In a recent report (1) we have postulated the existence of helper cells in the cytotoxic T-cell (CTL)1 response to H-Y antigen: as shown here and also in other experiments (2, 3), at least one H-2b locus in the left-hand side of the H-2 complex controls H-Y-specific CTL responses. The response gene(s) do not encode restricting elements, because H-Y-specific CTL restricted to the left-hand side of H-2b could not be found (2, 3). We have postulated therefore that H-2b encodes a gene product that is recognized by helper cells and that is required for the CTL response to H-Y antigen.

Chimeras constructed by injecting bone marrow cells from B6 × CBA responder mice in irradiated CBA nonresponder recipients did not respond to H-Y antigen, even though H-2b encodes restricting elements which can be recognized by H-Y-specific CTL. We have argued that nonresponsiveness was a result of the fact that differentiating lymphocytes in B6 × CBA → CBA chimeras did not encounter H-2b gene products during differentiation in the thymus and therefore that H-2b-restricted helper cells, required in the H-Y-specific CTL response, would not be generated (1).

The following experiments provide direct evidence for the existence of such helper cells.

Materials and Methods

Mice. C57BL/6 (B6), CBA/J, DBA/2, and B6 × CBA F1 hybrid mice were obtained from the Institut für medizinische Forschung, A.G., Füllinsdorf, Switzerland. B10.A (5R) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Bone Marrow Chimeras. Bone marrow chimeras were prepared by injecting lethally irradiated (880 rad) mice with 2 × 10^7 anti-Thy 1.2-treated bone marrow cells, as previously described (4). The chimeras were used 2- to 3-mo after reconstitution.

Cell Cultures. H-Y-specific CTL were obtained in vitro as previously described (2).

Adoptive Transfer Experiments. B6 × CBA F1 recipient mice were irradiated with 750 rad. 4 h later, they received i.v. either 5 × 10^7 or 10^8 spleen cells from various mice together with 2 × 10^7 irradiated (2,000 rad) B6 × CBA male cells. The mice were killed 10-14 d after transfer and spleen cells were stimulated in vitro with irradiated (2,200 rad) B6 × CBA male cells.

Anti H-2 Sera. B10.BR anti-B10 and B10 anti-B10.BR sera were obtained from Searle Diagnostic, High Wycombe, Buckinghamshire, England. A 1:10 dilution was used at a cell concentration of 10^7 cells per ml. Appropriate cytotoxicity controls with guinea pig complement were included.

Results

The Response of Various F1 Hybrid → Parent Chimeras to Male Antigen. Anti-Thy 1.2-treated bone marrow cells from female B6 × CBA mice were injected i.v. in either

1 Abbreviation used in this paper: CTL, cytotoxic T cell.
Fig. 1. CTL responses of cells from B6 × CBA → B6, B6 × CBA → CBA, and B6 × CBA → B10.A (5R) chimeras. All chimeras were immunized by injecting them with 2 × 10^7 x-irradiated (2,000 rad) B6 × CBA male cells. B6 × CBA male cells were also used for stimulation in vitro. In control cultures allogeneic DBA/2 stimulators were used. Numbers on the abscissa represent numbers of initially cultured cells, the descendants of which lyse various targets: k, H-2^k; b, H-2^b; d, H-2^d.

B6, CBA, or B10.A (5R) irradiated female recipients. The mice were primed 2½ mo after reconstitution with 2 × 10^7 irradiated male cells from B6 × CBA mice. 14 d later, spleen cells were prepared and stimulated in vitro with B6 × CBA male cells. As shown in Fig. 1, only spleen cells from B6 × CBA → B6 chimeras generated male-specific CTL. This response was entirely restricted to H-2^b, whereas B6 × CBA cells...
FIG. 2. CTL responses (targets: [A], H-2^k; [B], H-2^b; [C], H-2^d) of cells from adoptively transferred mice receiving either B6 × CBA F1 (k × b, column 1) hybrid cells or cells from B6 × CBA → CBA as well as B6 × CBA → B6 chimeras (k × b → b + k × b → k, column 2), from B6 × CBA → CBA as well as B6 × CBA → B10.A (5R) chimeras (k × b → i5 + k × b → k, column 3), from B6 × CBA → B10.A (5R) chimeras alone (k × b → i5, column 4), from B6 × CBA →
from normal mice usually kill male H-2^k targets better than male H-2^b targets. Thus, the radioreistant tissue of the recipients, most likely the thymus (5, 6, 7) entirely determined the restriction specificity of male-specific CTL. As reported previously (1), cells from neither B6 × CBA → CBA, nor B6 × CBA → B10.A (5R) chimeras could be induced to lyse male targets. These results were obtained in four independent experiments. Cells from all chimeras when stimulated with DBA/2 cells were capable of lysing allogeneic DBA/2 target cells (Fig. 1, last column).

