Genes within the I-region of the major histocompatibility complex (MHC) 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 F₁ 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 \([\text{T,G}-\text{A--L}]\) (5). The activity of \((\text{T,G})\)-A--L primed helper T cells taken from high × low responder F₁ 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 F₁ 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
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 F1 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 F1 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)F1 (B6AF1) 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 60Co 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, B6AF1 → B10.A indicates an irradiated B10.A host reconstituted with B6AF1 bone marrow, and B10 → B6AF1; B10.A indicates an irradiated B6AF1 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 Bellwether 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 × 10^6 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).
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For in vitro immunizations each milliliter of culture medium was supplemented with either 0.1 \( \mu \)g TNP-KLH, \( 2 \times 10^5 \) TNP-SRBC, or \( 2 \times 10^5 \) 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 \( 3 \times 10^8 \) 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-\( H-2^b \)) and B10 anti-B10.A (anti-\( H-2^a \)) sera were prepared by skin grafting followed by biweekly i.p. injections of 1.5 \( \times 10^7 \) 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-\( H-2^a \) sera eliminated approximately 90% of the activity of B10.A or B6AF1, helper T cells with little effect on B10. Likewise, the anti-\( H-2^b \) sera eliminated approximately 90% of the activity of B10 and B6AF1, helper T cells with little effect on B10.A.

**Results**

**Experiments with F1 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 B6AF1 mice [high \( H-2^a \)] 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-\( H-2^b \) serum plus C to isolate B10.A cells, anti-\( H-2^a \) serum plus C to isolate B10.
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° or anti-H-2′ 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° serum plus C to isolate T cells carrying the (low responder) H-2° 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 F1 hosts the Ir genotype of (T,G)-A--L specific helper T cells did not determine the Jr phenotype observed.

In confirming these results we prepared chimeras consisting of irradiated B6AF1 mice, reconstituted with B10.A bone marrow only. To avoid complications due to the possible lack of H-2° antigen-presenting Mφ in these chimeras they were given 10⁸ T-cell-depleted B6AF1 spleen cells as a source of H-2° 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° serum plus C to eliminate the possibility of contaminating B6AF1 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

| Exp. | T-cell source | Priming antigen | Treatment of T cells | In vitro antigen | Helper activity ± SE (anti- TNP PFC/culture/10⁶ T cells) tested on |
|------|--------------|----------------|---------------------|----------------|---------------------------------------------------------------|
|      |              |                |                     |                | B10 B cells + Mφ | B10.A B cells + Mφ |
| I    | B10--, B6AF1 -- B10.A* | KLH | C' only | TNP-KLH | 692 ± 117 | 862 ± 157 |
|      |              | Anti-H-2° + C' |        |        | 636 ± 130 | 532 ± 134 |
|      |              | Anti-H-2° + C' |        |        | 372 ± 68 | 303 ± 95 |
|      |              | Anti-H-2° + anti-H-2° + C' | | | 59 ± 17 | 93 ± 27 |
|      | B10--; B6AF1 -- B10.A‡ | SRBC | C' only | TNP-SRBC | 749 ± 53 | 656 ± 110 |
|      |              | Anti-H-2° + C' |        |        | 210 ± 23 | 245 ± 25 |
|      |              | Anti-H-2° + C' |        |        | 286 ± 50 | 129 ± 13 |
|      |              | Anti-H-2° + anti-H-2° + C' | | | 0 ± 3 | 14 ± 4 |
| II   | B10--; B6AF1 -- B10.A‡ | (T,G)-A--L | Anti-H-2° + C' | TNP-(T,G)-A--L | 545 ± 104 | ND |
|      | normal B6AF1 | C' only |        | C' only | 252 ± 36 | ND |
|      |              | Anti-H-2° + C' |        | | 4 ± 1 | ND |

* Pooled from two mice.
‡ Pooled from five mice.
§ Pooled from nine mice.
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TABLE II

| 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 ± Mø | B10.A B cells ± Mø |
| I    | B10.A → B6AF₁*†§                  | Anti-H-2^b + C'     | TNP-(T,G)-A--L  | 1,432 ± 208 ND  |
|      | Normal B6AF₁§                     | C' only             | "            | 252 ± 36 ND  |
|      |                                   | Anti-H-2^b + C'     | "            | 4 ± 1 ND  |
| II   | B10.A → B6AF₁*†§                  | Anti-H-2^b + C'     | TNP-(T,G)-A--L  | 2,254 ± 215 12 ± 12 |
|      | Normal B6AF₁§                     | C' only             | "            | 896 ± 159 0 ± 3  |
|      |                                   | Anti-H-2^b + C'     | "            | 200 ± 16 30 ± 8  |

* Mice given 1 × 10^6 anti-T serum + C' treated B6AF₁ 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 Mø. 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 Mø.

