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 (Mφ) during helper T-cell priming (7, 8), and by both B cells and Mφ 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-2b, 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 B6AF1 animals, were primed with antigen in the presence of high responder Mφ, and were tested for activity with high responder B cells and Mφ.

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-2b. We chose to study H-2d 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-2b mice (12, 13). Our results show that Ir genes for anti-(TG)-A—L responses are expressed in H-2d helper T cells, a result which is in contrast to that obtained with H-2b helper T cells.

Materials and Methods

Mice. C57BL/10.Sn (B10) (H-2b) mice were obtained from The Jackson Laboratories (Bar Harbor, Maine). B10.M (H-2d) breeding pairs were supplied to us kindly by Dr. Mariana Cherry (The Jackson Laboratory). B10.M and (B10 × B10.M)F1 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 × B10.M)F1 recipients were depleted of recirculating T cells by intraperitoneal injection of 0.04 ml antithymocyte serum (ATS, Microbiological Associates, Walkersville, Md.) 2 d before use (1). After receiving 900 rads, recipients were given 2–3 × 10^7 donor bone marrow cells intravenously (i.v.), and rested for at least 8 wk before use. Donors, recipients, and chimeras (B10.M → (B10 × B10.M)F1) were maintained on acidified water and given 400 μg 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 supported by U. S. Public Health Service research grants AI-11558 and CA-11198 and American Cancer Society research grant IM-49.

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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 → (B10 × B10.M)F1 chimeric mice, these animals were given one mouse equivalent each of (B10 × B10.M)F1 or B10 B cells and Mφ i.v. on the day of priming. B cells and Mφ 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 F1 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 × B10.M)F1 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^t lymphocytes. Erythrocytes were removed from splenic T-cell preparations before antiserum and complement treatment using modified Gey's solution (14). B cells and Mφ were obtained from the spleens of TNP-LPS-primed mice by treatment with anti-T-cell serum and complement (10). Mφ 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 × 10^8 TNP-primed B cells and Mφ and TNP-KLH to a concentration of 1 μg/ml or 10^5 TNP-(TG)-A-L-pulsed Mφ as antigen. Antigen-pulsed Mφ were always syngeneic to the B cells and Mφ 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/10^5 T cells ± 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 → (B10 × B10.M)F1 chimeric mice to help responses to TNP-(TG)-A-L, and compared this activity with that of (B10 × B10.M)F1, high responder and B10.M H-2^b, low responder T cells. On the day of priming, chimeric mice were given one mouse equivalent each of splenic and peritoneal B cells and Mφ from (B10 × B10.M)F1 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 × B10.M)F1 T cells were able to help anti-TNP-(TG)-A-L responses of B10 B cells and Mφ very well, but the anti-TNP-(TG)-A-L responses of B10.M B cells and Mφ very poorly, in agreement with our previous experiments (11). B10.M T cells stimulated a poor response in B10.M B cells and Mφ. To our surprise, B10.M → (B10 × B10.M)F1 T cells also stimulated poor anti-TNP-(TG)-A-L responses, even when tested on high responder B10 B cells and Mφ.
Fig. 1. Expression of Ir genes in T cells. (B10 × B10.M)F1, high responder, and B10.M, low responder, mice were primed with (TG)-A---L. B10.M → (B10 × B10.M)F1 chimeric animals were given B10, high responder, antigen-presenting cells and were also primed with (TG)-A---L. Their T cells were subsequently titrated for helper activity in anti-TNP-(TG)-A---L responses of B cells and MΦ from B10 or B10.M mice. Before titration, aliquots of (B10 × B10.M)F1 or B10.M → (B10 × B10.M)F1 T cells were treated with anti-H-2b and complement to establish the origin of these T cells. Results shown are the helper activities, expressed as anti-TNP PFC/10^6 T cells/culture ± SE for different helper T-cell populations. Similar results were obtained in three other identical experiments.

experiments, chimeric and F1 T cells were treated with anti-H-2b serum and complement to prove that the response observed in chimeric animals was indeed a result of T cells bearing H-2f antigens only.

We were concerned that we had not successfully reconstituted the chimeric animals with high responder H-2b-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)F1 chimeric mice given H-2b-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)F1 animals (Fig. 2). Again, controls after T-cell treatment with anti-H-2b 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-2b, × low responder)F1 bone marrow chimeric mice. Thus far, we have tested two low responder haplotypes, H-2a (1), and in the present report, H-2f, with dramatically contrasting results. H-2a T cells from chimeric mice were good responders to (T,G)-A---L provided they were primed in the presence of H-2b-bearing MΦ and tested for helper activity with H-2b-bearing B cells and MΦ. On the other hand as reported here, H-2f T cells from chimeric mice were poor
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FIG. 2. H-2b-bearing antigen presenting cells are present in the chimeric mice. (B10 × B10.M)F1 mice were primed with KLH. B10.M → (B10 × B10.M)F1 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~ from B10 or B10.M mice. Before titration, aliquots of these T cells were treated with anti-H-2b and complement to establish their origin. Results shown are the helper activities expressed as anti-TNP PFC/10^6 T cells/culture ± 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-2b B cells and Mφ. These results suggest that for the H-2f, but not H-2a, 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-2a and H-2f haplotypes, Ir-genes were expressed in B cells and Mφ 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 Mφ and deficient in both the H-2a and H-2f haplotype and a second, expressed in T cells and defective in the H-2f but not H-2a haplotype. In this respect, our results are reminiscent of those of Munro and Tausig (12) who reported that H-2a, but not H-2f, 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 Mφ in a manner consistent with the idea that these genes control the recognition of B-cell- or Mφ-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-2f 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-2f 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 \((B10, \text{high responder} \times \text{low responder}) F_1\) 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 Mφ 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 Mφ 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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