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] 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-2a, H-2b, and H-2k 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-2b 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-2k 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-2k strains (7), and (b) bone marrow cells from (T,G)-A--L nonresponder H-2a and H-2k strains, and from (H,G)-A--L nonresponder H-2b strains, were unable to absorb or to respond to antigen-specific T-cell replacing activity.

Abbreviations used in this paper: BSA, bovine serum albumin; C', guinea pig complement; FACS-II, fluorescence-activated cell sorter; Fl-F(ab')2aFab, fluorescein isothiocyanate-conjugated rabbit F(ab')2 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; RaMB, 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)F1, B10.A(4R), B10.A(5R), A/J, A.BY, and (A/J × A.BY)F1 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 530188; 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 (61.1 ± 2.4% before G-10 passage and 53.0 ± 2.8% after G-10 passage); the percentage of T (rabbit anti-mouse brain+[RaMB+]) 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 1-3% 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%) RaMB+ cells, and 15-25% nonphagocytic cells negative for latex, sIg, and RaMB ("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-oFab) 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, 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 × 10⁵ normal spleen cells or mixtures of 5 × 10⁵ 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% CO₂-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 × 10⁻¹ 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/10⁷ cultured cells, and are expressed as the geometric mean (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 (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)F₁, Spleen Cells to TNP-(T,G)-A--L, TNP-(H,G)-A--L, and TNP-KLH.** To examine the possibility that I₂ 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)F₁ 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-

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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.
The in Vitro Anti-Hapten PFC Responses of Normal (B10 x B10.A)F1, 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 x B10.A)F1 Spleen cells (5 x 10⁶ culture) | No. of (B10 x B10.A)F1 spleen adherent cells added per culture | Direct PFC/10⁶ 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 0 772(1.10) 749(1.12) 3741(1.29) |
|      | G-10-Passed 2.5 x 10⁴                       | 0                                               | 782(1.10) 754(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 x 10⁴                       | 0                                               | 54(1.65) 40(1.49) 1153(1.11) |
|      | G-10-Passed 2.5 x 10⁴ 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 F1-F(ab')2 and depleted of sIg+ cells using the FACS-II. Such cells were 52% latex+ and none (0/600) were sIg+.

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-2b strains are responders to TNP-(T,G)-A-L, but not TNP-(H,G)-A-L, whereas H-2a strains are responders to TNP-(H,G)-A-L but not TNP-(T,G)-A-L; (H-2b x H-2a)F1, mice are phenotypically responders to both antigens. The question was then asked...
Spleen Adherent Cells from the Nonresponder Parental Strain Do Not Reconstitute the Responses of (B10 × B10.A)F1, Lymphocytes to TNP-(T.G)-A--L or TNP-(H,G)-A--L

| (B10 × B10.A)F1 | No. of spleen adherent cells added per culture | Strain of spleen adherent cells | No antigen | Direct PFC/10^7 cultured cells* | TNP-(T.G)-A--L | P1 | TNP-(H.G)-A--L | P |
|-----------------|---------------------------------------------|-------------------------------|------------|-------------------------------|----------------|----|----------------|----|
| Untreated       | 0                                           | 0                             | 0          | 452(1.04)                     | <0.01          | 384(1.12) | <0.01 |
|                  | 0                                           | 0                             | 0          | 476(1.26)                     | <0.01          | 331(1.07) | <0.01 |
| G-10-Passed     | 2 × 10^4 (B10 × B10.A)F1                    | 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)F1                    | B10                           | 0          | 210(1.50)                     | <0.05          | 404(1.26) | <0.01 |
|                 |                                              | B10.A                         | 0          | 358(1.15)                     | <0.01          | 251(1.25) | NS   |
|                 |                                              | 0                             | NS         | 189(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)F1 spleen cells.

