THE ROLE OF *H-2* LINKED GENES IN HELPER T-CELL FUNCTION

IV. Importance of T-Cell Genotype and Host Environment in *I*-Region and *Ir* Gene Expression*

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Genes within the *I*-region of the major histocompatibility complex (MHC)[1] have been shown to control the activity of helper T cells. In the mouse this control has been demonstrated in a number of ways. *H-2*-linked immune response (*Ir*) genes have been shown to determine the activity of helper T cells in the response to specific antigens (1–7). Interactions between helper T cells and antigen-presenting macrophages (Mϕ) or B cells have been shown to be restricted by *I*-region genes even in response to antigens for which *Ir* genes are not readily demonstrable (8–13). Finally, helper factors of various sorts have been shown to bear *I*-region associated antigenic determinants (14–16). Immunologists have been attempting over the past several years to consolidate these different *I*-region controlled phenomena under a single underlying mechanism of action of *I*-region gene products (7).

One approach to this problem has been to study the activity of helper T cells taken from F_1 mice whose parents differ at *H-2*, since these T cells could then be tested for activity using various combinations of other cell types of parental *H-2* type without fear of complicating allogeneic effects. For instance, we have used this approach to study the cellular expression of *Ir* genes which control the response to poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys [(T,G)-A--L] (5). The activity of (T,G)-A--L primed helper T cells taken from high × low responder F_1 mice was manifest only in cultures containing both B cells and Mϕ of high responder origin, thus, demonstrating the expression of *Ir* genes in both B cells and Mϕ. Similar experiments from this (4, 10, 11, 17) and other (3, 13, 18–20) laboratories using F_1 T cells and a variety of antigens, have also supported the idea that *I*-region genes expressed in either B cells or Mϕ are important in antigen recognition by helper T cells.

Such experiments have led to the conclusion that during their initial interaction with antigen helper T cells are selectively primed on the basis of their specificity for both antigen and products of *I*-region genes (including *Ir* genes) present on the surface of the antigen-presenting cell. Subsequent recognition of the antigen on the surface of a B cell or Mϕ by the helper T cell

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[1] *Abbreviations used in this paper:* B6AF$_1$, (C57BL/6 × A/J)F$_1$, B10, C57BL/10 Sn; BSS, balanced salt solution; C, complement; CFA; complete Freund's adjuvant; FCS, fetal calf serum; HRBC, horse erythrocytes; *Ir*, immune response; KLH, keyhole limpet hemocyanin; LPS, *Escherichia coli* lipopolysaccharide; Mϕ, macrophage; PFC, plaque-forming cells; SRBC, sheep erythrocytes; (T,G)-A--L, poly-(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys; TNP, trinitrophenyl(ated).

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requires the simultaneous recognition of this *I*-region product present on the B cell or Mϕ.

Deciding whether this mechanism can account for all *I*-region controlled phenomena has been a more difficult problem. A number of laboratories have proposed in addition the expression of *I*-region genes in the helper T cell itself (3, 8, 14–16, 21–23) or in the host environment (24–26) during helper T-cell development. Questions of this sort are not easily addressed using T cells from F_1 animals; however, several laboratories have shown that they can be studied using T cells from either tetraparental (21, 27) or irradiated, bone marrow reconstituted mice (25, 26, 28).

We report here on the properties of helper T cells primed in irradiated, bone marrow reconstituted mice constructed from various combinations of F_1 and parental bone marrow and host. Our results support the conclusion that *I*-region genes including *Ir* genes expressed in the host during T-cell development determine the subsequent activity of these T cells with B cells and Mϕ. Under the conditions of our experiments these genes were not expressed in the helper T cell itself. Our results further emphasize the similarities between *I*-region and *Ir* gene controlled phenomena.

Materials and Methods

Mice. C57BL/10Sn (B10), B10.A, B10.A(5R), and (C57BL/6 × A/J)F_1 ($B6AF_1$) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. B10.A(4R) mice were bred in our own facility from breeding pairs kindly provided by Dr. Chella David, Mayo Medical School, Rochester, Minn.

