MAJOR HISTOCOMPATIBILITY COMPLEX-RESTRICTED SELF RECOGNITION

A Monoclonal Anti-I-A^k Reagent Blocks Helper T Cell Recognition of Self Major Histocompatibility Complex Determinants

By RICHARD J. HODES, KAREN S. HATHCOCK, AND ALFRED SINGER

From the Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Major histocompatibility complex (MHC)[1] genes, in particular those encoded in the *I* region of the murine *H-2*, have been shown to be of functional importance in several aspects of immune response regulation. Thus, *I* region-encoded immune response (*Ir*) genes determine responsiveness or unresponsiveness to a number of antigens (1); and T cell recognition of *I* region gene products is critical for the *H-2* restricted cell interactions that mediate a number of immune responses (2–6). Although these *I* region gene functions are well established, the precise identification of the gene products mediating these functions remains uncertain. In contrast, there exists a class of *I* region gene products, the Ia antigens, for which extensive serologic and biochemical characterization has been accomplished (7–9). The functional role of Ia antigens in immune responses remains undetermined, however, and has been a subject of wide speculation. The present report represents an attempt to assess the functional significance of Ia antigens in the regulation of antibody responses studied in vitro.

A system of primary in vitro antibody responses has been characterized in which responses to soluble trinitrophenyl (TNP)-protein conjugates require the cooperation of T cells, B cells, and accessory cells (6). In these responses, a strict requirement has previously been demonstrated for helper T cell recognition of *K* or *I-A* encoded determinants expressed on accessory cells (6, 10). In the present studies, the functional role of serologically defined cell surface Ia antigens has been investigated. Using Ia-specific serologic reagents as probes, the present study demonstrates that: (*a*) a monoclonal anti-Ia reagent specific for a product of $I\text{-}A^k$ is capable of inhibiting antibody responses in vitro; and that (*b*) such inhibition occurs at the level of an $I\text{-}A^k$ product expressed by accessory cells. Moreover, (*c*) inhibition by this anti-Ia reagent occurs only for responses in which the *I-A* product on accessory cells is actively recognized by helper T cells as self; and (*d*) for *Ir* gene controlled responses, inhibition of response occurs only if the anti-I-A^k reagent is directed against determinants

[1] *Abbreviations used in this paper:* B10, C57BL/10; C′, complement; F_1, (B10 × B10.A)F_1; FCS, fetal calf serum; Ia, *I* region associated; *Ir*, immune response; KLH, keyhole limpet hemocyanin; MEM, Eagle's minimal essential medium; MHC, major histocompatibility complex; PFC, plaque-forming cell; RAMB, rabbit anti-mouse brain serum; SAC, spleen adherent cell; SRBC, sheep erythrocyte; TNP, trinitrophenyl.

encoded by the responder haplotype. These findings are interpreted as evidence for a unique and essential role of accessory cell Ia determinants that must be recognized by T helper cells for the generation of antibody responses.

Materials and Methods

Animals. C57BL/10 (B10), B10.A, and (B10 × B10.A)F_1 (F_1) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. B10.A(4R), B10.A(5R), and B10.MBR mice were generously provided from the colony of Dr. David Sachs (National Institutes of Health, Bethesda, Md.). Adult males 2–5 mo old were used in all experiments.

Chimeras. Recipient mice were irradiated with 950R X-ray and reconstituted 2–6 h later with 15 × 10^6 bone marrow cells that had been pretreated with rabbit anti-mouse brain serum (RAMB) and complement (C′). The RAMB reagent employed has been extensively characterized and is cytotoxic for T cells (11) with no detectable anti-stem cell activity (6). Chimeras are designated as bone marrow donor → irradiated recipient. Spleen cells were obtained from each chimera no earlier than 2 mo postirradiation, and were individually typed by indirect immunofluorescence using *H-2* specific reagents as previously described (6). By such testing, spleen cells from each chimera employed were of donor origin without detectable (<5%) cells of host origin.

Antigens. Keyhole limpet hemocyanin (KLH) (lot 530195, Calbiochem Behring Corp., American Hoechst Corp., San Diego, Calif.) was conjugated with 2,4,6-trinitrobenzene sulfonate (Pierce Chemical Co., Rockford, Ill.) as previously described (11). The degree of TNP substitution was 19 TNP residues/100,000 daltons KLH (TNP-KLH). TNP-KLH was used in culture at a final concentration of 10 μg/ml.

Anti-H-2 Reagent. (B10.A × A/J)F_1 anti-B10 immune ascites was prepared (12) and characterized (13) as previously described, and was generously provided by Dr. D. Sachs.

T CELLS. T cells were prepared by passage of spleen cells over nylon fiber columns and collection of the nylon nonadherent eluate. The purity of each cell population was functionally determined as previously described (11).

(B + ACCESSORY) CELLS. (B + accessory) cells were prepared by depleting spleen cells of T cells by pretreatment with a T cell-specific cytotoxic RAMB reagent as previously described in detail (11), or with a hybridoma anti-thy-1.2 (15) reagent that was the gift of Dr. P. Lake (University College, London, England). The T cell specificities of these reagents were monitored as previously described (11).

B CELLS. B cells were prepared by first depleting spleen cells of adherent cells by passage over Sephadex G-10 columns, and then by depleting the G-10-passed spleen cells of T cells by treatment with RAMB or anti-thy-1.2 + C′.

SPLEEN ADHERENT CELLS (SAC). 2-h glass-adherent cells were prepared as previously described (11). All SAC preparations were pretreated with RAMB + C′, irradiated with 1,000 R, and precultured at 10^7/ml on a roller drum overnight before addition to antibody cultures. Such populations have previously been characterized (11) and consist of 50–80% latex-ingesting cells, 8–15% nonphagocytic surface Ig^+ cells, <0.3% thy-1.2^+ cells, and 15–25% cells negative for all markers. The accessory cell activity of spleen cell populations has been shown to reside in radiation-resistant non-T, non-B, glass-adherent cells (11) that express *I-A* and *I-E/C* region encoded determinants (13), and that have recently been shown to phagocytose latex (16).

Anti-I-A^k Hybridoma. Cells of the hybridoma designated 10-2.16, described by Oi et al. (14) were obtained from the Cell Distribution Center of the Salk Institute (La Jolla, Calif.). Cells were maintained in in vitro passage and the supernate of such cultures was employed in the present studies as monoclonal anti-I-A^k reagent. Previous characterization has shown the product of this cell line to be an antibody of the IgG_{2b} subclass with specificity for a product of *I-A^k* (Ia.17) (14). Further characterization of the hybridoma product employed in the current studies (anti-I-A^k) confirmed the *I-A^k* specificity of this preparation by microcytotoxicity testing, and confirmed specificity for Ia antigen by immunoprecipitation (data not shown).

