IN A FULLY H-2 INCOMPATIBLE CHIMERA, T CELLS OF DONOR ORIGIN CAN RESPOND TO MINOR HISTOCOMPATIBILITY ANTIGENS IN ASSOCIATION WITH EITHER DONOR OR HOST H-2 TYPE*

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Despite much recent interest and effort, the role played by major histocompatibility complex products in the regulation of T-cell responses remains perplexing. In 1972 it was observed that mouse T and B cells would only cooperate in an antibody reponse if they shared certain regions of H-2 (1). Subsequently, H-2 gene products were also found to be involved in cytotoxic T-cell reactions, and it was postulated that the killer T cell must bear H-2 molecules in common with those of its target in order to effect lysis (2-6). Later studies with radiation chimeras showed that this is not the case, but that the H-2 region must be shared between the cells used to stimulate the response and the targets; a killer T cell that was itself H-2 type A, after having grown up in an (A × B)F$_1$ could be stimulated to lyse H-2 type B virus-infected or trinitrophenyl-modified targets (7-9). Such chimeras were also found to contain A type helper T cells which can cooperate with B type B cells (10). It was then postulated that T-cell precursors "learn" to recognize the H-2 type of the host as self (11). Recent evidence shows that the host H-2 type of a chimera does distinctly influence the specificity of the responding T-cell population (12, 13) and that it is the H-2 type of the thymus that is important (13). Most of this work has been done with semiallogeneic chimeras (e.g., "A" bone marrow into an irradiated [A × B]F$_1$, or [A × B]F$_1$ bone marrow into an "A" or [A × C]F$_1$) where the responses were very strongly restricted by the H-2 type of the host. A small number of completely allogeneic chimeras was tested (e.g., "A" bone marrow into "B") and appeared to be immunoincompetent. The virtually absolute restriction of the semiallogeneic chimeras as well as the immunoincompetence of the fully allogeneic chimeras has led to much speculation and has been quoted as suggestive evidence for the dual recognition model of T-cell receptors (13).

We report here that in contrast to the results with virus-infected mice, fully allogeneic chimeras made by repopulating irradiated BALB/c(H-2^d) mice with BALB.B(H-2^b) bone marrow are well able to respond to minor histocompatibility

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[1] *Abbreviations used in this paper*: B10, C57BL/10Sn; C, BALB/c; C.B, BALB.B; C.K., BALB.K; Con A, concanavalin A; CTL, cytotoxic T lymphocyte; H antigen, histocompatibility antigen.

$(H)^2$ antigens, and that the killer T cells that are themselves $H-2^b$ can recognize minor H antigens on either $H-2^b$ or $H-2^d$ targets.

Materials and Methods

Mice. C57BL/10Sn($H-2^b$) (B10), B10.D2/nSn($H-2^d$), B10.G($H-2^q$), B10.BR/SgSn($H-2^k$), and D1.C($H-2^d$) mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. The BALB/cKe($H-2^d$) (C), and BALB.B($H-2^b$) (C.B.) mice used for making the chimeras were bred at the Salk Institute, San Diego, Calif. The C, C.B, BALB.K($H-2^k$) (C.K), and (BALB/c × BALB.B) (C × C.B) used for target and stimulator cells were bred at the University of California San Diego, from breeding pairs generously given to us by Dr. Frank Lilly (Albert Einstein Medical College, N.Y.).

Relationship of B10 Series, BALB Series, and DBA/1 Series Mice. B10, B10.D2, B10.BR, and B10.G differ genetically by a small segment of chromosome 17 which carries the H-2 gene complex and Tla, and are otherwise probably identical (14, 15). D1.C is a congenic line made by putting the $H-2^d$ of BALB/c on the DBA/1 background. D1.C has some minor H antigens in common with the B10 series mice that are not expressed by BALB/c, and was sometimes used as a substitute for B10.D2 which were in short supply.

