MULTIPLE H-2-LINKED IMMUNE RESPONSE GENE
CONTROL OF H-2D-ASSOCIATED T-CELL-MEDIATED
LYMPHOLYSIS TO TRINITROPHENYL-MODIFIED
AUTOLOGOUS CELLS: Ir-LIKE GENES MAPPING TO THE
LEFT OF I-A AND WITHIN THE I REGION

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One of the more recent associations of the murine H-2 major histocompatibility complex (MHC) with immune function has been the finding that cytotoxic T-effector cells generated by sensitization with viral-infected (1-6), chemically modified (7-9), or weak transplantation antigen-associated (10, 11) syngeneic cells can efficiently lyse target cells which express the same viral, chemical, or weak antigenic agent, and which share the H-2K and/or H-2D regions of the MHC with the responding and/or stimulating cells. Furthermore, an additional contribution of a gene(s) within the H-2 complex has been demonstrated which controls immune response potential (Ir genes) in the generation of cytotoxic effector cells to trinitrophenyl (TNP)-modified self components (12, 13). In such studies it was found that certain B10 congenic strains generated good cytotoxic responses to both TNP-modified H-2K and H-2D region products, whereas other B10 congenic strains exhibited preferential or exclusive reactivity against TNP-modified H-2K region products. Some of these recombinant strains differing in response potential to TNP-modified H-2D products expressed the same haplotype at the D end, but differed at the K end of H-2. The low responsiveness observed in the B10.A strain to TNP-modified H-2D<sup>d</sup> when compared to B10.D2 and (B10.A × B10.D2)<sup>F<sub>1</sub></sup>, for the same specificity, suggested a role of dominant Ir genes which map in K, I-A, I-B, I-J, and/or I-E (12, 14).

In the present report an attempt was made to further map within the MHC the Ir gene(s) controlling cell-mediated lympholysis (CML) to TNP-modified H-2D<sup>d</sup>, by using recombinant mouse strains on the A and B10 backgrounds. Irrespective of the genetic background, the s and k haplotypes at the K end generated high and low cytotoxic responses, respectively, to H-2D<sup>d</sup>-TNP. The intermediate responder and low responder status of the A.TL and A.AL strains, respectively, indicated that a gene mapping in the K region of H-2 influences response potential. Furthermore, the differences in the levels of cytotoxicity detected in the A.TH and A.TL strains suggested an additional I region influence. Taken together these findings raise the possibility that multiple genes mapping within different regions of the MHC control the level of T-cell-mediated cytotoxicity to chemically modified autologous cells.
Materials and Methods

Mice. The mice used in the experiments were 6-9 wk of age and of both sexes. The B10.A, B10.BR, and SJL/J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. The A.TH, A.TL, A.AL, B10.S, and B10.HTT mice were kindly provided by Dr. C. David, Department of Genetics, Washington University, St. Louis, Mo., and from Dr. D. Sachs, Immunology Branch, National Cancer Institute, Bethesda, Md.

In Vitro Cell Culture, TNP Modification of Spleen Cells, and 51Cr-Release Assay. The methods employed for the primary 5-day in vitro CML sensitization, the TNP modification of stimulating and target cells, and the 4-h 51Cr-release cytotoxic assay have been described in earlier publications (7, 8). The target cells used in the 51Cr-release assay were either phytohemagglutinin (PHA)-stimulated spleen cells or the P-815 mastocytoma or LSTRA lymphoid tumor lines (both H-2d).

Results

In order to map the gene or genes that control CML responsiveness to TNP-modified H-2D<sup>d</sup> products within the left half of the H-2 complex, comparisons were made of the cytotoxic responses generated by splenic lymphocytes from the A.TH, A.TL, A.AL, B10.A, and B10.HTT mouse strains. The responding lymphocytes of these five strains were sensitized in vitro with TNP-modified spleen cells and assayed on TNP-modified blast or tumor target cells of the H-2<sup>d</sup>, H-2<sup>s</sup>, and H-2<sup>k</sup> haplotypes. Cytotoxic data were obtained at effector:target cell ratios of 40:1, 20:1, and 10:1 and the results obtained at ratios of 40:1 and 10:1 are summarized in Table I.

Spleen cells from the A.TH, A.TL, and B10.HTT strains generated effectors that lysed TNP-modified H-2<sup>d</sup> targets. These targets share the H-2 subregions K to S, the K, and the K, I-A, I-B, and I-J regions with the A.TH, A.TL, and B10.HTT strains, respectively. It may be noteworthy that in two of three experiments the A.TL cells generated a weaker response detected on H-2<sup>d</sup>-TNP targets than did cells from either A.TH or B10.HTT mice. Effector cells from A.TH and A.TL donors did not lyse H-2<sup>s</sup>-TNP targets, verifying the H-2K- or H-2D-associated specificity of the CML. However, B10.HTT effector cells did lyse to some extent the H-2<sup>k</sup>-TNP targets, indicating some lack of exclusive association with K or D private specificites. Effector cells from both the A.AL and B10.A strains lysed H-2<sup>k</sup>-TNP targets, whereas no appreciable lysis was detected on H-2<sup>s</sup>-TNP targets. These results verify reactivity against a specificity associated with the left half of the H-2 complex.

