The male specific antigen, H-Y, which is encoded or controlled by gene(s) situated in the Y chromosome (1), has its probable physiological function in the development of maleness (2). But it is also a weak transplantation antigen which may cause skin graft rejection and the production of cytotoxic T cells (1, 3). The cytotoxic response to H-Y in mice is H-2-restricted in the manner first described by Doherty et al. (4) for viruses, and Shearer et al. (5) for hapten-modified autologous cells. The cytotoxic responses against other minor histocompatibility antigens seem to have similar requirements of syngeneity for cytotoxicity to occur (6).

The production of cytotoxic cells against H-Y antigens is under the control of Ir genes, and there appear to be two kinds of gene regulating this response: the H-2b haplotype contains gene(s) which enable the production of cytotoxic cells in in vitro secondary responses (7), whereas mice of all other independent haplotypes tested so far have failed to respond (3). This trait of the H-2b haplotype seems to be dominant: F1 hybrids with one H-2b parent are able to produce anti-H-Y cytotoxic cells against both the H-2b parent and the nonresponder parent (8). The mating of two nonresponder strains may also produce F1 mice which are responders, thus indicating the presence of complementary Ir genes (9, 10). We have previously shown that both the dominant gene(s) of the H-2b haplotype and the complementary genes of other haplotypes are located in the H-2 complex and at least one of the complementary genes is in the IC region (10).

The mapping of these Ir genes is complicated by the H-2K and/or H-2D syngeneity requirements in the induction and effector phase of the anti-H-Y cytotoxic response, as well as by the lack of immunogenicity of H-Y antigen associated with certain K and D end antigens (10). But by using appropriate F1 hybrids, we are now able to demonstrate that the dominant Ir gene(s) of the H-2b haplotype is located in the IA region, whereas at least some of the complementary Ir genes are located in the IC region.
Mice were primed to H-Y antigen by the intraperitoneal injection of $1 \times 10^{6}$ viable male spleen cells 3 wk-4 mo before use. The $H-2$ haplotypes of mice used are indicated in Table I.

**In Vitro Sensitizations and Cytotoxic Assay.** The method used to elicit and assay a cytotoxic T-cell response has been previously described (7, 11). Briefly, spleens from female mice primed in vivo were removed and teased apart, and the resulting cell suspension was washed once in a balanced salt solution (BSS)1, and then resuspended at $5 \times 10^6$ cells/ml in bicarbonate-buffered RPMI medium containing 10% fetal calf serum (FCS) with 10 mM N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (Hepes), glutamine, penicillin and streptomycin, and $5 \times 10^{-5}$ M 2-mercaptoethanol. The responding cells were co-cultured with an equal number of 2,000 R irradiated male spleen cells in 25-cm² plastic tissue culture flasks. After 5 days of incubation in a humidified, 10% CO₂ atmosphere, the cultures were harvested, washed once in BSS and then resuspended in Eagle’s minimal essential medium with 10% FCS and 10 mM Hepes. The cell concentration was adjusted and three doubling dilutions made. The various concentrations of attacking cells were then plated in triplicate in wells of a microtiter plate, and $(1-5 \times 10^4)$ $^{51}$Cr-labeled target cells were added per well. The target cells were spleen cells which had been cultured 48-72 h in the presence of 4 µg/ml concanavalin A, labeled for 90 min with $^{51}$Cr-sodium chromate and then washed twice. The attacker:target cell ratios normally used were 8:1, 4:1, 2:1, and 1:1. The plates were spun briefly and then incubated at 37°C in a 10% CO₂ atmosphere for 3 h before harvesting the supernates for gamma counting. Maximum release was that amount of $^{51}$Cr released from Triton-treated target cells; spontaneous release was that released by target cells incubated in medium alone. The corrected percent lysis was computed according to the formula of Wunderlich et al. (12). Regression lines were calculated from the percent of corrected lysis at the four attacker:target (A:T) cell ratios used, and from these lines the percent of corrected lysis at 4:1 A:T was taken. Only when the $r^2$ value for such regression lines lay between 0.9 and 1.0 was the percent of corrected lysis at 4:1 regarded as positive. Each experiment reported in the tables was repeated at least three times and concordant results were obtained. The values given are from representative experiments.

