IMMUNE RESPONSE GENE FUNCTION CORRELATES WITH THE EXPRESSION OF AN Ia ANTIGEN

II. A Quantitative Deficiency in A_e:E_α Complex Expression Causes a Corresponding Defect in Antigen-presenting Cell Function*

By LOUIS A. MATIS, PATRICIA P. JONES, DONAL B. MURPHY, STEPHEN M. HEDRICK, ETHAN A. LERNER,‡ CHARLES A. JANEWAY, JR.,§ JANET M. McNICHOLAS,‖ AND RONALD H. SCHWARTZ

From the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205, the Department of Biological Sciences, Stanford University, Stanford, California 94305, and the Departments of Pathology and Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510

Gene products of the major histocompatibility complex (MHC)[1] are intimately involved in regulating restriction specificity, antigenic fine specificity, and immune response (*Ir*) gene control of the T lymphocyte immune response (1, 2). Specifically, for *I*-region-linked murine regulatory T cell (helper, delayed-type hypersensitivity, proliferative) responses, it has been proposed that the Ia antigens are the *Ir* gene products (1–3). Several lines of experimental evidence, including studies with anti-Ia antibodies, *I* region mutant strains, and T lymphocytes from chimeric animals, support this contention (1, 3–5).

Previous work in our laboratory (6–8) has demonstrated the requirement for two complementing MHC-linked *Ir* genes in the murine T lymphocyte proliferative response to the synthetic polypeptide, poly-$(Glu^{56}Lys^{35}Phe^{9})_n$ (GLϕ), in B10.A(5R) mice, and to the globular protein, pigeon cytochrome *c*, in two responding strains, the B10.A and B10.S(9R). The response to both antigens is marked by the requirement for the two high responder alleles to be present in a single antigen-presenting cell (APC) (8, 9). In the case of GLϕ, experiments with chimeric animals have also revealed that neither high-responder allele need be present in the proliferative T cell to generate an immune response to GLϕ if the T cell has matured in a high-responder environment (10). These data in the complementing *Ir* gene systems have been interpreted in the context of the biochemical studies of Ia antigens to reflect the

* Supported by grants AI-15732, AI-14349, CA-16359, and AI-14579 from the National Institutes of Health.

‡ Predoctoral trainee of the Medical Scientist Training Program at Yale University.

§ Investigator, Howard Hughes Medical Institute.

‖ Postdoctoral fellow of the Arthritis Foundation.

[1] *Abbreviations used in this paper:* APC, antigen-presenting cell; CFA, complete Freund's adjuvant; EHAA, Eagle's Hanks' amino acid; FCS, fetal calf serum; GLϕ, poly-$(Glu^{56}Lys^{35}Phe^{9})_n$; *Ir*, immune response; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; PETLES, peritoneal exudate T lymphocyte-enriched subpopulation; PPD, purified protein derivative; SAS, saturated ammonium sulfate; TCGF, T cell growth factor.

requirement for cell surface expression and function of a two chain Ia molecule (A_e:E_α) encoded in both the *I-A* and *I-E* subregions of the MHC (11, 12). Thus, in dual *Ir* gene-controlled immune responses, an *I-E* subregion-encoded α chain and an *I-A* subregion-encoded β chain combine in the cytoplasm to form a single two-chain restriction element (A_e:E_α) that is expressed at the surface of the APC.

One testable prediction arising from this model, and from the observed structural homogeneity of the E_α chains from several haplotypes studied to date, is that the α chains might be functionally equivalent (13). This hypothesis was first addressed by Schwartz et al. (14) in the response to GLϕ by the demonstration that primed B10.A(5R) (*I-A*^{b}) T cells could be stimulated in vitro by antigen-pulsed spleen cells from F_1 hybrids between B10 mice (GLϕ nonresponder but histocompatible with B10.A(5R) at *I-A*) and several non-*I-A*^{b} parental strains, all encoding an E_α chain and thus possessing the serologic specificity Ia.7. These studies demonstrated that histocompatibility at *I-A*, but not *I-E*, was required for antigen presentation of GLϕ to primed B10.A(5R) T cells. However, all the *I-E* histoincompatible strains that did complement for presentation expressed structurally similar E_α chains. Therefore, it was concluded that the E_α chains were functionally equivalent.

In this and the accompanying report (15), we present genetic, serologic, biochemical, and functional evidence that correlates in a quantitative fashion the level of APC function in the T cell proliferative response to pigeon cytochrome *c* with the level of cell surface expression of certain A_e:E_α complexes. In addition, we demonstrate a quantitative relationship between the degree of inhibition of the T cell proliferative response by a monoclonal anti-Ia antibody specific for the A_e:E_α combinatorial determinant Ia-m44, and the amount of this A_e:E_α determinant present on the APC surface.

Materials and Methods

Animals. Strains C57BL/10Sn (B10), B10.A/SgSn (B10.A), and B10.A(5R)/SgSn were obtained from The Jackson Laboratory, Bar Harbor, Maine. B10.A(2R)/SnCu were obtained from Sprague Dawley, Madison, Wis. B10.D2/nSnN were obtained from the National Institutes of Health small animal breeding facility. B10.A(4R)/Sg were obtained from Simonsen Labs, Gilroy, Calif. B10.RIII/Sg, B10.S/Sg, B10.P/Sg, and B10.PL/Sg were the progeny of breeding pairs generously provided by Dr. Jack Stimpfling, McLaughlin Research Institute, Great Falls, Mont. The B10.S(9R)/SfSx mice were the progeny of breeding pairs kindly provided by Dr. David Sachs, National Cancer Institute, Bethesda, Md. F_1 strains were bred in our animal colony from these B10 congenic lines. Mice of either sex between 2 and 12 mo of age were used for experiments.

Antigens and Immunization. Cytochrome *c* was either purchased from the Sigma Chemical Co., St. Louis, Mo., or was the gift of Dr. C. Hannum and Dr. E. Margoliash, Northwestern University, Evanston, Ill. It was purified by column chromatography on carboxymethyl cellulose to remove contaminants such as polymeric and deamidated forms of the molecule (16). The preparation of cyanogen bromide cleavage fragments of cytochrome *c* has been described previously (17).

For peritoneal exudate T lymphocyte-enriched subpopulation (PETLES) experiments, mice were immunized in the hind footpads with 2.4 nmol of pigeon cytochrome *c* emulsified 1:1 in complete Freund's adjuvant (CFA) that contained 1 mg/ml of killed Mycobacterium tuberculosis, strain H37Ra, (Difco Laboratories, Detroit, Mich.) in a total volume per animal of 0.1 ml. For purification of T cells from lymph nodes, mice were primed with 8 nmol of pigeon cytochrome *c* or pigeon cytochrome *c* fragment 81-104 in the hind footpads.

Cell Cultures. The preparation of PETLES and their in vitro culture with antigen are described in detail elsewhere (18). Briefly, 100 μl of Eagle's Hanks' amino acid (EHAA) medium

supplemented with 10% fetal calf serum (FCS) containing 2×10^5 to 3×10^5 PETLES were placed in each well of a sterile, U-bottomed microculture plate. Either medium alone or antigen was then added to the cultures to achieve desired final concentrations in a total of 200 μl. In some experiments, antigen-pulsed nonimmune spleen cells were used to present antigen to primed T cells. This procedure has been detailed elsewhere (19). In short, 1×10^7 irradiated (2,000 rad) spleen cells were incubated with 200 μg pigeon cytochrome *c* or 100 μg PPD in 1 ml of RPMI 1640 plus 10% FCS for 1 h at 37°C. The cells were then washed five times with cold RPMI 1640 without FCS to remove unbound antigen, and counted and mixed with the immune T cells in appropriate numbers. Nonantigen-pulsed irradiated spleen cells were added as controls. Cultures were incubated at 37°C for 5 d. Stimulation was assessed by measuring the incorporation of a 1 μCi pulse of tritiated thymidine (6.7 Ci/mmol, New England Nuclear, Boston, Mass.) 16–18 h before harvesting the cultures. Determinations were done in triplicate, and the data were expressed as the arithmetic mean ± SEM. Δ cpm represents the difference between antigen-stimulated and nonstimulated control means. Student's *t*-test was used to ascertain significant differences.

