ANTIGEN-SPECIFIC T CELL CLONES
RESTRICTED TO UNIQUE
F₁ MAJOR HISTOCOMPATIBILITY COMPLEX DETERMINANTS

Inhibition of Proliferation with a Monoclonal Anti-Ia Antibody

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The earliest experiments to suggest the existence of unique F_1-specific immune responses were ones in which two low or intermediate responder mice were crossed to produce a high responder (1-3). These were soon followed by more convincing "all or none" experiments with antigens such as poly(Glu^{55}Lys^{36}Phe^9)$_n$ (GLϕ),[1] in which two nonresponder strains were crossed to produce an F_1 responder (4, 5). In these studies, responder strains were also identified that were genetic recombinants between the two nonresponder strains, demonstrating that two separate genetic elements controlled the immune response (4-6). Further mapping studies placed both genes within the *I* region of the major histocompatibility complex (MHC), one in *I-A*, the other in *I-E/C* (7). Subsequently, other forms of gene complementation were found in which one genetic element mapped outside of the MHC (8) or in which both genetic elements mapped to the *I-A* subregion (β-β complementation) (6, 9, 10).

The first evidence that these immune responses involved the recognition of unique F_1 antigenic determinants came from studies of the mixed lymphocyte reaction (11) in which A/J anti-(B6A)F_1 T lymphocyte populations were found to have a greater proliferative response to (B6A)F_1 stimulators than to B6 stimulators. This concept was extended to gene complementation for soluble antigen responses through the use of radiation-induced, bone marrow chimeras (12, 13) and antigen-presentation experiments (12-14). The chimera experiments showed that for the proliferative response to GLϕ, both gene products had to be expressed in the same antigen-presenting cell and that neither gene had to be present in the responding T lymphocyte. The antigen-presentation experiments provided functional evidence that Ia antigens were the structural elements required for GLϕ presentation by confirming the prediction from biochemical studies (15-17) that the F_1-specific restriction element was composed of an α-chain coded for in the *I-E* subregion and a β-chain coded for in the *I-A* subregion.

Overall, the experiments provided the strongest correlative evidence to date that the Ia antigen-bearing molecules are in fact the *Ir* gene products.

The general model that has emerged from these studies is one in which T lymphocytes possess receptors for antigenic determinants unique to certain F_1 or recombinant strain MHC products expressed on antigen-presenting cells, and that recognition of these determinants is essential for T cell stimulation. Furthermore, the model predicts that only certain T lymphocytes in the responder population will possess such receptors, because if every T cell had receptors for all MHC products, previous studies (18–21) would not have been able to demonstrate MHC restriction (i.e., separate populations of antigen-specific T cells in F_1 mice restricted to only one of the parental MHC haplotypes). With the recent development of the technology for cloning T lymphocytes, it has now become possible to test directly the prediction that individual T cells are restricted to recognizing only one restriction element and that clones specific for F_1 structures do exist. For alloreactive T cells it was shown that individual clones recognize either unique F_1 MHC determinants or determinants shared with the allogeneic parent (22).

In this paper we show that for the soluble protein antigen poly(Glu^{60}Ala^{30}Tyr^{10})$_n$ (GAT) three types of T cell clones can be found in (A × B)F_1 mice, one specific for GAT in association with parent A MHC products, one specific for GAT in association with parent B MHC products, and one specific for GAT in association with unique F_1 MHC products. Furthermore, we isolate B10.A(5R) clones reactive to GLϕ and prove at the single-cell level our previous conclusion that stimulation requires F_1 antigen-presenting cells expressing both high responder *Ir* gene products and the Ia.7-bearing α_E-chain. Finally, we demonstrate that a monoclonal antibody directed against the β_{AE}-α_E Ia molecule can completely inhibit the F_1 antigen-presenting cell-induced GLϕ proliferative response of the B10.A(5R) clone, although it has no effect on the response of a PPD-specific B10.A(5R) colony or a GAT-specific (B10.A × B10)F_1 clone, which are both restricted to recognizing their antigen in association with β_A-α_A Ia molecules. These results demonstrate that the biological basis of *Ir* gene complementation is the existence of T cell clones whose receptors are restricted to recognition of antigen in association with determinants found only on unique F_1 (or recombinant) Ia molecules on antigen-presenting cells.

Materials and Methods

Animals. Murine strains C57BL/10Sn(B10), B10.A/SgSn, (B10.A × B10)F_1, and B10.A(5R)/SgSn were purchased from The Jackson Laboratory, Bar Harbor, Maine. B10.D2/nSnN mice were purchased from the Division of Research Services, National Institutes of Health, Bethesda, Md. B10.RIII/Sg and B10.Q/Sg mice were the progeny of breeding pairs kindly provided by Dr. Jack Stimpfling, McLaughlin Research Institute, Great Falls, Mont. The B10.S/Sx mice were the progeny of breeding pairs kindly provided by Dr. David Sachs, National Cancer Institute, National Institutes of Health. All 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. GAT and GLϕ were purchased from Miles-Yeda Laboratories, Inc., Research Products Division, Elkhart, Ind. The polymers were dissolved in 1% Na₂CO₃ in saline and then adjusted to pH 7.2 with 1 N HCl. Purified protein derivative of *Mycobacterium tuberculosis* (PPD) was purchased from Connaught Laboratories, Toronto, Ontario, as a 2-mg/ml solution in saline. Mice were immunized in the hind foot pads and the base of the tail with 100 μg of GAT or GLϕ in saline emulsified in complete Freund's adjuvant containing 1 mg/ml of killed *M. tuberculosis*, strain H37Ra (Difco Laboratories, Detroit, Mich.).

Cell Cultures.   T cells from lymph nodes draining the sites of immunization were prepared as previously described (23) by passage over nylon-wool columns and cultured in Click's medium with 100 µg/ml of antigen and irradiated (2,000 rad) spleen cells as a source of antigen-presenting cells.

Soft Agar Colony Formation. 2×10^6 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 described previously (23). After 5 d, colonies were picked and expanded in liquid culture with the aid of T cell growth factor (TCGF) derived from 24-h supernatant fluids of concanavalin A-stimulated DBA/2 splenocytes (23, 24). Cloning from these lines was carried out by distributing 200 cells in soft agar, as described in detail elsewhere (23).

Quantitation of Clonal Responses to Antigen.   T cells from the colonies were examined for reactivity in a 6-d proliferation assay using [^{3}H]thymidine incorporation (23, 25). 10^3–5×10^4 T cells were cultured in Click's or RPMI-1640 medium plus 10% fetal calf serum and 10–20% TCGF with various concentrations of antigens and different sources of irradiated (2,000 rad) spleen cells (10^4–10^5) in a total volume of 100 µl. After 3 d, 50 µl of culture medium not containing any cells was removed from each culture and replaced with 50–100 µl of fresh medium containing 10–20% TCGF, antigen, and irradiated spleen cells. After an additional 2 d, the cultures were pulsed with 1 µCi of [^{3}H]thymidine (6.7 Ci/mmol, New England Nuclear, Boston, Mass.) in 10 µl of saline and harvested 16–18 h later using a MASH II (Microbiological Associates, Walkersville, Md.). Incorporation of thymidine was measured in a β scintillation counter (Beckman Instruments, Inc., Fullerton, Calif.) with an efficiency of 50–60%. The results are expressed as counts per minute (cpm) ± the standard error of the mean (SEM) for triplicate determinations or as the difference between antigen-stimulated and nonstimulated control means (Δcpm) ± the square root of the sum of the squares of the antigen-stimulated and nonstimulated SEM.

