CELLULAR AND GENETIC CONTROL OF ANTIBODY RESPONSES IN VITRO

III. Immune Response Gene Regulation of Accessory Cell Function

By ALFRED SINGER, CAROL COWING, KAREN S. HATHCOCK, HOWARD B. DICKLER, AND RICHARD J. HODES

(From the Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014)

Antibody responses to a variety of antigens, including certain synthetic polypeptides such as poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L][1] and poly-L-(His,Glu)-poly-D,L-Ala--poly-L-Lys [(H,G)-A--L] are regulated in the mouse by immune response (*Ir*) genes which are located within the murine major histocompatibility complex (*H-2*) (1). Since these *Ir* gene-controlled antibody responses require interactions among several cell populations, it is important for an understanding of the mode of action of *Ir* genes to determine the cell type(s) which express *Ir* gene function. Despite much research effort, the identity of such cell type(s) remains controversial (2, 3).

The cell type(s) involved in *Ir* gene control has been most extensively studied in the responses to (T,G)-A--L and (H,G)-A--L of *H-2*^{a}, *H-2*^{b}, and *H-2*^{k} mouse strains. Some investigators have implicated T lymphocytes as the cell type which expressed *Ir* genes in these responses since: (*a*) the ability of responder *H-2*^{b} strains to produce (T,G)-A--L-specific IgG antibodies and to generate (T,G)-A--L-specific memory was abolished by neonatal thymectomy (4), and (*b*) B lymphocytes from nonresponder *H-2*^{k} strains were capable of producing (T,G)-A--L-specific antibodies when immunized with (T,G)-A--L complexed to methylated bovine serum albumin (5) or when stimulated by nonspecific graft-versus-host reactions (6). Conversely, other investigators have implicated B lymphocytes as the cell type which expresses *Ir* genes in these same responses since: (*a*) limiting dilution experiments demonstrated that reactive (T,G)-A--L-specific bone marrow cells, not thymocytes, were the limiting cell type in nonresponder *H-2*^{k} strains (7), and (*b*) bone marrow cells from (T,G)-A--L nonresponder *H-2*^{a} and *H-2*^{k} strains, and from (H,G)-A--L nonresponder *H-2*^{b} strains, were unable to absorb or to respond to antigen-specific T-cell replacing

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; C', guinea pig complement; FACS-II, fluorescence-activated cell sorter; Fl-F(ab')$_2\alpha$Fab, fluorescein isothiocyanate-conjugated rabbit F(ab')$_2\alpha$anti-mouse Fab; (H,G)-A--L, poly-L-(His,Glu)-poly-D,L-Ala--poly-L-Lys; *Ir*, immune response; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cells; RαMB, rabbit anti-mouse brain serum; sIg, surface immunoglobulin; SRBC, sheep erythrocytes; (T,G)-A--L, poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys; TNP, trinitrophenyl.

factors, even though educated thymocytes from these nonresponder strains were competent to produce such (T,G)-A--L- and (H,G)-A--L-specific factors (8, 9). However, the possibility was not excluded in these previous studies that *Ir* genes were expressed in cells other than T or B cells which were required for the generation of antibody responses, i.e. accessory cells.

Recently, an in vitro primary IgM antibody-forming cell assay using normal spleen cells was characterized (10, 11). This assay could potentially be utilized to determine which cell type(s) express *Ir* genes, since responses to trinitrophenyl (TNP) conjugates of (T,G)-A--L and (H,G)-A--L in this system were under autosomal dominant, *H-2*-linked *Ir* gene control, and required the presence of adherent accessory cells, T cells, and B cells (10, 11). In this report, the possibility was examined that accessory cells participate in the *Ir* gene control of primary plaque-forming cell (PFC) responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L.

Materials and Methods

Animals. C57BL/10 (B10), B10.A, (B10 × B10.A)F_1, B10.A(4R), B10.A(5R), A/J, A.BY, and (A/J × A.BY)F_1 mice of both sexes were obtained from The Jackson Laboratory, Bar Harbor, Maine. All mice were used at 6–12 wk of age, and were sex matched in each experiment.

Antigens. (T,G)-A--L (lot MC-3; Yeda Research and Development Co., Ltd., Rehovot, Israel), (H,G)-A--L (the generous gift of Dr. Edna Mozes, Weizmann Institute of Science, Rehovot, Israel), and Keyhole limpet hemocyanin (KLH) (lot 530195; Calbiochem, San Diego, Calif.) were conjugated with 2,4,6-trinitrobenzene sulfonate (Pierce Chemical Co., Rockford, Ill.) as previously described (10). The degree of TNP modification was 8 TNP groups per 100,000 daltons (T,G)-A--L, 5 TNP groups per 100,000 daltons (H,G)-A--L, and 12 TNP groups per 100,000 daltons KLH. The final concentration used in culture was the optimum concentration for each antigen preparation and was 100–250 μg/ml TNP-(T,G)-A--L, 100–250 μg/ml TNP-(H,G)-A--L, and 5–10 μg/ml TNP-KLH (10, 11).