Preparation of Antigen-specific T Cell Colonies. 7–8 d after antigen priming, T cells from draining lymph nodes were prepared as previously described by passage over nylon wool columns and cultured in EHAA medium with 200 μg/ml of pigeon cytochrome *c* and irradiated (2,000 rads) syngeneic spleen cells as a source of APC. 2×10^6 viable cultured cells were collected after 3 d of in vitro stimulation, washed, and distributed in the upper layer of a two-layer soft agar system in 30-mm wells as previously described (20). After 4 d, colonies were picked and expanded in liquid culture medium supplemented with antigen, syngeneic spleen cells, and a partially purified interleukin 2 (IL-2) derived from a 48-h, phorbol myristate acetate- (Sigma Chemical Co., St. Louis, Mo.) induced, supernatant fluid of an IL-2-producing mouse EL-4 T lymphoma subline (the generous gift of Dr. John Farrar, National Institute of Dental Research). Additional antigen-specific T cell lines were propagated by serial restimulation of lymph node-derived T cells in vitro as described by Kimoto and Fathman (21).

The proliferative responses of antigen-specific T cell colonies and lines were assayed in a manner similar to that of whole T cell populations. 10^4–2×10^4 T cells were cultured in microtiter wells (Falcon 3040, Falcon Labware, Oxnard, Calif.) in a 1:1 mixture of EHAA medium and RPMI 1640 plus 10% FCS, 2% (vol/vol) T cell growth factor (TCGF) (not required for assaying T cell lines), antigen (or antigen-pulsed spleen cells), and varying numbers (10^5–5×10^5) of irradiated spleen cells to achieve a final volume of 200 μl. After 2–3 d, cultures were pulsed with 1 μCi of [^{3}H]thymidine and harvested 12–18 h later.

Monoclonal Antibody and Blocking Studies. The preparation and characterization of the monoclonal anti-Ia antibody, Y-17, has been previously described (3). To assess blocking of antigen-induced T cell proliferation, a 12 mg/ml solution from a saturated ammonium sulfate (SAS) precipitate of ascites containing Y-17 was dialyzed against RPMI 1640 and then added to microculture wells to achieve a final dilution of 1% (vol/vol). Percent inhibition by Y-17 was calculated by dividing the antigen stimulation (Δ cpm) in the presence of the antibody by the Δ cpm in its absence, subtracting the ratio from 1, and multiplying by 100.

Results

Functional Equivalence of Most I-E-encoded Pigeon Cytochrome c Ir Genes from Various Ia.7-positive Strains. The immune response to pigeon cytochrome *c* is controlled by two complementing *Ir* genes, one mapping in the *I-A* subregion, the other in the *I-E* subregion. Previously, we have presented evidence from anti-Ia blocking studies to suggest that the *Ir* gene product controlled by the *I-A* subregion is the A_e chain and the *Ir* gene product controlled by the *I-E* subregion is the E_α chain (3). To explore more directly the role played by E_α chains in the dual *Ir* gene controlled response to pigeon cytochrome *c*, we crossed the nonresponder B10.A(4R) strain, which possesses a cytoplasmic form of the high responder A_e^k chain, with a number of Ia.7-positive low or nonresponder strains, which express structurally similar E_α chains. The various

F_1 hybrid strains were immunized with pigeon cytochrome *c*, and their T cell proliferative responses were assessed (Table I).

With one surprising exception, all the F_1 strains bearing E_α chains responded to pigeon cytochrome *c*. F_1 strains not expressing an E_α chain failed to respond. These results suggested that most *I-E*-encoded *Ir* genes were functionally equivalent. However, the B10.PL ($H\text{-}2^u$) strain, which is Ia.7 positive and possesses an E_α protein, failed to complement with the B10.A(4R) to generate a pigeon cytochrome *c*-specific proliferative response. This was not due to a general state of unresponsiveness in these mice as they responded normally to purified protein derivative (PPD).

The Ir Defect in the (B10.A(4R) × B10.PL)F_1 Is also Seen in the (B10.S × B10.PL)F_1. The B10.S strain ($H\text{-}2^s$) is a low as opposed to nonresponder to pigeon cytochrome *c*, although the specificity of this small response is different from that of a responder strain in that pigeon cytochrome *c*-primed B10.S T cells show an equal stimulation with mouse cytochrome *c* (8). However, as shown in Table II, when B10.S was crossed with any of a number of E_α chain-bearing, nonresponder mice, the resulting F_1 hybrids generated a much greater proliferative response that was highly specific for the pigeon cytochrome *c* immunogen. As was the case with the B10.A(4R), the only exception was the Ia.7-positive B10.PL, which failed to complement with the B10.S to produce a large response to pigeon cytochrome *c*.

The Defect in the (B10.A(4R) × B10.PL)F_1 and (B10.S × B10.PL)F_1 Strains Is Expressed in the APC. To assess the potential role of the APC as a cellular site for the *Ir* gene defect seen in the (B10.A(4R) × B10.PL)F_1 and (B10.S × B10.PL)F_1 strains, we assayed the in vitro stimulation of primed B10.A or B10.S(9R) T cells by irradiated nonimmune antigen-pulsed parental or F_1 spleen cells (Tables III and IV). As shown

TABLE I

*Gene Complementation in the T Lymphocyte Proliferative Response of F_1 Hybrids between B10.A(4R) ($I\text{-}A^k$) and H-2 Congenic Strains Expressing Different E_α Chains**

F_1 strains‡	*I* subregions§		Ia.7-positive E_α chain‖	Thymidine incorporation (cpm ± SEM) in response to			
	I-A	*I-E*		Medium	Pigeon	Mouse	PPD
B10.A(4R) × B10.D2	k/d	b/d	d	800 (±170)	23,800 (±800)	2,700 (±320)	46,200 (±1500)
B10.A(4R) × B10.P	k/p	b/p	p	1,600 (±400)	40,000 (±1000)	2,700 (±900)	67,000 (±7800)
B10.A(4R) × B10.A(3R)	k/b	b/k	k	1,300 (±500)	31,700 (±5600)	5,800 (±1100)	33,500 (±1300)
B10.A(4R) × B10.A(5R)	k/b	b/k	k	5,100 (±700)	20,900 (±3000)	11,000 (±500)	28,500 (±370)
B10.A(4R) × B10.RIII	k/r	b/r	r	3,200 (±600)	35,000 (±3,100)	5,000 (±2,100)	100,500 (±6,200)
B10.A(4R) × B10.PL	k/u	b/u	u	2,500 (±370)	2,600 (±300)	2,100 (±700)	70,700 (±4700)
B10.A(4R) × B10	k/b	b/b	—	2,300 (±300)	3,100 (±750)	4,400 (±600)	52,300 (±2,500)
B10.A(4R) × B10.S	k/s	b/s	—	1,800 (±550)	4,000 (±570)	4,600 (±800)	28,300 (±150)

* 2×10^5 PETLES from mice primed with 2.4 nmol of pigeon cytochrome *c* were cultured in vitro, as described in Materials and Methods, and stimulated with various concentrations of pigeon cytochrome *c*, mouse cytochrome *c*, or PPD. The data are expressed as the arithmetic means of triplicate cultures (± SEM). Only the maximum stimulation from a dose response curve is shown for each antigen.