Monoclonal Antibodies and Blocking Studies.   A monoclonal anti-Thy 1.2 antibody was purchased in ascites form from New England Nuclear. 20 µl was used to treat 10^8 erythrocyte-lysed spleen cells in 10 ml of Hanks' balanced salt solution containing 5% heat-inactivated fetal calf serum. After 30 min at room temperature, the cells were pelleted and resuspended in 4 ml of a 1:3 dilution of guinea pig complement (previously adsorbed with mouse spleen cells). After 45 min at 37°C, the cells were again pelleted, washed three times with phosphate-buffered saline, suspended in culture medium, and counted. Viability was generally 50–60% compared with the starting population. Using this procedure to eliminate T cells from a normal spleen cell population, we routinely reduced the proliferative response of such cells to concanavalin A by 90% or more.

The monoclonal antibody Y-17 has been shown previously to react with the $\beta_{AE}^{b,k,r,s}$-$\alpha_E^{k,d,p,r}$ Ia molecules on B cells and macrophages and to inhibit specifically the T cell proliferative response of GLφ and pigeon cytochrome *c*-primed lymph node cells and peritoneal exudate T lymphocyte-enriched subpopulations (26). In the present studies, a 1% vol/vol final concentration of an NH_4SO_4 cut of an ascites fluid was added to the wells at the beginning of the 6-d culture and again at 3 d with the supplemental feeding. Inhibition was measured relative to cultures without Y-17 and is expressed as a percentage according to the following formula:

$$\left(1 - \frac{\Delta\text{cpm with Y-17}}{\Delta\text{cpm without Y-17}}\right) \times 100.$$

Results

GAT-specific T Cell Colonies from F_1 Mice.   In earlier studies from this laboratory on the proliferative response to GAT (27), it was noted that primed T cells from (H-2^a × H-2^s) and (H-2^b × H-2^s)F_1 mice consistently gave a greater response to GAT when the antigen was presented on F_1 spleen cells than when the antigen was presented on high responder *H-2^a* or *H-2^b* parental spleen cells. Because the low responder *H-2^s* spleen cell population did not present GAT at all and because mixtures of *H-2^b* and *H-2^s* spleen cells were no better than *H-2^b* spleen cells alone, the data suggested that

there were some T lymphocytes recognizing GAT in association with F₁-specific *I*-region gene products. With the recent development of the technology for cloning antigen-specific T lymphocytes (28–33), it finally became possible to isolate and unequivocally identify such F₁-specific T cells, even in populations in which they might represent only a minority of the total antigen-specific lymphocytes.

(B10.A × B10)F₁ mice were primed with GAT, the draining lymph node T lymphocytes were restimulated in vitro with GAT, and the cells were cloned in soft agar. 14 colonies were picked, expanded in liquid culture, and tested for antigen specificity in a proliferation assay in the presence of B10, B10.A, (B10.A × B10)F₁, or B10 plus B10.A irradiated spleen cells. As shown in Table I, four distinct patterns of responsiveness were found. Four of the colonies (numbers 1, 4, 8, and 12) responded to GAT in the presence of spleen cells bearing B10.A MHC gene products [i.e., B10.A and (B10.A × B10)F₁], but not in the presence of spleen cells bearing only B10 MHC gene products. The failure of B10 spleen cells to stimulate was not due to a nonspecific suppression phenomenon caused by these cells, because a mixture of equal numbers of B10 and B10.A spleen cells elicited as much of a proliferative response as B10.A spleen cells alone. Instead, it appeared that T cells from these four colonies were restricted to recognizing GAT only in association with B10.A MHC gene products. Six other colonies (numbers 2, 3, 5, 9, 10, and 13) appeared to have the opposite restriction specificity. They responded to GAT in the presence of B10 and (B10.A × B10)F₁ spleen cells but not B10.A spleen cells. Again, mixing experiments did not show any nonspecific suppression, suggesting that T cells from these six colonies were restricted to recognizing GAT only in association with B10 MHC gene products. The 10 colonies in these two groups represent the expected two subsets of F₁ T lymphocytes, restricted to recognizing the antigen in association with one of the parental haplotypes, which have been identified in uncloned T cell populations by several research laboratories (18–21).

The remaining four colonies represent the new subsets revealed by T cell cloning.

TABLE I

Different Types of MHC Restriction of GAT-specific T Cell Colonies from (B10.A × B10)F₁ Mice

Colony number	Proliferative response (cpm ± SEM) to GAT with spleen cells from					Specificity
	None	B10.A	B10	(B10.A × B10)F₁	B10.A + B10	
1	120 (±20)	36,453 (±2,664)	83 (±6)	37,423 (±10,961)	34,450 (±3,836)	B10.A
4	70 (±20)	36,473 (±834)	70 (±5)	48,193 (±9,068)	36,206 (±913)	B10.A
8	100 (±30)	16,876 (±844)	76 (±17)	26,403 (±5,853)	19,950 (±3,291)	B10.A
12	80 (±40)	21,090 (±4,738)	110 (±11)	33,590 (±3,415)	35,013 (±3,790)	B10.A
2	165 (±25)	183 (±16)	41,350 (±3,055)	39,986 (±1,356)	36,533 (±2,254)	B10
3	75 (±5)	106 (±13)	39,186 (±1,228)	34,033 (±5,038)	36,563 (±1,722)	B10
5	65 (±25)	86 (±14)	40,060 (±1,769)	42,040 (±2,483)	36,513 (±1,831)	B10
9	95 (±5)	110 (±10)	30,240 (±6,986)	26,760 (±3,569)	29,926 (±7,271)	B10
10	155 (±55)	160 (±11)	27,553 (±5,721)	32,773 (±3,526)	21,716 (±5,989)	B10
13	170 (±10)	86 (±14)	16,243 (±2,739)	20,456 (±1,279)	18,566 (±4,734)	B10
6	167 (±25)	183 (±29)	133 (±48)	41,773 (±2,410)	176 (±12)	(B10.A × B10)F₁
11	105 (±5)	156 (±17)	143 (±29)	25,430 (±3,990)	106 (±31)	(B10.A × B10)F₁
14	170 (±10)	186 (±20)	103 (±311)	32,140 (±6,189)	200 (±72)	(B10.A × B10)F₁
7	165 (±75)	17,496 (±1,790)	18,833 (±1,912)	30,150 (±2,911)	38,490 (±1,617)	B10.A or B10

(B10.A × B10)F₁ mice were immunized with GAT and T lymphocytes from the draining nodes were stimulated in vitro with GAT and cloned in soft agar. 14 colonies were picked and expanded in liquid culture. 2×10^4 T cells from each colony were stimulated with 100 µg/ml of GAT in a 6-d thymidine-incorporation assay (see Materials and Methods) in the presence of 10^5 irradiated (2,000 rad) spleen cells from B10, B10.A, or (B10.A × B10)F₁ mice. In the mixing experiment (B10.A + B10), 10^5 spleen cells of each type were added. The restriction specificity observed for each colony is listed in the last column.

Three of the colonies (numbers 6, 11, and 14) appeared to be restricted to recognizing GAT in association with unique F_1 MHC gene products. Thus, neither the B10 nor the B10.A parental spleen cell population supported a GAT response by these colonies. Furthermore, a mixture of the two parental spleen cell populations was not sufficient. Only F_1 spleen cells were capable of supporting a GAT response. The fact that F_1 spleen cells worked, whereas a mixture of B10 and B10.A parental spleen cells failed, even though both populations contain the same total genetic information, suggests that the $H-2^b$ and $H-2^a$ gene products must interact within the same cell to generate the unique F_1 restriction elements that these three colonies recognize.