For all of the five strains compared here, the specificity of TNP-modified H-2D<sup>d</sup> products involves the d haplotype. Both the B10.A [as previously shown (12, 13)] and A.AL strains were poor responders to H-2D<sup>d</sup>-TNP. In contrast, spleen cells from A.TH, A.TL, and B10.HTT mice generated appreciable CML responses to H-2D<sup>d</sup>-TNP.

The possibility exists that the CML response generated against H-2D<sup>d</sup>-TNP was due to cross-reactivity with H-2<sup>s</sup>-TNP in those strains expressing the H-2<sup>s</sup> haplotype at the K end of H-2. In order to test this possibility, cytotoxic experiments were performed in which the specificity of A.TH effector cells assayed on H-2<sup>d</sup>-TNP targets was tested by blocking the lytic phase with nonradioactive B10.D2 (H-2<sup>d</sup>), SJL/J (H-2<sup>s</sup>), or B10.BR (H-2<sup>k</sup>) TNP-modified cells. The results, summarized in Table II indicate that lysis was inhibited only with B10.D2-TNP blockers, and not by either SJL/J-TNP or B10.BR-TNP cells. Those strains expressing the H-2<sup>s</sup> haplotype at K only (A.TL); at K, I-A, I-B,
TABLE I
Comparison of In Vitro T-Cell-Mediated Cytotoxic Responses to TNP-Modified Autologous Cells in Different Inbred Mouse Strains
on the A and C57BL/10 Genetic Backgrounds

| Responder strain | Immunogen  | H-2 haplotype at: | H-2 haplotype of TNP-modified target cells | % Specific lysis ± SE |
|------------------|------------|-------------------|------------------------------------------|----------------------|
|                  |            | K I-A I-B I-1 E L C S D | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 |
|                  |            |                   | 40:1* | 10:1 | 40:1 | 10:1 | 40:1 | 10:1 | 40:1 | 10:1 | 20:1 | 10:1 |
| A.TH             | A.TH-TNP   | s s s s s s s d   | H-2*  | 36.0 ± 1.6§ | 19.7 ± 0.5 | 43.5 ± 1.2§ | 27.8 ± 1.2 | 33.3 ± 1.4§ | 20.0 ± 1.6 | NT   |       |
| A.TL             | A.TL-TNP   | k k k k k k k d   | H-2*  | 21.6 ± 0.8§ | 6.2 ± 1.7 | 22.9 ± 1.7§ | 12.0 ± 1.1 | 19.9 ± 1.1 § | 11.9 ± 1.3 | NT   |       |
| A.AL             | A.AL-TNP   | k k k k k k k d   | H-2*  | 8.2 ± 1.6§ | 1.8 ± 1.0 | 9.8 ± 0.8§ | 2.9 ± 1.0 | NT   |       |
| B10.HTT          | B10.HTT-TNP| s s s s k k k d   | H-2*  | NT        | NT       | 38.0 ± 1.46 | 17.4 ± 1.2 | 32.7 ± 2.3 | 26.6 ± 4.5 |       |
| B10.A            | B10.A-TNP  | k k k k k d d d   | H-2*  | 3.3 ± 0.4§ | 1.5 ± 1.0 | 10.0 ± 1.2 | 5.5 ± 0.7 | 6.6 ± 1.2§ | 2.2 ± 1.1 | 5.6 ± 1.6§ | 7.2 ± 2.3 |       |

* Effector:target ratio. §H-2 subregions as taken from reference 14; H-2 subregions common to responder, stimulator, and target cells are shown in italics. ¶H-2* tumor LSTRA. II. ||H-2* B10.D2 PHA-stimulated blast spleen cells. †H-2* SJL/J PHA-stimulated blast spleen cells. **H-2* B10.S PHA-stimulated blast spleen cells. ‡H-2* B10.BR PHA-stimulated blast spleen cells.
TABLE II

Analysis of the Specificity of the A.TH-Responding Cells Reactive against TNP Modified H-2d Tumor Cells by Nonradioactive Cell Blocking of the Lytic Phase

| Responding cell* | Immunogen | Strain of origin of blocking spleen cells | % Specific lysis ± SE§ of 51Cr H-2d-TNP tumor cells in the presence of blocking cells |
|------------------|-----------|-----------------------------------------|-------------------------------------------------------------------------------------|
| A.TH             | A.TH-TNP  | B10.D2                                  | 23.2 ± 0.8 22.7 ± 0.7 25.4 ± 1.4                                                  |
|                  |           | dd.dd.dd.dd.d-TNP                       |                                                                                     |
|                  |           | SJL                                     | 7.0 ± 0.4 9.2 ± 0.6 16.4 ± 1.8                                                    |
|                  |           | dd.dd.dd.dd.d-TNP                       |                                                                                     |
|                  |           | SJL-TNP                                 | 24.3 ± 1.0 25.8 ± 1.4 22.4 ± 1.5                                                  |
|                  |           | dd.dd.dd dd dd dd-TNP                   |                                                                                     |
|                  |           | B10.BR                                  | 25.4 ± 0.4 25.3 ± 0.6 25.5 ± 0.6                                                  |
|                  |           | kkkkkkkkkkkkkk-kkkkkkkkkkkkkk-TNP       |                                                                                     |
|                  |           | B10.BR-TNP                              | 19.1 ± 0.8 23.1 ± 0.7 23.0 ± 1.3                                                  |
|                  |           | kkkkkkkkkkkk-kkkkkkkkkkkkkk-TNP         |                                                                                     |