Preparation of Cells

SEPHADEX G-10 PASSAGE OF SPLEEN CELLS. Spleen cells were passed sequentially over two Sephadex G-10 columns (lot 9067; Pharmacia, Inc., Piscataway, N. J.) by a modification of the method of Ly and Mishell (12) described elsewhere (10). Total cell recovery after two sequential Sephadex G-10 passages ranged from 40 to 60%. In a series of 16 consecutive experiments, the percentage of B (surface $Ig^+[sIg^+]$) cells (10) in the cell population was not significantly affected by the Sephadex G-10 passages (51.1 ± 2.4% before G-10 passage and 53.0 ± 2.8% after G-10 passage); the percentage of T (rabbit anti-mouse $brain^+[R\alpha MB^+]$) cells (10) was somewhat increased (28.5 ± 1.8% before G-10 passage and 39.4 ± 2.8% after G-10 passage). Conversely, Sephadex G-10 passage markedly reduced the number of phagocytic cells as measured by latex ingestion (6.9 ± 1.2% before G-10 passage to 0.73 ± 0.1% after G-10 passage) (13).

PREPARATION AND CHARACTERIZATION OF SPLEEN ADHERENT CELLS (SAC). The preparation of 2-h glass-adherent spleen cells has been described in detail previously (10, 13). All adherent cell populations were sequentially treated with anti-Thy 1.2 serum (lot 231-72-6; Litton Bionetics, Kensington, Md.), guinea pig complement (C′) (Flow Laboratories, Inc., Rockville, Md.), and irradiation with 1,000 R. The adherent cells were then precultured overnight on a roller drum at 37°C and added to cell cultures the next day (10).

The final viable cell recovery of adherent, radiation-resistant, anti-Thy 1.2-treated cells was ≅1% of the initial spleen cell population and, in a large number of experiments, consisted of 50–80% phagocytic cells, 8–15% nonphagocytic sIg^+ cells, no detectable (<0.3%) $R\alpha MB^+$ cells, and 15–25% nonphagocytic cells negative for latex, sIg, and RαMB ("null" cells).

DEPLETION OF SIG^+ CELLS FROM THE SPLEEN ADHERENT CELL POPULATION USING THE FLUORESCENCE-ACTIVATED CELL SORTER (FACS-II). Spleen adherent cells were prepared as above but the medium lacked phenol red to diminish autofluorescence. After the overnight preincubation, 5 × 10^6 adherent cells were resuspended in 0.05 M phosphate-buffered saline, pH 7.2, containing 2%

bovine serum albumin (BSA), and reacted with 0.2 μg of an affinity-purified fluorescein isothiocyanate-conjugated rabbit $F(ab')_2$ anti-mouse Fab reagent [Fl-$F(ab')_2\alpha$Fab] which was made against Fab fragments derived from a pool of IgG obtained from a wide variety of mouse strains. This reagent was predominantly reactive with kappa light chains, it has been extensively characterized on the FACS-II[2], and it was the generous gift of Dr. Thomas Chused, National Institutes of Health, Bethesda, Md. The cells were then applied to the FACS-II (Becton, Dickinson & Co., Rutherford, N. J.) for sorting according to a procedure previously described by others (14). Fluorescence-negative cells (50% of the unsorted population) were pooled, washed in the cold, and resuspended for culture. 52% of such cells were latex$^+$ and none of the latex$^+$ (0/300) nor latex$^-$ (0/300) cells were sIg$^+$ when analyzed by fluorescence microscopy with or without restaining with fluorescein isothiocyanate-conjugated rabbit anti-mouse Ig serum.

Culture Conditions. 5×10^5 normal spleen cells or mixtures of 5×10^5 Sephadex G-10-passed spleen cells plus graded numbers of spleen adherent cells were cultured for 4 days in a total volume of 200 μl per flat-bottom well of microtiter plates at 37°C in a 5% CO_2-humidified air atmosphere as previously described (10, 11). Viable cell recovery was generally >50%, and was not significantly affected by the presence or absence of antigen, the passage of spleen cells over Sephadex G-10, or the addition of spleen-adherent cells (10, 11). Two to four parallel cultures were pooled to constitute one culture group; three replicate culture groups were used in all experiments.