C, C.B, and C.K also differ only at the H-2 gene complex. The C mice from Salk Institute differ from those of Lilly in that they possess some minor H antigen differences which were discovered when some Salk C mice immunized with B10.D2 showed slight activity on C mice from Lilly. In general, such differences were not a problem although every so often we came across a chimera which had slight cytotoxic activity on Lilly C, C.B, or (C × C.B)F$_1$ targets.

Chimeras. 20 C ♂ mice were given 900 rads from a Cobalt 60 source and immediately given an i.v. injection of 10^7 C.B pooled ♀ + ♂ viable bone marrow cells. T cells were removed from the marrow by treatment with AKR anti-C3H (anti-Thy 1.2) serum (15 min, 4°C) followed by agarose-absorbed guinea pig complement (45 min, 37°C). 11 of these chimeras were used between 5 wk and 13 mo after reconstitution. A total of 17 chimeras survived for more than 4 mo.

Testing Chimerism. The presence of host cells in chimeric spleen cell suspensions was assayed before in vitro culture with a cytotoxic antiserum (C57BL/6 anti-P815 ($H-2^d$) (kindly given us by Joseph Coha, University of California San Diego). Spleen cells were incubated with antiserum and selected nontoxic rabbit complement for 45 min at 37°C. The ratio of viable to dead cells was determined using trypan blue. On each test, positive and negative control cells were included to ensure the specificity and activity of the antiserum.

The H-2 type of the cytotoxic effector cells generated in vitro was tested just before the ^{51}Cr release assay, using antisera produced, absorbed, and extensively tested in functional assays by Dr. Michael Bevan, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Mass. We used his C.B anti-B10.D2($\alpha H-2^d$) and C3H × DBA/2 anti-C.B($\alpha H-2^b$). The test cells were washed, resuspended to 12 × 10^6 viable cells/ml, incubated with antiserum at 4°C for 30 min, centrifuged, and resuspended in guinea pig C for 45 min at 37°C. They were then washed twice, resuspended in equal volumes, and titrated against ^{51}Cr-labeled targets.

Immunizations. Chimeras were primed by injection of 10^7 viable spleen cells i.p. in Hanks' balanced salt solution and tested in culture from 3 wk to 12 mo later without apparent differences in response.

Cytotoxic Assay. Spleen cells from primed chimeras were cultured for 5 days at a ratio of 4:1 with mitomycin C-treated stimulator spleen cells as described (4), and were then washed once before use in the ^{51}Cr release assay.

Serial dilutions of such in vitro-boosted cells were then titrated against 4 × 10^4 ^{51}Cr-labeled concanavalin A (Con A) blasts (4, 16). The percentage of specific release after 3 to 5 h of incubation was calculated as:

$$\% \text{ specific release} = \frac{\text{experimental release (cpm)} - \text{spontaneous release (cpm)}}{\text{total incorporated (cpm)} - \text{spontaneous release (cpm)}} \times 100.$$

Spontaneous release varied from 9.6 to 35.6% of total in different experiments. The greatest differences we saw in spontaneous release for different targets within a single experiment was 8%. When effector:target ratio is given, the effector number is based on the number of primed
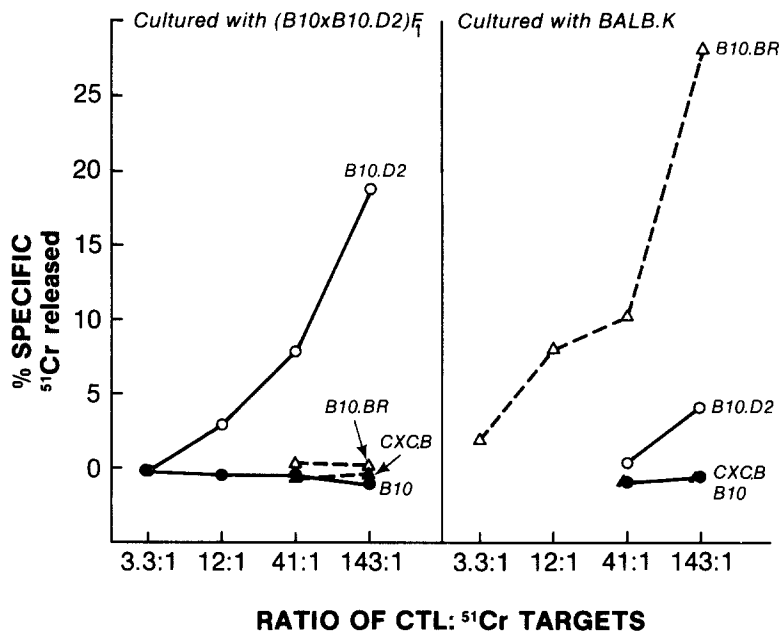
Fig. 1. Cytotoxic activity of C.B → C chimeric spleen cells primed in vivo with D1.C, cultured against (B10 × B10.D2)F₁ or BALB.K (C.K) stimulator spleen cells, and tested on ⁵¹Cr-labeled B10.D2 (O), B10 (●), B10.BR (△), or (C × C.B)F₁ (▲) Con A blasts.