‡ Each of the parental strains has been shown to be a low or nonresponder to pigeon cytochrome *c*.

§ Letters represent the haplotype source of origin of the indicated *I* subregion alleles contributed by each of the parental strains.

‖ Haplotype source of origin of the gene coding for the E_α chain.

TABLE II

*Gene Complementation in the T Cell Proliferative Response to Pigeon Cytochrome c of F_1 Hybrids between B10.S(I-A^s) and H-2 Congenic Strains Expressing Different E_α Chains**

F_1 strains	*I* subregions‡		Ia.7-positive E_α chain§	Thymidine incorporation (cpm ± SEM) in response to			
	I-A	*I-E*		Medium	Pigeon	Mouse	PPD
B10.S × B10.A(5R)	s/b	s/k	k	3,600 (±550)	25,500 (±3700)	4,000 (±300)	36,600 (±3000)
B10.S × B10.D2	s/d	s/d	d	1,700 (±900)	39,900 (±4700)	3,300 (±1000)	33,800 (±5700)
B10.S × B10.P	s/p	s/p	p	5,900 (±400)	37,400 (±4200)	8,000 (±650)	61,800 (±7700)
B10.S × B10.RIII	s/r	s/r	r	5,900 (±800)	30,400 (±1000)	6,900 (±1300)	71,200 (±8700)
B10.S × B10.PL	s/u	s/u	u	1,700 (±100)	2,400 (±200)	1,900 (±900)	98,200 (±1000)
B10.S × B10	s/b	s/b	—	5,500 (±900)	6,900 (±800)	4,900 (±1500)	56,900 (±600)

* 2×10^5 PETLES frrm mice primed with 2.4 nmol of pigeon cytochrome *c* were stimulated in vitro with various concentrations of pigeon cytochrome *c*, mouse cytochrome *c*, or PPD. Only the maximum stimulation from each dose-response curve is shown.

‡ See legend to Table I.

§ See legend to Table I.

TABLE III

Presentation of Pigeon Cytochrome c to Primed B10.A T Cells by Parental or F_1 Hybrid APC

Spleen cells	*I* subregions*		Ia.7-positive E_α chain‡	Thymidine incorporation (cpm ± SEM) in response to			Δ cpm§	
	I-A	*I-E*		Nonpulsed spleen	Cytochrome *c* pulsed	PPD pulsed	Cytochrome *c*	PPD
B10.A	k	k	k	200 (±15)	14,000 (±800)	73,900 (±8000)	13,800	73,700
B10.A(4R)	k	b	—	1,000 (±300)	700 (±30)	133,400 (±7000)	−300	132,400
B10.D2	d	d	d	8,900 (±1800)	9,000 (±100)	9,900 (±200)	100	1,000
B10.A(4R) × B10.D2	k/d	b/d	d	8,600 (±100)	45,600 (±500)	105,500 (±22,700)	37,000	96,900
B10.P	p	p	p	15,200 (±1000)	17,700 (±1300)	19,600 (±2900)	2,500	4,200
B10.A(4R) × B10.P	k/p	b/p	p	6,600 (±900)	23,400 (±4500)	92,000 (±3,400)	17,800	85,400
B10.A(3R)	b	k	k	5,400 (±1100)	5,100 (±1200)	8,500 (±800)	−300	3,100
B10.A(4R) × B10.A(3R)	k/b	b/k	k	4,300 (±500)	25,300 (±1700)	15,100 (1400)	21,000	10,800
B10.RIII	r	r	r	6,900 (±400)	8,100 (±1500)	5,700 (±300)	1,200	−200
B10.A(4R) × B10.RIII	k/r	b/r	r	7,400 (±600)	38,800 (±7600)	21,200 (±1000)	31,400	13,800
B10.PL	u	u	u	10,300 (±3100)	9,200 (±1300)	13,400 (±1800)	−800	3,100
B10.A(4R) × B10.PL	k/u	b/u	u	14,800 (±1800)	18,000 (±2200)	56,000 (±7700)	3,200	41,200
B10.A + B10.A(4R) × B10.PL			k + u	7,500 (±700)	22,200 (±1200)	76,500 (±200)	14,800	69,000
B10.A(4R) × B10	k/b	b/b	—	11,400	8,300	67,200	−3,100	55,800

2×10^5 B10.A PETLES primed to pigeon cytochrome *c* in CFA were stimulated in vitro with 10^5 pigeon cytochrome *c*-pulsed, PPD-pulsed, or nonpulsed irradiated (2,000 rad) nonimmune spleen cells as a source of APC. Stimulation was assessed 5 d later by measuring the incorporation of tritiated thymidine as described in Materials and Methods.

* See legend to Table I.

‡ See legend to Table I.

§ Δ cpm represents the difference between antigen-pulsed and nonpulsed (MLR) stimulation. Statistically significant values are underlined.

in Table III, syngeneic antigen-pulsed B10.A spleen cells stimulated responses to pigeon cytochrome *c*, as well as to the positive control antigen, PPD, in primed B10.A T cells ($A_e^k E_\alpha^k$). In contrast, pulsed B10.A(4R) spleen cells, sharing *I-A*^{k} with B10.A

TABLE IV

Presentation of Pigeon Cytochrome c to Primed B10.S(9R) T Cells by F_1 Hybrid or Parental APC

Spleen cells	I subregions*		Ia.7-positive E_α chain‡	Thymidine incorporation (cpm ± SEM) in response to			Δ cpm§	
	I-A	*I-E*		Nonpulsed spleen	Cytochrome *c* pulsed	PPD pulsed	Cytochrome *c*	PPD
B10.S(9R)	s	k	k	1,100 (±200)	32,400 (±1700)	43,700 (±5000)	31,300	42,600
B10.S	s	s	—	1,300 (±200)	1,800 (±500)	22,600 (±3300)	500	21,300
B10.A(5R)	b	k	k	3,800 (±900)	3,500 (±200)	5,800 (±600)	−300	2,000
B10.S × B10.A(5R)	s/b	s/k	k	2,900 (±400)	23,300 (±3500)	9,900 (±1000)	20,400	7,000
B10.D2	d	d	d	6,100 (±500)	8,100 (±700)	7,000 (±500)	2,000	900
B10.S × B10.D2	s/d	s/d	d	3,600 (±300)	20,300 (±1200)	21,200 (±400)	16,700	17,600
B10.RIII	r	r	r	4,300 (±100)	3,500 (±900)	4,100 (±1200)	−800	−200
B10.S × B10.RIII	s/r	s/r	r	7,300 (±600)	38,400 (±1700)	33,000 (±2100)	31,100	25,700
B10.P	p	p	p	7,100 (±700)	9,400 (±2500)	9,800 (±900)	2,300	2,700
B10.S × B10.P	s/p	s/p	p	4,700 (±1700)	29,500 (±2000)	14,400 (±1800)	24,800	9,700
B10.PL	u	u	u	3,200 (±500)	3,800 (±800)	5,100 (±4900)	600	1,900
B10.S × B10.PL	s/u	s/u	u	4,400 (±200)	7,100 (±700)	16,500 (±11200)	2,700	12,100
B10.S × B10	s/b	s/b	—	5,600 (±800)	9,300 (±400)	29,500 (±300)	3,700	23,900

Conditions are as in Table III except responding T cells are from B10.S(9R) strain.

* See legend to Table I.

‡ See legend to Table I.