**Involvement of Helper Cells in the Male-specific CTL Response.** That cells from B6 × CBA → B10.A (5R) and B6 × CBA → CBA could not be induced to lyse male targets may be explained in the following way (1): cells differentiating in B10.A (5R) mice encounter nonpermissive K and D products for H-Y-specific CTL, but permissive H-2^b gene products for H-Y-specific help. (Nonpermissive gene products means that either H-Y-specific CTL or helper cells cannot be restricted to products encoded by these alleles. In Fig. 1 we have encircled the alleles permissive for H-Y-specific CTL, K^k, D^k, D^b, and the allele permissive for helper cells, provisionally, IA^b.) Cells differentiating in a H-2^k host would encounter permissive K^k and D^k alleles for H-Y-specific CTL, but a nonpermissive IA^k allele for helper cells. If this interpretation were correct, cells from B6 × CBA → B6 or B6 × CBA → B10.A (5R) on the one hand (helper) and B6 × CBA → CBA chimeras on the other (H-2^k-restricted CTL precursor) should complement each other to produce a H-Y-specific CTL response restricted to H-2^k. We therefore transferred spleen cells from B6 × CBA → B6 as well as B6 × CBA → B10.A (5R) either alone or together with spleen cells from B6 × CBA → CBA female recipients and primed the mice with B6 × CBA male cells. 10–14 d after transfer, spleen cells from the recipients were cultured in vitro with male B6 × CBA stimulator cells. In separate cultures, cells from various recipients were stimulated with H-2^d cells. In control experiments, we transferred cells from normal B6 × CBA F1 hybrids.

Representative examples of these transfer experiments are shown in Fig. 2 A and B: 10^8 (circles) or 5 × 10^7 (triangles) B6 × CBA female spleen cells were transferred in x-irradiated B6 × CBA recipients and cultured subsequently with B6 × CBA male stimulator cells; cells from all four transferred mice lysed male H-2^k targets and two out of the four also lysed male H-2^b targets (Fig. 2 A and B, first column). Another group of four mice received 5 × 10^7 cells from B6 × CBA → B6 chimeras as well as 5 × 10^7 cells from B6 × CBA → CBA chimeras. Cells from all four transferred mice lysed male H-2^k targets. Lysis of male H-2^b targets was not observed here (Fig. 2 B, second column). Similarly, cells from all recipients of 5 × 10^7 cells from B6 × CBA → B10.A (5R) as well as 5 × 10^7 cells from B6 × CBA → CBA chimeras lysed male H-2^k targets but not H-2^b targets (Fig. 2 A and B, third column). Although lysis of H-2^k targets was equally effective with cells from recipients of

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CBA alone (k × b → k, column 5), and from B6 × CBA → B6 alone (k × b → b, column 6). Adoptively transferred B6 × CBA mice were primed with 2 × 10^7 x-irradiated (2,000 rad) B6 × CBA male cells. B6 × CBA F1 hybrid male cells were also used to stimulate cells in vitro. In control cultures, allogeneic DBA/2 stimulators were used. For numbers on abscissa, see Fig. 1. The data were obtained in two independent experiments represented either by circles or triangles. A total of four mice per group was transferred. Spleen cells from two mice per group were cultured 10 d, the other two, 14 d after transfer. In both cases, the responses were similar. The data shown were obtained 14 d after transfer.
only F1 or a mixture of chimeric cells (Fig. 2A, columns 1–3, circles) in one experiment cells from mice receiving normal F1 cells killed male H-2k targets significantly better than the mixture of chimeric cells (Fig. 2A, columns 1, 2, triangles).

Recipients injected with either $10^8$ (Fig. 2A, B, and C, fourth column, circles) cells from B6 × CBA B10.A (5R) alone or, $10^8$ (circles) or $5 \times 10^7$ cells (triangles) from B6 × CBA → CBA chimeras alone could not be induced to lyse H-2b or H-2k male targets, but responded well to H-2d alloantigens (Fig. 2A, B, and C, fifth column).

