

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

VI. Expression of *Ir* Genes by Helper T Cells\*

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In past experiments we and others have shown that *I* region and immune response (*Ir*) genes are expressed in the thymus during T-cell maturation (1–6), by macrophages (M $\phi$ ) during helper T-cell priming (7, 8), and by both B cells and M $\phi$  during the effector stage of helper T-cell activity (7–11). In a previous paper we showed that *I* region and *Ir*-gene activity could not be detected in helper T cells themselves (1). In the strain combination we studied, for example, B10.A, *H-2<sup>a</sup>*, low responder T cells provided good helper activity in responses to trinitrophenylated-poly-L-(Tyr,Glu)-poly-D,L-Ala—poly-L-Lys(TNP-(TG)-A—L), providing they differentiated in high responder B6AF<sub>1</sub> animals, were primed with antigen in the presence of high responder M $\phi$ , and were tested for activity with high responder B cells and M $\phi$ .

Because of several reports that the sites of *Ir*-gene action may be different in low responder mice of different haplotypes (12, 13) we decided to investigate the expression of (TG)-A—L-specific *Ir* genes in low responder helper T cells of a haplotype other than *H-2<sup>a</sup>*. We chose to study *H-2<sup>f</sup>* helper T cells, because mice of this haplotype had previously been shown to have a lesion in anti-(TG)-A—L response at a site different from that of *H-2<sup>a</sup>* mice (12, 13). Our results show that *Ir* genes for anti-(TG)-A—L responses are expressed in *H-2<sup>f</sup>* helper T cells, a result which is in contrast to that obtained with *H-2<sup>a</sup>* helper T cells.

**Materials and Methods**

*Mice.* C57BL/10.Sn (B10) (*H-2<sup>b</sup>*) mice were obtained from The Jackson Laboratories (Bar Harbor, Maine). B10.M (*H-2<sup>f</sup>*) breeding pairs were supplied to us kindly by Dr. Mariana Cherry (The Jackson Laboratory). B10.M and (B10  $\times$  B10.M)F<sub>1</sub> mice were bred in our facilities.

*Preparation of Irradiated, Bone Marrow Reconstituted Mice.* Bone marrow chimeric mice were prepared as previously described (1). Briefly, both B10.M donors and (B10  $\times$  B10.M)F<sub>1</sub> recipients were depleted of recirculating T cells by intraperitoneal injection of 0.04 ml anti-thymocyte serum (ATS, Microbiological Associates, Walkersville, Md.) 2 d before use (1). After receiving 900 rads, recipients were given 2–3  $\times 10^7$  donor bone marrow cells intravenously (i.v.), and rested for at least 8 wk before use. Donors, recipients, and chimeras (B10.M  $\rightarrow$  (B10  $\times$  B10.M)F<sub>1</sub>) were maintained on acidified water and given 400  $\mu$ g gentamicin sulfate (Schering Corp., Kenilworth, N. J.) on the day before, day of, and day after irradiation.

*Antigens.* Keyhole limpet hemocyanin (KLH) was purchased from CalBiochem-Behring Corp., American Hoechst Corp., San Diego, Calif., (TG)-A—L (batch number MC8) was

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purchased from Miles Laboratories Inc., Miles Research Products, Elkhart, Ind. and *Escherichia coli* lipopolysaccharide (LPS) was bought from Difco Laboratories, Detroit, Mich. Horse erythrocytes (HRBC) from a single animal were provided by the Colorado Serum Co., Denver, Colo. A trinitrophenylated form of each of these antigens was prepared as previously described (11).

**Immunizations.** KLH- or (TG)-A—L-specific helper T cells were obtained from animals immunized as previously described (11). To ensure that the appropriate antigen-presenting cells were present in B10.M  $\rightarrow$  (B10  $\times$  B10.M) $F_1$  chimeric mice, these animals were given one mouse equivalent each of (B10  $\times$  B10.M) $F_1$  or B10 B cells and M $\phi$  i.v. on the day of priming. B cells and M $\phi$  were prepared free of T cells by injection of donor animals with 0.04 ml ATS i.p. 2 d before transfer, and treatment of combined splenic and peritoneal cells with anti-T serum and complement (10) immediately before transfer. This is a procedure which we have shown in the past supplies antigen-presenting cells of the required haplotype to parental  $\rightarrow$   $F_1$  chimeric mice (1). For in vitro immunizations antigen was added as previously described (11).