Hemolytic PFC Assay. The number of TNP-specific direct PFC was determined for each individual culture group as the difference in number of PFC observed with TNP-conjugated sheep erythrocytes (SRBC) in the absence and presence of TNP-BSA as a specific inhibiting reagent. Greater than 80% of the number of PFC in each responding culture group was inhibited by the concentration of TNP-BSA used (5×10^{-5} M final concentration of TNP, a concentration which does not inhibit anti-SRBC PFC) (10). A variable number of parallel cultures were pooled to constitute one culture group to allow a reasonable number of PFC (>30) to be counted on each slide. Therefore, results were corrected to the number of PFC/10^7 cultured cells, and are expressed as the geometric mean $\times/\div$ (standard error) of TNP-inhibitable PFC of triplicate culture groups.

Statistics. Statistical analyses were performed using the two-tailed Student's t test on the geometric means $\times/\div$ (standard errors) of the uncorrected number of TNP-inhibitable PFC of the culture groups compared, i.e. on the numbers of TNP-inhibitable PFC actually counted in each culture group.

Results

Ability of Spleen Adherent Cells to Function as Accessory Cells in the in Vitro Anti-Hapten PFC Responses of Normal (B10 × B10.A)F_1 Spleen Cells to TNP-(T,G)-A--L, TNP-(H,G)-A--L, and TNP-KLH. To examine the possibility that *Ir* genes regulate the function of accessory cells in the PFC responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L, it was necessary to demonstrate the requirement for such cells in the in vitro responses to these antigens. Normal (B10 × B10.A)F_1 spleen cells responded to TNP-(T,G)-A--L, TNP-(H,G)-A--L, and TNP-KLH (Table I). Passage of these spleen cells over Sephadex G-10 columns markedly reduced the percentage of phagocytic cells, did not alter the percentage of B cells, and somewhat increased the percentage of T cells (Materials and Methods). Sephadex G-10 passage also abrogated the responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L, and significantly diminished ($P < 0.01$) the response to TNP-KLH (Table I). The persistent though significantly diminished response to TNP-KLH was considered to be due to either an accessory cell-independent component of the response to this antigen, or to the residual accessory cells present in the Sephadex G-10-passed population which might have been sufficient to support partial responsiveness to a multi-deter-

[2] T. M. Chused, S. S. Kassan, and S. O. Sharrow. Increased frequency of cells with low density surface immunoglobulin in NZB mice. Manuscript in preparation.

TABLE I

The in Vitro Anti-Hapten PFC Responses of Normal (B10 × B10.A)F_1 Lymphocytes to TNP-(T,G)-A--L and TNP-(H,G)-A--L are Abolished by Sephadex G-10 Passage and Restored by the Addition of Spleen Adherent Cells

Exp.	(B10 × B10.A)F_1 Spleen cells (5×10^5/culture)	No. of (B10 × B10.A)F_1 spleen adherent cells added per culture	Direct PFC/10^7 cultured cells*			
			No antigen	TNP-(T,G)-A--L	TNP-(H,G)-A--L	TNP-KLH
1	Untreated	0	48(1.76)	752(1.10)	627(1.29)	5754(1.09)
	G-10-Passed	0	0	0	0	1080(1.18)
	G-10-Passed	2.5×10^4	0	772(1.10)	742(1.12)	3741(1.29)
2	Untreated	0	28(1.44)	559(1.02)	317(1.09)	2060(1.10)
	G-10-Passed	0	0	54(1.65)	40(1.49)	1153(1.11)
	G-10-Passed	2.5×10^4	41(1.44)	514(1.10)	275(1.22)	2659(1.18)
		2.5×10^4				
	G-10-Passed	Depleted of sIg^+ cells‡	0	1148(1.04)	371(1.16)	2418(1.40)

* Geometric mean (SE) of TNP-inhibitable PFC of parallel triplicate culture groups.

‡ Stained for surface Ig with Fl-F(ab')$_2\alpha$Fab and depleted of sIg^+ cells using the FACS-II. Such cells were 52% latex$^+$ and none (0/600) were sIg^+.

minant antigen such as TNP-KLH, but not sufficient to support any response to antigens with more limited numbers of antigenic determinants such as TNP-(T,G)-A--L or TNP-(H,G)-A--L. The responses of G-10-passed spleen cells to all three antigens were reconstituted by the addition of small numbers (2.5×10^4 or 5% of the total cultured cells) of syngeneic (B10 × B10.A)F_1 spleen adherent cells as accessory cells (Table I).