Anti-I-A^k + C′ Treatment of Cells. For treatment with anti-I-A^k + C′, cells were suspended at 5 × 10^6/ml in Eagle's minimal essential medium (MEM) + 10% fetal calf serum (FCS) (MEM-FCS) containing anti-I-A^k at a dilution of ¼ and incubated for 30 min at 37°C. Cells

were then pelleted by centrifugation, resuspended to the starting volume with rabbit C′ (Type 3, Pel-Freeze Biologicals, Inc., Rogers, Ark.) at a 1/10 dilution in MEM-FCS and incubated for an additional 30 min at 37°C. Cells were then washed with MEM-FCS and resuspended in incubation medium for culture.

Preparation of Cells

ADHERENT CELL-DEPLETED SPLEEN (T + B) CELLS. Spleen cells were depleted of adherent accessory cells by passage over G-10 Sephadex columns as previously described (13). This procedure markedly reduces the percentage of latex-ingesting cells while not significantly altering the percentage of T cells or B cells (11).

CULTURE CONDITIONS. All cultures were performed in a vol of 200 μl/flat-bottomed well of microtiter plates, and were incubated for 4 d in a 5% CO_2-humidified air atmosphere. The medium employed was MEM-FCS supplemented as previously described (11). Cells were harvested by repeated pipetting, washed, resuspended in Hanks' balanced salt solution, and individual cultures assayed for plaque-forming cells (PFC). The cell number of each individual population employed is indicated for each experiment.

PFC ASSAY. Sheep erythrocytes (SRBC) were conjugated with TNP (TNP-SRBC) by the method of Rittenberg and Pratt (17). Direct IgM PFC to TNP-SRBC were assayed by the slide modification of the Jerne hemolytic plaque technique (18). The TNP specificity of the PFC generated under these conditions has been described (11). All points shown in each experiment represent the geometric mean responses of three replicate cultures.

Results

Anti-H-2 and Anti-Ia Reagents Specifically Inhibit Primary Antibody Responses to TNP-KLH. To assess the functional effect of anti-MHC antibodies on in vitro antibody responses, a broadly specific anti-H-2 reagent was first employed. An anti-H-2^b reagent, with demonstrated specificities for products of K^b, I^b, and D^b, was found to completely inhibit the primary response of unprimed B10 (H-2^b) spleen cells to TNP-KLH, while having no effect on the response of B10.A spleen cells (Fig. 1). The same anti-H-2^b reagent has known cross-reactivity for a single product of the H-2^d haplotype, identified as a product of I-A^d (Ia.8) (7), and this reagent was found to inhibit the response of B10.D2 (H-2^d) spleen cells to TNP-KLH (Fig. 1). These findings demonstrate that antibodies to MHC products are capable of inhibiting antibody responses. Moreover, they suggest that antibodies directed at more restricted *H-2* products, possibly even a single *I-A* determinant, may be sufficient to inhibit responses. This possibility was further evaluated by investigating the effect of a monoclonal hybridoma anti-Ia reagent with specificity for a single *I-A* subregion determinant.

A Monoclonal Anti-I-A^k Reagent Specifically Inhibits Primary Antibody Responses to TNP-KLH. The effect of a hybridoma anti-I-A^k product on primary antibody responses to TNP-KLH was determined for unprimed spleen cells from B10, B10.A, and the recombinants B10.A(4R), B10.A(5R), and B10.MBR. The *H-2* haplotypes of these strains are summarized in Table I. Serial dilutions of the monoclonal anti-I-A^k produced profound inhibition of the responses by B10.A, B10.A(4R), and B10.MBR spleen cells, whereas there was no significant effect on responses by B10 or B10.A(5R) (Table I). These data map the susceptibility to inhibition by anti-I-A^k to the *I-A* subregion, consistent with the serologic specificity of this reagent. Thus, a monoclonal anti-Ia reagent with specificity for a single serologically and biochemically characterized product of I-A^k was sufficient to specifically inhibit antibody responses by spleen cells of the I-A^k haplotype.

Inhibition by Anti-I-A^k Occurs through Interaction with B Cells and/or Accessory Cells, and

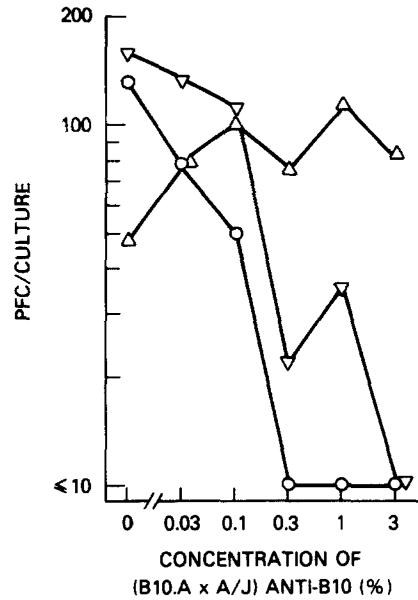

FIG. 1. (B10.A × A/J)F_1 anti B10 immune ascites inhibits the in vitro antibody responses of B10 or B10.D2 spleen cells to TNP-KLH. Immune ascites at the indicated final concentrations was present throughout the 4-d cultures of 5 × 10^5 B10 (○), B10.D2 (▽), or B10.A (△) spleen cells with 10 μg/ml TNP-KLH. <10 PFC/culture were observed in the absence of antigen.

TABLE I

Monoclonal Anti-Ia Reagent Inhibits the Primary In Vitro Antibody Responses of Spleen Cells Expressing the I-A^k Haplotype

Concentration^{-1} of anti-I-A^k*	PFC/culture‡				
	B10§	B10.A	B10.A(4R)	B10.A(5R)	B10.MBR
	bbbbbbbb	kkkkkddd	kkbbbbbb	bbbkkddd	bkkkkkkq
—	141 (1.17)‖	101 (1.26)	117 (1.34)	186 (1.21)	227 (1.21)
4	130 (1.31)	7 (1.92)	3 (1.31)	164 (1.22)	14 (1.44)
8	142 (1.35)	8 (1.31)	5 (1.33)	218 (1.21)	62 (1.86)
16	118 (1.32)	37 (1.63)	15 (1.32)	197 (1.17)	55 (1.17)
32	132 (1.25)	47 (1.95)	98 (1.26)	239 (1.01)	146 (1.14)
64	123 (1.15)	79 (1.05)	53 (1.66)	174 (1.12)	383 (1.04)

* Reciprocal of the concentration of monoclonal anti-I-A^k reagent in culture.
‡ A culture consisted of 5 × 10^5 spleen cells.
§ Strain of spleen cells cultured and MHC haplotype designated as K ABJEC S D.
‖ Geometric mean (geometric SE) of triplicate cultures.