**Preparation of T Cells, B Cells, and Macrophages.** T cells were isolated from nylon fiber columns as previously described (10). Where necessary these cells were depleted of B10 or (B10  $\times$  B10.M) $F_1$  cells by treatment with B10.A anti-B10 serum followed by washing and incubation with rabbit complement (1). The antiserum selected for this treatment had a high cytotoxic titer on  $H-2^b$  lymphocytes and no discernable cytotoxicity on  $H-2^f$  lymphocytes. Erythrocytes were removed from splenic T-cell preparations before antiserum and complement treatment using modified Gey's solution (14). B cells and M $\phi$  were obtained from the spleens of TNP-LPS-primed mice by treatment with anti-T-cell serum and complement (10). M $\phi$  for presentation of TNP-(TG)-A—L were obtained from the peritoneal washings of normal mice, and pulsed with TNP-(TG)-A—L as previously described (11).

**Assay of Helper T-Cell Activities.** Cells were cultured by modifications of the methods of Mishell and Dutton (10) in Linbro FB16-24TC culture trays (Linbro Chemical Co., Hamden, Conn.). Each culture contained  $3 \times 10^5$  TNP-primed B cells and M $\phi$  and TNP-KLH to a concentration of 1  $\mu$ g/ml or  $10^5$  TNP-(TG)-A—L-pulsed M $\phi$  as antigen. Antigen-pulsed M $\phi$  were always syngeneic to the B cells and M $\phi$  in vitro. KLH- or (TG)-A—L-primed T cells were titrated into these cultures, and carrier-specific helper activity quantitated as previously described (10). Briefly, the number of anti-TNP plaque-forming cells (PFC) observed per culture after 4 d incubation 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 was 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  $\pm$  the standard error (SE). When T cells were treated with antisera, the T-cell activities reported are based on the original number of T cells before treatment.

Anti-TNP PFC were assayed from triplicate cultures using the slide modification of the hemolytic plaque assay (10). Parallel determinations were made with HRBC and TNP-HRBC and the difference recorded as the number of anti-TNP specific PFC.

## Results

We tested the ability of T cells from B10.M  $\rightarrow$  (B10  $\times$  B10.M) $F_1$  chimeric mice to help responses to TNP-(TG)-A—L, and compared this activity with that of (B10  $\times$  B10.M) $F_1$ , high responder and B10.M  $H-2^f$ , low responder T cells. On the day of priming, chimeric mice were given one mouse equivalent each of splenic and peritoneal B cells and M $\phi$  from (B10  $\times$  B10.M) $F_1$  or B10 animals, to provide antigen-presenting cells bearing the  $H-2^b$ , high responder, haplotype in these animals. As shown in Fig. 1, (B10  $\times$  B10.M) $F_1$  T cells were able to help anti-TNP-(TG)-A—L responses of B10 B cells and M $\phi$  very well, but the anti-TNP-(TG)-A—L responses of B10.M B cells and M $\phi$  poorly, in agreement with our previous experiments (11). B10.M T cells stimulated a poor response in B10.M B cells and M $\phi$ . To our surprise, B10.M  $\rightarrow$  (B10  $\times$  B10.M) $F_1$  T cells also stimulated poor anti-TNP-(TG)-A—L responses, even when tested on high responder B10 B cells and M $\phi$ . In control