Recipients receiving $10^8$ (circles) or $5 \times 10^7$ (triangles) cells from B6 × CBA → B6 chimeras alone could be induced to lyse male H-2b targets but not male H-2k targets (Fig. 2A and B, sixth column).

In no combination was lysis observed on either H-2b or H-2k female targets. Results like those given in Fig. 2 were obtained in four independent experiments. In two other experiments, we failed to induce H-Y-specific CTL, even with cells from recipients injected with normal B6 × CBA F1 hybrid cells. In no case did normal F1 hybrid cells respond when the mixture of chimeric cells did not. Thus, the results indicate that cells from two types of chimeras (helper cells and precursors for CTL) can combine to generate a male-specific CTL response.

Are Helper Cells Restricted in Their Interaction with CTL or CTL Precursors? The results in Fig. 2 indicate that T helper cells for H-Y-specific CTL are generated only in mice that express in their radioresistant tissue H-2b alleles encoded in the left-hand side of the H-2 complex. This however does not seem to be the only requirement for their operation: we noted previously (1) that cells from B10.A (5R) → B6 × CBA but not from CBA → B6 × CBA, or B10.BR → B6 × CBA chimeras could be induced to generate H-Y-specific CTL. Thus, there seemed to be a requirement for the expression of H-2b products by the responding lymphocytes themselves. This requirement could be interpreted in several ways. First, the interaction of T helper cells with CTL or their precursors requires that CTL express IA b antigens to which the helper cells are restricted (1, 8). This would be analogous to T-B-cell collaboration (9). Second, H-2k cells may not express receptors for male cells or responses to male cells may be suppressed in H-2k mice. Third, in H-2k → H-2k × H-2b chimeras there is no sufficient presentation of H-Y antigen in the context of IA b, because the vast majority of hemopoietic cells express H-2k antigens only. (The hemopoietic cells of the recipient may be required to present H-Y antigen to T helper cells.) To distinguish between possibilities one and two, on the one hand, and three on the other, we reconstituted irradiated B6 × CBA recipients with 12 × 106 B6 and 6 × 106 CBA bone marrow cells. 2 mo after reconstitution, ~60% of the lymphocytes were H-2k, whereas 40% were H-2b. The chimeras were immunized with male B6 × CBA cells and stimulated in vitro by the same cells and, in separate cultures, by DBA/2 cells. As shown in Fig. 3, >90% of the H-Y-specific CTL could be killed by an anti-H-2b serum. Anti-H-2k serum had no additional effect and, in other experiments, did not affect the H-Y-specific CTL response (not shown). In contrast, <50% of anti-H-2k CTL from these chimeras were killed by anti-H-2b serum, whereas anti-H-2k plus anti-H-2k together eliminated >90% (Fig. 3). This indicates that only a few, if any, H-Y-specific CTL were derived from H-2k donor cells, whereas a substantial proportion of H-2k-specific CTL expressed H-2k antigens.

A failure of antigen presentation in the context of IA b cannot account for the unresponsiveness of H-2k lymphocytes in this case. We favor therefore the idea that,
in vivo, the interaction of helper and killer cells is possible only when killer cells express antigens encoded in the left end of the H-2$^b$ haplotype.

Discussion

At present, the CTL response to H-Y is one of the few systems where several nonpermissive H-2 alleles, and therefore, several nonresponder strains, have been clearly documented (1, 2, 3). Haas et al. (10), and Mullbacher and Blanden (11) have recently made similar observations in a CTL response to haptenated cells or to virus-infected cells, identifying several nonresponder strains. In addition, the cytotoxic H-Y response is the only system where mice were nonresponders despite the fact that they expressed K and D products to which H-Y-specific CTL could be restricted (1, 2, 3). Thus, additional permissive H-2 gene products are required, and in several mouse strain combinations these have been shown to map in the left-hand side of the H-2$^b$ complex, possibly IAb. We found an absolute requirement for H-2$^b$ gene products to see male-specific responses, i.e., many other mouse strains, including k × a, k × d, and k × s F1 hybrids, did not generate H-Y-specific CTL.

