days later, spleen cells from these mice were incubated with graded numbers of C57BL/6 or DBA/1 GAT-Mϕ or 5 μg soluble GAT. Normal DBA/1 Mϕ were added to some cultures for control purposes. Spleen cells from mice immunized with C57BL/6 GAT-Mϕ developed secondary PFC responses to GAT when incubated with soluble GAT or C57BL/6 GAT-Mϕ, but not when incubated with allogeneic DBA/1 GAT-Mϕ (A). By contrast, spleen cells from mice immunized with DBA/1 GAT-Mϕ developed secondary PFC responses to GAT when incubated with DBA/1 GAT-Mϕ, but not when incubated with soluble GAT or C57BL/6 GAT-Mϕ (B). Spleen cells from mice immunized with both C57BL/6 and DBA/1 GAT-Mϕ developed comparable secondary PFC responses when stimulated with soluble GAT, C57BL/6, or DBA/1 GAT-Mϕ (C). As a control, unprimed C57BL/6 spleen cells developed comparable primary PFC responses when stimulated with soluble GAT, C57BL/6, or DBA/1 GAT-Mϕ (E).

To evaluate possible effects due to sensitization of C57BL/6 lymphocytes by DBA/1 Mϕ, C57BL/6 mice were immunized with C57BL/6 GAT-Mϕ plus normal

TABLE I

PFC Responses of GAT-Primed Lymphoid Cells Stimulated by Syngeneic and Allogeneic Mφ

GAT-Mφ/culture	Day 5 IgG GAT-specific PFC/culture			
	10^7 virgin lymphoid cells – C57BL/6 (*H-2^b*)		10^7 GAT-primed lymphoid cells* – C57BL/6 (*H-2^b*)	
	C57BL/6 Mφ‡ (*H-2^b*)	DBA/1 Mφ‡ (*H-2^q*)	C57BL/6 Mφ‡ (*H-2^b*)	DBA/1 Mφ‡ (*H-2^q*)
7 × 10^4	2,950	2,445	3,980	1,260
3.5 × 10^4	530	1,195	3,310	710
1 × 10^4	<10	105	1,130	105
No Mφ – 5 μg GAT	<10		205	

Mφ/culture + 10^7 SRBC	Day 5 anti-SRBC PFC/culture			
	10^7 GAT-primed lymphoid cells* – C57BL/6 (*H-2^b*)			
	C57BL/6 Mφ§		DBA/1 Mφ§	
	IgM	IgG	IgM	IgG
7 × 10^4	1,535	490	1,340	490
3.5 × 10^4	1,095	245	675	150
1 × 10^4	270	60	405	110
No Mφ + 10^7 SRBC	195	85	–	–

* Lymphoid cells were prepared from spleen cell suspensions from C57BL/6 mice immunized 53 days previously by i.p. injection of 10 μg GAT in Maalox-pertussis. At culture initiation, these cells had 25 IgG GAT-specific PFC per 10^7 cells.

‡ C57BL/6 Mφ, 1.20 ng GAT/10^5 cells; DBA/1 Mφ, 1.25 ng GAT/10^5 cells.

§ Normal, non-GAT-bearing Mφ.

DBA/1 Mφ (D). Further, cultures with both C57BL/6 GAT-Mφ and normal DBA/1 Mφ provide critical control information. Normal DBA/1 Mφ had no significant effect on responses stimulated by C57BL/6 GAT-Mφ in cultures of spleen cells from mice immunized with C57BL/6 GAT-Mφ (A), C57BL/6 GAT-Mφ and DBA/1 GAT-Mφ (C), C57BL/6 GAT-Mφ and normal DBA/1 Mφ (D), or unprimed spleen cells (E). These observations indicated that DBA/1 Mφ did not stimulate a suppressive effect on secondary responses of appropriately immunized spleen cells to C57BL/6 GAT-Mφ. Further, DBA/1 Mφ added to cultures of spleen cells from mice immunized with DBA/1 GAT-Mφ did not enhance the responses in cultures containing C57BL/6 GAT-Mφ (B). This suggested that allogeneic Mφ did not stimulate a nonspecific enhancing effect and, more importantly, that any transfer of GAT from C57BL/6 to DBA/1 Mφ was insufficient to stimulate a secondary PFC response to GAT.