§ See legend to Table III.

but lacking an *I-E* subregion-encoded E_α chain, presented PPD but not pigeon cytochrome *c*. Spleen cells from F_1 hybrids between B10.A(4R) and B10.D2, B10.P, B10.A(3R), and B10.RIII strains clearly stimulated both pigeon cytochrome *c* and PPD antigen-specific responses above the responses to the nonpulsed controls (which represent the mixed lymphocyte reaction (MLR) induced by the semi-histoincompatible F_1 spleens). However, [B10.A(4R) × B10.PL]F_1 spleens, though Ia.7 positive (E_α chain-bearing) and sharing $I\text{-}A^k$, failed to significantly stimulate primed B10.A T cells when pulsed with pigeon cytochrome *c*, although they were competent to induce a PPD response. That this failure to stimulate a pigeon cytochrome *c* response was not due to the induction of suppressor T cells was suggested by the finding that simultaneous addition of cytochrome-pulsed (B10.A(4R) × B10.PL)F_1 spleen cells to similarly pulsed B10.A spleen cells failed to suppress the proliferative response of B10.A T cells. Finally, Ia.7-negative (non-E_α-bearing) (B10.A(4R) × B10)F_1 spleen cells also failed to induce a significant response to pigeon cytochrome *c*.

Entirely analogous results were obtained by stimulating pigeon cytochrome *c* primed B10.S(9R) (A^s_e:E^k_α) T cells with in vitro antigen-pulsed parental and F_1 spleen cells (Table IV). Again, syngeneic B10.S(9R) spleen cells stimulated responses to both pigeon cytochrome *c* and PPD, while *I-A*-compatible, non-E_α-bearing spleen cells induced a response to PPD but not to pigeon cytochrome *c*. Fully histoincompatible parental spleen cells failed to stimulate any significant antigen-specific proliferation above unpulsed controls, while spleen cells from F_1 hybrids between *I-A* compatible B10.S and Ia.7-positive (E_α-bearing) B10.A(5R), B10.D2, B10.RIII, or B10.P strains induced both pigeon cytochrome *c* and PPD responses. Again, (B10.S × B10.PL)F_1

spleen cells behaved like the Ia.7-negative, non-E_α chain-bearing control (B10.S × B10)F_1, in only stimulating a PPD response.

Overall, these functional gene complementation studies in the response to pigeon cytochrome *c*, in concert with the previous studies with GLϕ, strongly support the concept that *Ir* gene-controlled responsiveness to these antigens requires the assembly of an appropriate A_e chain with any E_α chain to form a functional restriction element on the surface of the APC (11). In the following sections we will demonstrate that the apparent exception to this conclusion observed with E_α^u chains represents a quantitative rather than a qualitative effect.

Functional Complementation Can Be Demonstrated between E_α^u Chains and A_e^k (β) Chains from B10.A(4R) Mice. To further investigate the failure of the E_α^u chain to complement with $A_e^{k,s}$ chains in the response to pigeon cytochrome *c*, a series of biochemical and serological studies were performed that are detailed in the accompanying report (15). Briefly, these studies revealed that, indeed, A_e:E_α complexes do form between the B10.PL E_α chain and the A_e chains from B10, B10.A(4R), and B10.S. Furthermore, they are expressed on the cell surface. However, $A_e^{b,k,s}$:E_α^u molecules were expressed in reduced amounts relative to corresponding $A_e^{b,k,s}$:$E_\alpha^{d,k,p}$ molecules. Thus, the demonstration of the cell surface expression of $A_e^{k,s}$:E_α^u molecules met our initial criterion for the generation of an immune response to pigeon cytochrome *c* and, consequently, appeared to contradict the functional data indicating an absence of a pigeon cytochrome *c* response in primed (B10.A(4R) × B10.PL)F_1 and (B10.S × B10.PL)F_1 mice, as well as a lack of APC function for pigeon cytochrome *c* in these strains. However, the finding of only low amounts of $A_e^{k,s}$:E_α^u chains in these mice suggested that the functional deficiency might be quantitative in nature. Therefore, in an attempt to demonstrate antigen presenting function, we performed a series of experiments with pigeon cytochrome *c*-specific T cell colonies and long-term T cell lines. These responding cell populations are highly enriched for antigen-specific T cells and are depleted of alloreactive potential and syngeneic presenting cells, which we reasoned represented optimal conditions for testing for antigen-specific presenting activity with soluble antigen.

Pigeon cytochrome *c*-primed lymph node T cells from B10.A and B10.A(2R) mice ($I\text{-}A^k$, $I\text{-}E^k$) were restimulated with antigen in vitro and then cloned in soft agar (as described in Materials and Methods). Colonies were picked, expanded in liquid culture, and tested for antigen specificity. Representative results obtained with one such colony are shown in Table V. In the presence of syngeneic B10.A(2R) spleen cells, these T cells mount a significant antigen-dependent proliferative response. Whereas neither the *I-A* histocompatible only B10.A(4R) nor the *I-E* histocompatible only B10.A(5R) spleen cells were able to mediate a pigeon cytochrome *c*-specific proliferative response, (B10.A(4R) × B10.A(5R))F_1 spleen cells were able to mediate such a response. No significant alloreactivity was observed, permitting a clear demonstration of gene complementation. Thus, the genetic requirements for stimulating a pigeon cytochrome *c*-specific colony, like unselected whole T cell populations, indicate that both high responder alleles must be expressed in the same APC. However, in contrast to the results with whole primed T cell populations, the functional competence of (B10.A(4R) × B10.PL)F_1 APC was clearly demonstrated in this experiment. Thus, B10.A(2R) T cells, which recognize pigeon cytochrome *c* in the context of the A_e^k E_α^k molecule on the syngeneic APC, were also able to respond in

TABLE V

*Functional Competence of (B10.A(4R) × B10.PL)F₁ APC**

Spleen cells	*I* subregions		Proliferative response of B10.A(2R) colony D6 (cpm ± SEM)		
	I-A	*I-E*	Antigen concentration		
			0 μM	*8 μM*	*24 μM*
B10.A(2R)	k	k	1,700 (±300)	18,400 (±1000)	15,200 (±1600)
B10.A(4R)	k	b	300 (±60)	400 (±100)	300 (±70)
B10.A(5R)	b	k	800 (±100)	1,400 (±300)	
B10.A(4R) × B10.A(5R)	$\frac{k}{b}$	$\frac{b}{k}$	2,100 (±600)	13,800 (±1,400)	
B10.A(4R) × B10.PL	$\frac{k}{u}$	$\frac{b}{u}$	600 (±200)	10,500 (±1300)	12,700 (±800)

* B10.A(2R) mice were immunized with pigeon cytochrome *c* and T lymphocytes from the draining lymph nodes were stimulated in vitro with pigeon cytochrome *c* and cloned in soft agar. Colonies were picked and expanded in liquid culture. 10^4 cloned T cells were stimulated with antigen or medium alone in the presence of 10^5 irradiated (2,000 rads) spleen cells from nonimmune mice and 2% (vol/vol) of TCGF. Proliferation was assessed by measuring incorporation of [^{3}H]thymidine in a 3-d assay.

the context of the $A_e^k E_\alpha^u$ complex (Table V). Entirely analogous results were obtained with (B10.S × B10.PL)F_1 APC in their ability to stimulate pigeon cytochrome *c*-specific responses of A_e^s:E_α^k-restricted B10.S(9R) T cell colonies (data not shown).