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^b 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 F₁ 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 Mø. 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 F₁ 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 F₁ 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 Mø 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 B6AF₁ 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 Mø of various H-2 types. KLH-primed T cells were tested with B cells and Mø from B10, B10.A, B10.A(4R), B10.A(5R), and B6AF₁ mice (Table III). When the chimera host was B10, these T cells had high activity with B10, B10.A(5R), and B6AF₁ B cells and Mø, but low
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 B6AF1 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 F1 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, alloaggressive 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 B6AF1 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).
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TABLE IV
Helper T Cells Primed to (T,G)-A--L in B6AF, → B10 and B6AF, → B10.A Chimeric Mice

| Source | Ir-responder type | Chimera source | Helper activity ± SE (anti-TNP PFC/culture/10^6 T cells) |
|--------|------------------|----------------|-----------------------------------------------------|
|        |                  | (T,G)-A--L primed T cells* |                                                     |
|        |                  |                |                                       |
| B10    | High             | B6AF, → B10    | High 864 ± 39                                    |
|        |                  | B6AF, → B10.A  | Low 15 ± 2                                      |
|        |                  | B6AF, → B10 + B6AF, → B10.A | 347 ± 7                                      |
|        |                  |                | (1:1)                                             |
| B10.A  | Low              | B6AF, → B10    | High 1 ± 0                                       |
|        |                  | B6AF, → B10.A  | Low 36 ± 6                                       |
| B6AF,  | High             | B6AF, → B10    | High 385 ± 113                                   |
|        |                  | B6AF, → B10.A  | Low 38 ± 6                                       |

* B6AF, → B10 T cells pooled from seven mice; B6AF, → 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, 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 F1 animals. High activity was seen with either B10 or B6AF, cells and Mφ, but low activity with B10.A B cells and Mφ. Similar results were obtained in a second experiment. Thus, F1 T cells differentiating in a high responder host behaved as normal high responder F1 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, F1 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 F1 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 F1 mice as hosts and bone marrow donors. The parental H-2 haplotypes were selected such that one, H-2\(^a\), determined low responsiveness to (T,G)-A\(^--\)L and the other, H-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 \(\rightarrow\) F1 and in F1 \(\rightarrow\) 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 F1 hosts cooperated equally well with B cells and Mφ of either parental H-2 type. However, KLH or SRBC-specific F1 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 F1 host induced high anti-TNP-(T,G)-A\(^--\)L responses in B cells and Mφ of high responder type. Finally, F1 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 F1 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-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 radioresistant 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. 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 F1 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 F1 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 F1 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 F1 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) F1 hosts induced high responses in high responder B cells and Mφ. (T,G)-A--L-specific F1 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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References

1. Green, I., W. E. Paul, and B. Benacerraf. 1966. The behavior of hapten-poly-L-Lysine conjugates as complete antigens in genetic responder and as haptens in nonresponder guinea pigs. J. Exp. Med. 123:859.
2. McDevitt, H. O. 1968. Genetic control of the antibody response. III. Quantitative and qualitative characteristics of the antibody response to (T,G)-A--L in CBA and C57 mice. J. Immunol. 100:485.
3. Katz, D. H., T. Hamaoka, M. D. Dorf, P. H. Maurer, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. IV. Involvement of the...
immune response (I) gene in the control of lymphocyte interactions in responses controlled by the gene. J. Exp. Med. 138:734.

4. Kappler, J. W., and P. C. Marrack. 1977. The role of H-2 linked genes in helper T cell function. I. In vitro expression in B cells of immune response genes controlling helper T activity. J. Exp. Med. 145:1748.

5. Marrack, P., and J. W. Kappler. 1978. The role of H-2-linked genes in helper T cell function. III. Expression of immune response genes for trinitrophenyl conjugates of poly-L-(Tyr, Glu)-poly-D,L-Ala-poly-L-Lys in B cells and macrophages. J. Exp. Med. 147:1596.

6. Benacerraf, B., and D. H. Katz. 1975. The histocompatibility-linked immune response genes. Adv. Cancer Res. 21:121.

7. Paul, W. E., and B. Benacerraf. 1977. Functional specificity of thymus-dependent lymphocytes. Science (Wash. D. C.). 195:1293.

8. Katz, D. H., T. Hamaoka, M. E. Dorf, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. III. Demonstration that the H-2 gene complex determines successful physiologic lymphocyte interactions. Proc. Natl. Acad. Sci. U. S. A. 70:2624.

9. Erb, P., and M. Feldmann. 1975. The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage-T cell interaction for helper cell induction with soluble antigens. J. Exp. Med. 142:460.