cells originally put into the boosting culture, not viable cells recovered. Recovery varied from 20 to 60% in different experiments.

Results

Can Fully Allogeneic Chimeras Respond to Minor H Antigens?   Spleen cells from a C.B → C chimera, primed with 15×10^6 D1.C spleen cells 5 wk after reconstitution, were checked for the presence of residual host $H\text{-}2^d$ cells and were found to contain <1%. The cells were then cultured with (B10 × B10.D2)F₁ or C.K stimulatory cells for 5 days before testing on B10, B10.D2, B10.BR, or (C × C.B)F₁ targets. As illustrated in Fig. 1, the cytotoxic T lymphocytes (CTL) cultured with (B10 × B10.D2)F₁ stimulators lysed B10.D2 targets very well and had no activity on (C × C.B)F₁, B10, or B10.BR targets. Thus these CTL are specific for the combination of minor H + $H\text{-}2^d$. The CTL cultured with C.K lysed B10.BR specifically, showed a slight cross reaction on B10.D2, and had no activity on B10 or (C × C.B)F₁. A similar cross reaction has been noticed before in normal C mice primed against B10.D2 and tested on B10.BR or C.K targets (16), and has been extensively studied elsewhere (17). It appears then that a fully allogeneic chimera can respond to minor H antigens and is tolerant to both marrow donor and host type cells.

Can These Chimeras Respond to Minor H Antigens in Combination with Either $H\text{-}2^b$ or $H\text{-}2^d$?   In the previous experiment, cross priming should have led to priming of anti-B10 CTL, which should then have been boosted in the culture with (B10 × B10.D2)F₁ stimulators (16); however, no killing was seen on B10 targets. This could have been explained in three ways. Either (a) there really were no CTL precursors capable of responding to B10, (b) anti-B10 CTL
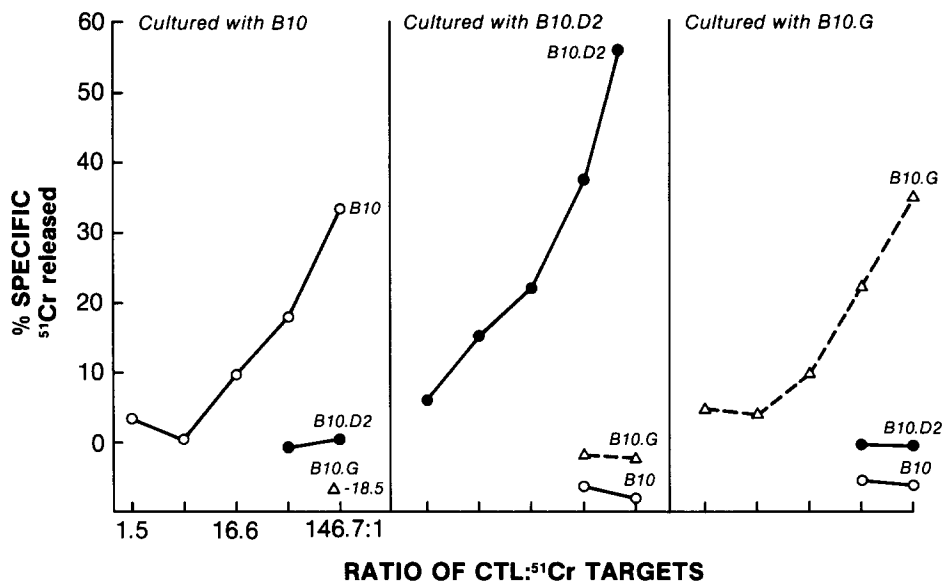
FIG. 2. Cytotoxic activity of C.B → C chimeric spleen cells primed in vivo with D1.C, cultured with B10, B10.D2, or B10.G spleen cell stimulators, and tested on ^{51}Cr-labeled B10 (O), B10.D2 (●), or B10.G (△) Con A blasts.

