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The Two High Responder Haplotypes to (TG)-A--L Do Not Cross-React at the Helper T Cell Level[1]

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The idea that T cells recognize antigen in association with products of the major histocompatibility complex was suggested first by the experiments of a number of groups studying cytotoxic T cells (1–3). The principle was rapidly applied to the study of helper T cells and led to a similar conclusion, that helper T cells recognize antigen in association with self *I* region products, usually a product of the *I-A* subregion (4–7). In later experiments, again analagous to those performed with cytotoxic T cells, it was shown that helper T cells can only recognize antigen in association with *I* region haplotypes to which they were exposed in the thymus (5, 6, 8–10).

In many respects some immune response (*Ir*)[3] genes behave like especially restricted examples of *I-A* plus antigen recognition by helper T cells. *Ir* genes for poly-L(Tyr,Glu)-poly-D,L-Ala--poly-L-Lys ((TG)-A--L), for example, map to the *K* or *I-A* regions of *H-2* and are expressed during helper T cell development by the thymus and antigen-presenting cells, and not always by the T cells themselves (9, 11–13).

A number of theories have been proposed to explain how helper T cells recognize antigen in association with *I* products (14–17). Any theory, however, must account for the observed properties of helper T cells, for example, their great specificity not only for antigen, but also for *I* of the appropriate haplotype. Thus, even though *I* region products of different haplotypes have considerable structural, sequence, and immunochemical similarities to each other (18–22), helper T cells usually do not recognize cross-reacting determinants between *I* of different haplotypes (6, 7, 10).

It has been suggested, however, that helper T cell cross-reactivities might be observed for antigens, with few determinants, under *Ir* gene control (23). In support of this has been the work of Tada (24) and Kapp *et al.* (25) on suppressor factors in which keyhole limpet hemocyanin (KLH) specific suppressor factors do not cross-react between different *H-2* haplotypes, but poly (Glu, Ala, Tyr) (GAT)-specific suppressor factors do. Anti-GAT, but not anti-KLH, responses are under *Ir* gene control. We wished to determine whether such an observation could also be made for the *Ir* gene controlled-antigen (TG)-A--L, for which there are only two known independent high responder *H-2* haplotypes, and helper T cells recognizing it.

The experiments reported here show that *H-2*^{b} and *H-2*^{d}, the two high responders to (TG)-A--L, do not cross-react when presenting this antigen to helper T cells. They therefore show that extensive *H-2* cross-reactivities are not necessarily observed by helper T cells, even for an antigen with few determinants.

MATERIALS AND METHODS

Normal female BDF_1 (*H-2*^{b} × *H-2*^{d}) C57BL/10 Sn (B10, *H-2*^{b}) and B10.D2/nSn (B10.D2, *H-2*^{d}) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. B10 and B10.D2 mice were irradiated with 950 rads from a ^{60}Co source and reconstituted by i.v. injection of 10^7 bone marrow cells from BDF_1 animals. Both donors and recipients were depleted of recirculating T cells 2 days before use by i.p. administration of 0.04 ml rabbit anti-mouse thymocyte serum (Microbiological Associates, Bethesda, Md.). Recipients were given antibiotics and maintained on acidified water to prevent post-irradiation infection as previously described (9). Chimeric mice (BDF_1 → B10 or BDF_1 → B10.D2) were left for 8 to 12 weeks after irradiation to recover. When they were killed, lymphocytes from their mesenteric lymph nodes were typed in cytotoxic assays with anti-*H-2*^{b} or anti-*H-2*^{d} sera plus rabbit complement (C). In every case lymphocytes from these animals were at least 95% of donor origin.

In order to assay (TG)-A--L-specific helper activity, chimeric and normal BDF_1 animals were primed with 100 μg (TG)-A--L (Miles Laboratories, Elkhart, Ind., Lot MC9) in complete Freund's adjuvant in the base of the tail. Seven days later T cells were isolated from their inguinal and periaortic lymph nodes by passing cell suspensions from these nodes over nylon fibre columns (11). B cells and macrophages (Mϕ) for helper assays were obtained from the spleens of animals primed 7 days previously i.p. with 1 μg of trinitrophenylated lipopolysaccharide (TNP-LPS, 9). T cells were removed from these cell suspensions by treatment with anti-Thy 1 hybridoma antibody (T24/40.7, kindly given to us by Dr. Ian Trowbridge, Salk Institute) plus C. TNP-(TG)-A--L was added as antigen to assay cultures bound to normal BDF_1 peritoneal cells, and the (TG)-A--L-specific helper activity of various T cell populations was measured by titrating the T cells into cultures containing constant numbers of splenic B cells and Mϕ, and TNP-(TG)-A--L-pulsed peritoneal cells as previously described (11). After 4 days culture anti-TNP direct plaque-forming cells (PFC) were assayed by using TNP-horse red blood cells (HRBC) as indi-

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[3] Abbreviations used in this paper: B10, C57BL/10; GAT, poly (Glu, Ala, Tyr); HRBC, horse red blood cells; *Ir*, immune response; KLH, keyhole limpet hemocyanin; Mϕ, macrophage; (TG)-A--L, poly-L-(Tyr, Glu)-poly-D,L-Ala--poly-L-Lys.

cator cells (26). Background PFC on HRBC were subtracted.