Preparation of Irradiated, Bone Marrow Reconstituted Mice. Both recipients and donors of bone marrow were given 0.04 ml of anti-thymocyte serum (Microbiological Associates, Walkersville, Md.) i.p. two days previously to deplete them of recirculating T cells (29). Single cell suspensions of bone marrow were prepared from the tibias and femurs of donors. After receiving 800–900 rads from a ^{60}Co source, recipients were given 2×10^7 bone marrow cells i.v. in 0.2 ml of balanced salt solution (BSS). When recipients received bone marrow from two different donors, 1×10^7 cells were given from each donor. Recipients were given 400 μg of gentamicin sulfate (Schering Pharmaceutical Corp., Kenilworth, N. J.) i.p. on the day before, day of, and day after irradiation. For 3 wk after irradiation drinking water was supplemented with 400 mg/liter tetracycline (American Cyanamic Co., Princeton, N. J.). Greater than 80% of the animals survived 8 wk or longer under these conditions. Animals were not used in experiments until at least 7 wk after irradiation and reconstitution. In referring to a particular chimera an arrow (→) is used to designate the relation between bone marrow donor and irradiated host. For example, $B6AF_1 \rightarrow$ B10.A indicates an irradiated B10.A host reconstituted with $B6AF_1$ bone marrow, and B10 → $B6AF_1$ ← B10.A indicates an irradiated $B6AF_1$ host reconstituted with a mixture of B10 and B10.A bone marrow.

Antigens. Keyhole limpet hemocyanin (KLH) was purchased from Calbiochem, La Jolla, Calif. Poly-L-(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys (T,G)-A--L (batch numbers MC6 and MC8) was purchased from Miles Laboratories, Inc., Miles Research Products, Elkhart, Ind. *Escherichia coli* lipopolysaccharide (LPS) was purchased from Difco Laboratories, Detroit, Mich. Sheep erythrocyte (SRBC) from a single animal were obtained from Bellwhether Farms, Palmyra, N. Y. Horse RBC (HRBC) from a single animal were purchased from the Colorado Serum Co., Denver, Colo. A trinitrophenylated (TNP) form of each of these antigens was prepared as previously described (30, 31).

Immunizations. Immunizations for the priming of helper T cells were as follows: mice were given 50 μg of KLH i.p. emulsified in 0.2 ml of complete Freund's adjuvant (CFA). 7 days later their spleen cells were used as a source of KLH-specific helper cells (32). Mice were given 4×10^5 SRBC i.v. in 0.2 ml of BSS (33). 4 days later their spleen cells were used as a source of SRBC-specific helper cells. Mice were given 100 μg of (T,G)-A--L subcutaneously at the base of the tail emulsified in 40 μl of CFA. 7 days later draining lymph nodes were used as a source of (T,G)-A--L-specific helper T cells (5).

Mice were immunized i.p. with 1 μg TNP-LPS in 0.2 ml of BSS. 7 days later their spleen cells were used as a source of TNP-primed B cells (32).

For in vitro immunizations each milliliter of culture medium was supplemented with either 0.1 μg TNP-KLH, 2×10^5 TNP-SRBC, or 2×10^5 peritoneal cells carrying approximately 30 ng of surface bound TNP-(T,G)-A--L.

Preparation of T Cells, B Cells, and Macrophages (Mϕ). T cells were isolated from either spleen or lymph node suspensions using nylon fiber columns as previously described (4, 34). TNP-primed spleen cells were depleted of T cells using anti-T-cell serum and complement (C) as previously described (32). These preparations contained both functional B cells and Mϕ. Peritoneal washings from normal mice served as a source of antigen presenting Mϕ. These cells were pulsed with TNP-(T,G)-A--L as previously described (5). In all cases these peritoneal cells were syngeneic to the B cell and Mϕ preparation with which they were tested.

Culture Conditions. Cells were cultured by the methods of Mishell and Dutton (35) with some modifications (4) in Linbro FB16-24TC culture trays in 0.5 ml of culture medium. Each culture well contained 3×10^6 T-cell depleted TNP-primed spleen cells, a fixed concentration of TNP-(T,G)-A--L-pulsed peritoneal cells, TNP-KLH or TNP-SRBC as antigen and a varying number of carrier-primed T cells.