Comparable results have been obtained when P/J (*H-2^p*) or BALB/c (*H-2^d*) Mφ were substituted for DBA/1 Mφ throughout the experiment. Further, significant DNA synthetic responses ($E/C < 1.5$) were not detected in cultures of spleen cells from mice immunized with allogeneic Mφ and *H-2*-identical alloge-

TABLE II

Secondary PFC Responses to GAT Stimulated by Syngeneic and Allogeneic Mφ in Cultures of Spleen Cells Primed with GAT-Mφ

GAT-Mφ used to prime spleen cells*	GAT-Mφ/culture	Day 5 IgG GAT-specific PFC/culture 10^7 spleen cells – C57BL/6 (*H-2^b*)		
		C57BL/6 Mφ‡ (*H-2^b*)	DBA/1 Mφ‡ (*H-2^q*)	5 μg GAT
A C57BL/6 (*H-2^b*)	5×10^4	425	<10	
	2.5×10^4	265	<10	680
	2.5×10^4§	420	–	
B DBA/1 (*H-2^q*)	5×10^4	20	1,020	
	2.5×10^4	30	760	<10
	2.5×10^4§	30	–	
C C57BL/6 + DBA/1	5×10^4	1,510	850	
	2.5×10^4	1,040	910	1,270
	2.5×10^4§	1,020	–	
D C57BL/6 + normal DBA/1	5×10^4	355	<10	
	2.5×10^4	840	<10	1,020
	2.5×10^4§	350	–	
E None (unprimed spleen cells)	5×10^4	1,110	870	
	2.5×10^4	410	520	1,255
	2.5×10^4§	1,520	–	

* C57BL/6 mice were immunized 28 days previously by i.p. injection of 4×10^6 of the indicated Mφ. C57BL/6 Mφ, 33.5 ng GAT/10^6 cells; DBA/1 Mφ, 27.0 ng GAT/10^6 cells. At culture initiation, these spleen cells had <25 IgG GAT-specific PFC per 10^7 cells.

‡ C57BL/6 Mφ, 2.78 ng GAT/10^5 cells; DBA/1 Mφ, 3.15 ng GAT/10^5 cells.

§ 2.5×10^4 non-GAT-bearing DBA/1 Mφ were added to cultures containing 2.5×10^4 C57BL/6 GAT-Mφ.

neic Mφ. These genetic restrictions regulating efficient Mφ-lymphoid cell interactions in secondary antibody responses cannot be demonstrated before 2 wk and disappear gradually 8 wk after a single immunization with GAT-Mφ.

Localization of Genetic Restrictions Regulating Macrophage-Lymphoid Cell Interactions in Secondary PFC Responses to the H-2 Complex. The next experiments were designed to determine if the restrictions regulating Mφ-lymphoid cell interactions in secondary PFC responses were controlled by the *H-2* gene complex. C57BL/6 mice were immunized with syngeneic or allogeneic (DBA/1) GAT-Mφ and 24 days later spleen cells from these mice were stimulated with GAT-Mφ from the five strains of mice indicated in Table III. Primary PFC responses to GAT by virgin spleen cells demonstrated that all the GAT-Mφ were capable of stimulating antibody responses (A). Spleen cells from mice immunized with C57BL/6 GAT-Mφ developed secondary PFC responses to GAT preferentially when stimulated with C57BL/6 (*H-2^b*), D1.LP (*H-2^b* on DBA/1 background), and (C57BL/6 × DBA/1)F_1 (*H-2^{b/q}*) GAT-Mφ (B). Further, spleen cells from mice immunized with allogeneic DBA/1 GAT-Mφ developed secondary PFC responses preferentially when stimulated with DBA/1 (*H-2^q*), B10.G

TABLE III
Secondary PFC Responses to GAT by C57BL/6 Spleen Cells Primed with GAT-Mϕ Depend on the H-2 Haplotype of the Stimulating Macrophages

	GAT-Mϕ used to prime spleen cells*	GAT-Mϕ/ culture	Day 5 IgG GAT-specific PFC/culture 10^7 spleen cells – C57BL/6 (*H-2*^{b})				
			C57BL/6 Mϕ‡ (*H-2*^{b})	DBA/1 Mϕ‡ (*H-2*^{q})	B10.G Mϕ‡ (*H-2*^{q})	D1.LP Mϕ‡ (*H-2*^{b})	(B6 × D1)F_1 Mϕ‡ (*H-2*$^{b/q}$)
A	None (unprimed spleen cells)	7 × 10^4	740	800	940	500	330
		5 × 10^4	980	850	760	500	1,050
		2.5 × 10^4	970	630	710	380	240
		None	<10	–	–	–	–
B	C57BL/6 (*H-2*^{b})	7 × 10^4	210	<10	20	330	570
		5 × 10^4	350	<10	<10	350	230
		2.5 × 10^4	40	<10	50	240	200
		None	40	–	–	–	–
C	DBA/1 (*H-2*^{q})	7 × 10^4	30	210	480	40	430
		5 × 10^4	20	150	260	40	310
		2.5 × 10^4	90	220	320	40	<10
		None	30	–	–	–	–