The initial slopes of anti-TNP PFC/culture plotted *vs* T cell added were approximately linear, and could therefore be determined by using a modified linear regression program. These slopes, expressed as anti-TNP PFC/culture/10^6 T cells ± standard error, were taken as measures of the helper activities of the different T cell populations.

RESULTS AND DISCUSSION

In order to measure the degree to which helper T cells recognizing (TG)-A--L in association with the high responder haplotype, *H-2*^{b}, could recognize the same antigen in association with the other known responder haplotype, *H-2*^{d}, BDF_1 T cells, which were tolerant to both *H-2*^{b} and *H-2*^{d}, were prepared and primed to (TG)-A--L in $BDF_1 \rightarrow$ B10 bone marrow chimeric mice. The BDF_1 T cells were thus restricted to recognize antigen in the context of *H-2*^{b} (9). Normal BDF_1 T cells were also primed with (TG)-A--L. Both types of T cell were titrated for helper activity in cultures containing B10, B10.D2, or BDF_1 B cells and Mϕ, and TNP-(TG)-A--L-pulsed BDF_1 peritoneal cells. As shown in Table I, normal BDF_1 T cells helped responses of all three types of B cells very well, but $BDF_1 \rightarrow$ B10 T cells only cooperated with B10 or BDF_1 B cells and interacted poorly with B10.D2 B cells. Similar results were obtained in two repeats of this experiment.

The reciprocal experiment was performed with $BDF_1 \rightarrow$ B10.D2 and BDF_1 T cells primed with (TG)-A--L. As shown in Table II, $BDF_1 \rightarrow$ B10.D2 T cells cooperated very well with B10.D2 or BDF_1 B cells, but poorly with B10 B cells. Similar results were obtained in a repeat of this experiment.

These results led to three conclusions. First, T cells from $F_1 \rightarrow$ parent bone marrow chimeras could only cooperate well in anti-TNP-(TG)-A--L responses with cells bearing the recipient parental *H-2* type. Their activity with cells bearing only the other parental *H-2* type was less than 3% of controls. Thus, even though *H-2*^{b} and *H-2*^{d} are both responders to (TG)-A--L, an antigen which is perhaps somewhat limited in the ways in which it can be presented to helper T cells, helper T cells do not recognize (TG)-A--L in association with structures cross-reacting between the two haplotypes. Second, the restriction observed in these chimeric T cells was not due to suppression of the response of B cells bearing the inappropriate haplotype, since the responses of F_1 B cells were stimulated by all three types of helper T cells studied. Finally, $F_1 \rightarrow$ parent T cells, once induced, were restricted at least by the haplotype of the B cells in culture, since all cultures contained antigen-pulsed F_1 peritoneal cells, which we have previously shown contain functional Mϕ (11). This result agrees with our previous findings that the *I* region and/or *Ir* gene type of the B cell at least restricts helper T cell activity in our cultures, and implies that B cells at least present antigen to effector helper T cells (4–7, 9, 11).

In conclusion, our results show that helper T cells specific for (TG)-A--L do not recognize the antigen in association with cross-reacting structures of the two high responder haplotypes, and suggest that a search for products of *Ir* genes that involves the identification of similar structures between different high responder haplotypes may be misleading.

TABLE I

$BDF_1 \rightarrow$ B10 T cells only cooperate in anti-TNP-(TG)-A--L responses with H-2^b*-bearing B cells*

Cells in Culture			Helper Activity[a] (Anti-TNP PFC/ Culture/10^6 T Cells)
B cells + MØ	TNP-(TG)-A--L-pulsed MØ	T Cells	
B10	BDF_1	$BDF_1 \rightarrow$ B10	2330 ± 180
B10.D2	BDF_1	$BDF_1 \rightarrow$ B10	61 ± 1
BDF_1	BDF_1	$BDF_1 \rightarrow$ B10	1920 ± 270
B10	BDF_1	BDF_1	465 ± 75
B10.D2	BDF_1	BDF_1	362 ± 44
BDF_1	BDF_1	BDF_1	1050 ± 210

[a] Initial slope of titration line ± S.E.

TABLE II

$BDF_1 \rightarrow$ B10.D2 T cells only cooperate in anti-TNP-(TG)-A--L responses with H-2^d*-bearing B cells*

Cells in Culture			Helper Activity[a] (Anti-TNP PFC/ Culture/10^6 T Cells)
B cells + Mϕ	TNP-(TG)-A--L-pulsed Mϕ	T Cells	
B10	BDF_1	$BDF_1 \rightarrow$ B10.D2	2 ± 3
B10.D2	BDF_1	$BDF_1 \rightarrow$ B10.D2	812 ± 95
BDF_1	BDF_1	$BDF_1 \rightarrow$ B10.D2	913 ± 129
B10	BDF_1	BDF_1	343 ± 52
B10.D2	BDF_1	BDF_1	110 ± 52
BDF_1	BDF_1	BDF_1	842 ± 137

[a] Initial slope of titration line ± S.E.

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