The last pattern of responsiveness, which was defined by only one of the 14 colonies (number 7), consisted of GAT stimulation in the presence of all the spleen cell populations tested (Table I). This was potentially the most interesting colony, since the data suggested that its cells could respond to GAT in association with either B10.A or B10 MHC gene products; i.e., the T cells were not restricted. However, in contrast to the other colonies, these cells showed unusual quantitative differences in the magnitude of the GAT stimulation achieved, depending on which spleen cell population was added. Using (B10.A × B10)F_1 spleen cells as stimulators, the thymidine incorporation was 30,000 cpm, whereas addition of either the B10 or B10.A parental spleen cell population alone resulted in ~60% of that amount of stimulation. Furthermore, mixing the two parental populations gave an additive result. These data suggest that the colony is not a clone of cells with a single receptor capable of recognizing GAT in association with either parental haplotype but rather is a colony composed of at least two clones, one specific for GAT in association with B10.A MHC gene products, and one specific for GAT in association with B10 MHC gene products. However, definitive proof of this conclusion required recloning to isolate the two putative subpopulations.

200 cells from colony 7 were recloned with 10^5 irradiated F_1 spleen cells over a lower agar layer containing 100 μg/ml of GAT and 25% vol/vol TCGF. From six similar platings, an average of 187 ± 5 colonies emerged for a plating efficiency of 93.5%. 10 of these colonies were picked, expanded in liquid culture with GAT and F_1 spleen cells, and tested for antigen specificity in the presence of B10.A, B10, and (B10.A × B10)F_1 spleen cells. As shown in Table II, two patterns of responsiveness were found. Three of the clones (7.3, 7.5, and 7.10) were specific for GAT in association with B10.A MHC gene products, and the other seven were specific for GAT in association with B10 MHC gene products. None of the clones showed specificity for GAT in association with both B10 and B10.A MHC gene products, which was the property of the original colony. Thus, we conclude that the original colony was not a clone but instead was composed of two different GAT-specific T cell populations, one restricted to B10.A, the other to B10 MHC gene products. This demonstration of a colony derived from more than one antigen-specific cell illustrates why we have previously been cautious in treating the initial colonies in soft agar as true clones (23, 24). Before drawing any conclusions from unusual results, it is essential that the population be recloned with high-plating efficiency at least once.

GLφ-specific T Cell Clones. The immune response to GLφ is the best-studied example at the population level for the existence of T cell clones that recognize unique F_1 (or recombinant) histocompatibility molecules (7). Because of the relative structural simplicity of the antigen, only a few inbred strains of mice respond to its antigenic

TABLE II

Recloning at High-Plating Efficiency of the (B10.A × B10)F₁ T Cells Specific for GAT in Association with B10.A or B10 MHC Products Reveals That the Original Colony Was a Mixture of Two T Cell Clones with Different Restriction Specificities

Clone number	Specificity	Proliferative response (cpm ± SEM) to GAT with spleen cells from				
		None	B10.A	B10	(B10.A × B10)F₁	B10.A + B10
7.1	B10	200 (±45)	283 (±76)	11,350 (±605)	12,986 (±1,356)	12,533 (±1,254)
7.2	B10	75 (±35)	186 (±44)	10,060 (±1,769)	12,040 (±1,483)	12,513 (±1,831)
7.3	B10.A	170 (±30)	16,473 (±834)	70 (±15)	18,193 (±1,068)	16,200 (±913)
7.4	B10	258 (±75)	260 (±22)	13,553 (±1,721)	12,060 (±1,026)	12,848 (±1,124)
7.5	B10.A	174 (±40)	11,290 (±1,730)	212 (±21)	13,370 (±1,215)	13,942 (±1,520)
7.6	B10	192 (±51)	227 (±62)	10,152 (±1,350)	11,731 (±1,320)	10,951 (±1,610)
7.7	B10	217 (±78)	307 (±86)	14,691 (±1,782)	12,719 (±1,517)	13,800 (±1,920)
7.8	B10	143 (±21)	278 (±102)	12,345 (±991)	13,812 (±1,253)	14,070 (±1,315)
7.9	B10	183 (±47)	124 (±61)	10,795 (±1,042)	9,948 (±840)	11,068 (±980)
7.10	B10.A	220 (±80)	14,273 (±1,126)	180 (±27)	13,575 (±1,096)	13,978 (±1,836)

200 T cells from colony 7 of Table I were recloned at high-plating efficiency (93.5%) and 10 colonies were picked and expanded in liquid culture. In this experiment, 10⁴ T cells from each clone were stimulated with 100 μg/ml of GAT in a 6-d thymidine-incorporation assay (see Materials and Methods) in the presence of 10⁵ irradiated spleen cells from B10, B10.A, or (B10.A × B10)F₁ mice. In the mixing experiment (B10.A + B10), 10⁵ spleen cells of each type were added. Two types of restriction specificities were found: seven clones having T cells that recognize GAT in association with B10 MHC gene products, and three clones having T cells that recognize GAT in association with B10.A MHC products. No clones were found having T cells that had the joint recognition properties of cells from the original colony.

determinants. Furthermore, strains of only one haplotype appear to be capable of using solely the *I-A*-subregion gene products to generate an immune response against GLϕ (*H-2^q*). All the other responder strains require gene products in both *I-E* and *I-A*. Thus, it was possible even before T cell cloning to demonstrate the need for both *I*-region gene products to be expressed in the same antigen-presenting cell (12, 13) and the requirement for the T cell population to recognize such F₁-specific histocompatibility structures to generate a GLϕ response (14). From these data and special experiments with chimeric mice designed to exclude two T cell models (13), we concluded that the existence of clones of T cells specific for F₁ (or recombinant) MHC gene products was the only way to explain the results. However, unequivocal proof of this hypothesis required isolation of GLϕ-specific T cell clones and demonstration that these cells had all the recognition properties of the original T cell population.

B10.A(5R) mice, the GLϕ-specific high responder recombinant strain derived from a crossover event between the low responders A/WySn (*H-2^a*) and C57BL/10Sn (*H-2^b*) during the derivation of the B10.A congenic line, were immunized with 100 μg of GLϕ. 7 d later, the T lymphocytes from the draining lymph nodes were prepared and stimulated in vitro with 100 μg/ml of GLϕ. 3 d later 2 × 10⁶ cells (six replicates) were cloned in soft agar in the presence of antigen. After another 5 d, an average of 45 ± 6 colonies were observed, representing a clonal frequency of 2.3 × 10⁻⁵. This is similar to the clonal frequencies we have observed previously for the antigens PPD and the dinitrophenyl derivative of ovalbumin (23, 34). 20 of these colonies were picked, nine were successfully expanded in liquid culture, and six of them were tested for antigen specificity. Cells from all six colonies were shown to be GLϕ-specific using a 6-d proliferation assay (data not shown). 200 cells from one of the colonies (number 6) were recloned in soft agar in the presence of antigen but without TCGF (six replicates). An average of 173 ± 5 colonies appeared after 5 d for a cloning efficiency of 86.5%. 10 of these clones were picked, nine of them were successfully expanded in liquid culture, and five were tested for antigen specificity. Again, all the colonies were GLϕ

specific. Finally, to be statistically confident at the 5% level that we were dealing with true clones of T cells, we subcloned again, this time in the presence of 25% TCGF but without antigen. From six replicate plates, each seeded with 200 cells of clone 6.2, we recovered an average of 183 ± 3 colonies for a cloning efficiency of 91.5%. 10 of these colonies were picked, all of them were successfully expanded in liquid culture, and five were tested and shown to be GLϕ specific (some of the data showing responsiveness to GLϕ are shown in Tables IV–VII as controls for those experiments). The probability that any of these subclones were derived from a single cell, making the most stringent possible assumptions, is

$$\left(1 - \left[\frac{13.5}{86.5} \times \frac{9.5}{91.5}\right] = 0.984\right)$$

(see reference 23 for the mathematical basis of this calculation).