* C57BL/6 mice were immunized 24 days previously by i.p. injection of 3 × 10^6 of the indicated Mϕ. C57BL/6 Mϕ, 33.5 ng/10^6 cells; DBA/1 Mϕ, 25.0 ng/10^6 cells. At culture initiation these spleen cells had <30 IgG GAT-specific PFC per 10^7 cells.

‡ C57BL/6 Mϕ, 2.25 ng GAT/10^5 cells; DBA/1 Mϕ, 1.75 ng GAT/10^5 cells; B10.G Mϕ, 2.00 ng GAT/10^5 cells; D1.LP Mϕ, 2.00 ng GAT/10^5 cells; (C57BL/6 × DBA/1)F_1, (B6 × D1)F_1 Mϕ, 1.85 ng GAT/10^5 cells.

(*H-2*^{q} on C57BL/10 background), and (C57BL/6 × DBA/1)F_1 (*H-2*$^{b/q}$) GAT-Mϕ (C).

Thus, secondary PFC responses to GAT are elicited preferentially by Mϕ syngeneic or semisyngeneic at the *H-2* complex with the Mϕ which presented GAT to the lymphocytes during the in vivo immunization process. The experiment in Table IV demonstrates the involvement of the *H-2* complex in this phenomenon again, using spleen cells from B10.A (*H-2*^{a}) mice immunized with B10.A GAT-Mϕ. These spleen cells developed secondary PFC responses when stimulated with B10.A or A/J (*H-2*^{a}) GAT-Mϕ, but not when stimulated with allogeneic B10 (*H-2*^{b}) or P/J (*H-2*^{p}) GAT-Mϕ. It should be noted in both Tables III and IV that GAT-Mϕ from strains with the same genetic background and differing only at the *H-2* complex from Mϕ used in the immunization process, failed to stimulate significant secondary PFC responses to GAT in vitro.

Discussion

These experiments illustrate some major points concerning genetic restrictions regulating efficient Mϕ-lymphoid cell interactions in the development of primary and secondary antibody responses in vitro. First, in primary antibody responses, no genetic restrictions have been demonstrated; syngeneic and allogeneic Mϕ support development of comparable responses. Second, in the second-

TABLE IV

Secondary PFC Responses to GAT by B10.A Spleen Cells Primed with GAT-Mφ Depend on the H-2 Haplotype of Stimulating Macrophages

GAT-Mφ used to prime spleen cells*	GAT-Mφ/ culture	Day 5 IgG GAT-specific PFC/culture 10^7 spleen cells – B10.A (*H-2^a*)			
		B10.A Mφ‡ (*H-2^a*)	A/J Mφ‡ (*H-2^a*)	B10 Mφ‡ (*H-2^b*)	P/J Mφ‡ (*H-2^p*)
None (unprimed spleen cells)	7×10^4	590	450	580	470
	5×10^4	1,110	400	450	340
	None	<10	–	–	–
B10.A (*H-2^a*)	7×10^4	760	670	<10	<10
	5×10^4	660	780	60	60
	None	70	–	–	–

* B10.A mice were immunized 15 days previously by i.p. injection of 3×10^6 B10.A Mφ bearing 17.0 ng GAT/10^6 cells. At culture initiation, these cells had 30 IgG GAT-specific PFC per 10^7 cells.

‡ B10.A Mφ, 2.00 ng GAT/10^5 cells; A/J Mφ, 2.50 ng GAT/10^5 cells; B10 Mφ, 2.00 ng GAT/10^5 cells; P/J Mφ, 1.80 ng GAT/10^5 cells.

ary antibody response, genetic restrictions are operative; immunized spleen cells develop antibody responses preferentially when stimulated with antigen-bearing Mφ syngeneic with the Mφ which presented antigen to the virgin lymphocytes during the immunization process in vivo. Third, these genetic restrictions are controlled by the *H-2* complex. Fourth, they can be demonstrated only during a limited period, from 2 to 8 wk, after a single immunization with limiting quantities of antigen on Mφ. Fifth, these restrictions in secondary antibody responses are antigen specific; responses of the same primed lymphoid cells to another antigen do not exhibit genetic restrictions. Sixth, these genetic restrictions appear to operate at the level of the immune T cell, although restrictions at the level of the B cell have not yet been rigorously excluded (19).