not through Interaction with Helper T Cells. To further assess the role of Ia antigens in antibody responses, the mechanism of anti-I-A^k-mediated inhibition of responses to TNP-KLH was analyzed. Because in vitro PFC responses to TNP-KLH require the cooperation of T cells, B cells, and accessory cells (11), it was possible that the inhibition of antibody responses by monoclonal anti-I-A^k reagent was mediated by its effect on any one or more of these populations. To identify the cellular site(s) at which inhibition by anti-I-A^k was occuring, a series of cell fractionation experiments was carried out in which only one of the cooperating cell populations was of the

appropriate genotype to bind the anti-I-A^k monoclonal reagent. First *H-2*^{b} T cells from B10 → (B10 × B10.A)F_1 chimeras, which were tolerant to both B10 (H-2^b) and B10.A (H-2^a) haplotypes (data not shown), cooperated equally well with B10 or B10.A (B + accessory) cells for responses to TNP-KLH (Fig. 2 A and B). The addition of anti-I-A^k at a final concentration of 1/10 had no effect upon the ability of these B10 → (B10.A)F_1 T cells to cooperate with B10 (B + accessory) cells (Fig. 2 A) as expected because all of these cells expressed only H-2^b determinants. In contrast, addition of anti-I-A^k profoundly inhibited the ability of the same chimera T cells to cooperate with B10.A (B + accessory) cells (Fig. 2 B). Because this reagent was only specific for determinants expressed by the B10.A (B + accessory) cells in these cultures, these findings demonstrate that anti-Ia-mediated inhibition can occur by binding to determinants expressed on cells in the B and/or accessory cell populations. Such results do not, however, exclude the possibility that inhibition could also occur at the level of T cells of the appropriate (*I-A*^{k}) haplotype. This possibility was next assessed

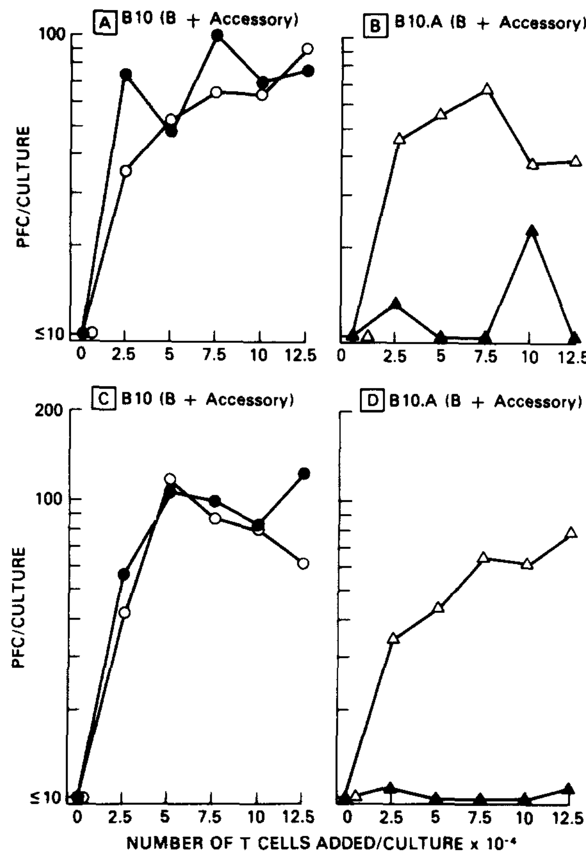

FIG. 2. Monoclonal anti-I-A^k antibody inhibits the in vitro antibody responses of *H-2*^{a} but not *H-2*^{b} (B + accessory) cells. 4 × 10^5 anti-thy-1.2 + C′-treated B10 (○ ●) or B10.A (△ ▲) (B + accessory) cells were cultured in the presence (closed symbols) or absence (open symbols) of anti-I-A^k reagent (final concentration 1/10), and with graded numbers of T cells from B10 → (B10 × B10.A)F_1 (panels A and B), or B10.A → (B10 × B10.A)F_1 (panels C and D) chimeras. TNP-KLH was present at 10 μg/ml. <10 PFC/culture were observed in the absence of antigen, or in cultures containing TNP-KLH and T cells alone.

by examining the responses resulting from the cooperation of B10.A → (B10 × B10.A)F_1 splenic T cells (*H-2*^{a} and therefore *I-A*^{k}) with B10 or B10.A (B + accessory) cells. However, even though anti-I-A^k inhibited the cooperation of these T cells with B10.A (B + accessory) cells, it did not inhibit the cooperation of these *H-2*^{a} T cells with B10 (B + accessory) cells (Fig. 2C and D). Thus, there is no evidence that inhibition by anti-I-A^k occurs by binding to determinants expressed by helper T cells; rather, inhibition results from the binding of anti-I-A^k to determinants expressed on cells within the (B + accessory) cell population.

One possible mechanism for the inhibition of response by anti-I-A^k antibody is the generation of suppressor cells by the interaction of this I-A^k-specific reagent with the I-A^k determinants expressed on B10.A (B + accessory) cells. However, even though anti-I-A^k inhibited the response of B10.A (B + accessory) cells, it did not suppress the ability of B10 (B + accessory) cells to respond when equal cell numbers of these populations were present together in culture (Fig. 3). Thus, the response of B10 (B + accessory) cells was not inhibited by the simultaneous presence of B10.A (B + accessory) cells and anti-I-A^k antibody, demonstrating that nonspecific suppression was not the mechanism by which anti-I-A^k inhibited the generation of antibody responses.

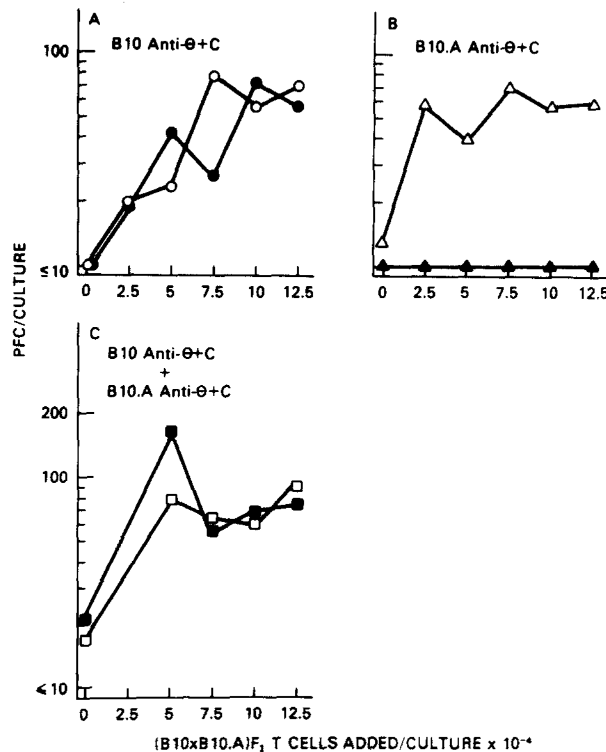

FIG. 3. Inhibition by anti-I-A^k antibody is not mediated by nonspecific suppression. 4 × 10^5 anti-thy-1.2 + C′-treated B10 (○ ●) or B10.A (△ ▲) (B + accessory) cells, or an equal mix of these populations (□ ■) was cultured in the presence (closed symbols) or absence (open symbols) of anti-I-A^k reagent (final concentration 1/10), and with graded numbers of (B10 × B10.A)F_1 T cells. TNP-KLH was present at 10 μg/ml. <10 PFC/culture were observed in the absence of antigen or in cultures containing TNP-KLH and T cells alone.