Plaque-Forming Cell (PFC) Assay. After 4 days identical culture wells were pooled and assayed for PFC using the slide modification of the hemolytic plaque assay (35). Parallel determinations were made with TNP-HRBC and HRBC and the difference calculated as the number of anti-TNP-specific PFC (30, 36). This correction was always very small.

Quantitation of Helper Activity. Carrier-specific helper activity was quantitated as previously described (4, 5, 11, 17, 29, 32, 37). Briefly, the number of anti-TNP PFC observed per culture was plotted versus the number of carrier-primed T cells added. A straight line was fit to the initial linear portion of this titration and the slope of this line taken as a relative measure of the activity of the T-cell preparation. This slope and its standard error are reported in units of anti-TNP PFC/culture/10^6 T cells ± the standard error (SE). When T cells were treated with antisera the T-cell activities are reported based on the original number of T cells before treatment.

Preparation of and Treatment with Anti-H-2-Antisera. B10.A anti-B10 (anti-$H\text{-}2^b$) and B10 anti-B10.A (anti-$H\text{-}2^a$) sera were prepared by skin grafting followed by biweekly i.p. injections of 1.5×10^7 spleen cells after the rejection of the grafts. Mice were bled on alternate weeks after the third immunization. In the experiments reported here nylon purified T cells were treated at a concentration of 10^7/ml with a 1:5 dilution of antisera for 30 min at 0°C. The cells were then centrifuged, washed once with BSS, and then resuspended to the original volume in 1:15 rabbit complement (Grand Island Biological Co., Grand Island, N. Y.) using a batch of complement which had been prescreened for low cytotoxicity on murine lymphocytes. After 30 min at 37°C the T cells were centrifuged, washed once with BSS, resuspended in culture medium, and tested for helper activity. The specificity and titer of the batches of antisera used in these studies were established in control experiments. Under these conditions the anti-$H\text{-}2^a$ sera eliminated approximately 90% of the activity of B10.A or $B6AF_1$ helper T cells with little effect on B10. Likewise, the anti-$H\text{-}2^b$ sera eliminated approximately 90% of the activity of B10 and $B6AF_1$ helper T cells with little effect on B10.A.

Results

Experiments with F_1 Mice Reconstituted with Bone Marrow of Parental H-2 Type. We wished to determine whether the inability of helper T cells to cooperate with B cells and Mϕ of a different *H-2* type was a property inherent in the genotype of the T cell. Likewise, we wished to determine whether the ability of T cells to induce high responses to TNP-(T,G)-A--L was controlled by the genotype of the helper T cell. We therefore constructed chimeras consisting of irradiated $B6AF_1$ mice [high × low responder to (T,G)-A--L], reconstituted with equal numbers of bone marrow cells from B10 mice [high responder to (T,G)-A--L] and B10.A mice [low responder to (T,G)-A--L]. These chimeras were then immunized with KLH, SRBC, or (T,G)-A--L.

KLH or SRBC primed splenic T cells from the chimeras were treated with C alone, anti-$H\text{-}2^b$ serum plus C to isolate B10.A cells, anti-$H\text{-}2^a$ serum plus C to isolate B10

TABLE I

Helper T Cells Primed in B10 → B6AF$_1$ ← B10.A Chimeric Mice

Exp.	T-cell source	Priming antigen	Treatment of T cells	In vitro antigen	Helper activity ± SE (anti-TNP PFC/culture/10^6 T cells) tested on	
					B10 B cells + Mϕ	B10.A B cells + Mϕ
I	B10 → B6AF$_1$ ← B10.A*	KLH	C′ only	TNP-KLH	692 ± 117	862 ± 157
	"	"	Anti-H-2^b + C′	"	638 ± 158	532 ± 134
	"	"	Anti-H-2^a + C′	"	372 ± 68	303 ± 95
	"	"	Anti-H-2^b + anti-H-2^a + C′	"	59 ± 17	93 ± 27
	B10 → B6AF$_1$ ← B10.A‡	SRBC	C′ only	TNP-SRBC	749 ± 53	656 ± 110
	"	"	Anti-H-2^b + C′	"	210 ± 23	245 ± 25
	"	"	Anti-H-2^a + C′	"	268 ± 50	129 ± 13
	"	"	Anti-H-2^b + anti-H-2^a + C′	"	0 ± 3	14 ± 4
II	B10 → B6AF$_1$ ← B10.A‡	(T,G)-A--L	Anti-H-2^b + C′	TNP-(T,G)-A--L	545 ± 104	ND
	normal B6AF$_1$§	"	C′ only	"	252 ± 36	ND
	"	"	Anti-H-2^b + C′	"	4 ± 1	ND

* Pooled from two mice.