The antigen used in these experiments was the synthetic linear random terpolymer of GAT. Antibody responses to GAT are under *H-2*-linked, immune response gene control; mice of the $H\text{-}2^{a,b,d,f,j,k,r,u,v}$ haplotypes are "responders" (20); spleen cells from these mice develop IgG GAT-specific PFC responses when stimulated with soluble GAT or GAT-Mφ in Mishell-Dutton cultures. Spleen cells from mice of the $H\text{-}2^{n,p,q,s}$ haplotypes fail to respond to GAT and are "nonresponders" (16, 20). Mφ and helper T cells are required for development of antibody responses to GAT by responder B cells in vitro, and the defect in nonresponder mice is not in Mφ (13). Mφ from peptone-induced peritoneal exudates of responder and nonresponder mice bind approximately equal quantities of GAT, and these Mφ bearing nanogram quantities of GAT stimulate comparable PFC responses by responder lymphoid cells (13, 14, 17).

The genetic restrictions regulating efficient Mφ-lymphoid cell interactions in secondary antibody responses have been investigated, to date, only with GAT. However, these restrictions have been demonstrated with three types of responder spleen or lymphoid cells: C57BL/6 (*H-2^b*), BALB/c (*H-2^d*), and B10.A (*H-2^a*); and with Mφ from several murine strains, which are both responders and nonresponders to GAT and which may or may not share specificities

encoded by the *H-2* complex with the responder lymphoid or spleen cells. Thus, the observations appear to reflect a general phenomenon in secondary responses to this antigen and are not restricted to limited combinations of Mϕ and lymphoid cells.

It should be pointed out, however, that the observed genetic restrictions in secondary antibody responses are the only criteria of successful in vivo immunization in these experiments available at this time. IgG GAT-specific PFC responses in spleens of mice immunized with GAT-Mϕ are insignificant compared to those elicited by 10 μg of GAT. The secondary PFC responses to GAT in vitro have the same kinetics of development and are of the same general magnitude as primary PFC responses. The possibility that immunization with GAT-Mϕ may lead to a significant shift in the Ig class of the PFC from IgG_1 to IgG_2 in the secondary response is currently under investigation.

Another observation which deserves comment at this juncture is that spleen cells from mice immunized with allogeneic GAT-Mϕ, although developing secondary PFC responses when stimulated with the same allogeneic GAT-Mϕ, do not develop significant primary PFC responses when stimulated with soluble GAT or syngeneic GAT-Mϕ. In part, this phenomenon may be related to the problem of criteria of immunization discussed above. Since these spleen cells do respond to the GAT-Mϕ used for immunization, it is clear that the immune apparatus is intact, i.e., functional specific T cells and B cells are present. Since other studies have indicated that the genetic restrictions operate at the level of the T cell and not the B cell (19), this observation implies that virgin T cells were either not present in spleens of mice immunized with GAT-Mϕ, or that, if present, their function was either pre-empted or suppressed by the primed T cells. At this time, we have no data to permit a choice among these possibilities.

The observations that secondary antibody responses by immunized lymphoid cells develop preferentially with Mϕ syngeneic with those used for immunization and that these genetic restrictions appear to operate at the level of the immune T cells, provide an explanation for the genetic restrictions in Mϕ-lymphocyte interactions observed in DNA synthetic responses to antigen by guinea pig cells (6, 7). The lymphocytes in these experiments were from animals immunized in the presence of syngeneic Mϕ. The fact that these lymphocytes develop DNA synthetic responses preferentially in the presence of syngeneic Mϕ is, therefore, entirely consistent with the present findings. More recent observations involving transfer of delayed hypersensitivity in mice indicate that sensitized T cells elicit these responses only when transferred to hosts whose Mϕ are syngeneic at the *I* region of the *H-2* complex with those present during the sensitization of the T cells (21, 22). These observations emphasize the critical nature of the Mϕ membrane-antigen complex in the sensitization of T cells and in eliciting subsequent responses by immune T cells.