**Results**

The dominance of the Ir gene(s) of the $H-2^b$ haplotype was again shown using (B10 × B10.S)F₁ and (B10 × B10.G)F₁ females as responders, and B10, B10.S, and B10.G males as the stimulator cells. In this respect, these haplotypes ($s$ and $q$) did not seem to be different from those previously reported ($H-2^a$, $H-2^b,$ $H-2^c$).

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1Abbreviations used in this paper: A:T, attacker:target cell ratio; BSS, balanced salt solution; FCS, fetal calf serum; Hepes, N-2-hydroxyethylpiperazin-N'-2-ethane sulfonic acid.
### Table II

**Mapping of the Dominant Ir Gene(s) of the H-2<sup>h</sup> Haplotype in Anti-H-Y Cytotoxic Responses**

| Responder ♀ | Antigen ♂ | Target | Target haplotype (H-2<sup>K,<sub>1</sub> H-2D<sup>+</sup>) | Corrected lysis ± SE<sup>†</sup> | Mapping of HY association |
|-------------|-----------|--------|-------------------------------------------------------------|---------------------------------|-----------------------------|
| B10         | B10       | B10    | K<sup>b</sup>, D<sup*h</sup>                               | 31.4 ± 1.6                      | D<sup>b</sup>                |
|             |           | B10♀   | K<sup>o</sup>, D<sup*h</sup>                               | 1.0 ± 0.5                       |                             |
|             |           | D2.GD♂ | K<sup>o</sup>, D<sup*h</sup>                               | 34.7 ± 0.8                      |                             |
|             |           | B10.A(5R)♂ | K<sup>b</sup>, D<sup*d</sup>              | 3.5 ± 0.4                       |                             |
| (B10 × B10.S)F<sub>1</sub> | B10     | B10    | K<sup>b</sup>, D<sup*h</sup>                               | 24.1 ± 1.3                      | D<sup>b</sup>                |
|             |           | B10♀   | K<sup>o</sup>, D<sup*h</sup>                               | 1.1 ± 0.1                       |                             |
|             |           | D2.GD♂ | K<sup>o</sup>, D<sup*h</sup>                               | 18.6 ± 0.8                      |                             |
|             |           | B10.A(5R)♂ | K<sup>b</sup>, D<sup*d</sup>              | 5.1 ± 1.6                       |                             |
| (B10 × B10.S)F<sub>1</sub> | B10.S    | B10.S♂ | K<sup>s</sup>, D<sup*u</sup>                               | 38.3 ± 0.4                      | D<sup>†</sup>                |
|             |           | B10.S♀ | K<sup>s</sup>, D<sup*u</sup>                               | 0.4 ± 0.2                       |                             |
|             |           | A.TH♂   | K<sup>s</sup>, D<sup*u</sup>                               | -2.7 ± 0.7                      |                             |
| (B10 × B10.G)F<sub>1</sub> | B10     | B10    | K<sup>b</sup>, D<sup*h</sup>                               | 20.2 ± 0.5                      | D<sup>†</sup>                |
|             |           | B10♀   | K<sup>o</sup>, D<sup*h</sup>                               | 3.8 ± 0.2                       |                             |
|             |           | D2.GD♂ | K<sup>o</sup>, D<sup*h</sup>                               | 18.4 ± 0.9                      |                             |
|             |           | B10.A(5R)♂ | K<sup>b</sup>, D<sup*d</sup>              | 1.3 ± 0.4                       |                             |
| (B10 × B10.G)F<sub>1</sub> | B10.G    | B10.G♂ | K<sup>o</sup>, D<sup*h</sup>                               | 6.8 ± 0.9                       | D<sup>s</sup>                |
|             |           | B10.G♀ | K<sup>o</sup>, D<sup*h</sup>                               | 2.9 ± 1.8                       |                             |
|             |           | AQR♂    | K<sup>o</sup>, D<sup*h</sup>                               | -0.9 ± 0.6                      |                             |
| (B10.A(2R) × B10.A(5R))F<sub>1</sub> | B10.A(2R) | B10    | K<sup>b</sup>, D<sup*h</sup>                               | 29.5 ± 1.1                      | D<sup>s</sup>                |
|             |           | B10♀   | K<sup>o</sup>, D<sup*h</sup>                               | 1.6 ± 0.7                       |                             |
|             |           | D2.GD♂ | K<sup>o</sup>, D<sup*h</sup>                               | 32.2 ± 1.3                      |                             |
|             |           | B10.A(5R)♂ | K<sup>b</sup>, D<sup*d</sup>              | -0.4 ± 0.2                      |                             |
| (B10.A(5R) × BALB/c)F<sub>1</sub> | BALB/c  | BALB/c♂ | K<sup>s</sup>, D<sup*u</sup>                               | 22.3 ± 2.3                      | K<sup>s</sup>                |
|             |           | BALB/c♀ | K<sup>s</sup>, D<sup*u</sup>                               | 0.8 ± 0.2                       |                             |
|             |           | D2.GD♂ | K<sup>s</sup>, D<sup*u</sup>                               | 14.7 ± 1.3                      |                             |
|             |           | B10.A♂   | K<sup>s</sup>, D<sup*u</sup>                               | 3.8 ± 0.1                       |                             |
| (B10.A(4R) × BALB/c)F<sub>1</sub> | BALB/c  | BALB/c♂ | K<sup>s</sup>, D<sup*u</sup>                               | -0.9 ± 1.2                      |                             |
|             |           | BALB/c♀ | K<sup>s</sup>, D<sup*u</sup>                               | -0.6 ± 0.1                      |                             |