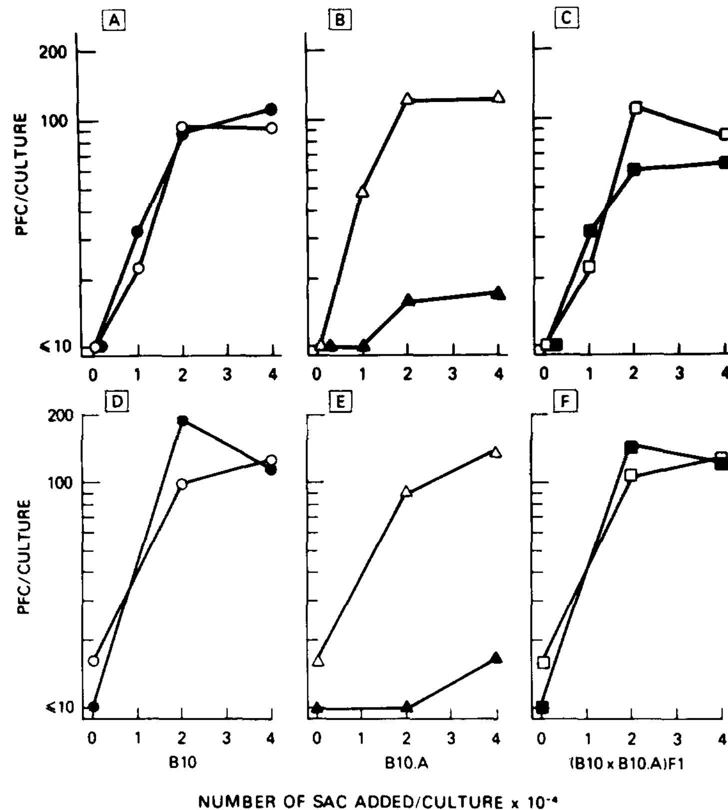

FIG. 4. Monoclonal anti-I-A^k antibody inhibits in vitro antibody responses mediated by *H-2*^{a} but not *H-2*^{b} accessory cells. 5 × 10^5 Sephadex G-10 passed B10 → (B10 × B10.A)F_1 (panels A–C) or B10.A → (B10 × B10.A)F_1 (panels D–F) chimera spleen cells were cultured with graded numbers of B10 (○ ●), B10.A (△ ▲), or (B10 × B10.A)F_1 (□ ■) SAC in the presence (closed symbols) or absence (open symbols) of anti-I-A^k reagent (final concentration ⅒). TNP-KLH was present at 10 μg/ml. <10 PFC/culture were observed in the absence of antigen.

Inhibition of Primary In Vitro Responses by Anti-I-A^k Occurs through Interaction with Accessory Cells, and Does not Occur at the Level of T Cells or B Cells. To further define the cellular site of anti-Ia inhibition, cell mixing experiments were carried out employing Sephadex G-10 passed spleen (T + B) cells from B10 → (B10 × B10.A)F_1 chimeras and accessory SAC populations from B10, B10.A, or (B10 × B10.A)F_1 mice. Although the responsiveness of these G-10 passed cells was fully reconstituted by each accessory population, the presence of anti-I-A^k inhibited only reconstitution by B10.A SAC (Fig. 4A–C). Because the B10.A SAC were the only cells in these latter cultures that could bind the anti-I-A^k reagent, these findings demonstrate that binding of the reagent to appropriate I-A^k determinants on homozygous accessory cells is sufficient to inhibit responsiveness. Moreover, the failure to observe inhibition of the responses reconstituted by (B10 × B10.A)F_1 accessory cells that can also bind the anti-I-A^k reagent, but which additionally express I-A^b determinants, strongly suggested that inhibition by anti-I-A^k was not mediated by nonspecific inhibition of accessory cell function.

Although the presence of *I-A*^{k} accessory cells was shown by these studies to be

sufficient for anti-I-A^k-mediated inhibition, these results do not exclude the possibility that inhibition could also occur by binding of the anti-I-A^k reagent to appropriate determinants expressed on the B cell. Indeed, it is important to note that *H-2*^{a} B cells, as well as accessory cells, express determinants recognized by this monoclonal anti-I-A^k reagent, as pretreatment with anti-IA^k + C′ resulted in complete elimination of B cells functional in this response, as well as complete elimination of accessory cell function (data not shown). To assess the ability of anti-I-A^k to inhibit response through interaction with *I-A*^{k} product expressed by B cells, the effect of this reagent on responses generated by G-10 passed B10.A → F_1 (*H-2*^{a}) spleen cells and B10, B10.A, or F_1 SAC was determined. Responses to TNP-KLH were inhibited only when reconstitution was mediated by B10.A accessory cells; no inhibition was observed for responses supported by B10 or (B10 × B10.A)F_1 SAC (Fig. 4 D–F). Because the B cells (as well as the T cells) in these cultures were *H-2*^{a} and could bind the anti-I-A^k reagent, the failure to inhibit responses with all three SAC populations demonstrated that binding to B cell I-A^k determinants was not sufficient to inhibit responsiveness. Because the B cells in these experiments were of chimera origin, the effect of anti-I-A^k upon responses of normal (nonchimeric) *H-2*^{a} B cells was also determined. It was observed that anti-I-A^k antibody did not inhibit responses mediated by normal B10.A B cells in the presence of B10 accessory cells (Fig. 5), as it had not inhibited the response of chimeric B10.A B cells. Together, these results demonstrate that recognition by anti-I-A^k antibody of determinants expressed on accessory cells is both necessary and sufficient for inhibition of PFC responses. In contrast, interaction of the same antibody with determinants that were expressed on B cells was neither sufficient nor necessary for such inhibition.