The present findings appear to be in conflict with recent observations that helper T cells develop in vitro only in the presence of Mϕ sharing specificities encoded by the *I* region of the *H-2* complex with the T cells (5). However, in the assay of helper T-cell activity, Mϕ syngeneic with the T cells, but not syngeneic with Mϕ used to generate the helper T cells, have been employed. On the basis of the present findings, it could be predicted that, if Mϕ syngeneic with those

used to generate helper T cells were used in the assay, the helper T cells would function preferentially with these Mϕ. If the genetic restrictions involved in the generation of helper T cells are indeed explained on the basis of the secondary response restrictions demonstrated in the present experiments, both phenomena should be attributable to *I*-region gene products expressed on Mϕ. Studies mapping the genetic restrictions in the present system are in progress.

The observations that specifically sensitized T cells respond preferentially when confronted with the same Mϕ-antigen complex used for immunization have considerable implications for the understanding of fundamental mechanisms in immune responses to T-cell-dependent antigens. First, these observations provide evidence for a more actively specific role for Mϕ in immunizing T cells and eliciting subsequent specific responses by T cells than just presentation of antigen in a highly immunogenic form. Moreover, a role for antigens encoded by the *H-2* complex in this process is demonstrated. Thus, immune T cells appear to recognize and respond to specific antigen selectively or preferentially when confronted with that antigen in the context of Mϕ-membrane molecules encoded by the *H-2* complex. Whether Mϕ-membrane molecules are modified in some way when presenting antigen, i.e. "altered-self concept," or whether T cells recognize antigen only in the context of specific, unaltered membrane molecules, i.e. "linked-recognition concept," is not known at this time (23).

Nevertheless, the Mϕ membrane-antigen complex involved in these phenomena may be analogous to the hapten-carrier complex involved in stimulating B cells, i.e., Mϕ membrane molecules may function as a "carrier" for T-cell antigen recognition. Since the genetic restrictions regulating Mϕ-lymphoid cell interactions in secondary antibody responses are determined by the *H-2* complex, these phenomena may represent an expanded capacity of T cells to recognize antigens encoded by the *I* region of the *H-2* complex, analogous to the mixed lymphocyte reaction. Recent observations indicate that T cells participating in the mixed lymphocyte response can also participate in responses to nonhistocompatibility antigens (24). From an evolutionary point of view, recognition of histocompatibility antigens is a primitive mechanism. It is possible that during evolution T cells have expanded the library of antigens which they recognize and respond to by "seeing" these antigens displayed on Mϕ membranes in the context of histocompatibility antigens. This ability to recognize new antigens would provide obvious survival advantages for the species.

Summary

The ability of antigen-bearing syngeneic and allogeneic peptone-induced peritoneal exudate macrophages to support development of primary and secondary antibody responses by murine lymphoid or spleen cells in vitro has been investigated. The antigen used was the terpolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT). Syngeneic and allogeneic macrophages supported development of comparable primary antibody responses to GAT, indicating that genetic restrictions do not limit efficient macrophage-lymphocyte interactions in primary responses. By contrast, immunized spleen or lymphoid cells developed secondary antibody responses preferentially when stimulated in vitro with GAT on macrophages syngeneic to the macrophages used to present GAT during in

vivo immunization. Thus, genetic restrictions regulate efficient macrophage-lymphocyte interactions in secondary antibody responses. These restrictions have been demonstrated from 2 to 8 wk after a single immunization with limiting quantities of GAT and are controlled by the *H-2* gene complex. The implications that immune lymphocytes selectively recognize and respond to antigen presented in the context of the macrophage membrane-antigen complex which sensitized the lymphocytes initially are considered.