*Spleen cells from female mice primed in vivo and challenged in mixed lymphocyte culture with the male cells (antigen) shown were assayed against 51Cr-labeled target cells. A:T = 4:1.

and H-2<sup>o</sup> (10): (B10 × B10.S)F<sub>1</sub> or (B10 × B10.G)F<sub>1</sub> female mice immunized in vivo with B10 male cells and restimulated in vitro with the same cells gave an anti-H-Y cytotoxic response indistinguishable from the response of B10 females (Table II). (B10 × B10.S)F<sub>1</sub> female cells stimulated with B10.S male cells gave
an $H-2D^b$-associated cytotoxic response, and the association was with $H-2D^e$ in (B10 × B10.G)$F_1$ females stimulated with B10.G male cells. In the latter case, the cytotoxic response was relatively small (Table II).

The recombinational event in B10.A(5R) strain is to the right of IB (Table I), offering the possibility of localizing the Ir gene(s) of the $H-2^b$ haplotype in IA or IB, but this strain does not produce anti-H-Y cytotoxic cells after in vivo priming and in vitro restimulation with syngeneic male cells (9). Lack of cytotoxicity may be due to the lack of proper associative antigen: we have not found an anti-H-Y cytotoxic response associated with $H-2K^b$ or $H-2D^d$. In (B10.A(5R) × B10.A(2R))$F_1$ hybrids, the B10.A(2R) parent introduces the necessary $H-2D^b$ coded antigen(s), but is a nonresponder. These $F_1$ female mice stimulated with B10.A(2R) male cells gave an $H-2D^b$-associated anti-H-Y response (Table II), thus localizing the dominant Ir gene(s) in IA or IB. This was confirmed by using (B10.A(5R) × BALB/c)$F_1$ hybrid females which gave an $H-2K^b$-associated anti-H-Y cytotoxic response when stimulated with BALB/c male cells (Table II). (B10.A(4R) × BALB/c)$F_1$ female mice did not respond to stimulation with BALB/c male cells, excluding the possibility that the dominant gene is to the right of the IA region (Table II). This negative result also indicated that B10.A(4R) mice do not possess Ir genes capable of complementing the Ir genes in the $H-2^d$ haplotype.

In a previous publication (10) we demonstrated that (CBA × B10.S)$F_1$ female mice are responders, but when CBA was replaced by B10.A(2R) (having IC$^b$, S$^b$, G$^b$, and D$^b$) in making the $F_1$ hybrid, we got a nonresponder, indicating that IC$^b$ is probably mandatory in H-2$^b$/H-2$^b$ complementation. We have now studied this complementation further (Table III). We confirmed the location of Ir gene(s) in the H-2 complex and particularly in the IC$^a$ region by demonstrating that (B10.HTT × B10.S)$F_1$ female mice were also able to respond to B10.S male cell stimulation (Table III). Despite the fact that IC$^a$ Ir genes (in B10.A(2R)) did not complement with the Ir genes of $H-2^b$ haplotype, we found that (B10.D2 × B10.S)$F_1$ females were responders, although only when stimulated with B10.S cells. Reactivity to only male cells of one parental strain (B10.S) was also noticed in (B10.S × B10.G)$F_1$ females (Table III).