‡ Pooled from five mice.

§ Pooled from nine mice.

T cells or both antisera plus C to insure that such a double treatment eliminated all T-cell activity. The various preparations were then tested for helper activity using B10 and B10.A B cells and Mϕ. The results are shown in Table I. KLH or SRBC primed T cells from these chimeras treated with C alone had equivalent activity when tested with either B10 or B10.A B cells and Mϕ. Treatment with either anti-*H-2^b* or anti-*H-2^a* serum plus C eliminated part of this activity; however, the residual B10 or B10.A T cells also did not discriminate between B10 and B10.A B cells and Mϕ. Finally, treatment with both antisera plus C eliminated virtually all helper activity confirming that the helper T-cell population in these animals was indeed chimeric.

These results confirm those previously reported by von Boehmer and Sprent (28) and Waldmann et al. (27) demonstrating that the ability of helper T cells to cooperate with B cells and Mϕ is not necessarily a function of the T cell genotype.

Similarly, (T,G)-A--L primed lymph node T cells from these chimeric animals were treated with anti-*H-2^b* serum plus C to isolate T cells carrying the (low responder) *H-2^a* genotype. When these T cells were then tested for helper activity with B cells and Mϕ from high responder B10 mice a high anti-TNP-(T,G)-A--L response was seen (Table I). Thus when they had developed in F$_1$ hosts the *Ir* genotype of (T,G)-A--L specific helper T cells did not determine the *Ir* phenotype observed.

In confirming these results we prepared chimeras consisting of irradiated B6AF$_1$ mice, reconstituted with B10.A bone marrow only. To avoid complications due to the possible lack of *H-2^b* antigen-presenting Mϕ in these chimeras they were given 10^8 T-cell-depleted B6AF$_1$ spleen cells as a source of *H-2^b* bearing Mϕ at the time of priming with (T,G,)-A--L. Primed lymph node T cells from these mice were treated as above with anti-*H-2^b* serum plus C to eliminate the possibility of contaminating B6AF$_1$ T cells. The resulting B10.A T cells were tested for helper activity using both B10 and B10.A B cells and Mϕ. The results of two experiments are shown in Table II. Confirming the results shown in Table I, B10.A T cells from these chimeras induced

TABLE II

Helper T Cells Primed to (T,G)-A--L in B10.A → B6AF$_1$ Chimeric Mice

Exp.	Source of (T,G)-A--L primed T cells	Treatment of T cells	In vitro Ag	Helper activity ± SE (anti-TNP PFC/culture/10^6 T cells) tested on	
				B10 B cells ± Mϕ	B10.A B cells ± Mϕ
I	B10.A → B6AF$_1$*‡	Anti-H-2^b + C′	TNP-(T,G)-A--L	1,432 ± 208	ND
	Normal B6AF$_1$§	C′ only	"	252 ± 36	ND
	"	Anti-H-2^b + C′	"	4 ± 1	ND
II	B10.A → B6AF$_1$*‖	Anti-H-2^b + C′	TNP-(T,G)-A--L	2,254 ± 215	12 ± 12
	Normal B6AF$_1$§	C′ only	"	896 ± 159	0 ± 3
	"	Anti-H-2^b + C′	"	200 ± 16	30 ± 8

* Mice given 1 × 10^8 anti-T serum + C′ treated B6AF$_1$ spleen cells at the time of priming with (T,G)-A--L.

‡ Pooled from five mice.

§ Pooled from nine mice.

‖ Pooled from four mice.

high response when tested with high responder B10 B cells and Mϕ. Their activity, however, was still under *Ir* gene control as shown by the low response seen when they were tested with low responder B10.A B cells and Mϕ.