Having generated a series of GLϕ-specific T cell clones and subclones, we next examined the genetic requirements for antigen presentation by varying the source of irradiated spleen cells added in the proliferation assay. As shown in Table III, the T cells at all stages of their cloning (colony, clone, subclone) could be stimulated with GLϕ in the presence of the high responder (B10.A × B10)F$_1$ spleen cells in addition to the syngeneic B10.A(5R) spleen cells. In contrast, they could not be stimulated at all in the presence of either the low responder B10 (Ir-GLϕ $\alpha^-\beta^+$) or B10.A (Ir-GLϕ $\alpha^+\beta^-$) parental spleen cells. Even more important is that a mixture of B10 and B10.A spleen cells was totally nonstimulatory. This result (as discussed for GAT earlier) demonstrates that both high responder *Ir* genes must be expressed in the same antigen-presenting cell. Overall, the results confirm the findings made with uncloned T cell populations (12). In addition, because absolutely no stimulation was seen with the low responder parental spleen cells for any of the six independently derived colonies, we can conclude that it is likely that the small stimulations seen in the original experiments, which used uncloned T cells, were due to antigen carryover to residual F$_1$ or 5R antigen-presenting cells in the responding T cell population rather than to

TABLE III

Stimulation of GLϕ-specific B10.A(5R) T Cell Clones Requires Spleen Cells from High Responder Mice Expressing Both Ir-GLϕ Gene Products in the Same Cell*

Antigen-presenting spleen cells	Ir-GLϕ alleles‡		Proliferative response (cpm ± SEM) to GLϕ by B10.A(5R)					
	α	β	Colony 1	Colony 6	Clone 6.2	Clone 6.4	Subclone 6.2.3	Subclone 6.2.4
None			ND§	ND	67 (±28)	173 (±52)	160 (±49)	72 (±16)
B10.A(5R)	+	+	35,740 (±2,884)	38,846 (±2,547)	7,447 (±61)	9,243 (±1,774)	12,730 (±515)	9,220 (±1,753)
B10.A	+	−	90 (±55)	63 (±19)	60 (±15)	67 (±15)	110 (±15)	87 (±15)
B10	−	+	37 (±12)	77 (±12)	70 (±10)	87 (±20)	47 (±9)	67 (±27)
B10.A + B10	±	∓	203 (±168)	85 (±15)	73 (±22)	63 (±23)	73 (±88)	70 (±15)
(B10.A × B10)F$_1$	+	+	38,820 (±980)	34,693 (±2,819)	10,177 (±1,492)	9,497 (±1,514)	9,410 (±808)	10,197 (±1,351)

* B10.A(5R) mice were immunized with GLϕ and T cells from the draining lymph nodes were isolated and stimulated with GLϕ in vitro. After 3 d, the cells were cloned in soft agar. Six colonies were picked and expanded in liquid culture. T cells from colony 6 were recloned in soft agar from which five clones were isolated and expanded for testing. One of these clones, 6.2, was recloned again and five subclones were isolated and expanded for testing. This table shows results from experiments in which 2 × 10^4 T cells from colonies 1 and 6, 5 × 10^3 T cells from clones 6.2 and 6.4, and 5 × 10^3 T cells from subclones 6.2.3 and 6.2.4 were stimulated with 100 μg/ml of GLϕ in the presence of 10^5 irradiated (2,000 rad) spleen cells from B10.A(5R), B10.A, B10, or (B10.A × B10)F$_1$ mice. In the mixing experiment, 10^5 B10.A and 10^5 B10 spleen cells were added. Stimulation was assessed by measuring the incorporation of tritiated thymidine in a 6-d assay as described in the Materials and Methods.
‡ + indicates a high responder allele; − indicates a low responder allele at either the Ir-GLϕ-α or Ir-GLϕ-β locus.
§ Not determined.

clones of T cells in those populations capable of seeing GLϕ in association with low responder B10.A or B10 MHC gene products.

Recent structural studies on Ia antigens using one- and two-dimensional gels (15–17) and peptide mapping (35–37) have provided a potential biochemical basis for the functional two-gene complementation. These studies revealed that the I-E subregion encodes only the larger α-chain (α_E) of an Ia molecule, whereas a gene in I-A codes for the physically associated, smaller β-chain (β_{AE}). The assembly of these two chains to form a functional molecule (β_{AE}-α_E) can only occur when both gene products are expressed in the same cell. Thus, if one were to equate Ia-bearing molecules with Ir gene products, one could then predict that the β_{AE}-α_E Ia molecules found only in certain F₁ and recombinant strains were the restriction elements on the surface of the antigen-presenting cells that were required for stimulating GLϕ-primed T lymphocytes. In other words, Ir gene defects were simply the absence of (or failure to stimulate) T cells capable of recognizing particular antigens in association with particular MHC gene products.

To test this prediction, we examined the genetic requirements at the I-E subregion for spleen cells to present GLϕ to primed B10.A(5R) T lymphocytes (14). The B10.A(5R) strain possesses I-A^b and I-E^k alleles. To examine MHC restriction at the I-E subregion only, a series of F₁ mice were bred in which one parent was always a B10 (I-A^b, I-E^b). Thus, when spleen cells from these F₁ mice were used to present GLϕ, they all shared I-A^b with the responding B10.A(5R) T lymphocytes and only varied at I-E. The results using uncloned T cell populations as responders revealed that spleen cells from H-2^b by H-2^a, H-2^k, H-2^d, H-2^p, or H-2^r F₁'s were all capable of presenting GLϕ, whereas spleen cells from H-2^b by H-2^f, H-2^q, or H-2^s could not present GLϕ (14). The results using the GLϕ-specific B10.A(5R) T cell clones are shown in Table IV. At all stages of their cloning the specificity of the T cells was identical to that found for the whole population, i.e., they responded to GLϕ in the presence of H-2^b by H-2^a, H-2^d, or H-2^r F₁ spleen cells but not in the presence of H-2^b by H-2^q, or H-2^s F₁ spleen cells. In addition, using clones as the responding population eliminated the need to derive the GLϕ response data by subtracting the MLR-induced stimulation, which was always present when uncloned T cell populations were used as responders (14). The absence of any alloreactivity by the T cell clones allowed a conclusive demonstration that the H-2^q and H-2^s haplotypes could not complement at all for GLϕ presentation, whereas the H-2^d and H-2^r haplotypes could complement. Furthermore, the H-2^d and H-2^r I-E alleles appeared to be functionally equivalent to the syngeneic H-2^k allele, as judged by the magnitude of the proliferative response. These results correlate perfectly with the biochemical studies on the I-E subregion Ia antigens in which the α_E-chains coded for by the H-2^k, H-2^d, H-2^p, and H-2^r haplotypes were shown by peptide mapping to be structurally very similar (35–37) and in which no α_E-chain could be found in spleen cell lysates from H-2^f-, H-2^q-, and H-2^s-bearing mice (15). Thus, the data demonstrate that the B10.A(5R) T cell population contains clones of T cells that were selected on the basis of their ability to recognize GLϕ in association with the $\beta_{AE}{}^b$-$\alpha_E{}^k$ molecule on the surface of the antigen-presenting cell and that these T cell clones are capable of responding when any of the other similar α_E-chains are substituted for $\alpha_E{}^k$. This perfect correlation between structure and function provides strong evidence in support of the hypothesis that Ia-bearing molecules are the Ir gene products.