By using B10.G mice instead of B10.S mice in producing some of the hybrids mentioned in Table III, we obtained $F_1$ females whose responsiveness indicates the importance of IC$^a$ also in H-2$^b$/H-2$^a$ complementation: (CBA × B10.G)$F_1$ females responded to male cells of both parental strains, whereas (B10.A(2R) × B10.G) were nonresponders (Table IV). For mapping the Ir genes in H-2$^b$/H-2$^a$ complementation, we used (CBA × A)$F_1$ hybrids, and the positive response of these mice to CBA male cell stimulation indicates that both of the complementary genes are to the right of the IE region (Table IV).

Discussion

The cytotoxic response against the H-Y antigen represents those T-cell responses which have revealed the importance of the association of T-cell cytotoxicity and the antigens encoded by the major histocompatibility complex. It was first demonstrated that the anti-H-Y cytotoxic cell and the male target cell must share H-2K and/or H-2D coded antigens for the target cell lysis to occur (7). These kinds of syngeneity requirements seem to be also necessary in
### Table III

Mapping of the Complementary Ir Genes in H-2<sup>+</sup>/H-2<sup>-</sup> Complementation*  

| Responder ♀ | Antigen ♂ | Target haplotype (H-2<sup>K</sup>, H-2<sup>D</sup>) | Corrected lysis ± SE‡ | Mapping of H-Y association |
|------------|-----------|---------------------------------|----------------------|---------------------------|
| (CBA x B10.S)F<sub>1</sub> | B10.S | B10.S, K<sup>+</sup>, D<sup>+</sup> | 42.7 ± 2.8 | D<sup>+</sup> |
| | | B10.S, K<sup>+</sup>, D<sup>+</sup> | 3.8 ± 0.9 | |
| | | A.TH, K<sup>+</sup>, D<sup>+</sup> | 2.3 ± 1.1 | |
| (B10.A(2R) x B10.S)F<sub>1</sub> | B10.S | B10.S, K<sup>+</sup>, D<sup>+</sup> | 1.1 ± 1.6 | |
| | | B10.S, K<sup>+</sup>, D<sup>+</sup> | 0.7 ± 0.5 | |
| (B10.H2 × B10.S)F<sub>1</sub> | B10.S | B10.S, K<sup>+</sup>, D<sup>+</sup> | 10.0 ± 2.6 | D<sup>+</sup> |
| | | B10.S, K<sup>+</sup>, D<sup>+</sup> | 2.2 ± 1.2 | |
| | | A.TH, K<sup>+</sup>, D<sup>+</sup> | 2.9 ± 1.4 | |
| (B10.D2 × B10.S)F<sub>1</sub> | B10.S | B10.S, K<sup>+</sup>, D<sup>+</sup> | 42.6 ± 1.4 | D<sup>+</sup> |
| | | B10.S, K<sup>+</sup>, D<sup>+</sup> | 0.2 ± 0.1 | |
| | | A.TH, K<sup>+</sup>, D<sup>+</sup> | 1.8 ± 0.3 | |
| (B10.D2 × B10.S)F<sub>1</sub> | B10.D2 | B10.D2, K<sup>+</sup>, D<sup>+</sup> | 3.9 ± 0.4 | |
| | | B10.D2, K<sup>+</sup>, D<sup>+</sup> | 3.7 ± 0.1 | |
| | | B10.A, K<sup>+</sup>, D<sup>+</sup> | 0.9 ± 0.1 | |
| | | D2, K<sup>+</sup>, D<sup>+</sup> | 0.3 ± 0.6 | |
| (B10.G × B10.S)F<sub>1</sub> | B10.S | B10.S, K<sup>+</sup>, D<sup>+</sup> | 18.6 ± 0.7 | D<sup>+</sup> |
| | | B10.S, K<sup>+</sup>, D<sup>+</sup> | 3.3 ± 0.2 | |
| | | A.TH, K<sup>+</sup>, D<sup>+</sup> | 2.4 ± 0.3 | |
| (B10.G × B10.S)F<sub>1</sub> | B10.G | B10.G, K<sup>+</sup>, D<sup>+</sup> | 1.9 ± 0.1 | |
| | | B10.G, K<sup>+</sup>, D<sup>+</sup> | 5.1 ± 0.7 | |
| | | AQR, K<sup>+</sup>, D<sup>+</sup> | 2.8 ± 1.3 | |