precursors did exist but cross priming had not occurred, or (c) such CTL did exist and were primed but were such a minority population that they were not boosted well by the (B10 × B10.D2)F_1 stimulators. We therefore decided to boost with B10 and B10.D2 separately to see whether we could raise a population of effector cells against B10.

Spleen cells from a chimera primed with D1.C were checked for the presence of H-2^d positive cells, found to be <1% positive, and then cultured with B10, B10.D2, or B10.G stimulators for 5 days before testing on B10, B10.D2, and B10.G targets. Fig. 2 shows that the CTL cultured with B10 stimulators lysed B10 cells, CTL cultured with B10.D2 lysed B10.D2 cells, and those cultured with B10.G lysed B10.G. The activity seen on B10, although sixfold lower than that on B10.D2 targets, is quite good. It seemed that we had two responding populations in the chimeras primed with D1.C, a large (or very active) set of CTL precursors against B10.D2 and a smaller (or less active) set against B10. This shows that C.B → C chimeric T cells that have grown up under the influence of an H-2^d thymus can nevertheless react against minor H antigens plus H-2^b. It also indicates that the type of boost given may be important. When the population of T cells one is looking for is likely to be small, it may be best to immunize in a way that will expand that population preferentially over other possible responders.

Table I is a summary of results from eight chimeras primed against B10 minor H antigens and boosted in vitro with either (B10 × B10.D2)F_1 or B10 and B10.D2 separately. In lines 1–4 there is virtually no activity on B10 targets whereas B10.D2 targets are lysed very well. From such data we could conclude that these chimeras are only able to respond to minors presented with H-2^d (even though cross priming should have led to activity on B10). However, lines

TABLE I

The H-2 Type of Cells Used to Prime and Boost Influences the Cytotoxic Responses Detected in H-2 Incompatible C.B → C Chimeras*

Experi-ment‡	Primed with	Boosted with	Aggressor: target ra-tio	Specific ^{51}Cr release	
				B10	B10.D2
75	B10.D2	(B10 × B10.D2)	8:1	0.6	63
75a	B10.D2	"	6:1	3.3	60.4
47	D1.C	"	142:1	−2.0	18.5
67	D1.C	"	50:1	3.6	76.0
62	D1.C	B10	147:1	33.2	0.4
		B10.D2	147:1	−8.0	56.0
59	D1.C	B10	97:1	23.0	−2.4
		B10.D2	49:1	1.5	65.2
67a	B10	(B10 × B10.D2)	50:1	31.6	12.8
62a	B10	B10	147:1	15.0	−1.3
		B10.D2	147:1	−4.6	7.9

* Chimeras were primed in vivo and boosted in vitro (for 5 days) with spleen cells and tested in a 4-h ^{51}Cr release assay. Although each assay was done as a titration, we report only one ratio here for convenience. No significant activity was seen on C, C.B, or (C × C.B)F$_1$ targets.

‡ Each number represents an individual mouse.

5–11 negate such a conclusion and show that the antigen used to prime or boost can definitely influence the response. Mice primed with minor + H-2^d and boosted with B10 definitely respond to B10, as do mice primed with B10 and boosted with the F$_1$(B10 × B10.D2). It appears that CTL precursors reactive to B10 do exist and can be seen if their numbers are expanded preferentially either in the priming or boosting immunizations.