(B10.A(4R) × B10.PL)F₁ APC, Though Functionally Competent, Manifest a Relative Defect. Biochemical and serological analysis of a number of parental and F_1 strains clearly indicated that $A_e^{b,k,s}$:E_α^u complexes were present on the cell surface, though in diminished amounts relative to $A_e^{b,k,s}$:$E_\alpha^{d,k,b}$ complexes (15). Consequently, we performed a series of experiments designed to test whether this quantitative difference in cell surface expression of the Ia molecule could similarly be manifested at the functional level.

In one such experiment, we compared the antigen dose-response curves for a B10.A pigeon cytochrome *c*-specific T lymphocyte cell line in the presence of splenic APC from different strains (Fig. 1). The proliferative response at varying soluble antigen doses was measured in the presence of constant numbers of responding B10.A T cells and APC from each haplotype. The large magnitude of the response generated under these conditions enabled us to demonstrate quite clearly an ~10-fold shift to higher antigen concentration of the dose-response curve in the presence of irradiated (B10.A(4R) × B10.PL)F_1 APC relative to (B10.A(4R) × B10.D2)F_1 or B10.A APC. Additionally, stimulation of this same population of antigen-specific T cells by in vitro antigen-pulsed (16 μM) irradiated splenic APC revealed that (B10.A(4R) × B10.PL)F_1 spleen cells could induce a significant response (Δ cpm = 28,300), but that this response was ~10-fold lower in magnitude than the corresponding stimulation induced by pigeon cytochrome *c*-pulsed (B10.A(4R) × B10.D2)F_1 splenic APC (Δ cpm = 212,400) (Fig. 1). Interestingly, the relative responses induced by pigeon cytochrome *c*-pulsed (B10.A(4R) × B10.D2)F_1 and (B10.A(4R) × B10.PL)F_1 APC would correspond very closely to the responses to an antigen concentration of 0.8 μM on the soluble antigen dose-response curve (Fig. 1), suggesting that in this case pulsed spleen cells were 1/20 as effective as continuous antigen.

Pigeon Cytochrome c-specific Antigen-presenting Function of (B10.A(4R) × B10.PL)F₁

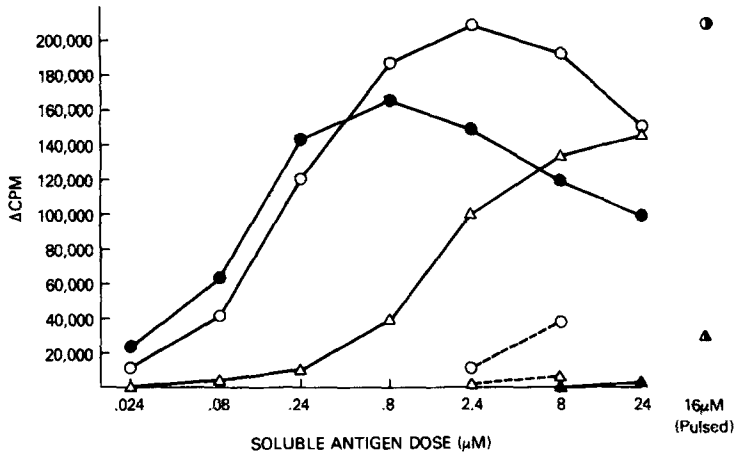

FIG. 1. Long-term B10.A T cell lines specific for pigeon cytochrome *c* were propagated in vitro by the method of Kimoto and Fathman (21). In this experiment 10^4 B10.A T cells were restimulated in culture with 5×10^5 irradiated (2,000 rad) spleen cells as a source of APC in the presence of varying doses of pigeon cytochrome *c* or medium alone. Dose response curves are shown in the presence of spleen cells from B10.A (●——●), (B10.A(4R) × B10.D2)F_1 (○——○), (B10.A(4R) × B10.PL)F_1 (Δ——Δ), B10.D2 (▲——▲), (B10.A(4R) × B10.D2)F_1 + 1% Y-17 (○- - -○), and (B10.A(4R) × B10.PL)F_1 + 1% Y-17 (Δ- - -Δ). Medium controls were B10.A spleen, 100 ± 5; (B10.A(4R) × B10.D2)F_1 spleen, 989 ± 54; (B10.A(4R) × B10.PL)F_1 spleen, 4,469 ± 910, and B10.D2 spleen, 2,356 ± 547. The proliferation of the same T cells in the presence of in vitro antigen-pulsed (16 μM) (B10.A(4R) × B10.D2)F_1 (◑) and (B10.A(4R) × B10.PL)F_1 (◭) irradiated spleen cells is shown in the far right side of the figure. Nonpulsed controls were 1,400 ± 600 for (B10.A(4R) × B10.D2)F_1 spleens, and 2,800 ± 700 for (B10.A(4R) × B10.PL)F_1 spleens. The proliferative response was assessed in a 4-d tritiated thymidine incorporation assay.

Spleens Is Inhibited by a Monoclonal Anti-Ia Antibody-specific for the A_e:E_α Complex. As detailed in the accompanying report, Y-17 is a monoclonal antibody which recognizes a combinatorial or conformational determinant present on certain two-chain A_e:E_α Ia molecules (15). Moreover, this antibody has been shown to specifically inhibit the T cell proliferative responses to the antigens pigeon cytochrome *c* and GLØ, both of which require the cell surface expression of the A_e:E_α complex, while it fails to inhibit significantly the responses to poly-$(Glu^{60}Ala^{30}Tyr^{10})_n$ or poly(Tyr,Glu)-poly D,L-Ala-poly Lys, antigens whose responses are controlled by Ir genes mapping solely within the *I-A* subregion (3, L. Matis and R. Schwartz, unpublished observations). Therefore, a demonstration that Y-17 could inhibit the antigen-presenting function of (B10.A(4R) × B10.PL)F_1 spleen cells would very strongly suggest that the A_e^k:E_α^u Ia molecule on the APC surface in fact functions as the restriction element recognized by the responding pigeon cytochrome *c*-specific T cells.

The data outlined in Table VI address this question. Two pigeon cytochrome *c*-specific colonies from B10.A mice were stimulated with antigen in the presence of either (B10.A(4R) × B10.PL)F_1 or (B10.A(4R) × B10.D2)F_1 irradiated spleen cells, with or without 1% Y-17 added to the cultures. In both experiments the partial but significant inhibition by Y-17 of the B10.A T cell proliferation in the presence of (B10.A(4R) × B10.D2)F_1 APC at the higher antigen dose (8 μM) was enhanced when the antigen concentration was lowered to 2.4 μM, although the magnitude of the response in the absence of inhibiting antibody was clearly on a plateau in the range encompassed by the two doses shown. Virtually complete inhibition of the prolifera-

TABLE VI

*The Proliferative Response of Cloned B10.A T Cells to Pigeon Cytochrome c in the Presence of (B10.A(4R) × B10.PL)F_1 APC Is Blocked by Monoclonal Anti-Ia Antibody, Y-17**

Spleen cells	Proliferative response (cpm ± SEM)							
	Antigen dose							
	0 μM		*2.4 μM*			*8 μM*		
	No Y-17	*1% Y-17*	*No Y-17*	*1% Y-17*	*(% inhib.)*	*No Y-17*	*1% Y-17*	*(% inhib.)*
Experiment 1								
B10.A colony C10								
B10.A(4R) × B10.D2	600 (±100)	300 (±100)	9,200 (±1400)	3,500 (±900)	63	8,400 (±500)	6,300 (±1400)	23
B10.A(4R) × B10.PL	400 (±100)	300 (±100)	4,200 (±600)	400 (±70)	97	11,700 (±300)	1,100 (±200)	93
Experiment 2								
B10.A colony G7								
B10.A(4R) × B10.D2	600 (±100)	200 (±50)	10,900 (±600)	1,000 (±500)	92	10,300 (±1000)	2,200 (±500)	79
B10.A(4R) × B10.PL	300 (±20)	200 (±20)	900 (±100)	400 (±30)	—	4,700 (±100)	400 (±30)	95