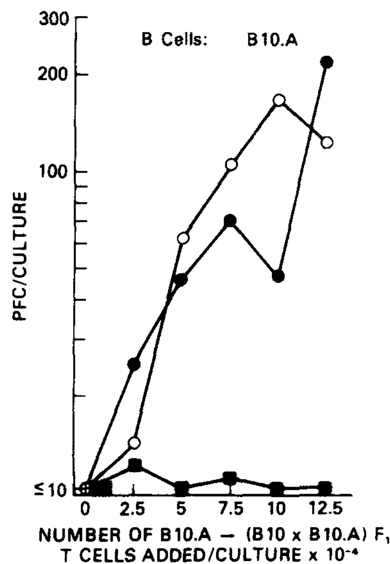

FIG. 5. Monoclonal anti-I-A^k antibody does not inhibit responses mediated by *I-A*^{k} B cells in the absence of *I-A*^{k} accessory cells. 4 × 10^5 Sephadex G-10 passed and anti-thy-1.2 + C′-treated B10.A spleen (B) cells were cultured with graded numbers of B10.A → (B10 × B10.A)F_1 T cells and either no SAC (■) or 4 × 10^4 B10 SAC (○ ●) in the presence (●) or absence (○) of anti-I-A^k reagent (final concentration 1/10). TNP-KLH was present at 10 μg/ml. <10 PFC/culture were observed in the absence of antigen or in cultures devoid of B cells.

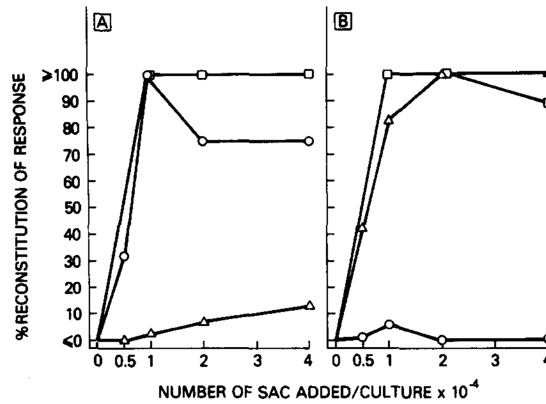

FIG. 6. Accessory cell-depleted (A × B)F1 → parentA chimera spleen cells cooperate with accessory cells expressing parentA but not parentB H-2 determinants. 5 × 10^5 Sephadex G-10 passed (B10 × B10.A)F1 → B10 or (B10 × B10.A)F1 → B10.A chimera spleen cells were cultured with graded numbers of B10 (○), B10.A (△), or (B10 × B10.A)F1 (□) SAC in the presence of 10 μg/ml TNP-KLH. <10 PFC/culture were observed in the absence of antigen.

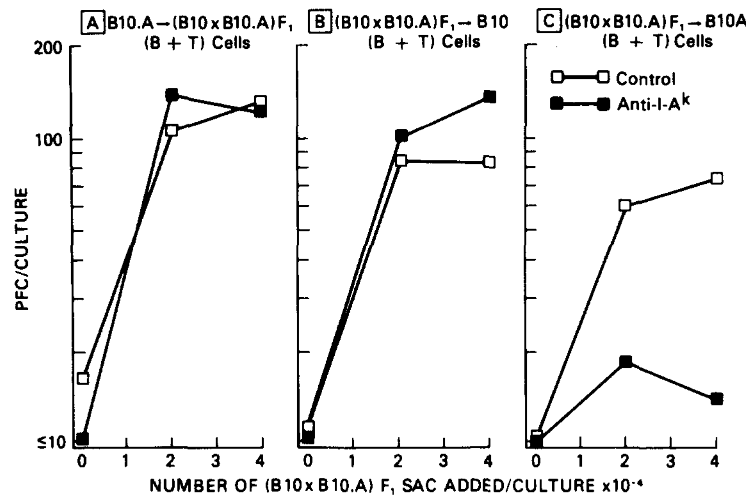

FIG. 7. Inhibition by anti-I-A^k antibody is dependent upon active recognition of *I-A*^{k} encoded determinants expressed on accessory cells. 5 × 10^5 Sephadex G-10 passed B10.A → (B10 × B10.A)F1, (B10 × B10.A)F1 → B10, or (B10 × B10.A)F1 → B10.A chimera spleen cells were cultured with graded numbers of (B10 × B10.A)F1 SAC in the presence (■) or absence (□) of anti-I-A_k reagent (final concentration 1/10). TNP-KLH was present at 10 μg/ml. <10 PFC/culture were observed in the absence of antigen.

Inhibition by Anti-I-A^k is Dependent upon Helper T Cell Recognition of I-A^k Encoded Determinants Expressed on Accessory Cells. The data presented above demonstrated that an anti-I-A^k reagent is capable of inhibiting in vitro antibody responses by interacting with *I-A*^{k}-encoded determinants expressed on accessory cells. Because it has previously been reported that helper T cell recognition of *K*- or *I-A*-encoded accessory cell determinants is required for in vitro primary antibody responses (6), experiments were designed to test the possible relationship between anti-I-A^k blocking of antibody responses and MHC-restricted interactions between helper T cells and accessory cells.

It was previously reported for primary in vitro responses to TNP-KLH that helper

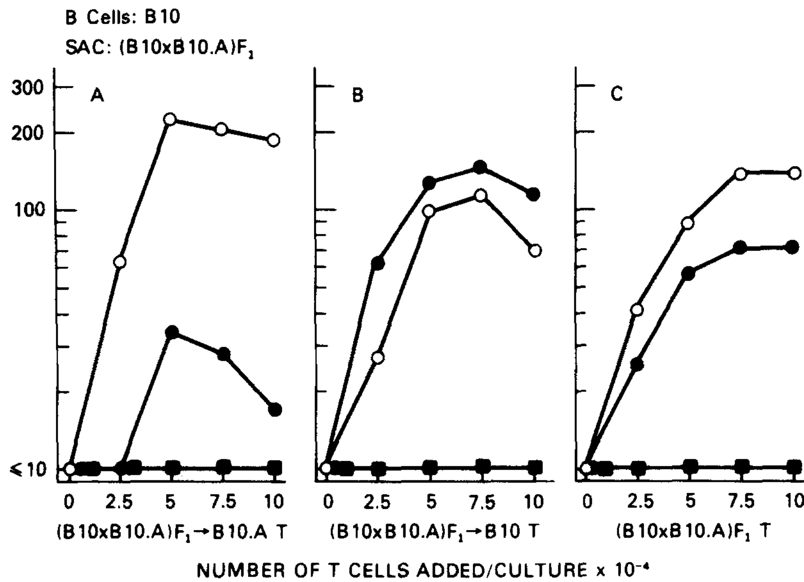

FIG. 8. Inhibition by anti-I-A^k antibody is dependent upon T cell recognition of *I-A*^{k} encoded determinants expressed on accessory cells. 4 × 10^5 Sephadex G-10 passed and anti-thy-1.2 + C′-treated B10 (B) cells were cultured with no SAC (■) or with 4 × 10^4 (B10 × B10.A)F_1 SAC and graded numbers of (B10 × B10.A)F_1 → B10.A, (B10 × B10.A)F_1 → B10, or (B10 × B10.A)F_1 T cells in the presence (●) or absence (○) of anti-I-A^k reagent (final concentration 1/10). TNP-KLH was present at 10 μg/ml. <10 PFC/culture were observed either in the absence of antigen or in cultures devoid of B cells.