* Spleen cells from female mice primed in vivo and challenged in mixed lymphocyte culture with the male cells (antigen) shown were assayed against 51Cr-labeled target cells.
‡ A:T = 4:1.

the induction phase of anti-H-Y cytotoxicity (10)<sup>2</sup>. In addition to this kind of genetical requirement for cytotoxicity, the anti-H-Y response in vivo and in vitro is regulated by other genes within the H-2 complex in mice, e.g. the rejection time of male skin grafts in female recipients of different inbred mouse strains varies from about 2 wk to permanent graft acceptance, and this is mainly the result of genes within H-2 (1).

From the earlier skin grafting studies it was evident that the mouse strains

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<sup>2</sup> Elizabeth Simpson, R. D. Gordon, and D. W. Bailey. Anti-H-Y responses of H-2<sup>+</sup> mutant mice. Manuscript submitted for publication.
Table IV
Mapping of the Complementary \( I_r \) Genes in \( H-2^b/H-2^k \) and \( H-2^r/H-2^d \) Complementations*

| Responder \( \hat{?} \) | Antigen \( \hat{\delta} \) | Target | Target haplotype \((H-2^K, H-2^D)\) | Corrected lysis \( \pm SE\hat{\delta} \) | Mapping of \( H-Y \) association |
|---------------------|----------------|--------|---------------------------------|-----------------|----------------------|
| (CBA \( \times \) B10.G)\( F_1 \) | B10.G | B10.G \( \hat{\delta} \) | \( K^r, D^r \) | 22.1 \( \pm 1.1 \) | \( D^p \) |
|                     |       | B10.G \( \hat{\gamma} \) | \( K^r, D^r \) | 3.9 \( \pm 0.2 \) |               |
|                     |       | AQR \( \hat{\delta} \) | \( K^r, D^d \) | -2.9 \( \pm 0.7 \) |               |
| (CBA \( \times \) B10.G)\( F_1 \) | CBA    | CBA \( \hat{\delta} \) | \( K^b, D^b \) | 16.0 \( \pm 0.5 \) | \( K^b, D^p \) |
|                     |       | CBA \( \hat{\gamma} \) | \( K^b, D^b \) | 3.8 \( \pm 0.3 \) |               |
|                     |       | B10.A \( \hat{\delta} \) | \( K^b, D^d \) | 13.7 \( \pm 0.4 \) |               |
|                     |       | C3H.OH \( \hat{\delta} \) | \( K^b, D^b \) | 12.5 \( \pm 1.4 \) |               |
| (B10.A(2R) \( \times \) B10.G)\( F_1 \) | B10.G | B10.G \( \hat{\delta} \) | \( K^r, D^r \) | -1.9 \( \pm 0.1 \) |               |
|                     |       | B10.G \( \hat{\gamma} \) | \( K^r, D^r \) | -1.1 \( \pm 0.7 \) |               |
| (B10.A(2R) \( \times \) B10.G)\( F_1 \) | B10.A(2R) | B10 \( \hat{\delta} \) | \( K^b, D^b \) | 1.7 \( \pm 0.8 \) |               |
|                     |       | B10 \( \hat{\gamma} \) | \( K^b, D^b \) | 0.8 \( \pm 0.1 \) |               |
| (CBA \( \times \) A)\( F_1 \) | CBA    | CBA \( \hat{\delta} \) | \( K^r, D^r \) | 14.2 \( \pm 0.5 \) | \( K^b, D^p \) |
|                     |       | CBA \( \hat{\gamma} \) | \( K^r, D^r \) | 0.7 \( \pm 0.9 \) |               |
|                     |       | A \( \hat{\delta} \) | \( K^r, D^d \) | 12.4 \( \pm 1.1 \) |               |
|                     |       | C3H.OH \( \hat{\delta} \) | \( K^r, D^p \) | 10.7 \( \pm 0.7 \) |               |