The reconstituting spleen adherent cell populations were prepared by 2-h adherence to glass, treatment with anti-Thy 1.2 serum + C', irradiation with 1,000 R, and contained three identifiable cell populations: (*a*) 50–80% phagocytic cells, (*b*) 8–15% nonphagocytic sIg^+ cells, and (*c*) 15–25% nonphagocytic, sIg^-, RαMB$^-$ cells ("null" cells). To determine whether the nonphagocytic sIg^+ cells were responsible for the accessory cell function of the spleen adherent cell populations, Ig-bearing spleen adherent cells were stained with Fl-F(ab')$_2\alpha$Fab and removed from the other spleen adherent cells using the FACS-II. The remaining cells fully reconstituted the responses to all three antigens tested, even though they contained no detectable sIg^+ cells (Table I, Exp. 2). Thus, a population consisting of sIg^- and radiation-resistant glass-adherent cells, devoid of both T and B cells, enriched in spleen phagocytic cells but also containing nonphagocytic "null" cells was sufficient to reconstitute the responses of Sephadex G-10-passed spleen cells to TNP-(T,G)-A--L, TNP-(H,G)-A--L, and TNP-KLH. It has not yet been determined whether the phagocytic cells or the nonphagocytic "null" cells are responsible for the accessory cell function of the spleen adherent cell populations.

Ability of Spleen Adherent Cells from the Responder, but not Nonresponder, Parent to Restore the Responses of Sephadex G-10-Passed (B10 × B10.A)F_1 Spleen Cells to TNP-(T,G)-A--L and TNP-(H,G)-A--L. It has been previously shown that the in vitro PFC responses of normal spleen cells to TNP-(T,G)-A--L and TNP-(H,G)-A--L are under autosomal dominant, *H-2*-linked *Ir* gene control which map within the *K* or *I-A* regions of *H-2* (11). *H-2^b* strains are responders to TNP-(T,G)-A--L but not TNP-(H,G)-A--L, whereas *H-2^a* strains are responders to TNP-(H,G)-A--L but not TNP-(T,G)-A--L; (*H-2^b* × *H-2^a*)F_1 mice are phenotypically responders to both antigens. The question was then asked

TABLE II

Spleen Adherent Cells from the Nonresponder Parental Strain Do Not Reconstitute the Responses of (B10 × B10.A)F_1 Lymphocytes to TNP-(T,G)-A--L or TNP-(H,G)-A--L

(B10 × B10.A)F_1 Spleen cells (5×10^5/culture)	No. of spleen adherent cells added per culture	Strain of spleen adherent cells	Direct PFC/10^7 cultured cells*				
			No antigen	TNP-(T,G)-A--L	P‡	TNP-(H,G)-A--L	P
Untreated	0	–	0	452(1.04)	<0.01	348(1.12)	<0.01
G-10-Passed	0	–	41(2.08)	36(1.37)		40(1.49)	
G-10-Passed	2×10^4	(B10 × B10.A)F_1	0	476(1.26)	<0.01	331(1.07)	<0.01
		B10	20(1.0)	337(1.09)	<0.01	41(1.44)	NS
		B10.A	0	60(1.78)	NS	325(1.05)	<0.01
G-10-Passed	1×10^4	(B10 × B10.A)F_1	0	218(1.50)	<0.05	404(1.26)	<0.01
		B10	0	358(1.15)	<0.01	25(1.25)	NS
		B10.A	0	0	NS	193(1.03)	<0.02

* Geometric mean (SE) of TNP-inhibitable PFC of parallel triplicate culture groups.

‡ Compared to responses of G-10-passed spleen cells alone. NS, not significant. ($P > 0.05$).

whether spleen adherent cells from both B10 (*H-2*^{b}) and B10.A(*H-2*^{a}) mice could function as accessory cells in the responses of Sephadex G-10-passed (B10 × B10.A)F_1 spleen cells.

The addition of F_1 spleen adherent cells to Sephadex G-10-passed (B10 × B10.A)F_1 lymphocytes restored the responses to both TNP-(T,G)-A--L and TNP-(H,G)-A--L (Tables I and II). However, the response to TNP-(T,G)-A--L was restored by spleen adherent cells from the responder (B10) parent, but not by such cells from the nonresponder (B10.A) parent (Table II). Similarly, the response to TNP-(H,G)-A--L was restored by spleen adherent cells from the responder (B10.A) parent, but not by such cells from the nonresponder (B10) parent (Table II). Other experiments showed that the ability of spleen adherent cells from responder strains, but not nonresponder strains, to restore these responses was consistent over the entire range of spleen adherent cell doses tested, from 5×10^3 to 1×10^5 per culture (1 to 20% of the total cultured cells). Analogous results were obtained with (A/J × A.BY)F_1 lymphocytes reconstituted with spleen adherent cells from syngeneic F_1, A/J (*H-2*^{a}), or A.BY(*H-2*^{b}) mice (data not shown).