* 10^4 T cells from pigeon cytochrome *c*-specific B10.A colonies were stimulated with medium alone or varying doses of pigeon cytochrome *c* in the presence of 10^5 irradiated (2,000 rad) spleen cells as a source of antigen-presenting cells and in the presence or absence of 1% (vol/vol) Y-17. Stimulation was assessed in a 3-d tritiated thymidine incorporation assay. Percent inhibition by Y-17 was calculated by dividing the Δ cpm in the presence of Y-17 by the Δ cpm in its absence, subtracting the quotient from 1 and multiplying by 100.

tion induced by (B10.A(4R) × B10.PL)F_1 splenic APC was observed in the presence of 1% Y-17, even at the higher concentration (8 μM) of pigeon cytochrome *c*. Similarly, marked inhibition by Y-17 of pigeon cytochrome *c*-induced proliferation was also seen with the antigen-specific long-term T cell lines (Fig. 1). Again, inhibition of the response in the presence of (B10.A(4R) × B10.PL)F_1 spleen cells was greater than the inhibition in the presence of (B10.A(4R) × B10.D2)F_1 spleen cells at the same concentration of Y-17 and antigen. Thus, the proliferative response of B10.A pigeon cytochrome *c*-specific T cells in the presence of (B10.A(4R) × B10.PL)F_1 APC can be completely inhibited by a monoclonal antibody specific for the A_e:E_α complex, indicating that the A^k_e:E^u_α Ia molecule is indeed functioning as the restriction element recognized by the B10.A T cells. Moreover, the more complete inhibition by Y-17 of (B10.A(4R) × B10.PL)F_1 APC-induced proliferation relative to that of (B10.A(4R) × B10.D2)F_1 APC at a constant antigen dose is consistent with the decreased cell surface expression of the relevant A_e:E_α complex on the (B10.A(4R) × B10.PL)F_1 APC.

Discussion

The essential role of APC-associated Ia antigens in the generation of regulatory T cell-dependent immune responses has been demonstrated in a number of experimental systems (3, 14, 22). Recently reported studies (23, 24) in chimeric animals have shown that immune responses mediated by interactions between genotypically distinct T cells and APC are specifically inhibited by monoclonal antibodies directed against Ia antigenic determinants on the APC, but not the T cell. In experiments with radiation-induced bone marrow chimeras studying the responses to antigens under *Ir* gene control, it has been demonstrated that the APC, but not the T cell, must be of high-responder genotype in order to generate an immune response (5). Results of this nature support the conclusion that the Ia antigens are the *Ir* gene products and that they are functionally expressed at the surface of the APC.

The data in this and the accompanying paper significantly extend previous work

in this area by the demonstration that immune response gene function, as assessed by the responsiveness of various F_1 hybrid strains immunized with pigeon cytochrome *c*, correlated exactly with antigen-presentation function by spleen cells from the same strains. Moreover, both responsiveness and APC function required the cell surface expression of the appropriate A_e:E_α complex (15). Finally, quantitative differences in the cell surface expression of this Ia molecule were directly mirrored in corresponding quantitative differences in APC function in the response to pigeon cytochrome *c*, an antigen under dual *Ir* gene control (Fig. 1). These functional and structural gene complementation studies are entirely consistent with the molecular model in which a two-chain complex (A_e:E_α) functions as a single restriction element at the APC surface.

These functional complementation studies with F_1 hybrid strains exactly parallel the biochemical and serological analysis of the same strains with Y-17, a monoclonal anti-Ia antibody directed against a combinatorial or conformational determinant formed by certain A_e:E_α complexes (15). Indeed, the functional role of the A_e:E_α complex in antigen presentation was initially demonstrated by the specific inhibition of MHC-linked, *Ir*-gene-controlled immune responses by Y-17 (3). Although functional blocking studies with anti-Ia antibodies are highly suggestive, we believe that the direct correlation between the amount of an Ia antigen expressed at the cell surface and the quantitative level of APC function provides strong independent evidence that Ia antigens are in fact the *Ir* gene products.

Several recent studies with mutant strains of mice have also provided direct evidence for a correlation of Ia antigen expression with immune response gene function (25–27). Rosenwasser and Huber (25) have shown, for example, that the response of H-2^b mice to beef insulin correlates with the expression of the private I-A^b specificity, Ia.W39 at the level of the APC. Similarly, functional *Ir* gene defects have been demonstrated in the mutant strain B6.C-H-2^{bm12}, in which an *I* subregion mutation has resulted in the expression of an altered *I-A*^{b} β chain on the cell surface (26, 27).[2] It is of interest that the biochemical and serological analysis of the bm12 mutant has revealed that some *I-A*^{b} subregion-encoded specificities are still detectable in the mutant, although they are quantitatively greatly reduced (27). In light of the data presented herein, it is possible that the Ir gene defects in the bm12 mutant could also be quantitative ones.

The use of antigen-specific T cell lines was a key factor in the successful demonstration of antigen-presenting function of (B10.A(4R) × B10.PL)F_1 and (B10.S × B10.PL)F_1 spleen cells. The advantages evident in the use of cloned T cells as the source of responding cells are several: (*a*) they are highly enriched for antigen-specific cells, (*b*) they facilitate the observation of MHC-restricted antigen-specific responsiveness due to the absence of concomitant alloreactivity, and (*c*) they are largely free of any endogenous APC activity, which permits the use of high doses of soluble antigen in the in vitro assay. In contrast, antigen-primed whole T cell responding populations are not entirely free of residual APC function and, therefore, necessitated the use of antigen-pulsed APC to assess stimulation. In this regard, the current results (Fig. 1) using both soluble antigen and antigen-pulsed spleens to stimulate pigeon cytochrome

[2] Lin, C.-C., A. S. Rosenthal, H. C. Passmore, and T. H. Hansen. Selective loss of an antigen-specific *Ir* gene function in the *I-A* mutant B6.C-*H-2*^{bm12} is an antigen-presenting cell defect. *Proc. Natl. Acad. Sci. U. S. A.* **78**:6406.

c-specific long-term T cell lines suggested that antigen pulsing may effectively correspond to a point on the soluble antigen dose response curve that is suboptimal for (B10.A(4R) × B10.PL)F_1 antigen presentation of pigeon cytochrome *c*. Therefore, the use of enriched T cell populations clearly enhanced the sensitivity of the proliferative assay, such that the functional competence of (B10.A(4R) × B10.PL)F_1 and (B10.S × B10.PL)F_1 APC was manifested despite the suboptimal cell surface expression of the *I*-region-encoded restriction element.

Similar considerations apply to the observation that (B10.A(4R) × B10.PL)F_1 and (B10.S × B10.PL)F_1 mice completely fail to respond when primed with pigeon cytochrome *c*. One possible explanation for this result is that induction of a T lymphocyte-dependent immune response requires a higher cell surface density of Ia antigens than does stimulation of already antigen-primed T cells. On the other hand, there is preliminary evidence to suggest that priming (B10.S × B10.PL)F_1 mice with high doses of pigeon cytochrome *c* fragment 81-104, which is a more potent immunogen than the native pigeon cytochrome *c*, but is subject to identical *Ir* gene control, does result in a small but significant antigen-specific T cell proliferative response (L. Matis and R. Schwartz, unpublished observations). Moreover, further evidence for reduced in vivo function of the A^k_e:E^u_α hybrid restriction element has recently been reported by Alpert and Sprent, who have shown that significant though suboptimal negative selection of sheep erythrocyte-specific, T cells of CBA strain mice restricted by the A^k_e:E^k_α Ia molecule does occur in irradiated (B10.A(4R) × B10.PL)F_1 hosts (28). All of these results are consistent with the idea that *Ir* gene defects are relative phenomena, i.e., they can, to some degree, be overcome by increasing the dose of antigen.