T cells from (A × B)F_1 → $parent_A$ chimeras are restricted to recognizing and cooperating with $parent_A$ but not $parent_B$ accessory cells (6). Consistent with such data, it was found that the response of G-10 passed (B10 × B10.A)F_1 → B10 (abbreviated F_1 → B10) spleen cells was reconstituted by B10 but not B10.A SAC, whereas G-10 passed F_1 → B10.A cells were reconstituted by B10.A but not B10 SAC (Fig. 6); the responses of both chimera populations were reconstituted by (B10 × B10.A)F_1 (F_1) SAC (Fig. 6). Because (A × B)F_1 → $parent_A$ spleen cells only recognized $parent_A$ accessory cells, it was reasoned that the cooperation of G-10 passed F_1 → B10 spleen cells with F_1 SAC might be mediated by recognition of H-2^b but not H-2^a determinants on these SAC, whereas cooperation of F_1 → B10.A cells with these same SAC might be mediated by recognition of H-2^a but not H-2^b determinants. To study the relationship between anti-I-A^k inhibition and MHC-restricted T cell recognition of accessory cells, the effect of anti-I-A^k was examined for responses resulting from cooperation between F_1 SAC and G-10 passed B10.A → F_1, F_1 → B10, or F_1 → B10.A spleen cells. Each of the F_1 → parent chimeric populations was of donor F_1 origin, but the two populations differed in their abilities to recognize the parental MHC determinants expressed on accessory cells (Fig. 6). When anti-I-A^k was added to cultures of G-10 passed B10.A → F_1 or F_1 → B10 spleen cells and F_1 SAC, there was no inhibition of the response to TNP-KLH (Fig. 7). In contrast, anti-I-A^k profoundly inhibited the response of G-10 passed F_1 → B10.A spleen cells with the same F_1 SAC (Fig. 7). It should be emphasized that the MHC (including *I-A*^{k}) phenotypes of both F_1 → parent chimera populations were identically F_1, so that they differed only in the

self MHC receptor repertoire which each functionally expressed. These results suggested that the inhibitory effect of anti-I-A^k resulted from its interference with MHC-restricted recognition by lymphocytes of accessory cell Ia determinants required in these antibody responses, so that inhibition with anti-I-A^k occured only when lymphocytes were restricted to recognizing $H\text{-}2^a$ products on accessory cells. When lymphocytes were able to recognize either of the two parental ($H\text{-}2^a$) or ($H\text{-}2^b$) products expressed on F_1 accessory cells, as were B10.A → F_1 cells, a reagent specific for the $I\text{-}A^k$ product alone would be expected to inhibit only that portion of the response mediated by recognition of the $I\text{-}A^k$ product, and not the component mediated by recognition of $I\text{-}A^b$. In such circumstances, partial inhibition of the overall PFC response was in fact observed to range from 0 to 50% of the total response (Figs. 4C and F, 7, and 8C).

To determine whether the ability of the anti-I-A^k reagent to inhibit responses mediated by F_1 SAC was specifically the result of its interference with helper T cell recognition of self MHC determinants expressed on accessory cells, F_1, F_1 → B10, or F_1 → B10.A T cells were cultured with B10 B cells and F_1 accessory cells. All three populations of T cells were F_1, and were indistinguishable by cell surface phenotyping, so that each of the cultures contained F_1 T cells, F_1 accessory cells, and B10 B cells (which were $H\text{-}2^b$ and did not bind the anti-I-A^k reagent). Anti-I-A^k inhibited profoundly those responses mediated by F_1 → B10.A chimera T cells, but had no effect upon responses of the same B cells and accessory cells that were mediated by normal F_1 or F_1 → B10 T cells (Fig. 8). These F_1 T cell populations differed only in their differentiation environments and in their self-recognition repertoires. Anti-I-A^k antibody completely inhibited their responses to TNP-KLH only when the F_1 helper T cell populations were restricted to recognizing $H\text{-}2^a$ ($I\text{-}A^k$) but not $H\text{-}2^b$-encoded determinants on F_1 accessory cells. These findings demonstrate that anti-I-A^k antibody inhibits the generation of responses to TNP-KLH by its interference with active helper T cell recognition of I-A^k determinants expressed on accessory cells.

Discussion

A series of experiments was performed investigating the functional role of cell surface Ia antigens in antibody responses. The function of Ia antigens was probed by analyzing the effect of anti-Ia antibodies on the generation in vitro of PFC responses to TNP-KLH. It was observed that a monoclonal anti-Ia reagent with specificity for a product of $I\text{-}A^k$ was capable of inhibiting in vitro primary antibody responses to TNP-KLH; and that such inhibition was dependent upon expression of $I\text{-}A^k$-encoded determinants by the responding spleen cells. Inhibition was mediated by the interaction of anti-Ia reagent with an $I\text{-}A^k$ product expressed by accessory cells, and was not mediated by binding to either T or B cells. Further, this anti-I-A^k-mediated inhibition resulted from the interference with active helper T cell recognition of accessory cell $I\text{-}A^k$ product, suggesting that the activation of T helper cells by recognition of accessory cell Ia antigen is an essential function of these determinants.

Because in vitro PFC responses to TNP-KLH require the cooperation of T cells, B cells, and accessory cells, it was possible that Ia antigen expression by any one of these cell populations might be functionally important for these responses. As a result, the inhibition of PFC responses by the monoclonal anti-I-A^k reagent could be mediated by its effect on any one or more of these populations. However, it was demonstrated

in the present studies that it was both necessary and sufficient for inhibition of antibody responses by anti-I-A^k that the *I-A*^{k} genotype be expressed on accessory cells. Conversely, the binding of anti-I-A^k to T cells and/or B cells was neither necessary nor sufficient for inhibition. Because treatment with the same monoclonal anti-I-A^k reagent in the presence of C′ resulted in complete elimination of functional B cells as well as accessory cells, it is clear that this monoclonal antibody was capable of recognizing a determinant expressed on both B cells and accessory cells, even though functional inhibition of antibody response was mediated only by its interaction with accessory cell determinants. These results suggest that Ia determinants expressed on accessory cells have a unique functional role in the in vitro responses being studied, one not necessarily shared by Ia on B cells.