Table IV

Stimulation of GLφ-specific B10.A(5R) T Cell Clones is Supported by Spleen Cells from (B10 × B10.A), (B10 × B10.D2), and (B10 × B10.RIII)F₁ Mice but Not from (B10 × B10.Q) and (B10 × B10.S)F₁ Mice *

Antigen-presenting spleen cells	MHC alleles‡		GLφ (100 μg/ml)	Proliferative response (cpm ± SEM) to GLφ by B10.A(5R)					
	I-A	I-E		Colony 1	Colony 6	Clone 6.2	Clone 6.5	Subclone 6.2.1	Subclone 6.2.3
B10.A(5R)	b	k	−	ND‖	ND	40 (±30)	57 (±19)	120 (±23)	180 (±37)
§			+	116,773 (±3,879)	136,875 (±10,900)	12,597 (±448)	11,207 (±1,758)	10,530 (±1,239)	10,710 (±1,395)
(B10 × B10.A)F₁	b/k	b/k	−	ND	ND	17 (±3)	58 (±9)	145 (±82)	210 (±87)
§			+	131,606 (±4,439)	138,766 (±9,031)	12,513 (±3,188)	9,993 (±2,002)	7,587 (±380)	8,063 (±700)
(B10 × B10.D2)F₁	b/d	b/d	−	ND	ND	13 (±3)	54 (±10)	60 (±10)	53 (±15)
§			+	126,435 (±4,981)	139,266 (±9,716)	8,183 (±742)	10,087 (±1,446)	10,773 (±715)	10,417 (±593)
(B10 × B10.RIII)F₁	b/r	b/r	−	ND	ND	23 (±3)	67 (±14)	87 (±42)	83 (±19)
§			+	113,533 (±2,376)	141,458 (±8,391)	7,997 (±348)	9,563 (±73)	10,193 (±603)	10,537 (±719)
(B10 × B10.Q)F₁	b/q	b/q	−	ND	ND	30 (±10)	126 (±142)	ND	ND
			+	80 (±25)	130 (±17)	53 (±7)	63 (±20)	50 (±6)	88 (±24)
(B10 × B10.S)F₁	b/s	b/s	−	ND	ND	20 (±6)	118 (±47)	ND	ND
			+	110 (±11)	106 (±24)	67 (±18)	70 (±27)	60 (±15)	53 (±15)

* GLφ clones were prepared as described in the legend to Table III. This table shows the results from experiments in which 5 × 10⁴ T cells from colonies 1 and 6, 5 × 10³ T cells from clones 6.2 and 6.5, and 5 × 10³ T cells from subclones 6.2.1 and 6.2.3 were cultured with or without 100 μg/ml of GLφ in the presence of 5 × 10⁵ (for the colonies) or 10⁵ (for the clones and subclones) spleen cells (2,000 rad irradiated) from B10.A(5R), (B10 × B10.A)F₁, (B10 × B10.D2)F₁, (B10 × B10.RIII)F₁, (B10 × B10.Q)F₁, or (B10 × B10.S)F₁ mice. Stimulation was assessed in a 6-d tritiated thymidine-incorporation assay as described in Materials and Methods.

‡ Letters refer to the haplotype source of origin of the alleles of the I-A and I-E subregions of the MHC possessed by each spleen cell donor.

§ indicates that these spleen cells bear Ia-specificity 7 and possess I-E subregion genes which code for similar α_E chains as detected by peptide mapping (I-E^k, I-E^d, I-E^r).

‖ Not determined.

Inhibition of Clonal T Cell Proliferation with a Monoclonal Antibody. Recently, a monoclonal anti-Ia antibody has been described that reacts with a determinant on the $\beta_{AE}^{b,k,r,s}$-$\alpha_E^{k,d,p,r}$ Ia molecule (26). This antibody was found to inhibit the T cell proliferative response of B10.A(5R) cells primed to GLφ and B10.A cells primed to pigeon cytochrome c (26), responses that require the β_{AE}-α_E molecule as a restriction element for antigen presentation (14, 38). In contrast, the antibody did not inhibit the T cell proliferative response of B10.A(5R) cells primed to (T,G)-A--L, a response which presumably utilizes only the β_A-α_A Ia molecule as a restriction element for antigen presentation (39). Although experiments with radiation-induced chimeras (40) have unequivocally shown that the antibodies have their effect on the antigen-presenting cell and not the T cell, the mechanism of this antigen-specific inhibition is not clear. It has been postulated that the antibody covers or strips off the relevant Ia-bearing molecules on the surface of the antigen-presenting cell, thus preventing antigen recognition (39, 41). However, the possibility has not been eliminated that the masking of the Ia antigens somehow leads to the development of suppressor T cells, which actually mediate the inhibition (42). To investigate this possibility we asked whether the antigen-induced proliferative responses of the T cell clones could also be inhibited with the monoclonal antibody.

As shown in Table V, the response of the GLφ-specific B10.A(5R) subclone, 6.2.3, was completely inhibited by the monoclonal antibody Y-17. In contrast, the proliferative response of cells from a B10.A(5R) colony specific for PPD was not significantly inhibited by 1% Y-17. The failure to inhibit the PPD response stems from the fact that these cells are restricted to recognizing the β_A-α_A pair of Ia molecules encoded solely in the I-A subregion (data not shown), whereas the antibody reacts with the β_{AE}-α_E Ia molecule. Because in both responses (GLφ and PPD) there are no suppressor

TABLE V

Inhibition of the Proliferative Response of T Cells from a GLφ-specific B10.A(5R) Subclone but Not T Cells from a PPD-specific Colony by the Monoclonal Antibody Y-17

T cells	Antigen	Experiment 1			Experiment 2		
		cpm ± SEM		Inhibi-tion	cpm ± SEM		Inhibi-tion
		No Y-17	With 1% Y-17		No Y-17	With 1% Y-17	
	μg/ml			*%*			*%*
Subclone 6.2.3	None	143 (±20)	183 (±30)	—	133 (±74)	170 (±6)	—
GLφ-specific	GLφ (50)	11,513 (±413)	150 (±21)	100	10,833 (±381)	127 (±33)	100
from B10.A(5R)	GLφ (100)	10,960 (±1,263)	237 (±30)	99.5	10,563 (±450)	150 (±25)	100
Colony 3	None	97 (±24)	137 (±20)	—	147 (±32)	193 (±20)	—
PPD-specific	PPD (25)	11,467 (±1,079)	10,095 (±115)	12.4	11,833 (±982)	11,220 (±546)	5.6
from B10.A(5R)	PPD (50)	9.820 (±1,059)	12,527 (±1,895)	0	11,103 (±1,217)	11,267 (±444)	0

5 × 10³ T cells from either the GLφ-specific B10.A(5R) subclone 6.2.3 or the PPD-specific B10.A(5R) colony 3 were stimulated with their respective antigens and 10⁵ B10.A(5R) irradiated (2,000 rad) spleen cells in the presence or absence of 1% vol/vol Y-17. Stimulation was assessed in a 6-d tritiated thymidine-incorporation assay. The data are expressed as counts per minute (±SEM) and as percent inhibition by Y-17, which was calculated as described in Materials and Methods.