One of the more striking aspects of the functional complementation data in this report is the clear demonstration that the magnitude of the T cell proliferative response is a function of the amount of Ia antigen expressed on the APC as well as of the concentration of nominal antigen. Thus, the proliferative response mediated by (B10.A(4R) × B10.PL)F_1 APC approaches the same magnitude as that mediated by (B10.A(4R) × B10.D2)F_1 or B10.A APC, but at an antigen dose one order of magnitude higher. Analogous observations were made with regard to the monoclonal anti-Ia (Y-17) inhibition data. The data in Table VI and Fig. 1 clearly demonstrate that inhibition of antigen-specific, MHC-restricted T cell proliferation by a monoclonal anti-Ia antibody may be overcome at a constant antigen dose by increasing the number of Ia molecules present on the APC (B10.A(4R) × B10.D2)F_1 APC vs. (B10.A(4R) × B10.PL)F_1 APC, or alternatively, by increasing the concentration of antigen in the presence of a constant number of APC. Finally, experiments comparing parental B10.A and (B10.A(4R) × B10.D2)F_1 APC have also revealed a small but reproducible difference in the antigen dose-response curves (Fig. 1) as well as differential effects of anti-Ia mediated blocking by Y-17 (L. Matis and R. Schwartz, unpublished observations). These results are what would be expected from gene dosage effects on the expression of Ia-m44 on parental versus F_1 APC.

Overall, these data can be discussed in the context of models of T cell recognition, which propose either an association of nominal antigen with Ia molecules on the APC surface (29–31), or alternatively, recognition of separate molecules of nominal antigen and Ia on the APC surface (32). Several groups of investigators have reported the existence of molecular complexes comprised of both Ia antigenic determinants and

nominal antigenic determinants which are capable of either stimulating or binding to antigen-specific T cells in an MHC-restricted fashion (33, 34). According to this model, responsiveness would depend primarily on the expression of sufficient amounts of the appropriate allelic forms of the Ia antigens, which would determine whether the Ia-nominal antigen interaction occurs. Immune response gene defects would, as has been previously proposed, reflect qualitatively or quantitatively suboptimal expression of the Ia-nominal antigenic complex at the APC surface (29–31). In this context, anti-Ia antibody-mediated inhibition of T cell proliferation would represent either competition with nominal antigen for binding sites on the Ia molecules, or alternatively, competition with the T cell receptor for binding to the Ia molecule in the process of formation of a trimolecular complex comprised of Ia molecule, T cell receptor(s), and nominal antigen (35).

The findings presented in this paper can also be interpreted according to associative recognition models in which T cell activation requires interdependent, though separate signalling by MHC products and nominal antigen on the APC surface (32). The efficiency of T cell activation would be a function of the availability of both nominal antigen and Ia to the T cell receptor(s), such that responsiveness might vary with the product of the independent concentrations of Ia and nominal antigen molecules on the APC. Anti-Ia antibody would effectively lower the concentration of available Ia antigenic determinants. Determining which of these models is correct awaits the actual isolation and characterization of the molecular complexes involved in MHC-restricted T cell recognition.

Summary

A series of experiments were performed to explore the role of complementing major histocompatability complex (MHC)-linked immune response *Ir* genes in the murine T cell proliferative response to the globular protein antigen pigeon cytochrome *c*. The functional equivalence of *I-E*-subregion-encoded, structurally homologous E_α chains from different haplotypes bearing the serologic specificity Ia.7 was demonstrated by the complementation for high responsiveness to pigeon cytochrome *c* of F_1 hybrids between low responder B10.A(4R) ($I\text{-}A^k$) or B10.S ($I\text{-}A^s$) mice and four low responder E_α-bearing haplotypes. Moreover, this *Ir* gene function correlated directly with both the ability of antigen-pulsed spleen cells from these same F_1 strains to stimulate pigeon cytochrome *c*-primed T cells from B10.A or B10.S(9R) mice, and with the cell surface expression of the two-chain Ia antigenic complex, A_e:E_α, bearing the conformational or combinatorial determinant recognized by the monoclonal anti-Ia antibody, Y-17.

The B10.PL strain ($H\text{-}2^u$), which expresses an Ia.7-positive *I-E*-subregion-encoded E_α chain, failed to complement with B10.A(4R) or B10.S mice in the response to pigeon cytochrome *c*. However, (B10.A(4R) × B10.PL)F_1 and (B10.S × B10.PL)F_1 mice do express A_e^k:E_α^u and A_e^s:E_α^u on their cell surface, although in reduced amounts relative to $A_e^{k,s}$:$E_\alpha^{k,d,p,r}$ complexes found in corresponding F_1 strains. This quantitative difference in Ia antigen expression correlated with a difference in the ability to present pigeon cytochrome *c* to B10.A and B10.S(9R) long-term T cell lines. Thus, (B10.A(4R) × B10.PL)F_1 spleen cells required a 10-fold higher antigen dose to induce the same stimulation as (B10.A(4R) × B10.D2)F_1 spleen cells. In addition, the monoclonal antibody, Y-17, which reacts with A_e:E_α molecules of several strains, had a greater inhibitory effect on the proliferative response to pigeon cytochrome *c* of B10.A T cells

in the presence of (B10.A(4R) × B10.PL)F_1 spleen cells than in the presence of (B10.A(4R) × B10.D2)F_1 spleen cells.

These functional data, in concert with the biochemical and serological data in the accompanying report, are consistent with the molecular model for *Ir* gene complementation in which appropriate two-chain Ia molecules function at the antigen-presenting cell (APC) surface as restriction elements. Moreover, they clearly demonstrate that the magnitude of the T cell proliferative response is a function of both the concentration of nominal antigen and of the amount of Ia antigen expressed on the APC. Finally, the direct correlation of a quantitative deficiency in cell surface expression of an Ia antigen with a corresponding relative defect in antigen-presenting function provides strong independent evidence that the *I*-region-encoded Ia antigens are the products of the MHC-linked *Ir* genes.

The authors would like to thank Dr. William E. Paul and Dr. Ethan M. Shevach for many valuable discussions during the course of this work. The editorial assistance of Ms. Shirley Starnes is gratefully acknowledged.

Received for publication 31 July 1981 and in revised form 13 October 1981.