Are the Effector Cells Entirely of Donor Origin?   Even though we could not detect any residual host cells in the chimeric spleens, the possibility remained that they did exist in small numbers, were expanded during culture, and were responsible for at least some of the activity we saw after 5 days. We therefore killed the effector cells just before the ^{51}Cr release assay with antiserum against H-2^b or H-2^d + C to see whether any activity was due to H-2^d positive cells.

The antisera were previously tested on C anti-C.B and C.B anti-C CTL using exactly the same protocol and reagents used here and were found to eliminate the activity of the appropriate effector cells completely (M. Bevan, personal communication).

Spleen cells from two chimeras that had been primed with B10.D2 7 mo after reconstitution (ample time for the host to regenerate if it were going to) were cultured 6 mo later with (B10 × B10.D2)F$_1$ or B10.G stimulators. After 5 days, a sample of each culture was assayed on B10, B10.D2, B10.G, C, and C.B targets and both chimeras were found to have some activity specific for B10, excellent activity on B10.D2 and B10.G targets, and none on C or C.B. The remaining B10.D2-boosted CTL were then treated with anti-H-2^b or anti-H-2^d plus C and titrated on B10.D2 targets.

Fig. 3 shows that CTL treated with anti-H-2^d + C were completely active
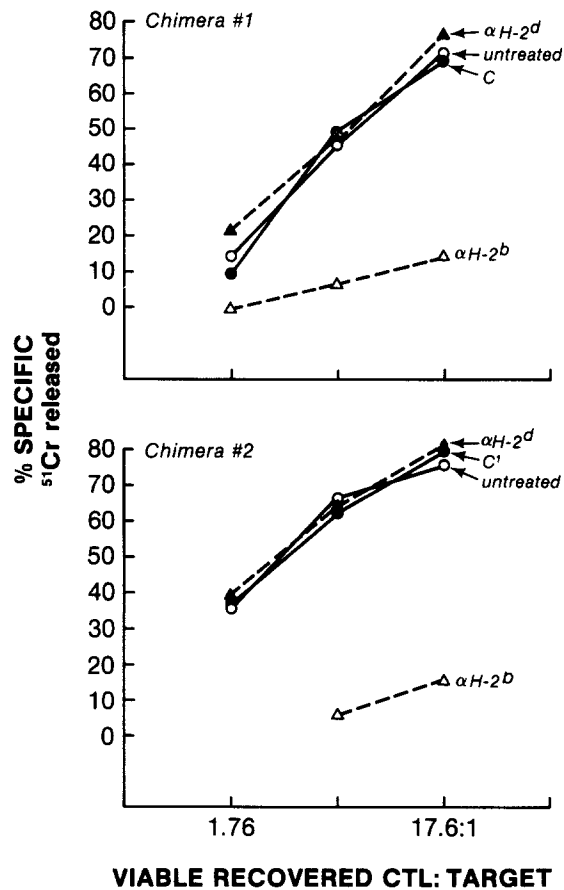
VIABLE RECOVERED CTL: TARGET

FIG. 3.   Effect of treatment with anti-H-2^b or anti-H-2^d serum + C on the ability of C.B →
C chimeric CTL to mediate lysis. These CTL were primed in vivo with B10.D2, cultured
with B10.D2 stimulators for 5 days, treated with antiserum + C, C alone, or left untreated
and then tested for their ability to lyse ^{51}Cr-labeled B10.D2 targets.

whereas those treated with anti-H-2^b + C were no longer able to function. Thus
there is no detectable contribution by the irradiated host to the CTL activity we
see. All the activity against B10.D2 must be due to H-2^b T cells.

Discussion

We began our experiments to ask whether H-2^b T cells could respond to minor
histocompatibility antigens associated with H-2^d in a fully allogeneic chimera,
and also to ask whether cross priming occurred in these animals. We found that
these chimeras do respond to minor H antigens on both H-2 types, they give
allogeneic reactions, they exhibit a type of cross-reactivity seen in normal C
and (C × C.B) mice, and they can be cross primed. In every aspect but one they
have the same CTL responses as a perfectly normal (C × C.B)F$_1$. The one
difference is that, although they have a population of CTL precursors which can
be activated against minor H + H-2^b (B10), they do show some preference for
minor + H-2^d (B10.D2). This, from the results of Bevan (12) and Zinkernagel et

al. (13) would be expected of T cells that have grown up in an H-2^d thymus.