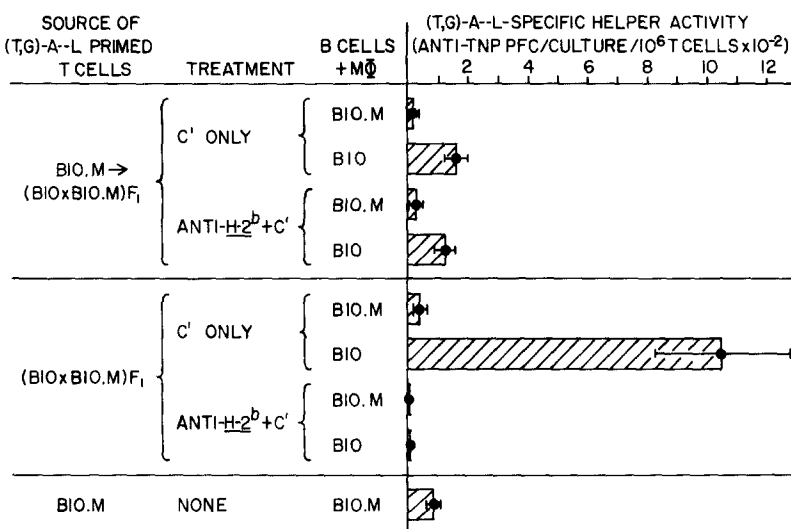


FIG. 1. Expression of *Ir* genes in T cells. (B10 × B10.M)F<sub>1</sub>, high responder, and B10.M, low responder, mice were primed with (T,G)-A—L. B10.M → (B10 × B10.M)F<sub>1</sub> chimeric animals were given B10, high responder, antigen-presenting cells and were also primed with (T,G)-A—L. Their T cells were subsequently titrated for helper activity in anti-TNP-(T,G)-A—L responses of B cells and Mφ from B10 or B10.M mice. Before titration, aliquots of (B10 × B10.M)F<sub>1</sub> or B10.M → (B10 × B10.M)F<sub>1</sub> T cells were treated with anti-*H-2<sup>b</sup>* and complement to establish the origin of these T cells. Results shown are the helper activities, expressed as anti-TNP PFC/10<sup>6</sup> T cells/culture ± SE for different helper T-cell populations. Similar results were obtained in three other identical experiments.

experiments, chimeric and F<sub>1</sub> T cells were treated with anti-*H-2<sup>b</sup>* serum and complement to prove that the response observed in chimeric animals was indeed a result of T cells bearing *H-2<sup>f</sup>* antigens only.

We were concerned that we had not successfully reconstituted the chimeric animals with high responder *H-2<sup>b</sup>*-bearing antigen-presenting cells. To prove that such cells were indeed functional we performed concomitant experiments to those described above, using B10.M → (B10 × B10.M)F<sub>1</sub> chimeric mice given *H-2<sup>b</sup>*-bearing B cells and Mφ on the day of immunization (see above) and primed with KLH. T cells from these animals were able to help anti-TNP-KLH responses of B10 or B10.M B cells and Mφ very well, and had as high a helper activity as KLH-primed cells from (B10 × B10.M)F<sub>1</sub> animals (Fig. 2). Again, controls after T-cell treatment with anti-*H-2<sup>b</sup>* serum and complement revealed that the KLH-specific helper activity in the chimeric mice was a result of T cells of donor origin.

### Discussion

We have been trying to determine whether *Ir*-genes are expressed in helper T cells specific for (T,G)-A—L by testing the properties of low responder T cells produced in low responder → (high responder, *H-2<sup>b</sup>*, × low responder)F<sub>1</sub> bone marrow chimeric mice. Thus far, we have tested two low responder haplotypes, *H-2<sup>a</sup>* (1), and in the present report, *H-2<sup>f</sup>*, with dramatically contrasting results. *H-2<sup>a</sup>* T cells from chimeric mice were good responders to (T,G)-A—L provided they were primed in the presence of *H-2<sup>b</sup>*-bearing Mφ and tested for helper activity with *H-2<sup>b</sup>*-bearing B cells and Mφ. On the other hand as reported here, *H-2<sup>f</sup>* T cells from chimeric mice were poor