TABLE VI

Inhibition of the Proliferative Response of T Cells from a GLφ-specific B10.A(5R) Subclone but Not T Cells from a GAT-specific (B10.A × B10)F₁ Clone by the Monoclonal Antibody Y-17 in the Presence of Either B10.A(5R)- or (B10.A × B10)F₁-presenting Cells

T cells	Spleen cells	Antigen	Proliferative response cpm (±SEM)		Inhibition
			No Y-17	With 1% Y-17	
		100 μg/ml			*%*
Subclone 6.2.3	B10.A(5R)	None	127 (±12)	93 (±13)	—
GLφ specific		GLφ	10,087 (±512)	803 (±25)	92.8
from B10.A(5R)	(B10.A × B10)F₁	None	90 (±12)	60 (±6)	—
		GLφ	10,080 (±1,011)	83 (±33)	100
Clone 7.8	B10.A(5R)	None	167 (±12)	57 (±17)	—
GAT specific		GAT	8,682 (±191)	10,505 (±1,184)	0
from (B10.A × B10)F₁	(B10.A × B10)F₁	None	120 (±47)	50 (±6)	—
		GAT	9,270 (±410)	9,490 (±43)	0

5 × 10³ T cells from either the GLφ-specific B10.A(5R) subclone 6.2.3 or the GAT-specific (B10.A × B10)F₁ clone 7.8 were stimulated with 100 μg/ml of their respective antigens in the presence of 10⁵ B10.A(5R) or (B10.A × B10)F₁ irradiated spleen cells. Proliferation was assessed in the presence or absence of 1% vol/vol Y-17, as described in the legend to Table V.

cells in the responding population and the antibody is reacting with the same antigen-presenting cell, it seems reasonable to conclude that the antigen-specific inhibition results from masking the relevant restriction element in some manner.

Further support for this model is shown in Table VI, in which the ability of the GLφ-specific B10.A(5R) subclone 6.2.3 to respond to GLφ in the presence of (B10.A × B10)F₁ as well as B10.A(5R) spleen cells was used. In this experiment, one of the GAT-specific clones (number 7.8) from (B10.A × B10)F₁ mice (see Table II) was used as a control. This clone was restricted to recognizing GAT in association with only *I-A*[b] MHC gene products, because it could be stimulated equally well by GAT in the presence of either B10 (Table II) or B10.A(5R) (Table VI) spleen cells. Y-17 completely inhibited the GLφ response of subclone 6.2.3 in the presence of either B10.A(5R) or (B10.A × B10)F₁ spleen cells. In contrast, the antibody had no effect on the GAT-induced proliferation of clone 7.8 in the presence of either spleen cell population. Thus, clonal proliferation can be inhibited by anti-Ia antibodies in an

antigen-specific manner, provided that the two antigens being examined use different restriction elements.

Although these experiments clearly indicate that suppressor T cells in the responding population cannot be the mechanism of anti-Ia antibody-induced suppression of T cell proliferative responses, it was still possible that T cells in the added spleen cell population accounted for the phenomenon. To investigate this possibility we repeated the blocking experiments using anti-Thy 1 and complement-treated spleen cells as a source of antigen-presenting cells. As shown in Table VII, this population presented GLϕ as well as whole spleen (compare with Table VI, which shows data with whole spleen cells from the same experiment), and this presentation was completely inhibited by Y-17. Thus, T cells in the spleen cell population are not required either for presentation of the antigen to the clone or for the monoclonal antibody Y-17 to produce its blocking effect. Overall, these results convincingly demonstrate that anti-Ia antibody inhibition is not mediated by suppressor T cells of any kind.

Discussion

The existence of T cells specific for antigens in association with unique F_1 or recombinant MHC gene products has been postulated for a number of years on the basis of work with whole T cell population responses to antigens such as GLϕ (7, 12–14). In this report we conclusively prove the existence of such T cells by isolating antigen-specific colonies from primed T cell populations, rigorously cloning the cells under conditions of high-plating efficiency, and demonstrating that such clones can only be stimulated with antigen in association with spleen cells bearing F_1 or recombinant MHC haplotypes. Neither parental spleen cell population alone nor the combination of the two supported a proliferative response. In the case of the GLϕ response of B10.A(5R) clones, it was possible to identify the unique MHC gene products from antigen presentation (Table IV) and anti-Ia blocking experiments (Tables V–VII) as consisting of an Ia.7-bearing α-chain encoded in the I-E subregion (α_E) and a β-chain encoded in the I-A subregion (β_{AE}). These results confirm the

TABLE VII

Depletion of T Cells from the Presenting Cell Population Does Not Affect the Ability of the Monoclonal Antibody Y-17 to Inhibit the Proliferative Response of T Cells from a GLϕ-specific B10.A(5R) Subclone

Spleen cells	GLϕ	Proliferative response (Δcpm ± SEM)		Inhibition
		No Y-17	With 1% Y-17	
	$\mu g/ml$			%
B10.A(5R)	50	9,500 (±700)	80 (±16)	99
	100	10,200 (±900)	−10 (±37)	100
(B10.A × B10)F$_1$	50	10,900 (±880)	50 (±50)	99.5
	100	9,300 (±1,300)	90 (±21)	99

5×10^3 T cells from the GLϕ-specific B10.A(5R) subclone 6.2.3 were stimulated with 50 or 100 μg/ml of GLϕ in the presence of 10^5 irradiated (2,000 rad) B10.A(5R) or (B10.A × B10)F$_1$ spleen cells previously treated with monoclonal anti-Thy 1 plus complement as described in Materials and Methods. Proliferation was assessed in the presence or absence of 1% vol/vol Y-17 as described in the legend to Table V. The data are expressed as Δcpm, the difference between cultures with and without antigen, ±SEM, the square root of the sum of the squares of the standard errors of the two means.

conclusions reached with whole T cell populations (12–14, 26) that GLϕ-specific B10.A(5R) T cell clones have dual specificity for antigen and the β_{AE}-α_E Ia molecule. In addition, they demonstrate that this restricted recognition mechanism is an intrinsic part of the responding T cell machinery and not a secondary result of an extrinsic suppressor mechanism by regulatory T cells. Why GLϕ is obligatorily linked to recognition of β_{AE}-α_E Ia molecules has not been elucidated by these studies. The two competing models that explain this phenomenon postulate either that the Ia molecules have some primitive form of binding site that discriminates between different antigens (43, 44) or the T cell population lacks certain clones of T cells, either because of their absence from the initial precursor pool or because such clones are deleted in the course of development of self tolerance and/or self recognition (45–50). At this time, there is no definitive evidence that would allow us to decide between these two general models.

The cloning technology has also allowed us to generalize the concept of T cells restricted to unique F_1 MHC products by demonstrating that such clones exist even in T cell populations responding to antigens that can be presented by either of the parental MHC gene products alone. The F_1-specific clones are difficult to detect in such whole-population responses, because they often represent only a minor subpopulation of the total responding cells. However, cloning allows one to isolate and expand even small subsets so that the specificity of the cells can be clearly delineated (51). In this regard it is interesting to note that of the 15 (H-2^a × H-2^b)F_1 colonies isolated that were specific for GAT (counting the mixed colony as two separate colonies), all of them were MHC restricted in one form or another. 33% of the colonies recognized GAT in association with H-2^a MHC gene products, 47% recognized GAT in association with H-2^b MHC gene products, and 20% recognized GAT in association with unique F_1 MHC gene products. None of the clones were capable of recognizing both H-2^a and H-2^b MHC gene products, i.e., none were unrestricted. Thus, MHC-restricted recognition of antigen appears to be the major, if not the sole, form of T cell recognition, at least for the proliferating T lymphocyte after priming.

The GAT-specific T cells restricted to recognizing unique F_1 MHC gene products showed the same pattern of restriction as the GLϕ-specific T cells (compare Tables I and III). However, this does not necessarily mean that the T cells from GAT colonies recognize the same F_1 structures as the GLϕ-specific T cells. In fact, recently published data from the laboratory of C. G. Fathman (33) suggest that the unique F_1 products seen by the GAT-specific T cells result from β-β complementation (7, 10), in which the α_A and β_A Ia products of the two I-A subregions rearrange to give the β_A^b-α_A^k and β_A^k-α_A^b combinations found only in the F_1. The reason that GAT recognition uses only these mixed molecules, whereas GLϕ recognition requires the β_{AE}-α_E molecule, is not clear.