* Effector cells, 2 × 10⁸.
† H-2 subregions common to TNP-modified stimulator and TNP-modified blocking cells are shown in italics.
§ % Specific lysis in the absence of blocking cells, 27.5 ± 1.5.
|| Target cells, 0.125 × 10⁸ LSTRA cells.

and I-J (B10.HTT); or at K, I-A, I-B, I-J, I-E, I-C, and S (A.TH), irrespective of the A or B10 background, are responders to H-2Dᵈ-TNP. Responsiveness to this specificity is therefore associated with an Ir-like gene or genes (i.e., whose influence is extended on the responding cell population), and is located to the left of the crossover, which in the A.TL strain has been mapped between K and I-A (15). Strains having the s haplotype in I-A, I-B, and I-J (B10.HTT) or from I-A through S (A.TH) consistently gave higher cytotoxicity associated with H-2Dᵈ-TNP than the strain having the k haplotype in I-A through S (A.TL). This would map an additional gene or genes controlling the generation of cytotoxic effector cells in the I-A, I-B, or I-J subregions.

Discussion

The finding that at least one of the genes controlling responsiveness to H-2Dᵈ-TNP maps to the left of the crossover which has been mapped between K and I-A (in the A.TL strain) is the first published example of an H-2-linked, Ir-like gene which appears to map outside of a known I subregion. This raises the possibilities that: (a) Ir genes might be randomly distributed so that not all of them would map in the I region; (b) some Ir genes which are usually associated with the I-A subregion, are situated to the left of the crossover that occurred in the A.TL strain; or (c) that functionally distinct immune response genes map in different regions of the MHC.

It is noteworthy that this is the only mapped Ir-like gene which controls the generation of cytotoxic T effector cells (12, 13). Recently, Ir-like genes have been postulated to exist which control strain-dependent differences in CML generated against H-2-associated syngeneic Friend virus-infected cells (6), and the H-Y antigen (16). Heretofore, Ir genes mapped within the I region controlled antibody production (17), probably at the level of the interaction between T-helper cells and B cells. Ir control of in vitro secondary T-cell proliferation in response to soluble antigens also maps within the I region (18). The presence of products
coded for by the I-A subregion on soluble factors mediating T-B cooperation (19) and their functional involvement in T-cell proliferation in response to soluble antigens as assessed by antisera blocking (18) suggest that products of genes mapping in the I region are involved in the cellular interactions required to generate those differentiated lymphoid functions. The differentiation from precursor cells into cytotoxic effector cells might involve different types of cell surface interaction structures, some of which could be coded for by genes mapping within H-2 in a region distinct from I. It may be significant that this Ir-like gene for cytotoxic function appears to map within a region that controls those cell surface products which are associated with the antigens recognized for cytotoxic function. In addition, there appears to be an influence of at least one other Ir-like gene which maps between I-A and I-J. Multiple and complementing Ir genes have been reported for control of antibody production (20), and F1 experiments are in progress to determine whether gene complementation also occurs for the control of CML against H-2D<sup>d</sup>-TNP.

The mechanisms by which these Ir-like genes influence the generation of cytotoxic effector cells is unknown. Since it appears that interacting cell types are involved in the generation of effectors in the autologous TNP-modified system (21), it is possible that these genes exert their influences on distinct cell populations and/or on their interactions. The cellular expression of such genetic influences could be exerted: (a) on a population of cytotoxic precursor cells, which might be expected to be specific for both the modifying agent and the associated H-2 haplotype; (b) on helper cells or cells which might augment selectively an H-2D-associated response or specifically an H-2D<sup>d</sup>-TNP response; and/or (c) on suppressor cells which could influence the level of cytotoxicity generated.

These results suggest a functional involvement by products of genes mapping within different H-2 regions in the generation of cytotoxic effector cells, irrespective of whether the dual recognition or altered self models (2) are considered. The failure to detect H-2-linked, Ir-like genetic effects in some of the viral-infected models (22) could be due to the complex and possible multiple specificities associated with a particular H-2 region product. Chemical modification of autologous cell surface products might provide a simpler model to detect H-2-linked immune response genetic influences than the modifications resulting from complex biological interactions between virus and host. Nevertheless, the chemically modified model appears to be mechanistically relevant for some viral-infected models, including those associated with neoplasms (6), but not for others (22).

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