It was concluded that (*a*) spleen adherent cells from the nonresponder parent strain are unable to function as accessory cells in the responses to TNP-(T,G)-A--L or TNP-(H,G)A--L, and that (*b*) the accessory cell function of spleen adherent cells in these responses is controlled by autosomal dominant, *H-2*-linked genes.

The ability of responder and nonresponder parental strain spleen adherent cells to cooperate with G-10-passed F_1 cells was also examined in experiments in which the spleen adherent cells were pulsed with antigen, thoroughly washed, and added as "antigen-presenting" cells without the addition of further antigen. In these experiments, spleen adherent cells from both parental strains presented TNP-KLH to F_1 lymphocytes, but only spleen adherent cells from the responder parent, and not the nonresponder parent, presented TNP-(T,G)-A--L or TNP-(H,G)-A--L (data not shown).

Subregion Mapping of the Genes Controlling the Ability of Spleen Adherent Cells to Restore the Responses of (B10 × B10.A)F_1 Lymphocytes to TNP-(T,G)-A--L and TNP-(H,G)-A--L. To further localize within *H-2* the gene(s) control-

TABLE III

Spleen Adherent Cells from the Nonresponder Recombinant Strain Do Not Reconstitute and Do Not Suppress the Responses of (B10 × B10.A)F$_1$ Lymphocytes to TNP-(T,G)-A--L and TNP-(H,G)-A--L

(B10 × B10.A)F$_1$ Spleen cells (5 × 10^5/culture)	No. of spleen adherent cells added per culture	Strain of spleen adherent cells	Direct PFC/10^7 cultured cells*					
			No antigen	TNP-(T,G)-A--L	*P*‡	TNP-(H,G)-A--L	*P*	TNP-KLH
Untreated	0	–	0	495(1.16)	<0.001	322(1.11)	<0.001	3474(1.06)
G-10-Passed	0	–	0	0		0		1143(1.80)
A. G-10-Passed	2 × 10^4	(B10 × B10.A)F$_1$	0	542(1.19)	<0.001	859(1.30)	<0.001	4650(1.21)
		B10	0	455(1.21)	<0.001	0	NS	4196(1.20)
		B10.A	0	45(1.68)	NS	401(1.34)	<0.01	3238(1.12)
		B10.A(4R)	0	28(1.44)	NS	378(1.06)	<0.001	4277(1.21)
		B10.A(5R)	0	889(1.11)	<0.001	66(2.38)	NS	3301(1.26)
G-10-Passed	1 × 10^4	(B10 × B10.A)F$_1$	45(1.52)	647(1.68)	<0.01	500(1.12)	<0.001	3424(1.02)
		B10	0	355(1 11)	<0.001	0	NS	2750(1.03)
		B10.A	0	0	NS	347(1.20)	<0.001	2839(1.03)
		B10.A(4R)	0	0	NS	450(1.15)	<0.001	2502(1.28)
		B10.A(5R)	0	375(1.27)	<0.01	0	NS	2739(1.15)
B. G-10-Passed	1 × 10^4 + 1 × 10^4	B10.A(4R) + B10.A(5R)	0	296(1.08)	<0.001	431(1.06)	<0.001	3919(1.09)

* Geometric mean (SE) of TNP-inhibitable PFC of parallel triplicate culture groups.

‡ Compared to responses of G-10-passed spleen cells alone. NS, not significant. ($P > 0.05$).

ling the ability of spleen adherent cells to function as accessory cells in the responses to TNP-(T,G)-A--L or TNP-(H,G)-A--L, spleen adherent cells from the *H-2* recombinant strains B10.A(4R) and B10.A(5R) were added to cultures with G-10-passed (B10 × B10.A)F$_1$ lymphocytes. The responses of Sephadex G-10-passed F$_1$ spleen cells to TNP-(T,G)-A--L were restored only by the addition of spleen adherent cells from responder strains (11) (F$_1$, B10, or B10.A(5R)), but were not restored by the addition of such cells from nonresponder strains (B10.A or B10.A(4R)) (Table III, A). Similarly, the responses of Sephadex G-10-passed F$_1$ spleen cells to TNP-(H,G)-A--L were restored only by the addition of spleen adherent cells from responder strains (11) (F$_1$, B10.A or B10.A(4R)), but were not restored by the addition of spleen adherent cells from nonresponder strains (B10 or B10.A(5R)) (Table III, A). Spleen adherent cells from each of these strains fully reconstituted the response of F$_1$ lymphocytes to TNP-KLH (Table III, A).