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 Corp., New York. 467.
2. Zinkernagel, R. M., and P. C. Doherty. 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction specificity, function, and responsiveness. *Adv. Immunol.* **27:**51.
3. Lerner, E. A., L. A. Matis, C. A. Janeway, Jr., P. P. Jones, R. H. Schwartz, and D. B. Murphy. 1980. A monoclonal antibody against an immune response (Ir) gene product? *J. Exp. Med.* **152:**1085.
4. Michaelides, M., M. Sandrin, G. Morgan, I. F. C. McKenzie, R. Ashman, and R. W. Melvold. 1981. Ir gene function in an I-A subregion mutant B6.C-H-2^{bm12}. *J. Exp. Med.* **153:**464.
5. Longo, D. L., L. A. Matis, and R. H. Schwartz. 1981. Insights into immune response gene function from experiments with chimeric animals. *Crit. Rev. Immunol.* **2:**83.
6. Schwartz, R. H., M. E. Dorf, B. Benacerraf, and W. E. Paul. 1976. The requirement for two complementing Ir-Glø immune response genes in the T-lymphocyte proliferative response to poly-($Glu^{53}Lys^{36}Phe^{11}$). *J. Exp. Med.* **143:**897.
7. Solinger, A. M., M. E. Ultee, E. Margoliash, and R. H. Schwartz. 1979. T-lymphocyte response to cytochrome *c*. I. Demonstration of a T-cell heteroclitic proliferative response and identification of a topographic antigenic determinant on pigeon cytochrome *c* whose immune recognition requires two complementing major histocompatibility complex-linked immune response genes. *J. Exp. Med.* **150:**830.
8. Matis, L. A., D. Lebwohl, M. Ultee, E. Margoliash, and R. H. Schwartz. 1980. Gene complementation in the T lymphocyte response to pigeon cytochrome *c*. *Fed. Proc.* **39:**1127.
9. Schwartz, R. H., A. Yano, J. H. Stimpfling, and W. E. Paul. 1979. Gene complementation in the T-lymphocyte proliferative response to poly ($Glu^{56}Lys^{36}Phe^{9}$)$_n$. A demonstration that both immune response gene products must be expressed in the same antigen-presenting cell. *J. Exp. Med.* **149:**40.
10. Longo, D. L., and R. H. Schwartz. 1980. Gene complementation. Neither Ir-Glø gene need

be present in the proliferative T cell to generate an immune response to poly $(Glu^{56}Lys^{35}Phe^{9})_n$. *J. Exp. Med.* **151:**1452.

11. Schwartz, R. H., A. M. Solinger, M. E. Ultree, E. Margoliash, A. Yano, J. H. Stimpfling, C. Chen, C. F. Merryman, P. H. Maurer, and W. E. Paul. 1979. Ir gene complementation in the murine T-lymphocyte proliferative response. *In* T and B lymphocytes: Recognition and Function. F. L. Bach, E. S. Vitetta, B. Bonavida, and C. F. Fox, editors. Academic Press Inc., New York. 261–275.
12. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1978. Two gene control of the expression of a murine Ia antigen. *J. Exp. Med.* **148:**925.
13. Uhr, J. W., J. D. Capra, E. S. Vitetta, and R. G. Cook. 1979. Organization of the immune response genes. Both subunits of murine I-A and I-E/C molecules are encoded within the I-region. *Science (Wash. D. C.).* **206:**293.
14. Schwartz, R. H., C. Chen, and W. E. Paul. 1980. Gene complementation in the T cell proliferative response to poly-$(Glu^{56}Lys^{35}Phe^{9})_n$. Functional evidence for a restriction element coded for by both the I-A and I-E subregion. *Eur. J. Immunol.* **10:**708.
15. McNicholas, J. M., D. B. Murphy, L. A. Matis, R. H. Schwartz, E. A. Lerner, C. A. Janeway, Jr., and P. P. Jones. 1982. Immune response gene function correlates with the expression of an Ia antigen. I. Preferential association of certain A_e and E_α chains results in a quantitative deficiency in expression of an A_e:E_α complex. *J. Exp. Med.* In press.
16. Brautigen, D. L., S. Ferguson-Miller, and E. Margoliash. 1978. Mitochondrial cytochrome *c*: preparation and activity of native and chemically modified cytochrome *c*. *Methods Enzymol.* **53:**128.
17. Corradin, G., and H. A. Harbury. 1970. Cleavage of cytochrome *c* with cyanogen bromide. *Biochem. Biophys. Acta.* **221:**489.
18. Schwartz, R. H., and W. E. Paul. 1976. T-lymphocyte-enriched murine peritoneal exudate cells. II. Genetic control of antigen-induced T-lymphocyte proliferation. *J. Exp. Med.* **143:** 529.
19. Yano, A., R. H. Schwartz, and W. E. Paul. 1977. Antigen presentation in the murine T-lymphocyte proliferative response. I. Requirement for genetic identity at the major histocompatibility complex. *J. Exp. Med.* **146:**828.
20. Sredni, B., H. Y. Tse, C. Chen, and R. H. Schwartz. 1981. Antigen-specific clones of proliferating T lymphocytes. I. Methodology, specificity and MHC restriction. *J. Immunol.* **126:**341.
21. Kimoto, M., and C. G. Fathman. 1980. Antigen reactive T cell clones. I. Transcomplementing hybrid I-A region gene products function effectively in antigen presentation. *J. Exp. Med.* **152:**759.
22. Sprent, J. 1980. Effects of blocking helper T cell induction in vivo with anti-Ia antibodies. Possible role of I-A/E hybrid molecule as restriction elements. *J. Exp. Med.* **152:**996.
23. Longo, D. L., and R. H. Schwartz. 1981. Inhibition of antigen-induced proliferation of T cells from radiation-induced bone marrow chimeras by a monoclonal antibody directed against an Ia determinant on the antigen-presenting cell. *Proc. Natl. Acad. Sci. U. S. A.* **78:** 514.
24. Hodes, R. J., K. S. Hathcock, and A. Singer. 1980. Major histocompatibility complex-restricted self recognition. A monoclonal anti-I-A^k reagent blocks helper T cell recognition of self major histocompatibility complex determinants. *J. Exp. Med.* **152:**1979.
25. Rosenwasser, L. J., and B. T. Huber. 1981. The *xid* gene controls Ia.W39-associated immune response gene function. *J. Exp. Med.* **153:**1113.
26. McKenzie, I. F. C., G. M. Morgan, M. S. Sandrin, M. M. Michaelides, R. W. Melvold, and H. I. Kohn. 1979. B6.C-H-2^{bm12}. A new H-2 mutation in the I region in the mouse. *J. Exp. Med.* **150:**1323.
27. McKean, D. J., R. W. Melvold, and C. David. 1981. Tryptic peptide comparison of Ia

antigen α and β and polypeptides from the I-A mutant B6.C-H-2^{bm12} and its congenic parental strain B6. *Immunogenetics.* **14:**41.

28. Alpert, B., and J. Sprent. 1982. Role of the *H-2* complex in induction of T helper cells in vivo. III. Contribution of the *I-E* subregion to restriction sites recognized by *I-A/I-E*-restricted T cells. *J. Exp. Med.* In press.
29. Rosenthal, A. S. 1978. Determinant selection and macrophage function in genetic control of the immune response. *Immunol. Rev.* **40:**136.
30. Benacerraf, B. 1978. An hypothesis to relate the specificity of T lymphocytes and the activity of I region-specific Ir genes in macrophages and B lymphocytes. *J. Immunol.* **120:** 1809.
31. Janeway, C. A., Jr., H. Wigzell, and H. Binz. 1976. Two different V_H gene products make up the T cell receptor. *Scand. J. Immunol.* **5:**993.
32. Langman, R. E. 1978. Cell-mediated immunity and the major histocompatibility complex. *Rev. Physiol. Biochem. Pharmacol.* **81:**1.
33. Erb, P., M. Feldman, and N. Hogg. 1976. Role of macrophages in the generation of T helper cells. IV. Nature of genetically related factor derived from macrophages incubated with soluble antigens. *Eur. J. Immunol.* **6:**365.
34. Lonai, P., J. Puri, and G. Hammerling. 1981. H-2-restricted antigen binding by a hybridoma clone that produces antigen-specific helper factor. *Proc. Natl. Acad. Sci. U. S. A.* **78:**549.
35. Eichman, K. 1980. Conclusion: A simple conservative model for antigen-specificity and MHC-restriction in lymphocyte communication. *Springer Semin. Immunopathol.* **3:**277.