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

11. Swierkosz, J. E., K. Rock, P. Marrack, and J. W. Kappler. 1978. The role of H-2 linked genes in helper T cell function. II. Isolation on antigen-pulsed macrophages of two separate populations of F1 helper T cells each specific for antigen and one set of parental H-2 products. J. Exp. Med. 147:554.

12. Pierce, C. W., J. A. Kapp, and B. Benacerraf. 1976. Regulation by the H-2 gene complex of macrophage-lymphoid cell interactions in secondary antibody responses in vitro. J. Exp. Med. 144:371.

13. Sprent, J. 1978. Restricted helper function of F1 hybrid T cells positively selected to heterologous erythrocytes in irradiated parental strain mice. II. Evidence for restrictions affecting helper cell induction and T-B collaboration, both mapping to the K-end of the H-2 complex. J. Exp. Med. 147:1159.

14. Taussig, M. J., A. J. Munro, R. Campbell, C. S. David, and N. A. Staines. 1975. Antigen-specific T-cell factor in cell cooperation. Mapping within the F-region of the H-2 complex and ability to cooperate across allogeneic barriers. J. Exp. Med. 142:694.

15. Howie, S., and M. Feldmann. 1977. In vitro studies of H-2-linked unresponsiveness to synthetic polypeptides. II. Production of an antigen-specific T helper cell factor to (T,G)-A--L. Eur. J. Immunol. 7:417.

16. McDougal, J., and D. S. Gordon. 1977. Generation of T-helper cells in vitro. II. Analysis of supernates derived from T-helper cell cultures. J. Exp. Med. 145:693.

17. Kappler, J. W., and P. Marrack. 1977. Simultaneous recognition of carrier antigens and products of the H-2 complex by helper cells. In Immune System: Genetics and Regulation. ICN-UCLA Symposia on Molecular and Cellular Biology. VI:439.

18. Pierce, C. W., R. N. Germain, J. A. Kapp, and B. Benacerraf. 1977. Secondary antibody responses in vitro to L-glytamic acid²⁰-L-alanine³⁰-L-tyrosine¹⁰ (GAT) by (responder × nonresponder) F1 spleen cells stimulated by parental GAT macrophages. J. Exp. Med. 146:1827.

19. McDougal, J. S., and S. P. Cort. 1978. Generation of T helper cells in vitro. IV. F1 T helper cells primed with antigen-pulsed parental macrophages and genetically restricted in their antigen-specific helper activity. J. Immunol. 120:445.