Since B10, and B10.A(5R) spleen adherent cells restored the response to TNP-(T,G)-A--L, whereas B10.A and B10.A(4R) adherent cells did not, the gene(s) controlling the accessory function of these cells in the response to TNP-(T,G)-A--L is located within the *K* or *I-A* region of the *H-2*^{b} responder haplotype. Similarly, since B10.A, and B10.A(4R) spleen adherent cells reconstituted the response to TNP-(H,G)-A--L, while B10 and B10.A(5R) adherent cells did not, the gene(s) controlling the accessory function of these cells in the response to TNP-(H,G)-A--L is located within the *K* or *I-A* regions of the *H-2*^{a} responder haplotype. Thus, the autosomal dominant *H-2*-linked genes controlling the accessory function of spleen adherent cells in the responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L are located within the same subregions of *H-2* as the autosomal dominant *Ir* genes controlling the responses of intact spleen cells to these antigens, suggesting that they may be the same genes.

Nonresponder Spleen Adherent Cells Do Not Suppress the Responses of (B10 × B10.A)F_1 Lymphocytes to TNP-(T,G)-A--L and TNP-(H,G)-A--L. The antigen-specific unresponsiveness of F_1 lymphocytes in the presence of spleen adherent cells from nonresponder strains could be due either to an inability of nonresponder spleen adherent cells to function as accessory cells for that antigen or, alternatively, to an active antigen-specific suppression induced by the nonresponder adherent cells. To distinguish between these two possibilities, G-10-passed F_1 lymphocytes were cultured with antigen and with a mixture of B10.A(4R) and B10.A(5R) spleen adherent cells (Table III, B). F_1 lymphocytes cultured with a mixture of spleen adherent cells from both strains, responded to both TNP-(T,G)-A--L and TNP-(H,G)-A--L, in addition to TNP-KLH (Table III, B), demonstrating that the responses to these antigens were not actively suppressed by the presence of nonresponder adherent cells.

Discussion

The present study demonstrates that the accessory function of non-T, non-B spleen adherent cells in the in vitro primary IgM anti-hapten PFC responses to the synthetic antigens TNP-(T,G)-A--L and TNP-(H,G)-A--L is regulated by autosomal dominant genes located in the *K* or *I-A* regions of the *H-2* complex. In the present in vitro experiments, unprimed ($H\text{-}2^b \times H\text{-}2^a$)$F_1$ spleen cells passed over Sephadex G-10 columns were unable to respond to TNP-(T,G)-A--L or TNP-(H,G)-A--L. The responses to these antigens were reconstituted by the addition of spleen adherent cells from the responder parental strain, but not from the nonresponder parental strain. The ability of spleen adherent cells to reconstitute these responses was shown to be antigen- and strain-specific in that: (*a*) spleen adherent cells from each parental strain reciprocally reconstituted the response to one synthetic antigen but not the other, and (*b*) spleen adherent cells from both parental strains reconstituted the response to TNP-KLH. Furthermore, experiments which utilized spleen adherent cells from parental *H-2* recombinant strains demonstrated that the ability of these cells to function as accessory cells in these responses was regulated by autosomal dominant, *H-2*-linked genes located in the *K* or *I-A* regions of the responder *H-2* complex. Since the *Ir* genes controlling responsiveness in vitro to TNP-(T,G)-A--L and TNP-(H,G)-A--L and those controlling responsiveness in vivo to (T,G)-A--L and (H,G)-A--L are also located in the *K* or *I-A* regions of the responder *H-2* complex, this result suggests that these *Ir* genes and the genes controlling accessory cell function in these responses may be the same.

The inability of spleen adherent cells from nonresponder strains to function as accessory cells in these responses cannot be due to histocompatibility barriers to cooperation between adherent cells and lymphocytes since (responder × nonresponder)F_1 lymphocytes were reconstituted with semi-syngeneic spleen adherent cells which restored the responses to both TNP-KLH and to at least one of the synthetic antigens. The possibility of antigen-nonspecific allogeneic effects was minimized by not using allogeneic spleen adherent cells and by always depleting the spleen adherent cell populations of T cells and irradiating them. Moreover, allogeneic effects cannot account for the present data since the results were specific for both antigen and the *H-2* type of the spleen adherent cells. The inability of spleen adherent cells from nonresponder strains to

reconstitute the responses of one of the synthetic antigens was also not due to antigen-specific suppression induced by these cells, since the mixing of spleen adherent cells from both responder and nonresponder strains did not suppress the responses to any of the antigens studied.