* Spleen cells from female mice primed in vivo and challenged in mixed lymphocyte culture with the male cells (antigen) shown were assayed against \( ^{51} \)Cr-labeled target cells.  
† A:T = 4:1.

with \( H-2^b \) haplotype were exceptional: the male skin graft rejection was most rapid in these mice, and the \( F_1 \) hybrids having one \( H-2^b \) parent were also able to reject the male skin from the nonresponder parent, thus indicating the dominance of the \( H-2^b \) haplotype genes (1). The production of cytotoxic cells is in accordance with these results: \( H-2^b \) strain females are the only ones able to produce cytotoxic cells after in vivo priming and in vitro restimulation with syngeneic male cells, and \( F_1 \) hybrid females with one \( H^b \) parent are able to produce cytotoxic cells against the male cells from both the \( H-2^b \) and the nonresponder parents (8, 10, Table II). The existing \( H-2 \) recombinant mouse strains are not suitable for mapping of the \( H-2^b I_r \) genes(s) regulating the formation of cytotoxic cells because of the lack of an appropriate associative antigen. Therefore, we produced \( F_1 \) hybrids where one parent had a part of the \( H-2^b \) haplotype, and the other parent introduced the associative antigen. The positive responses in \((B10.A(5R) \times B10.A(2R)) F_1\) females to \( B10.A(2R) \) male cell stimulation, and in \((B10.A(5R) \times BALB/c) F_1\) females to BALB/c male cell stimulation, localize this \( I_r \) gene in the \( IA \) or \( IB \) regions. Similar results were obtained recently by von Boehmer et al. (13). The lack of response in \((B10.A(4R) \times BALB/c) F_1\) females to BALB/c male cell stimulation excludes the \( IB \) region
and also indicates that the B10.A(4R) strain does not contain Ir genes able to complement the genes in the H-2^a haplotype. Thus, the gene regulating the formation of cytotoxic cells in the H-2^b haplotype is located in the IA region.

The other type of Ir genes enabling the production of anti-H-Y cytotoxic cells are the complementary genes; the F_1 hybrid between two nonresponder strains may be a responder (9, 10). By using H-2 congenic mice we have shown that these genes are also located in the H-2 complex (10). Mapping of these genes within H-2 has been based on the use of F_1 hybrids (trans-complementation). Only one of the recombinant strains tested so far has been positive (C3H.OH), and we think that this represents appropriate antigen presentation (which will be discussed later) rather than cis-complementation of Ir genes. The negative results in available recombinant strains do not, of course, indicate that cis-complementation does not exist in this response.

In H-2^v/H-2^v, H-2^b/H-2^b and H-2^b/H-2^b complementations we have been able to map one or both of the complementary genes. The only F_1 hybrid which shows that both of these complementary genes are in the IC region is (CBA × A)F_1. These F_1 females stimulated with CBA male cells give an H-2K^k-associated anti-H-Y cytotoxic response. The recombination in A strain is between IE and IC and this strain has the right associative antigen H-2K^k, but it is a nonresponder, indicating that genes in IC^x cannot complement the H-2^e haplotype genes to the left of IC. In H-2^v/H-2^v and H-2^b/H-2^b complementations, the Ir gene in IC^x seems to be mandatory, but the localization of the complementary genes in H-2^v and and H-2^v haplotypes is not known.

The mapping of the H-2^v complementing gene to the IC region was based on the lack of response in (B10.A(2R) × B10.S)F_1 and (B10.A(2R) × B10.G)F_1, female mice indicating that the Ir gene(s) in IC^x cannot complement the genes in H-2^v or H-2^v haplotype. Nevertheless, we have obtained a positive response in (B10.D2 × B10.S)F_1 hybrids, although only when stimulated with B10.S male cells. This can be explained by assuming that in this complementation, the H-2^v gene is not in IC; but another possibility is that in (B10.D2 × B10.S)F_1 mice, the B10.S parent is only introducing the right associative antigen (H-2D^d), whereas all the immune response genes come from the B10.D2 parent. This we call antigen presentation. Mice of H-2^v haplotype might possess Ir genes enabling the response against H-Y associated with H-2D^d (as well as with H-2D^k in the case of the recombinant strain, C3H.OH), but not against H-Y associated with its own H-2K- or H-2D-coded antigens. The negative response to B10.D2 male cell stimulation in (B10.D2 × B10.S)F_1 females may also be due to the lack of Ir gene complementation. Antigen presentation cannot be the explanation, for example, in the response of (CBA × A)F_1 females to CBA male cell stimulation because both strains already possess H-2K^k coded antigens and the anti-H-Y response in F_1 females is H-2K^k-associated. By using suitable chimeric mice it might be possible to separate the responder cells and test the question of antigen presentation.