Taken together these results emphasize the similarities between *I*-region and *Ir* gene control of helper T cells and indicate that under the conditions of these experiments this control is not determined by the genotype of the helper T cells.

We should emphasize that *H-2^a* is the only low responder haplotype which we have examined thus far in these chimeras. It is possible that other (T,G)-A--L low responder haplotypes might yield different results.

Experiments with Irradiated Mice of Parental H-2 Type Reconstituted with F$_1$ Bone Marrow. The results reported in the previous section argued against the control of helper T cells via the expression of *I*-region genes in these cells themselves and were consistent with previous work demonstrating the expression of these genes in B cells and Mϕ. However, they did not address the question of whether these genes were expressed in the non-bone marrow-derived portion of the host during T-cell differentiation, since the F$_1$ chimera host possessed and potentially expressed both sets of parental *I*-region genes.

Recently Zinkernagel et al. (25) and Sprent (26) have studied this question using chimeras consisting of irradiated, parental hosts reconstituted with F$_1$ bone marrow. In such chimeras T cells possess and are tolerant to both sets of parental H-2 gene products and both sets of H-2 gene products are expressed on the Mϕ of the chimeras, but T cells have differentiated in a host possessing only one set of *H-2* gene products.

We prepared chimeras of this sort consisting of irradiated B10 or B10.A hosts reconstituted with B6AF$_1$ bone marrow. They were primed with either KLH or (T,G)-A--L and their T cells tested for helper activity with B cells and Mϕ of various *H-2* types.

KLH-primed T cells were tested with B cells and Mϕ from B10, B10.A, B10.A(4R), B10.A(5R), and B6AF$_1$ mice (Table III). When the chimera host was B10, these T cells had high activity with B10, B10.A(5R), and B6AF$_1$ B cells and Mϕ, but low

TABLE III

Helper T Cells Primed to KLH in $B6AF_1 \rightarrow B10$ and $B6AF_1 \rightarrow B10.A$ Chimeric Mice

B cells + Mϕ		KLH-primed T cells*			
Source	H-2 subregions I / KABJECSGD	Chimera source	Treatment	H-2 subregions shared between chimera host and B cells + Mϕ	Helper activity ± SE (anti-TNP PFC/culture/10^6 T cells)
B10	bbbbbbbbb	$B6AF_1 \rightarrow B10$	C′ only	All	3,209 ± 205
		$B6AF_1 \rightarrow B10.A$	"	None	61 ± 10
B10.A	kkkkkdddd	$B6AF_1 \rightarrow B10$	C′ only	None	221 ± 50
		$B6AF_1 \rightarrow B10.A$	"	All	2,069 ± 50
B10.A(4R)	kkbbbbbbb	$B6AF_1 \rightarrow B10$	C′ only	IB-D	43 ± 10
		$B6AF_1 \rightarrow B10.A$	"	K-IA	731 ± 99
B10.A(5R)	bbbkkdddd	$B6AF_1 \rightarrow B10$	C′ only	K-IB	1,268 ± 84
		$B6AF_1 \rightarrow B10.A$	"	IJ-D	73 ± 21
$B6AF_1$	bbbbbbbbb / kkkkkdddd	$B6AF_1 \rightarrow B10$	C′ only	All	1,580 ± 221
			Anti-H-2^a + C′		197 ± 30
		$B6AF_1 \rightarrow B10.A$	C′ only	All	1,625 ± 84
			Anti-H-2^b + C′		332 ± 76

* T Cells pooled from three mice.

activity with B10.A and B10.A(4R) B cells and Mϕ. When the chimera host was B10.A, high activity was seen with B10.A, B10.(4R), and $B6AF_1$ B cells and Mϕ, but low activity with B10 and B10.A(5R) B cells and Mϕ. Treatment with the appropriate anti-*H-2* serum and complement demonstrated the donor origin of the majority of the helper T cells in these chimeras.