Before T cell cloning, the concept that MHC restriction was needed for the T cell to recognize self MHC antigens, in addition to the foreign antigen on the surface of antigen-presenting cells, was supported experimentally mainly by inhibition studies with antibodies directed against MHC gene products (39, 52). Although some of these studies showed that lymphocyte binding to macrophages (53, 54) could be inhibited with anti-Ia antibodies, suggesting that T cell recognition was being impaired, a recent publication describing experiments in a human system (42) demonstrated that the inhibition might be more complex and might involve the function of suppressor

T cells. To investigate this possibility in the better-defined murine model, we examined the ability of a monoclonal antibody Y-17 that reacts with the β_{AE}-α_E Ia molecule to inhibit the proliferative response of a GLϕ-specific B10.A(5R) T cell clone, which requires the presence of the β_{AE}-α_E Ia molecule on the antigen-presenting cell for stimulation. As shown here, the monoclonal antibody completely inhibited the proliferative response of the GLϕ-specific clone, even in the total absence of any other T cells to act as suppressor cells. The inhibition was shown to be absolutely specific for T cell clones that require the β_{AE}-α_E Ia molecule for stimulation, because the monoclonal antibody failed to inhibit the proliferative response of a PPD-specific colony and a GAT-specific clone, which both utilize the β_A-α_A Ia molecule as a restriction element. The result with the PPD colony was particularly interesting, because we had previously demonstrated that Y-17 could inhibit ~20–30% of the proliferative response to PPD of whole T cell populations from B10.A(5R) (26). The failure to inhibit the response of the PPD-specific colony at all supports our original conclusion that the whole population contains a mixture of clones, some of which recognize PPD in association with only β_A-α_A Ia molecules and some of which recognize PPD in association with only β_{AE}-α_E Ia molecules.

Overall, the blocking studies presented here completely eliminate the possiblity that suppressor T cells can account for the inhibitory effects seen with anti-Ia antibodies. Previous studies utilizing radiation-induced bone marrow chimeras and monoclonal antibodies have just as clearly shown that the site of action of these antibodies is the antigen-presenting cell and not the responding T lymphocyte (40). Thus, it appears that the most reasonable hypothesis to explain the inhibitory effects of anti-Ia antibodies is still that these antibodies mask or strip from the surface of antigen-presenting cells Ia determinants that must be recognized in conjunction with the foreign antigen in order to stimulate the T cell. The blocking with a monoclonal antibody suggests that it is the Ia-bearing molecules themselves that are essential for the presentation of antigen. However, the possibility of steric inhibition of a physically close, separate *Ir* gene product cannot be excluded.

Summary

The existence of T cells specific for soluble antigens in association with unique F_1 or recombinant major histocompatibility complex (MHC) gene products was first postulated from studies on the proliferative response of whole T cell populations to the antigen poly(Glu^{55}Lys^{36}Phe^9)$_n$ (GLϕ). In this paper we use the newly developed technology of T lymphocyte cloning to establish unequivocally the existence of such cells specific for GLϕ and to generalize their existence by showing that F_1-specific cells can be isolated from T cell populations primed to poly(Glu^{60}Ala^{30}Tyr^{10})$_n$ (GAT) where such clones represent only a minor subpopulation of cells. GLϕ-primed B10.A(5R) and GAT-primed (B10.A × B10)F_1 lymph node T cells were cloned in soft agar, and the colonies that developed were picked and expanded in liquid culture. The GLϕ-specific T cells were then recloned under conditions of high-plating efficiency to ensure that the final colonies originated from single cells. T cells from such rigorously cloned populations responded to stimulation with GLϕ but only in the presence of nonimmune, irradiated spleen cells bearing (B10.A × B10)F_1 or the syngeneic B10.A(5R) recombinant MHC haplotype. Spleen cells from either the B10 or B10.A parental strains failed to support a proliferative response, even when added

together. (B10 × B10.D2)F$_1$ and (B10 × B10.RIII)F$_1$ spleen cells also supported a proliferative response but (B10 × B10.Q)F$_1$ and (B10 × B10.S)F$_1$ spleen cells did not. These results suggested that the T cell clones were specific for GLϕ in association with the β_{AE}^b-$\alpha_E^{k,d,r,}$ Ia molecule and that recognition required both gene products to be expressed in the same antigen-presenting cells. Support for this interpretation was obtained from inhibition experiments using the monoclonal antibody Y-17 specific for a determinant on the β_{AE}-α_E Ia molecule. Y-17 completely inhibited the proliferative response of a GLϕ-specific clone but had no effect on the response of either a PPD-specific or GAT-specific clone, both of which required the β_A-α_A Ia molecule as their restriction element. No evidence could be found for the involvement of suppressor T cells in this inhibition. We therefore conclude that the phenomenon of F$_1$-restricted recognition by proliferating T cells results from the presence of antigen-specific clones that must recognize unique F$_1$ or recombinant Ia molecules on the surface of antigen-presenting cells in addition to antigen in order to be stimulated.

References

1. Vaz N. M., C. M. De Souza, and L. C. S. Maia. 1974. Genetic control of immune responsiveness in mice. Responsiveness to ovalbumin in (C57BL × DBA/2)F$_1$ mice. *Int. Arch. Allergy Appl. Immunol.* **46:**275.

2. Zaleski, M., and F. Milgrom. 1973. Complementary genes controlling immune response to θ-AKR antigen in mice. *J. Immunol.* **110:**1238.

3. Günther, E., and E. Rüde. 1975. Genetic complementation of histocompatibility-linked Ir genes in the rat. *J. Immunol.* **115:**1387.

4. Dorf, M. E., J. H. Stimpfling, and B. Benacerraf. 1975. Requirement for two H-2 complex Ir genes for the immune response to the GLϕ terpolymer. *J. Exp. Med.* **141:**1459.

5. Schwartz, R. H., M. E. Dorf, B. Benacerraf, and W. E. Paul. 1976. The requirement for two complementing *Ir-GLϕ* immune response genes in the T-lymphocyte proliferative response to poly-(Glu^{53}Lys^{36}Phe^{11}). *J. Exp. Med.* **143:**897.

6. Dorf, M. E., and B. Benacerraf. 1975. Complementation of the H-2 linked Ir genes in the mouse. *Proc. Natl. Acad. Sci. U. S. A.* **72:**3671.

7. Dorf, M. E. 1978. Complementation of H-2-linked genes controlling immune responsiveness. *Springer Semin. Immunopathol.* **1:**171.

8. Mozes, E. 1975. Expression of immune response (*Ir*) genes in T and B cells. *Immunogenetics.* **2:**397.

9. Zaleski, M. B., and J. Klein. 1976. Immune response of mice to the Thy 1.1 antigen: intra-H-2 mapping of the complementary Ir-Thy-1 loci. *J. Immunol.* **117:**814.

10. Solinger, A. M., and R. H. Schwartz. 1980. The T lymphocyte proliferative response to poly-L-Glu-poly-D,L-Ala-poly-L-Lys. *J. Immunol.* **124:**2485.

11. Fathman, C. G., and M. Nabholz. 1977. In vitro secondary mixed leukocyte reaction (MLR). II. Interaction MLR determinants expressed by F$_1$ cells. *Eur. J. Immunol.* **7:**370.

12. 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^{35}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.