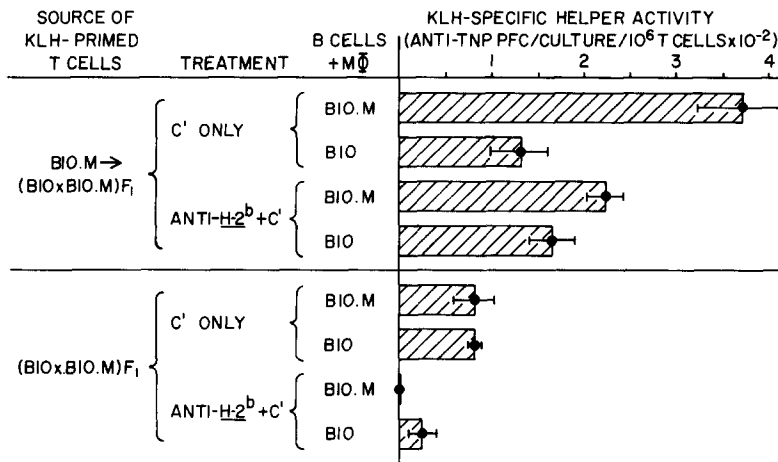


FIG. 2.  $H-2^b$ -bearing antigen presenting cells are present in the chimeric mice. (B10  $\times$  B10.M)<sub>F1</sub> mice were primed with KLH. B10.M  $\rightarrow$  (B10  $\times$  B10.M)<sub>F1</sub> animals were given B10 antigen-presenting cells and were also primed with KLH. Their T cells were subsequently titrated for helper activity in anti-TNP-KLH responses of B cells and M $\phi$  from B10 or B10.M mice. Before titration, aliquots of these T cells were treated with anti- $H-2^b$  and complement to establish their origin. Results shown are the helper activities expressed as anti-TNP PFC/10<sup>6</sup> T cells/culture  $\pm$  SE for different helper T-cell populations. Similar results were obtained in one other identical experiment.

responders to (T,G)-A—L even when primed and tested with  $H-2^b$  B cells and M $\phi$ . These results suggest that for the  $H-2^f$ , but not  $H-2^a$ , low responder haplotype an *Ir*-gene defect is expressed in helper T cells. Also, in this report and in our previous work we clearly established that for both the  $H-2^a$  and  $H-2^f$  haplotypes, *Ir*-genes were expressed in B cells and M $\phi$  during their interaction with primed helper T cells.

Taken together, these findings indicate at least two types of *Ir*-genes that control the response to (T,G)-A—L; one, expressed in B cells and M $\phi$  and deficient in both the  $H-2^a$  and  $H-2^f$  haplotype and a second, expressed in T cells and defective in the  $H-2^f$  but not  $H-2^a$  haplotype. In this respect, our results are reminiscent of those of Munro and Taussig (12) who reported that  $H-2^a$ , but not  $H-2^f$ , T cells were able to produce an antigen-specific helper factor in response to (T,G)-A—L.

There are a number of possible explanations for the expression of *Ir* genes in helper T cells. Many experiments from this (7, 9–11) and other (8, 15, 16) laboratories have shown that *Ir*-genes are expressed in B cells and M $\phi$  in a manner consistent with the idea that these genes control the recognition of B-cell- or M $\phi$ -bound antigen by T cells. By analogy, it may be, as suggested by Zinkernagel et al. (17) and von Boehmer et al. (5), that *Ir*-genes, expressed in helper T cells, control the recognition of helper T-cell-bound antigen by a second T cell whose activity is required for the response of the helper T cell. The attraction to us of such an explanation is it produces a unifying scheme for the mode of action of *Ir*-genes.

On the other hand, there are other explanations for our results which our data at present do not distinguish. For example, a second possibility is that some *Ir*-gene products are involved in the structure of the T-cell receptor for antigen and therefore, the expression of a low responder *Ir*-gene in  $H-2^f$  T cells indicates the lack of (T,G)-A—L-specific receptors in mice of this haplotype. A third possibility is that an *Ir*-gene controlling (T,G)-A—L-specific suppressor cells is expressed in mice of the  $H-2^f$  but

not  $H-2^a$  haplotype. Our future experiments will be aimed at distinguishing these various possibilities.

### Summary

We examined the expression of (TG)-A—L specific *Ir* genes in helper T cells using T cells from low responder  $\rightarrow$  (B10, high responder  $\times$  low responder)  $F_1$  chimeric mice. In this paper, the low responder strain studied was B10.M,  $H-2^f$ . B10.M T cells from these chimeric animals do not help anti-TNP-(TG)-A—L responses, even though they have matured in a high responder thymus and been primed and challenged with antigen on high responder  $M\phi$  and B cells. These findings indicate that in the  $H-2^f$  haplotype an *Ir*-gene controlling anti-(TG)-A—L activity is expressed in helper T cells. The findings are in contrast to those we have obtained and previously reported with T cells of another low responder haplotype,  $H-2^a$ .

Taken together with our previous findings that (TG)-A—L-specific *Ir* genes are expressed by B cells and  $M\phi$  of both the  $H-2^a$  and  $H-2^f$  haplotypes, the results indicate two sites of action for *Ir* genes, and suggest two different gene products acting at different stages of the response, both of which are defective in  $H-2^f$  cells, and only one of which is defective in  $H-2^a$  cells.

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