In conclusion, our results indicate the complexity of interaction between cytotoxic T cells and major histocompatibility complex-coded antigens in a response against a cell surface antigen, H-Y. First, T cells are able to 'see' this antigen only in the presence of self H-2K- and/or H-2D-coded antigens. In addition to this, Ir genes exert a regulatory function: there is a dominant Ir
gene in the IA region of the H-2\textsuperscript{b} haplotype and two complementary genes, both probably in IC, which enable the response in F\textsubscript{1} females derived from matings of certain two nonresponder parental strains. The syngeneity requirements for T-cell cytotoxicity are explainable by the assumption that there are two receptors on a T cell, one for the foreign antigen and the other for the H-2D- and H-2K-coded antigens (syngeneic or allogeneic). The data in favor of this dual recognition model has been reviewed recently by Janeway et al. (14). However, the notion of an altered self antigen, composed of H-Y plus self H-2, cannot be formally excluded.

The basic mechanism of action of Ir genes is still an open question and it seems that in different kinds of immune responses, the cellular locus of Ir gene function may be different (T cell, B cell, macrophage, or their interactions), as discussed recently by Katz (15). The fact that H-2K and/or H-2D regions restrict the activity of T-cell mediated cytotoxicity has led to the hypothesis that the controlling Ir genes may be found in these regions (16). Data from experiments using H-2\textsuperscript{b} K and D end mutant mouse strains which make anti-H-Y responses indistinguishable from wild strain H-2\textsuperscript{b} mice do not fit such a hypothesis.\textsuperscript{2} Other experiments show the failure to obtain a cytotoxic response against H-Y associated with certain K or D end antigens (e.g. H-2K\textsuperscript{b}, H-2D\textsuperscript{4}, and H-2K\textsuperscript{d}) (9, 10). This probably represents preferential association of H-Y antigen with certain structures rather than Ir gene function per se. It is more likely that Ir genes for cytotoxic responses map in the I region. The mechanism of action of Ir genes mapping in the I region is not readily explainable. There are data to suggest that collaboration between different T-cell subpopulations and also between macrophages is necessary in H-2-restricted cytotoxic responses (17-19) and this may offer the possibility of localizing the site of action of these genes (helper T cells?, macrophages?). The interactions of complementary Ir genes may be manifested in these cellular collaborations in the manner described by Munro and Taussig for T-cell-B-cell collaboration (20), or at the level of gene products (Ia antigens?), each gene coding, for example, one polypeptide chain in the IC region. The role of these H-2-restricted anti-H-Y cytotoxic cells in the male skin graft rejection has been studied by us and the results are shown in the accompanying paper (21).

Summary

The secondary cytotoxic responses to the male specific antigen (H-Y) in mice show H-2 restrictions so that cytotoxic female cells must share K and/or D end antigen with the male target cells. The production of cytotoxic cells is under the control of Ir genes, thus offering the possibility of studying the function of Ir genes in H-2-restricted cytotoxic responses. There are two kinds of Ir genes regulating this response; the dominant gene in the H-2\textsuperscript{b} haplotype and complementary genes in other haplotypes. Now we have been able to map the dominant gene and some of the complementary genes: the dominant gene is in IA\textsuperscript{b}, and in H-2\textsuperscript{b}/H-2\textsuperscript{a} complementation, the Ir genes are in IC\textsuperscript{a} and IC\textsuperscript{c}, and in H-2\textsuperscript{a}/H-2\textsuperscript{b} and H-2\textsuperscript{b}/H-2\textsuperscript{b} complementations, at least the H-2\textsuperscript{c} gene is in IC.

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