13. Longo, D. L., and R. H. Schwartz. 1980. Gene complementation. Neither *Ir*-GLϕ gene need be present in the proliferative T cell to generate an immune response to poly(Glu^{55}Lys^{36}Phe^9)$_n$. *J. Exp. Med.* **151:**1452.

14. Schwartz, R. H., C. Chen, and W. E. Paul. 1980. Gene complementation in the T-lymphocyte proliferative response to poly(Glu^{55}Lys^{36}Phe^9)$_n$. Functional evidence for a restriction element coded for by both the *I-A* and *I-E* subregions. *Eur. J. Immunol.* **10:**708.

15. 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.

16. Cook, R. G., E. S. Vitetta, J. W. Uhr, and J. D. Capra. 1979. Structural studies on the murine Ia alloantigens. V. Evidence that the structural gene for the I-E/C beta polypeptide is encoded within the *I-A* subregion. *J. Exp. Med.* **149:**981.

17. Silver, J. 1979. Trans gene complementation of I-E subregion antigens. *J. Immunol.* **123:**1423.

18. Paul, W. E., E. M. Shevach, S. Pickeral, D. W. Thomas, and A. S. Rosenthal. 1977. Independent populations of primed F_1 guinea pig T lymphocytes respond to antigen-pulsed parental peritoneal exudate cells. *J. Exp. Med.* **145:**618.

19. Zinkernagel, R. M., and P. C. Doherty. 1975. *H-2* compatibility requirement for T-cell-mediated lysis of target cells infected with lymphocyte choriomeningitis virus. Different cytotoxic T-cell specificities are associated with structures coded for in *H-2K* or *H-2D. J. Exp. Med.* **141:**1427.

20. Sprent, J. 1978. Role of H-2 gene products in the function of T helper cells from normal and chimeric mice *in vivo. Immunol. Rev.* **42:**108.

21. Kappler, J. W., and P. C. Marrack. 1976. Helper T cells recognize antigen and macrophage surface components simultaneously. *Nature (Lond.).* **262:**797.

22. Hengartner, H., and C. G. Fathman. 1980. Clones of alloreactive T cells. I. A unique homozygous MLR-stimulating determinant present on B6 stimulators. *Immunogenetics.* **10:**175.

23. 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.

24. Rosenberg, S. A., P. J. Spiess, and S. Schwarz. 1978. In vitro growth of murine T cells. I. Production of factors necessary for T cell growth. *J. Immunol.* **121:**1946.

25. 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.

26. 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.

27. Yano, A., R. H. Schwartz, and W. E. Paul. 1978. Antigen presentation in the murine T-lymphocyte proliferative response. II. Ir-GAT-controlled T lymphocyte responses require antigen-presenting cells from a high responder donor. *Eur. J. Immunol.* **8:**344.

28. Sredni, B., H. Y. Tse, and R. H. Schwartz. 1980. Direct cloning and extended culture of antigen-specific MHC-restricted proliferating T lymphocytes. *Nature (Lond.).* **283:**581.

29. von Boehmer, H., H. Hengartner, M. Nabholz, W. Lernhardt, M. H. Schreier, and W. Haas. 1979. Fine specificity of a continuously growing killer cell clone specific for H-Y antigen. *Eur. J. Immunol.* **9:**592.

30. Baker, P. E., S. Gillis, and K. A. Smith. 1979. Monoclonal cytolytic T-cell lines. *J. Exp. Med.* **149:**273.

31. Schreier, M. H., and R. Tees. 1980. Clonal induction of helper T cells: conversion of specific signals into nonspecific signals. *Int. Arch. Allergy Appl. Immunol.* **61:**227.

32. Lotze, M. T., J. L. Strausser, and S. A. Rosenberg. 1980. *In vitro* growth of cytotoxic human lymphocytes. II. Use of T cell growth factor (TCGF) to clone human T cells. *J. Immunol.* **124:**2972.

33. Kimoto, M., and C. G. Fathman. 1980. Antigen reactive T cell clones. I. Transcomple-

menting hybrid I-A region gene products function effectively in antigen presentation. *J. Exp. Med.* **152**:759.

34. Sredni, B., and R. H. Schwartz. 1980. Antigen-specific, proliferating T lymphocyte clones. Methodology, specificity, MHC restriction and alloreactivity. *Immunol. Rev.* In press.

35. Cook, R. G., E. S. Vitetta, J. W. Uhr, and J. D. Capra. 1979. Structural studies on the murine Ia alloantigens. III. Tryptic peptide comparisons of allelic products of the *I-E/C* sub-region. *Mol. Immunol.* **16**:29.

36. McMillan, M., J. M. Cecka, L. Hood, D. B. Murphy, and H. O. McDevitt. 1979. Peptide map analyses of murine Ia antigens of the I-E subregion using HPLC. *Nature (Lond.).* **277**: 663.

37. Freed, J. H., D. W. Sears, J. L. Brown, and S. G. Nathenson. 1979. Biochemical purification of detergent-solubilized H-2 alloantigens. *Mol. Immunol.* **16**:9.

38. 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.

39. 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.

40. 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.* In press.

41. Burger, R., and E. M. Shevach. 1980. Monoclonal antibodies to guinea pig Ia antigens. II. Effect on alloantigen-, antigen-, and mitogen-induced T lymphocyte proliferation in vitro. *J. Exp. Med.* **152**:1011.

42. Broder, S., D. L. Mann, and T. A. Waldmann. 1980. Participation of suppressor T cells in the immunosuppressive activity of a heteroantiserum to human Ia-like antigens (p23,30). *J. Exp. Med.* **151**:257.

43. Rosenthal, A. S. 1978. Determinant selection and macrophage function in genetic control of the immune response. *Immunol. Rev.* **40**:136.

44. Benacerraf, B. 1978. A 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.

45. Schwartz, R. H. 1978. A clonal deletion model for Ir gene control of the immune response. *Scand. J. Immunol.* **7**:3.

46. von Boehmer, H., W. Haas, and N. K. Jerne. 1978. Major histocompatibility complex-linked immune-responsiveness is acquired by lymphocytes of low-responder mice differentiating in thymus of high-responder mice. *Proc. Natl. Acad. Sci. U. S. A.* **75**:2439.

47. Cohn, M., and R. Epstein. 1978. T-cell inhibition of humoral responsiveness. II. Theory on the role of restrictive recognition in immune regulation. *Cell. Immunol.* **39**:125.

48. Janeway, C. A., Jr., H. Wigzell, and H. Binz. 1976. Two different V_H gene products make up the T-cell receptors. *Scand. J. Immunol.* **5**:993.

49. Langman, R. E. 1978. Cell-mediated immunity and the major histocompatibility complex. *Rev. Physiol. Biochem. Pharmacol.* **81**:1.

50. 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.

51. Sproviero, J. F., M. J. Imperiale, and M. Zauderer. 1980. Clonal analysis of F₁ hybrid helper T cells restricted to parental or F₁ hybrid major histocompatibility determinants. *J. Exp. Med.* **152**:920.

52. Shevach, E. M., W. E. Paul, and I. Green. 1972. Histocompatibility linked immune response gene functions in guinea pigs. Specific inhibition of antigen-induced lymphocyte proliferation by alloantisera. *J. Exp. Med.* **136:**1207.
53. Lyons, C. R., T. F. Tucker, and J. W. Uhr. 1979. Specific binding of T lymphocytes to macrophages. V. The role of Ia antigens on Mφ in the binding. *J. Immunol.* **122:**1598.
54. Werdelin, O., and E. M. Shevach. 1979. Role of nominal antigen and Ia antigen in the binding of antigen-specific T lymphocytes to macrophages. *J. Immunol.* **123:**2779.