The functional role of accessory cell Ia was further studied by evaluating the mechanism by which anti-I-A^k interaction with accessory cells leads to inhibition of in vitro antibody responses. The possibility that anti-I-A^k inhibits responses by activating nonspecific suppressor cells was considered, but could not be demonstrated. The possibility that interaction of anti-I-A^k with accessory cell Ia determinants nonspecifically interferes with accessory cell function was not supported, as anti-I-A^k failed to inhibit responses by (B10 × B10.A)F_1 cells to TNP-KLH. Because cell-cell interactions in the antibody responses being studied here have been shown to be *H-2* restricted (6), one model for the function of accessory cell Ia antigens would propose that these determinants are recognized by other cells participating in these responses, and that this recognition provides an activation signal necessary for responses to occur. A test of this hypothesis was based on the previous observation that helper T cells are required to recognize *K*- or *I-A*-encoded determinants expressed on accessory cells in order to allow the functional expression of T cell help (6). $F_1 \rightarrow$ parent chimera T cells were employed that were *H-2*-restricted in their recognition of accessory cells for the response to TNP-KLH. When the effects of anti-I-A^k were examined, it was observed that anti-I-A^k antibody inhibited responses only when helper T cells were restricted to recognizing *H-2*^{a} (*I-A*^{k}), but not *H-2*^{b}-encoded determinants on F_1 accessory cells. These results are consistent with the hypothesis that accessory cell Ia antigens must be recognized by helper T cells for helper T cell activation to occur, and that binding of specific antibodies to accessory cell Ia determinants interferes with this required recognition of accessory cell Ia determinants by T helper cells. Indeed, the most straightforward interpretation of these data is that the *I-A*^{k}-encoded determinant (Ia antigen) detected on accessory cells by monoclonal anti-Ia antibody and the accessory cell *I* region determinant that is recognized by helper T cells are expressed on the same molecule, and may represent identical or distinct sites on that molecule. Alternatively, if the Ia determinants detected by anti-I-A^k antibody are expressed on molecules distinct from those recognized by T cells, these molecules must be in steric proximity on the cell surface and encoded by closely linked *H-2* genes.

The inhibition of immune response by antibodies to Ia determinants has previously been reported by a number of investigators (19–26). Using conventional anti-Ia reagents, previous studies have reported inhibition of in vitro antibody responses to be mediated by reagents specific for products of the *I-A*, *I-C*, and *I-J* subregions, and this inhibition has been attributed to effects upon B cells (23) or accessory cells (24, 25). It is of interest that in the response system employed in the present studies, helper T cell recognition of accessory cell MHC products is required for antibody responses,

but that no such requirement exists for T cell recognition of MHC determinants on B cells (6). The demonstration that an anti-I-A^k reagent inhibited antibody responses as a consequence of binding to accessory cell, but not B cell, Ia antigens is thus consistent with the hypothesis that cell surface Ia antigens are of functional importance only when they function as targets for T cell recognition. These findings are also informative for the interpretation of the effects of anti-Ia antibodies on *Ir* gene controlled responses. It has been reported that anti-Ia antibody directed at responder haplotype products on (responder × nonresponder)F_1 cells inhibited responses under *Ir* gene control, a finding that suggests that in such responses T cells are restricted to recognizing antigen only in association with responder haplotype products, and that in such circumstances antibody directed against these products is sufficient to inhibit responses by blocking T cell recognition (1).

The present studies have also extended previous findings by demonstrating that a monoclonal hybridoma anti-Ia reagent specific for a single *I-A*^{k} specificity (Ia.17) is sufficient to inhibit antibody responses to a multideterminant antigen such as TNP-KLH. This finding can be interpreted in at least three ways: (*a*) It is possible that the monoclonal anti-Ia.17 reagent employed is site-specific for the unique sites on the *I-A*^{k} product molecule recognized by helper T cells for the antibody response to TNP-KLH. (*b*) Alternatively, the target sites for T cell recognition may be distinct from the site bound by anti-Ia.17, but their distribution on the accessory cell surface may be such that binding by anti-Ia.17 is sufficient to sterically block these recognition sites. (*c*) Finally, it is possible that T cell recognition of the *I-A*^{k} product requires recognition of multiple sites on the product molecule, and that blocking of any one of these sites is sufficient to block effective T cell recognition. If this last mechanism were in fact correct, it might be predicted that most or all antibodies with specificities for sites on the *I-A*^{k} product molecule would inhibit T cell recognition and would thus inhibit in vitro responses to TNP-KLH. Preliminary studies have demonstrated that not all monoclonal anti-I-A^k reagents are inhibitory for antibody responses (data not shown), failing to confirm this prediction. One interpretation of these findings is that the anti-Ia.17 reagent used in the present report may have site-specific effects on the *I-A*^{k} product molecule, although the observed differences among anti-I-A^k reagents may equally well be the result of differences in properties such as Ig subclass or antibody affinity. It should also be noted that the present findings do not exclude the possibility that reagents with specificities for non-*I-A* encoded *H-2* products might have similar inhibitory effects upon immune responses. Indeed, it has been observed that some reagents specific for *K* or *D* products do inhibit responses, but this inhibition appears to be mediated by mechanisms other than interference with T cell recognition of MHC encoded self determinants (data not shown). Thus, although alternative explanations exist, the results of the present study suggest that the monoclonal anti-I-A^k reagent employed might be site-specific for determinants on Ia molecules expressed by accessory cells that are recognized by helper T cells and are required for T cell activation.

The current studies have employed a monoclonal anti-I-A^k reagent to study the functional role of Ia antigens in in vitro primary antibody responses. This monoclonal reagent inhibited the responses of *I-A*^{k} spleen cells through interaction with accessory cell, but not B cell or T cell, *I-A*^{k} product. Moreover, this inhibition occurred only when helper T cells were restricted to recognizing products of the *I-A*^{k} haplotype

expressed on accessory cells. These findings demonstrate that helper T cells recognize Ia determinants on accessory cells, and that such recognition is required for the generation of T cell-dependent antibody responses.

Summary

The functional role of cell surface Ia antigens has been studied for in vitro antibody responses, using as a probe the ability of anti-Ia reagents to inhibit these responses. A hybridoma monoclonal anti-Ia reagent specific for a product of $I\text{-}A^k$ (Ia.17) profoundly inhibited in vitro antibody responses to TNP-KLH by spleen cells of the $I\text{-}A^k$ but not $I\text{-}A^b$ haplotype. This inhibition by anti-I-A^k was shown to occur as a result of interaction with an accessory cell $I\text{-}A^k$ product, but not by interaction with T or B cell product, in spite of the fact that functional B cells as well as accessory cells could be shown to express the determinant detected by this hybridoma reagent. These results suggest that the Ia expressed by accessory cells is of unique functional importance in these responses.