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References

1. Pierce, C. W., and J. A. Kapp. 1976. The role of macrophages in antibody responses *in vitro*. *In* Immunobiology of the Macrophage. D. S. Nelson, editor. Academic Press, Inc., New York. 1–33.
2. Unanue, E. R. 1972. The regulatory role of macrophages in antigenic stimulation. *Adv. Immunol.* 15:95.
3. Katz, D. H., M. Graves, M. E. Dorf, H. Dimuzio, and B. Benacerraf. 1975. Cell interactions between histoincompatible T and B lymphocytes. VIII. Cooperative responses between lymphocytes are controlled by genes in the *I* region of the *H-2* complex. *J. Exp. Med.* 141:263.
4. Pierce, S. K., and N. R. Klinman. 1975. The allogeneic bisection of carrier-specific enhancement of monoclonal B-cell responses. *J. Exp. Med.* 142:1165.
5. Erb, P., and M. Feldmann. 1975. The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage-T-cell interaction for helper cell induction with soluble antigens. *J. Exp. Med.* 142:460.
6. Rosenthal, A. S., and E. M. Shevach. 1973. The function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. Exp. Med.* 138:1194.
7. Shevach, E. M., and A. S. Rosenthal. 1973. The function of macrophages in antigen recognition by guinea pig T lymphocytes. II. Role of the macrophages in the regulation of genetic control of the immune response. *J. Exp. Med.* 138:1213.
8. Hartmann, K.-U., R. W. Dutton, M. McCarthy, and R. I. Mishell. 1970. Cell components in the immune response. II. Cell attachment separation of immune cells. *Cell. Immunol.* 1:182.
9. Haskill, J. S., P. Byrt, and J. Marbrook. 1970. In vitro and in vivo studies of the immune response to sheep erythrocytes using partially purified cell preparations. *J. Exp. Med.* 131:57.
10. Shortman, K., and J. Palmer. 1971. The requirement for macrophages in the *in vitro* immune response. *Cell. Immunol.* 2:399.
11. Cosenza, H., and L. D. Leserman. 1972. Cell interactions *in vitro*. I. Role of the third cell in the *in vitro* response of spleen cells to erythrocyte antigens. *J. Immunol.* 108:418.
12. Katz, D. H., and E. R. Unanue. 1973. Critical role of determinant presentation in the induction of specific responses in immunocompetent cells. *J. Exp. Med.* 137:967.
13. Kapp, J. A., C. W. Pierce, and B. Benacerraf. 1973. Genetic control of immune responses in vitro. II. Cellular requirements for the development of primary plaque-forming cell responses to the random terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) by mouse spleen cells in vitro. *J. Exp. Med.* 138:1121.

14. Pierce, C. W., J. A. Kapp, S. M. Solliday, M. E. Dorf, and B. Benacerraf. 1974. Immune responses in vitro. XI. Suppression of primary IgM and IgG plaque-forming cell responses in vitro by alloantisera against leukocyte alloantisera. *J. Exp. Med.* 140:921.
15. Pierce, C. W., B. M. Johnson, H. E. Gershon, and R. Asofsky. 1970. Immune responses in vitro. III. Development of primary γM, γG, and γA plaque-forming cell responses in mouse spleen cell cultures stimulated with heterologous erythrocytes. *J. Exp. Med.* 134:395.
16. Kapp, J. A., C. W. Pierce, and B. Benacerraf. 1973. Genetic control of immune responses in vitro. I. Development of primary and secondary plaque-forming cell responses to the random terpolymer L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) by mouse spleen cells in vitro. *J. Exp. Med.* 138:1107.
17. Pierce, C. W., J. A. Kapp, D. D. Wood, and B. Benacerraf. 1974. Immune responses *in vitro*. X. Functions of macrophages. *J. Immunol.* 112:1181.
18. Pierce, C. W. 1973. Immune responses *in vitro*. VI. Cell interactions in the development of primary IgM, IgG and IgA plaque-forming cell responses *in vitro*. *Cell. Immunol.* 9:453.
19. Pierce, C. W., J. A. Kapp, and B. Benacerraf. 1976. Stimulation of antibody responses *in vitro* by antigen-bearing syngeneic and allogeneic macrophages. *In* The Role of Products of the Histocompatibility Gene Complex in Immune Responses. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 391–401.
20. Benacerraf, B., and D. H. Katz. 1975. The histocompatibility-linked immune response genes. *Adv. Cancer Res.* 21:121.
21. Miller, J. F. A. P., M. A. Vadas, A. Whitelaw, and J. Gamble. 1975. H-2 gene complex restricts transfer of delayed-type hypersensitivity. *Proc. Natl. Acad Sci. U. S. A.* 72:5095.
22. Miller, J. F. A. P., M. A. Vadas, A. Whitelaw, and J. Gamble. 1975. H-2 linked Ir gene regulation of delayed-type hypersensitivity in mice. *In* The Role of Products of the Histocompatibility Gene Complex in Immune Responses. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 403–415.
23. Rosenthal, A. S., and E. M. Shevach. 1976. Macrophage-T lymphocyte interaction: the cellular basis for genetic control of antigen recognition. *In* The Role of Products of the Histocompatibility Gene Complex in Immune Responses. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 335–350.
24. Heber-Katz, E., and D. B. Wilson. 1976. Sheep red blood cell-specific helper activity in rat thoracic duct lymphocyte populations positively selected for reactivity to specific strong histocompatibility alloantigens. *J. Exp. Med.* 143:701.