These results demonstrate that tolerance to and priming in the presence of Mϕ bearing a particular set of *H-2* gene products are not sufficient conditions for the production of helper T cells capable of interacting with B cells and Mϕ of that *H-2* type. Also required is the expression of the relevant *H-2* gene products in the non-bone marrow derived portion of the host in which the helper T cells differentiate. The results obtained with B10.A(4R) and B10.A(5R) B cells and Mϕ indicate that the important genes are located at the K-IA end of the *H-2* complex. Similar results were obtained using SRBC as the priming antigen and carrier for TNP (data not shown).

One possible alternate explanation for these results might have been that F_1 T cells in the chimeras only appeared to be restricted by the host genotype because of their incomplete tolerance to the *H-2* antigens of the other parent. Thus, alloagressive T cells directed at these antigens might have suppressed the response of B cells and Mϕ carrying them (38). We feel that this possibility is highly unlikely for two reasons. First, when tested directly in mixed lymphocyte culture, T cells from these chimeras gave no response against either parental *H-2* antigens, but a normal response to third party *H-2* antigens (results not shown). Second, T cells from the chimeras had high activity with $B6AF_1$ B cells and Mϕ, which, of course, displayed a full set of both parental *H-2* antigens as potential targets for alloaggressive T cells (Table III).

TABLE IV

Helper T Cells Primed to (T,G)-A--L in B6AF$_1$ → B10 and B6AF$_1$ → B10.A Chimeric Mice

B cell + Mϕ		(T,G)-A--L primed T cells*		Helper activity ± SE (anti-TNP PFC/culture/10^6 T cells)
Source	Ir-responder type	Chimera source	Ir-responder type of chimera host	
B10	High	B6AF$_1$ → B10	High	864 ± 39
		B6AF$_1$ → B10.A	Low	15 ± 2
		B6AF$_1$ → B10 + B6AF$_1$ → B10.A (1:1)		547 ± 7
B10.A	Low	B6AF$_1$ → B10	High	1 ± 0
		B6AF$_1$ → B10.A	Low	36 ± 6
B6AF$_1$	High	B6AF$_1$ → B10	High	383 ± 113
		B6AF$_1$ → B10.A	Low	38 ± 6

* B6AF$_1$ → B10 T cells pooled from seven mice; B6AF$_1$ → B10.A T cells pooled from five mice.

A similar set of experiments was performed to test for the expression of *Ir* genes in the host environment during helper T-cell differentiation. (T,G)-A--L primed T cells from the chimeras were tested for activity with B10, B10.A and B6AF$_1$ B cells and Mϕ. The results of a representative experiment are shown in Table IV. When the chimera host was B10 (high responder) the results seen were similar to those we have previously reported for T cells from normal F$_1$ animals. High activity was seen with either B10 or B6AF$_1$ cells and Mϕ, but low activity with B10.A B cells and Mϕ. Similar results were obtained in a second experiment. Thus, F$_1$ T cells differentiating in a high responder host behaved as normal high responder F$_1$ T cells. The results obtained when the chimera host was B10.A (low responder) were strikingly different. In this case low activity was seen regardless of the origin of the B cell and Mϕ preparation. Similar results were obtained in three other experiments. Thus, despite their high responder genotype, F$_1$ T cells differentiating in a low responder host displayed the phenotype of the host even when tested with high responder B cells and Mϕ.

For the same reasons listed above, active suppression would seem an unlikely explanation for this result. However, as an additional test of this possibility, (T,G)-A--L-primed T cells from both types of chimeras were mixed 1:1 and tested on B10 B cells and Mϕ. The activity seen was no less than the average activity of the two T-cell preparations tested alone. Therefore, the failure of F$_1$ T cells which developed in a low responder host to cooperate with high responder B cells and Mϕ did not appear to be due to their suppression of the response.

All of our results taken together are consistent with the hypothesis that helper T cells simultaneously recognize antigen and products of *I*-region genes expressed in antigen presenting Mϕ and B cells. However, they indicate that this theory must be extended to accommodate a role for *I*-region genes expressed in the host during T-cell differentiation in determining the capacity of T cells to perform this dual recognition.