The present results are consistent with those reported in secondary guinea pig T-cell proliferation assays (15, 16) in which peritoneal exudate cells from the responder but not nonresponder parental strain effectively "presented" antigen to in vivo primed F_1 T cells. The present data extend these previous observations in several ways. First, these data demonstrate that accessory cells can participate in *Ir* gene regulation in antibody-forming cell responses. Second, since the PFC responses described in this report were generated by unprimed F_1 spleen cells, the observed results cannot be due to any effect of previously priming these cells in vivo in the presence of F_1 macrophages. The ability to avoid the potential restrictions on cell-cell collaboration induced by previously antigen-priming the responding lymphocytes is one of the advantages of examining an *Ir* gene-controlled primary IgM response. Third, as noted above, the genes controlling the accessory cell function of spleen adherent cells have been localized to the same region(s) of the *H-2* complex as the *Ir* genes which control in vitro and in vivo responsiveness to the same antigens and, hence, may be the same genes.

The present results do not conflict with previous reports that T cells or B cells express *Ir* genes in responses to (T,G)-A--L or (H,G)-A--L (4–9), since the present study does not examine the possibility that T cells or B cells also express *Ir* genes, and since these previous studies did not exclude the possibility that accessory cells express *Ir* genes. However, *Ir* gene control of accessory cell function could have accounted for these previous results. The present results do appear to conflict with those reported for the *Ir* gene-controlled primary IgG response to the antigen $Glu^{60}Ala^{30}Tyr^{10}$ (17, 18). The reasons for this discrepancy are unclear, but they may relate to the possibility that *Ir* gene defects for different antigens or different strains are expressed in different cell types. Studies to determine the factors actually responsible for these disparate results are in progress.

Several mechanisms have been proposed for the participation of accessory cells in *Ir* gene regulation (15, 16). *Ir* gene function could be inherent in accessory cells per se, and expressed as the ability of these cells to present antigenic determinants in an appropriate fashion for competent T cells or B cells (16). Alternatively, in order to maintain the more traditional concepts that *Ir* genes function in T or B lymphocytes, it can be hypothesized that *Ir* genes regulate the interaction of accessory cells with T or B lymphocytes (15). Regardless of which hypothesis is correct, the present data demonstrate that the localization to *H-2* of the genes controlling responsiveness to TNP-(T,G)-A--L and TNP-(H,G)-A--L could be solely due to the *H-2* region genes of the accessory cells. Consequently, it may not be necessary for lymphocyte receptors for antigen to themselves be *H-2*-region products in order to explain the linkage to *H-2* of the genes controlling the responses to these antigens.

The most straightforward interpretation of the data presented in this report is that the *H-2*-linked genes controlling the accessory cell function of spleen adherent cells in responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L are the

same as the *H-2*-linked *Ir* genes controlling in vitro and in vivo responsiveness to these antigens. These results do not exclude the possible expression of *Ir* genes by T or B lymphocytes as well.

Summary

The possibility was investigated that *Ir* genes regulate the function of cells other than T or B cells in the primary IgM responses to the synthetic antigens trinitrophenylated poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [TNP-(T,G)-A--L] and trinitrophenylated poly-L-(His,Glu)-poly-D,L-Ala--poly-L-Lys [TNP-(H,G)-A--L]. The primary responses of (B10 × B10.A)F_1 spleen cells to both antigens were abrogated by Sephadex G-10 passage, and restored by the addition of spleen adherent cells. The cell type in the spleen adherent cell population active in reconstituting the responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L was a non-T, non-B, radiation-resistant, glass-adherent spleen cell. The responses of Sephadex G-10-passed (responder × nonresponder)F_1 spleen cells to TNP-(T,G)-A--L or TNP-(H,G)-A--L were reconstituted by spleen adherent cells from only responder strains. Spleen adherent cells from F_1 mice reconstituted the responses to both antigens. Spleen adherent cells from each of the strains tested reconstituted the non-*Ir* gene-controlled response to a third antigen, TNP-keyhole limpet hemocyanin. The inability of spleen adherent cells from nonresponder strains to reconstitute the responses to either TNP-(T,G)-A--L or TNP-(H,G)-A--L was not a result of active suppression induced by the presence of nonresponder adherent cells, since a mixture of responder and nonresponder spleen adherent cells reconstituted the responses to both antigens. The use of spleen adherent cells from recombinant strains demonstrated that the autosomal dominant genes controlling the ability of spleen adherent cells to function as accessory cells in the responses to TNP-(T,G)-A--L and TNP-(H,G)-A--L are located in the *K* or *I-A* regions of the responder *H-2* complex, the same region(s) of *H-2* as the *Ir* genes controlling overall in vitro and in vivo responsiveness to these antigens.