To further characterize the function of Ia antigens in this response system, the mechanism of anti-I-A^k inhibition was determined. The inhibition resulting from interaction of anti-I-A^k with accessory cell Ia was not mediated by nonspecific suppressor cells, nor was there nonspecific interference with accessory cell function as a result of the binding of anti-Ia antibody. The relationship between anti-Ia inhibition and T helper cell recognition of self determinations on accessory cells was analyzed using T cells from radiation bone marrow chimeras. It was demonstrated that (B10 × B10.A)$F_1 \rightarrow$ B10 ($F_1 \rightarrow$ B10) chimera T cells were able to cooperate with B10 ($H\text{-}2^b$ and $I\text{-}A^b$) but not B10.A ($H\text{-}2^a$ and $I\text{-}A^k$) accessory cells for responses to TNP-KLH; $F_1 \rightarrow$ B10.A T cells were able to cooperate with B10.A but not B10 accessory cells; and both chimera populations were able to cooperate with (B10 × B10.A)F_1 (F_1) accessory cells. Monoclonal anti-I-A^k inhibited the cooperation of $F_1 \rightarrow$ B10.A T cells with F_1 accessory cells, but had no effect upon the cooperation of $F_1 \rightarrow$ B10 T cells with the same F_1 accessory cells. Thus, inhibition by anti-I-A^k is dependent upon active helper T cell recognition of $I\text{-}A^k$-encoded determinants expressed on accessory cells. These findings demonstrate that T cells recognize self Ia determinants expressed on accessory cells, and that such recognition is required for the generation of T cell-dependent antibody responses.

The authors thank Mr. J. Williams, Ms. C. Harrison, and Mr. S. Spence for their technical assistance, and Mr. W. Hinkle, Ms. D. Hernandez, Mr. F. Jones, and Mr. J. Israel for their care in maintaining animal colonies. The authors are grateful to Drs. H. Dickler, D. Longo, and R. Schwartz for their careful reading and criticism of this manuscript.

Received for publication 8 July 1980.

References

1. Berzofsky, J. A. 1980. Immune response genes in the regulation of mammalian immunity. *In* Biological Regulation and Development, R. F. Goldberger, editor. Plenum Publishing Corporation, New York. **2:**467.
2. Katz, D. H., T. Hamaoka, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. II. Failure of physiologic cooperative interactions between T

and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates. *J. Exp. Med.* **137:**1045.

3. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. *J. Exp. Med.* **138:**1194.
4. Sprent, J. 1978. Restricted helper function of F_1 hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. II. Evidence for restrictions affecting helper cell induction and T-B collaboration, both mapping to the K-end of the *H-2* complex. *J. Exp. Med.* **147:**1159.
5. Kappler, J. W., and P. Marrack. 1977. The role of *H-2* linked genes in helper T-cell function. I. In vitro expression in B cells of immune response genes controlling helper T cell activity. *J. Exp. Med.* **146:**1977.
6. Singer, A., K. S. Hathcock, and R. J. Hodes. 1979. Cellular and genetic control of antibody responses. V. Helper T-cell recognition of H-2 determinants on accessory cells but not B cells. *J. Exp. Med.* **149:**1208.
7. Sachs, D. H. 1976. The Ia antigens. *In* Contemporary Topics in Molecular Immunobiology. H. N. Eisen and R. A. Reisfeld, editors. Plenum Publishing Corporation, New York.
8. Klein, J., and V. Hauptfeld. 1976. Ia antigens: their serology, molecular relationships, and their role in allograft reactions. *Transplant. Rev.* **30:**83.
9. David, C. S. 1976. Serologic and genetic aspects of murine Ia antigens. *Transplant. Rev.* **30:** 299.
10. 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.
11. 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.
12. Sachs, D. H., and J. L. Cone. 1973. A mouse "B" cell alloantigen determined by gene(s) linked to the major histocompatibility complex. *J. Exp. Med.* **138:**1289.
13. Hodes, R. J,, G. B. Ahmann, K. S. Hathcock, H. B. Dickler, and A. Singer. 1978. Cellular and genetic control of antibody responses *in vitro.* IV. Expression of Ia antigens on accessory cells required for responses to soluble antigens including a response under *Ir* gene control. J. Immunol. **121:**1501.
14. Oi, V. T., P. P. Jones, J. W. Goding, L. A. Herzenberg, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2, and Ia antigens. *Curr. Top. Microbiol. Immunol.* **81:**115.
15. Lake, P., E. A. Clark, M. Khorshidi, and G. H. Sunshine. 1979. Production and characterization of cytotoxic Thy-1 antibody-secreting hybrid lines. Detection of T cell subsets. *Eur. J. Immunol.* **9:**875.
16. Dickler, H. B., C. Cowing, G. B. Ahmann, K. S. Hathcock, S. O. Sharrow, R. J. Hodes, and A. Singer. 1980. Characterization of the accessory cells required in T lymphocyte dependent antigen-specific immune responses. *In* Macrophage Regulation of Immunity. E. Unanue and A. Rosenthal, editors. Academic Press, Inc. New York. 265.
17. Rittenberg, M. B., and K. L. Pratt. 1969. Antinitrophenyl (TNP) plaque assay. Primary response of BALB/c mice to soluble and particulate immunogen. *Proc. Soc. Exp. Biol. Med.* **132:**575.
18. Jerne, N. K., and A. A. Nordin. 1963. Plaque formation in agar by single antibody-producing cells. *Science (Wash. D.C.).* **140:**405.
19. Shevach, E. M., W. E. Paul, and I. Green. 1972. Histocompatibility-linked immune response gene function in guinea pigs. Specific inhibition of antigen-induced lymphocyte proliferation by alloantisera. *J. Exp. Med.* **136:**1207.

20. 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 alloantigens. *J. Exp. Med.* **140:**921.
21. Schwartz, R. H., C. G. Fathman, and D. H. Sachs. 1976. Inhibition of stimulation in murine mixed lymphocyte cultures with an alloantiserum directed against a shared Ia determinant. *J. Immunol.* **116:**929.
22. Schwartz, R. H., C. S. David, D. H. Sachs, and W. E. Paul. 1976. T lymphocyte-enriched murine peritoneal exudate cells. III. Inhibition of antigen-induced T lymphocyte proliferation with anti-Ia antisera. *J. Immunol.* **117:**531.
23. Marrack, P., and J. W. Kappler. 1977. Anti-Ia inhibits the activity of B cells but not a T cell-derived helper mediator. *Immunogenetics.* **4:**541.
24. Niederhuber, J. E. 1978. The role of I region gene products in macrophage-T lymphocyte interaction. *Immunol. Rev.* **40:**28.
25. Niederhuber, J. E., and P. Allen. 1980. Role of *I*-region gene products in macrophage-induction of antibody response. II. Restriction at the level of T cell in recognition of *I-J*-subregion macrophage determinants. *J. Exp. Med.* **151:**1103.
26. Shevach, E. M., I. Green, and W. E. Paul. 1974. Alloantiserum-induced inhibition of immune response gene product function. II. Genetic analysis of target antigens. *J. Exp. Med.* **139:**679.