Discussion

In previous studies we (4, 5, 10, 11, 17) and others (6, 7) have presented evidence establishing that *I*-region genes (including *Ir* genes) which control helper T-cell

function are expressed in B cells and/or Mϕ during both the induction and effector phases of helper T cells. These results have supported the idea that the products of these genes are involved in the recognition of cell bound antigen by helper T cells, and that in many cases *I*-region and *Ir* gene phenomena can be considered manifestations of a single underlying mechanism.

The present study was designed to ask whether *I*-region genes function via their expression in other cell types as well, in particular in the helper T cell itself or in the non-bone-marrow-derived portion of the host in which helper T cells differentiate.

To address these questions we adapted the methods of others (25, 26, 28) and constructed irradiated, bone marrow reconstituted chimeric mice using various combinations of parent and F_1 mice as hosts and bone marrow donors. The parental *H-2* haplotypes were selected such that one, $H\text{-}2^a$, determined low responsiveness to (T,G)-A--L and the other, $H\text{-}2^b$, determined high responsiveness.

Our results lead us to several conclusions. First of all they confirm our previous findings (4, 5, 11, 17) that in the presence of appropriately primed T cells, it is the *I*-region or *Ir* type of B cells and Mϕ which determine the degree of response.

Secondly, our results show that in both parent → F_1 and in F_1 → parent chimeras, the phenotype of helper T cells specific for KLH, SRBC, or (T,G)-A--L is determined by the *I*-region genotype of the chimera host and not by the *I*-region genotype of the T cells themselves. Thus, KLH or SRBC-specific parental T cells produced in F_1 hosts cooperated equally well with B cells and Mϕ of either parental *H-2* type. However, KLH or SRBC-specific F_1 T cells produced in parental hosts cooperated only with B cells and Mϕ which shared the *K-IA* type of that parental host. Likewise, (T,G)-A--L specific parental T cells of low responder genotype produced in an F_1 host induced high anti-TNP-(T,G)-A--L responses in B cells and Mϕ of high responder type. Finally, F_1 T cells raised in high responder but not low responder hosts induced high responses in B cells and Mϕ of high responder type.

The finding that low responder T cells could be primed efficiently with (T,G)-A--L after developing in F_1 hosts was somewhat unexpected. The work of Feldmann et al. and Erb et al. (39, 40) has suggested that the induction of helper T cells requires their interaction with amplifier T cells analogous to T-cell/B-cell interactions and to T-cell/T-cell interactions in the induction of cytotoxic T cells. Therefore, by analogy, one might expect this interaction to be under *Ir* gene control such that the helper T cell must be of high responder genotype for antigen on its surface to be recognized by amplifier T cells. If such *Ir* gene controlled interactions exist, then our results show either that they are bypassed under our priming conditions (in vivo in CFA) or that the *Ir* gene controlling this T-cell/T-cell interaction is different than that controlling T-cell interactions with B cells and Mϕ. Perhaps future experiments utilizing chimeras having low responder haplotypes other than $H\text{-}2^a$ will shed some light on these possibilities.

Our results are consistent with several previous reports on the properties of T cells raised in chimeric mice in which the authors concluded that *I*-region restrictions on T-cell/B-cell interactions were not inherent in the genotype of the helper T cell (27, 28). The present study confirms this conclusion and extends it to cover an *Ir* gene controlled response.

The literature is somewhat cloudier on the question of whether the host environment during T-cell differentiation determines the *I*-region and *Ir* phenotype of helper T

cells. Such a possibility was originally proposed by Katz et al. (24, 41) based on their consistent failure to see cooperation between allogeneic T cells and B cells under a wide variety of conditions. Direct evidence for this possibility was obtained in an elegant series of experiments by Zinkernagel et al. (25, 42) in which the induction of cytotoxic T cells to vaccinia viral antigens was studied in irradiation chimeras. They found that the specificity of these T cells as well as the helper T cells involved in their induction was limited by the *K-IA* type of the host radioresistent thymic epithelium. Recently, Sprent (26) has reported similar findings for helper T cells specific for SRBC. Our results confirm these reports and demonstrate in addition that *Ir* genes expressed in the host environment determine the subsequent activity of helper T cells.