This set of phenomena differs from haplotype preference originally described by Schmitt-Verhulst and Shearer (12) and Gordon et al. (13). In their experiments, CTL restricted to certain K or D products dominate the response, whereas CTL restricted to other K or D alleles present in the same mouse strains make only a minor contribution to the response. In the case of the H-Y response in B6 × CBA F1 hybrids, H-Y-specific CTL restricted to H-2$^b$ dominate the response. We have shown that preference of H-Y-specific CTL for H-2$^b$ occurs also in B6 → B6 × CBA chimeras, i.e., that the radioreistant tissue of the chimeric hosts influences the preference of H-Y-specific CTL for certain H-2 alleles (14). Similar observations on allele preference of virus-specific CTL have been made by Zinkernagel et al. (15, 16), Doherty et al. (17), and Kurrle et al. (18). In the response to vaccinia virus, no CTL restricted to D$^k$
could ever be demonstrated. This failure may be a result of either the fact that CTL restricted to all K alleles tested dominate the response or that the Dk allele is nonpermissive for Dk-restricted vaccinia-specific CTL.

Our experiments point to the existence of distinct Ir genes for helper cells and T killer cells in the response to H-Y antigen. The B10.A (5R) strain expresses permissive alleles for helper cells but nonpermissive alleles for H-Y-specific CTL, whereas H-2k strains express nonpermissive alleles for helper cells, but permissive alleles for H-Y-specific CTL.

Kralova and Demant (19) reported that in an H-Y-specific host-versus-graft assay, female recipients responded significantly only when antigen was given in the context of H-2b antigen, i.e., B10.A (5R) male cells, and when recipient mice expressed antigens encoded in the left-hand side of H-2b. It appears that in these experiments, a proliferative response of T cells, which function as helper cells for generation of H-Y-specific CTL in our experimental system, was observed.

The radioresistant tissue, the thymus, of chimeric hosts determines the responder phenotype of lymphocytes, according to experiments by Zinkernagel (5), Bevan and Fink (6), and Waldmann et al. (7). Our results indicate that the two complementary cell types, helper cells on the one hand and CTL on the other, are restricted to different H-2 gene products. Thus, Ir gene products may be encoded by K, D, or I region alleles depending on what cell type is under study. The concept that Ir gene products are identical with the restricting elements is very well in line with early experiments reported by Katz et al. (20) and Shevach and Rosenthal (21) showing that in F1 hybrids between high- and low-responder mice T cells could only interact with cells from the high-, but not low-responder parental strain.

We are providing here the first direct evidence for participation of Ir gene-controlled T helper cells in CTL responses to non-H-2 antigens. H-2-restricted T helper cells in CTL responses have been postulated by Zinkernagel et al. (8) on the basis of experiments done in IA-incompatible allogeneic chimeras which did not generate any virus-specific CTL. It is well possible, even though not documented, that nonresponsiveness was the outcome of H-2-restricted helper cells being unable to interact with CTL in such mice. Our experiments on H-Y responses in double chimeras are compatible with the idea that T help requires the expression of permissive H-2 gene products on CTL and their precursors, even though other possibilities have not been formally ruled out.

Helper cells seemed essential for generation of H-Y-specific CTL in our experimental system. This does not mean that help would be required in CTL responses to all other antigens as well. We found that in completely allogeneic chimeras excellent CTL responses to H-2 antigens were obtained which, according to the suggestion of Zinkernagel et al. (8), would be independent of T-cell help.

Summary

The H-Y-specific cytotoxic T-cell response requires helper cells: cells from bone marrow chimeras B6 × CBA → B6, B6 × CBA → B10.A (5R), or B6 × CBA → CBA are each unable to respond to H-2k male cells. If, however, cells from B6 × CBA → B6 or B6 × CBA → B10.A (5R) chimeras are adoptively transferred together with cells from B6 × CBA → CBA chimeras, H-Y-specific CTL restricted to H-2k can be obtained. Thus, cells from B6 × CBA → B6 or B6 × CBA → B10.A (5R)
chimeras (restricted to the left end of the H-2<sup>b</sup> haplotype) can help CTL precursors from B6 × CBA → CBA chimeras (restricted to H-2<sup>b</sup>). The two classes of T cells required for the CTL response to H-Y antigen are controlled by different IR genes. All H-Y-specific CTL obtained from chimeras B6 + CBA → B6 × CBA were found to be of B6 origin. This suggests that CTL or their precursors must express antigens encoded in the left end of the H-2<sup>b</sup> haplotype for interaction with helper cells.

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