The addition of F1 spleen adherent cells to Sephadex G-10-passed (B10 × B10.A)F1 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 × 10^6 per culture (1 to 20% of the total cultured cells). Analogous results were obtained with (A/J × A.BY)F1 lymphocytes reconstituted with spleen adherent cells from syngeneic F1, A/J (H-2^a), or A.BY(H-2^a) 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 F1, 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 F1 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)F1, Lymphocytes to TNP-(T,G)-A--L and TNP-(H,G)-A--L. To further localize within H-2 the gene(s) control-
**Ir GENE REGULATION OF ACCESSORY CELL FUNCTION**

**Table III**

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

| (B10 × B10.A/F) | No. of spleen adherent cells added per culture | Strain of spleen adherent cells | Direct PFC/10^6 cultured cells* | No antigen | TNP-(T,G)-A--L | TNP-(H,G)-A--L | P | TNP-KLH |
|-----------------|---------------------------------|---------------------------------|-------------------------------|------------|----------------|----------------|---|----------|
| Untreated       | 0                               | 0                               | 495(1.16)                     | <0.001     | 3221(1.11)     | <0.001         | 3474(1.06)   |
| G-10-Passed     | 0                               | 0                               | 0                             |            |                |                |   |          |
| A. G-10-Passed  | 2 × 10^5                        | (B10 × B10.A/F)                 | 542(1.19)                     | <0.001     | 509(1.30)      | <0.001         | 465(1.21)    |
|                 |                                 | B10                             | 455(1.21)                     | <0.001     | 0              | NS             | 419(1.20)    |
|                 |                                 | B10.A                           | 45(1.68)                      | NS         | 401(1.34)      | <0.01          | 322(1.12)    |
|                 |                                 | B10.A(4R)                       | 28(1.44)                      | NS         | 378(1.06)      | <0.001         | 4277(1.21)   |
|                 |                                 | B10.A(5R)                       | 888(1.11)                     | <0.001     | 66(2.36)       | NS             | 330(1.28)    |
| G-10-Passed     | 1 × 10^5                        | (B10 × B10.A/F)                 | 45(1.52)                      | 647(1.68)  | <0.01          | 500(1.12)      | <0.001       | 349(1.02)  |
|                 |                                 | B10                             | 355(1.11)                     | <0.001     | 0              | NS             | 2700(1.03)   |
|                 |                                 | B10.A                           | 0                             | NS         | 347(1.23)      | <0.001         | 2569(1.03)   |
|                 |                                 | B10.A(4R)                       | 0                             | NS         | 450(1.15)      | <0.001         | 2502(1.28)   |
|                 |                                 | B10.A(5R)                       | 375(1.27)                     | <0.01      | 0              | NS             | 2729(1.15)   |
| B. G-10-Passed  | 1 × 10^5 + 1 × 10^6             | B10.A(4R)                       | 0                             | 296(1.08)  | <0.001         | 431(1.06)      | <0.001       | 3919(1.09) |
|                 |                                 | B10.A(5R)                       | 0                             | NS         | 347(1.20)      | <0.001         | 330(1.28)    |

* 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) lymphocytes. The responses of Sephadex G-10-passed F1 spleen cells to TNP-(T,G)-A--L were restored only by the addition of spleen adherent cells from responder strains (11) (F1, 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 F1 spleen cells to TNP-(H,G)-A--L were restored only by the addition of spleen adherent cells from responder strains (11) (F1, 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 F1 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 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 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)F1 Lymphocytes to TNP-(T,G)-A--L and TNP-(H,G)-A--L. The antigen-specific unresponsiveness of F1 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 F1 lymphocytes were cultured with antigen and with a mixture of B10.A(4R) and B10.A(5R) spleen adherent cells (Table III, B). F1 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-2b × H-2n)F1 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)F1 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 F₁ 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 F₁ spleen cells, the observed results cannot be due to any effect of previously priming these cells in vivo in the presence of F₁ 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 Glu₁₀Ala₃⁰Tyr₁₀ (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 \textit{H}-2-linked \textit{Ir} genes controlling in vitro and in vivo responsiveness to these antigens. These results do not exclude the possible expression of \textit{Ir} genes by T or B lymphocytes as well.

Summary

The possibility was investigated that \textit{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 \textit{[TNP-(T,G)-A--L]} and trinitrophenylated poly-L-(His,Glu)-poly-D,L-Ala--poly-L-Lys \textit{[TNP-(H,G)-A--L]}. The primary responses of (B10 × B10.A)F1 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)F1 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 F1 mice reconstituted the responses to both antigens. Spleen adherent cells from each of the strains tested reconstituted the non-\textit{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 \textit{K} or \textit{I-A} regions of the responder \textit{H}-2 complex, the same region(s) of \textit{H}-2 as the \textit{Ir} genes controlling overall in vitro and in vivo responsiveness to these antigens.

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