The consistency of these results notwithstanding there are reports in the literature which apparently exclude the host environment as a site of *I*-region or *Ir* gene expression. For example, Tyan et al. and Cheseboro et al. (43, 44) studying the in vivo IgG response to (T,G)-A--L, have reported a number of examples in which high responder fetal liver was used to reconstitute irradiated low responder hosts resulting in chimeras a portion of which produced high levels of anti-(T,G)-A--L antibody of high responder origin. In addition, there are a number of reports of successful interactions between T cells and either B cells or Mϕ taken from completely allogeneic animals (12, 38, 45, 46). In some of these studies the allogeneic interactions were revealed only after some manipulation to eliminate alloreactive T cells. This has led some investigators to conclude that the main obstacle to allogeneic T-cell/B-cell interaction is alloreactive suppressor T cells (38). Clearly these results are at odds with ours and with those of others mentioned above.

We are unable to offer an explanation which resolves all of these conflicting results. It may turn out that technical differences among the experiments explain some of the differences. One possibility which is admittedly difficult to assess in hindsight is that in some of these studies undetected residual alloreactive T cells bypassed normal *I*-region/*Ir* gene control via an enhancing allogeneic effect. This possibility is given some credence by the previous demonstration that in some cases low responder animals can convert to high responders in the presence of an allogeneic effect (47), and our recent finding that nonspecific helper factors similar to those produced as a result of allorecognition can bypass the requirement for high responder B cells in the anti-TNP-(T,G)-A--L response.[2] Another point is that some of these contradictory experiments were performed in vivo, where unforeseen limitations in the activities of cell types other than helper T cells may have masked underlying *Ir* gene or *I*-region effects.

All in all, the bulk of the evidence from this and other laboratories supports the idea that *I*-region genes including *Ir* genes function in cells which must present antigen to helper T cells. Their products operate in such a fashion that helper T cells have apparent specificity for both antigen and these gene products both during their initial induction and subsequent effector phase. A number of mechanisms have to be suggested to explain this apparent dual specificity (6, 7, 24, 25, 41, 42, 48–52) at a molecular level. These models propose various permutations of single versus dual T-cell receptors and positive versus negative selection of T-cell specificities in the thymus. The experiments reported here and previously by Zinkernagel et al. (25, 42) and

[2] P. Marrack et al. Evidence for two types of *Ir* gene restricted helper T cells. Manuscript in preparation.

Sprent (26) point out that each of these proposals is now constrained by the expression in the apparent absence of antigen of *I*-region genes including *Ir* genes in the host during the differentiation of helper T cells.

Summary

We have studied the properties of helper T cells specific for sheep erythrocytes (SRBC), keyhole limpet hemocyanin (KLH), or poly-L-(Tyr,Glu)-poly-DL-Ala--poly-L-Lys [(T,G)-A--L]. These T cells differentiated and were primed in vivo in irradiation chimeras constructed of various combinations of F_1 and parental bone marrow donors and irradiated recipients. Primed T cells were then tested for helper activity in the in vitro response of B cells and macrophages (Mϕ) of parental or F_1 origin to the hapten trinitrophenol coupled to the priming antigen.

When testing either SRBC or KLH-specific T cells of parental *H-2* type which had differentiated in F_1 hosts, we found that they cooperated equally well with B cells and Mϕ of either parental *H-2* type. On the other hand, when testing F_1 T cells which had differentiated in parental hosts, we found that they cooperated well only with B cells and Mϕ which had the *K-IA* region type of the parental host.

In similar experiments we found that (T,G)-A--L-specific T cells of low responder *H-2* type which had differentiated in (high responder × low responder) F_1 hosts induced high responses in high responder B cells and Mϕ. (T,G)-A--L-specific F_1 T cells which differentiated in high responder but not those which differentiated in low responder hosts induced high responses in high responder B cells and Mϕ. Low responder B cells and Mϕ yielded low responses in all cases regardless of the source of (T,G)-A--L-specific T cells with what they were tested.

Our results support the conclusion that *I*-region and *Ir* genes function via their expression in B cells and Mϕ and in the host environment during helper T-cell differentiation, but not, at least under the conditions of these experiments, via their expression in the helper T cell itself. These findings place constraints upon models which attempt to explain the apparent dual recognition of antigen and *I*-region gene products by helper T cells.

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