The present results differ from those reported by Zinkernagel et al. in two important and related respects. Firstly, like Bevan, we find a preference in CTL activity toward antigen in association with host H-2 rather than the virtually absolute restriction reported by Zinkernagel et al. Secondly, whereas fully allogeneic chimeras were reported to be immunoincompetent (13), we find that they are completely capable of responding to minor H antigens and to foreign H-2. There are technical differences between our systems that could lead to these disparities.

(a) Zinkernagel et al. looked at an in vivo primary response whereas we assay a secondary response in vitro. Perhaps a boost is required to raise effector cell levels to those that can be detected in a ^{51}Cr release assay. Thus the population capable of responding to B10 in C.B → C chimeras would be undetectable without specific priming or boosting (Table I, Fig. 2).

(b) After inoculation with live virus, host cells must present viral antigen to CTL precursors. A host antigen-presenting system may not be necessary for direct priming against minor H antigens on spleen cells and the priming may be more efficient as a consequence. Thus, a chimera may respond detectably to minor H antigens while it remains unresponsive to viral antigens.

(c) The apparent immunoincompetence of fully allogeneic chimeras in viral systems was explained by postulating that CTL precursors require a helper T cell to be activated (13). In our case, therefore, T helpers, having grown up in an H-2^d thymus, should be restricted to seeing antigen in the context of H-2^d and should not be able to give assistance to the H-2^b repopulating CTL precursors. It is possible that an injection of B10.D2 spleen cells leads to a positive allogeneic effect against the H-2^b CTL, thereby sidestepping any requirement for T helpers. We feel this is unlikely because it was previously shown (16) that an allogeneic effect is neither necessary nor sufficient for priming against minor H antigens. In any case, B10 spleen cells should not be able to give a positive allogeneic effect against the C.B. CTL, and yet they are perfectly able to prime these chimeras.

It is impossible to exclude altogether a role for mature T cells contaminating the marrow inoculum, despite the treatment with anti-Thy-1 + C. In theory, such mature helper T cells, having grown up in an H-2^b thymus, could retain complete restriction to H-2^b and thus provide help for contaminating mature H-2^b CTL precursors which were themselves H-2^b restricted, or for CTL precursors grown up in the host thymus, completely restricted to H-2^d. This interpretation seems unlikely on quantitative grounds, but must be borne in mind when absolute restrictions are not seen.

We feel rather that the restriction imposed by the thymus is profound but not absolute, so that an H-2^d mouse does have some T-cell precursors capable of reacting to antigen + H-2^b (or H-2^q or H-2^s etc.) and that these precursors will be found in all classes of T cells. Therefore, if T helpers are necessary for CTL function, our fully allogeneic chimeras must have an adequate supply of such T helpers.

In summary we have found that congenic, fully H-2 incompatible radiation chimeras, primed in vivo and boosted in vitro with cells bearing minor H

differences, produce H-2-restricted cytotoxic cells specific for the minor H antigens. The specificity of restriction can be either to donor or host H-2 and the activity observed depends on the H-2 type of the cells used to prime or boost.

Summary

Fully H-2 incompatible radiation chimeras were prepared using BALB congenic mice. Such chimeric mice were immunized in vivo against histocompatibility antigens of the C57BL/10Sn (B10) background in association with either of the parental H-2 haplotypes, and their spleen cells subsequently boosted in vitro with the same minor antigens. Strong H-2-restricted cytotoxic activity against minor antigens was detected, and the specificity of the restriction could be to the H-2 haplotype of the donor or the host depending on the cells used for priming or boosting. Cross priming could also be demonstrated in these mice. The results show that fully allogenic radiation chimeras can produce H-2-restricted T-cell responses to minor histocompatibility (H) antigens, and are discussed in relation to contrasting results recently obtained against viral antigens.

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