The authors particularly wish to thank Dr. Gene Shearer for his encouragement and suggestions throughout the course of this work; Dr. Edna Mozes for the generous gift of (H,G)-A--L; Dr. Thomas Chused for the gift of the Fl-F(ab')$_2$αFab reagent; Ms. Susan Sharrow for her expert operation of the FACS-II; Doctors William Terry, David Sachs, Alan Rosenthal, William Paul, Dinah Singer, and David Pisetsky for critically reviewing this manuscript; Mr. Walter Lyles and Mr. Francis Jones for their care of experimental animals; Mr. John Williams for his expert technical assistance; and Ms. Judy Steckel for the preparation of the manuscript.

Received for publication 14 November 1977.

References

1. McDevitt, H. O., B. D. Deak, D. C. Schreffler, J. Klein, J. H. Stimpfling, and G. D. Snell. 1972. Genetic control of the immune response. Mapping of the *Ir-1* locus. *J. Exp. Med.* 135:1259.
2. Mozes, E. 1975. Expression of immune response (Ir) genes in T and B cells. *Immunogenetics.* 2:397.
3. Benacerraf, B., and D. H. Katz. 1975. Histocompatibility linked immune response genes. *Adv. Cancer Res.* 21:121.
4. Mitchell, G. F., F. C. Grumet, and H. O. McDevitt. 1972. Genetic control of the

immune response. The effect of thymectomy on the primary and secondary antibody response of mice to poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys. *J. Exp. Med.* 135:126.

5. McDevitt, H. O. 1968. Genetic control of the antibody response. Qualitative and quantitative characterization of the antibody response to (T,G)-A--L in CBA and C57 mice. *J. Immunol.* 100:485.
6. Ordal, J. C., and F. C. Grumet. 1972. Genetic control of the immune response. The effect of graft-*versus*-host reaction on the antibody response to poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys in nonresponder mice. *J. Exp. Med.* 136:1195.
7. Lichtenberg, L., E. Mozes, G. M. Shearer, and M. Sela. 1974. The role of thymus cells in the immune response to poly(Tyr,Glu)-poly-D,L-Ala-poly-Lys as a function of the genetic constitution of the mouse strain. *Eur. J. Immunol.* 4:430.
8. Taussig, M. J., and A. J. Munro. 1976. Antigen-specific T-cell factor in cell cooperation and genetic control of the immune response. *Fed. Proc.* 35:2061.
9. Isac, R., and E. Mozes. 1977. Antigen-specific T cell factors: a fine analysis of specificity. *J. Immunol.* 118:584.
10. Hodes, R. J., and A. Singer. 1977. Cellular and genetic control of antibody responses in vitro. I. Cellular requirements for the generation of genetically controlled primary IgM responses to soluble antigens. *Eur. J. Immunol.* 7:892.
11. Singer, A., H. B. Dickler, and R. J. Hodes. 1977. Cellular and genetic control of antibody responses in vitro. II. Ir gene control of primary IgM responses to trinitrophenyl conjugates of poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys and poly-L-(His,Glu)-poly-D,L-Ala--poly-L-Lys. *J. Exp. Med.* 146:1096.
12. Ly, I. A., and R. J. Mishell. 1974. Separation of mouse spleen cells by passage through columns of Sephadex G-10. *J. Immunol. Methods.* 5:239.
13. Cowing, C., B. Schwartz, and H. B. Dickler. 1977. Macrophage Ia antigens. Macrophage populations differ in their expression of Ia antigens. *J. Immunol.* 120:378.
14. Loken, M. R., and L. A. Herzenberg. 1975. Analysis of cell populations with a fluorescence-activated cell sorter. *Ann. N. Y. Acad. Sci.* 54:163.
15. Shevach, E. M., and A. S. Rosenthal. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. II. Role of the macrophage in the regulation of genetic control of the immune response. *J. Exp. Med.* 138:1213.
16. Barcinski, M. A., and A. S. Rosenthal. 1977. Immune response gene control of determinant selection. I. Intramolecular mapping of the immunogenic sites on insulin recognized by guinea pig T and B cells. *J. Exp. Med.* 145:726.
17. 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.
18. Pierce, C. W., R. N. Germain, J. A. Kapp, and B. Benacerraf. 1977. Secondary antibody responses in vitro to L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) by (responder × nonresponder)F_1 spleen cells stimulated by parental GAT-macrophages. *J. Exp. Med.* 146:1827.