20. Singer, A., C. Cowing, K. S. Hathcock, H. Dickler, and R. Hodes. 1978. Cellular and genetic control of antibody responses in vitro. III. Immune response gene regulation of
accessory cell function. J. Exp. Med. 147:1611.
21. Bechtol, K. B., J. H. Freed, L. A. Herzenberg, and H. O. McDevitt. 1974. Genetic control of the antibody response to Poly-L(Tyr,Glu)-poly-D, L-Ala--poly-L-Lys in C3H ↔ CWB tetraparental mice. J. Exp. Med. 140:1660.
22. Shearer, G. M., E. Mozes, and M. Sela. 1972. Contribution of different cell types to the genetic control of immune responses as a function of the chemical nature of the polymeric side chains (poly-L-Prolyl and poly-DL-Alanyl) of synthetic immunogens. J. Exp. Med. 135:1009.
23. Munro, A. J., and M. J. Taussig. 1975. Two genes in the major histocompatibility complex control response. Nature (Lond.). 256:103.
24. Katz, D. H., N. Chiorazzi, J. McDonald, and L. R. Katz. 1976. Cell interactions between histoincompatible T and B lymphocytes. IX. The failure of histoincompatible cells is not due to suppression and cannot be circumvented by carrier-priming T cells with allogeneic macrophages. J. Immunol. 117: 1853.
25. R. M. Zinkernagel, G. N. Callahan, A. Althage, S. Cooper, and J. Klein. 1978. On the thymus in the differentiation of "H-2 self-recognition" by T cells: evidence for dual recognition? J. Exp. Med. 147:882.
26. J. Sprent. 1978. Restricted helper function of F1 → parent bone marrow chimeras controlled by K-end of H-2 complex. J. Exp. Med. 147:1838.
27. Waldmann, H., H. Pope, and A. J. Munro. 1975. Cooperation across the histocompatibility barrier. Nature (Lond.). 258:728.
28. Von Boehmer, H., and J. Sprent. 1976. T cell function in bone marrow chimeras: absence of host-reactive T cells and cooperation of helper T cells across allogeneic barriers. Transplant. Rev. 29:3.
29. Araneo, B. A., P. C. Marrack, and J. W. Kappler. 1975. Functional heterogeneity among the T-derived lymphocytes of the mouse. II. Sensitivity of subpopulations to anti-thymocyte serum. J. Immunol. 114:747.
30. Rittenberg, M. B., and K. L. Pratt. 1969. Primary response of BALB/c mice to soluble and particulate immunogens. Proc. Soc. Exp. Biol. Med. 132:575.
31. Jacobs, D. M., and D. C. Morrison. 1975. Stimulation of a T-independent primary anti-hapten response in vitro by TNP-lipopolysaccharide (TNP-LPS). J. Immunol. 114:560.
32. Marrack, P., and J. W. Kappler. 1976. Antigen-specific and non-specific mediators of T cell/B cell cooperation. II. Two helper T cells distinguished by their antigen sensitivities. J. Immunol. 116:1373.
33. Falkoff, R., and J. Kettman. 1972. Differential stimulation of precursor cells and carrier-specific thymus-derived cell activity in the in vivo response to heterologous erythrocytes in mice. J. Immunol. 108:54.
34. Julius, M. F., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3:645.
35. Mishell, R. I., and R. W. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. J. Exp. Med. 126:423.
36. Kettman, J., and R. W. Dutton. 1970. An in vitro primary immune response to TNP-substituted erythrocytes. Response against carrier and hapten. J. Immunol. 104:1558.
37. Hoffmann, M., and J. W. Kappler. 1973. Regulation of the immune response I. Qualitative and quantitative differences between thymus- and bone marrow-derived lymphocytes in the recognition of antigen. J. Exp. Med. 137:721.
38. Swain, S. L., P. E. Trefts, H. Y. S. Tse, and R. W. Dutton. 1977. The significance of T-B collaboration across haplotype barriers. Cold Spring Harbor Symp. Quant. Biol. 41:597.
39. Feldmann, M., P. C. L. Beverley, J. Woody, and I. F. C. McKenzie. 1977. T-T interactions in the induction of suppressor and helper T cells: analysis of membrane phenotype of precursor and amplifier cells. J. Exp. Med. 145:793.
generation of T helper cells. V. Evidence for differential activation of short-lived T₁ and long-lived T₂ lymphocytes by the macrophages factors GRF and NMF. *J. Immunol.* 119:206.

41. Katz, D. H. 1977. The role of the histocompatibility gene complex in lymphocyte differentiation. *Cold Spring Harbor Symp. Quant. Biol.* 41:611.

42. Zinkernagel, R. M., G. N. Callahan, A. Althage, S. Cooper, J. W. Streilein, and J. Klein. 1978. The lymphoreticular system in triggering virus plus self-specific cytotoxic T cells: evidence for T cell help. *J. Exp. Med.* 147:897.

43. Tyan, M. L., H. O. McDevitt, and L. A. Herzenberg. 1969. Genetic control of the antibody response to a synthetic polypeptide: transfer of response with spleen cells or lymphoid precursors. *Transplant. Proc.* 1:549.

44. Cheseboro, B. W., G. F. Mitchell, F. C. Grumet, L. A. Herzenberg, H. O. McDevitt, and T. G. Wegmann. 1972. Cell transfer studies in a genetically controlled immune response. *Eur. J. Immunol.* 2:243.

45. Heber-Katz, E., and D. B. Wilson. 1975. Collaboration of allogenic T and B lymphocytes in the primary antibody response to sheep erythrocytes in vitro. *J. Exp. Med.* 142:928.

46. Shevach, E. M., and D. W. Thomas. 1977. Genetic regulation of macrophage-T lymphocyte interaction. In *Regulatory genetics of the immune system* ICN-UCLA Symposia on Molecular and Cellular Biology VI:411.

47. Ordal, J. C., and F. C. Grumet. 1972. Genetic control of the immune response. The effect of graft-versus-host reaction on the antibody response to poly-L-(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys in nonresponder mice. *J. Exp. Med.* 136:1195.

48. Paul, W. E., E. M. Shevach, D. W. Thomas, S. F. Pickeral, and A. S. Rosenthal. 1977. Genetic restriction in T-lymphocyte activation by antigen-pulsed peritoneal exudate cells. *Cold Spring Harbor Symp. Quant. Biol.* 41:571.

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

50. Matzinger, P., and M. J. Bevan. 1977. Why do so many lymphocytes respond to major histocompatibility antigens? *Cell Immunol.* 29:1.

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

52. Blanden, R. V., and G. L. Ada. 1978. A dual recognition model for cytotoxic T cells based on thymic selection of precursors with low affinity for self *H-2* antigens. *Scand. J. Immunol.* 7:181.