

PRIMARY ANTI-VIRAL CYTOTOXIC T-CELL RESPONSES IN
SEMIALLOGENEIC CHIMERAS
ARE NOT ABSOLUTELY RESTRICTED TO HOST H-2 TYPE

BY ROBERT V. BLANDEN AND MARION E. ANDREW

From the Department of Microbiology, John Curtin School of Medical Research, Australian National University, Canberra City, A.C.T. Australia

In semiallogeneic murine chimeras produced by injecting (AXB) F_1 fetal liver or bone marrow stem cells into lethally irradiated type A or B hosts, cytotoxic T-cell responses to minor histocompatibility (H) antigens (1-3) or viruses (4) are restricted mainly or exclusively to recognition of H-2 antigens of host type. In the case of primary responses in vivo to viral infection, this restriction was apparently absolute (4); in contrast, secondary responses in vitro to minor H antigens showed host type bias, but not absolute restriction (1, 2). Since these observations have profound implications for the development and nature of T-cell recognition capability (3-7) it is important to resolve this discrepancy. We report here that in primary (in vivo) and secondary (in vitro) T_c cell responses to ectromelia virus infection a high proportion of (AXB) F_1 \rightarrow A type chimeras show bias but not absolute restriction to A type H-2 antigens. About 75% of such chimeras possessed detectable T_c cells which recognized virus plus B type H-2 antigens.

Materials and Methods

Mice. CBA/H, C56BL/6, BALB/c, (CBA/H \times C57BL/6) F_1 , and (BALB/c \times CBA/H) F_1 mice were bred at the John Curtin School.

Chimeras. Young adult CBA/H, C57BL/6 or BALB/c mice were irradiated (950 rads from a cobalt 60 source) and reconstituted 24 h later by i.v. injection of $2-4 \times 10^7$ fetal liver cells from 15-17-d embryos of appropriate F_1 hybrids. Surviving chimeras were used in experiments from 4 to 22 wk after reconstitution. Testing with anti-H-2 sera and complement indicated that spleen cells were >90% of donor type as found by others (1-4).

Immunization. Chimeras were immunized by i.v. injection of 2×10^4 of plaque-forming units attenuated ectromelia virus. Primary T_c -cell responses in the spleen were assayed at 5 d after injection. Secondary responses in vitro used as responders spleen cells from chimeras primed as above at least 2 wk previously, and as stimulators virus-infected, 2,000 rad-irradiated spleen cells of appropriate F_1 hybrids syngeneic with the fetal liver donor of the chimera. The basic method has been given in detail elsewhere (8).

Cytotoxicity Assay. Macrophage target cells were used throughout as described previously (9). Data given are the means of triplicates \pm SE and have had spontaneous ^{51}Cr release subtracted. Significance was determined by Student's *t* test.

Results and Discussion

Primary Response to Ectromelia Infection In Vivo and Secondary Response In Vitro. A total of 53 individual chimeras of the type (AXB) F_1 \rightarrow A or B were immunized i.v. with attenuated ectromelia virus at times ranging from 4 to 22 wk after irradiation and

TABLE I
 Primary (In Vivo) or Secondary (In Vitro) T_c-Cell Responses* To Ectromelia Virus Infection in (AXB)F₁ → A
 or B Chimeras

Chimera	Type of re- sponse	Killer: target ratio	Specific lysis of macrophage target cells‡					
			CBA		C57BL/6		BALB/c	
			Infected	Normal	Infected	Normal	Infected	Normal
%								
1. (CBA × C57BL/6)F ₁ → C57BL/6	Primary	30	38.4 ± 3.3	0	55.1 ± 2.5	0	15.6 ± 1.7	14.9 ± 3.0
		10	30.4 ± 0.2	0	48.6 ± 3.0	0	11.6 ± 0.6	10.0 ± 2.1
		3	2.0 ± 0.9	0	18.8 ± 4.4	0	5.2 ± 2.7	2.0 ± 0.8
2. (CBA × C57BL/6)F ₁ → C57BL/6	Primary	30	12.6 ± 0.7	1.7 ± 0.9	54.0 ± 2.4	0	12.2 ± 2.3	7.1 ± 3.6
		10	5.0 ± 2.4	4.0 ± 1.6	51.8 ± 2.0	0	12.3 ± 0.4	5.7 ± 2.2
		3	3.1 ± 0.8	2.8 ± 1.4	17.6 ± 3.2	0	4.9 ± 1.2	5.3 ± 1.5
3. (BALB/c × CBA)F ₁ → CBA	Primary	30	66.2 ± 1.0	5.9 ± 0.8	10.5 ± 1.2	12.2 ± 3.0	33.7 ± 3.6	1.4 ± 0.5
		10	60.3 ± 2.4	3.8 ± 1.2	7.8 ± 2.4	9.7 ± 2.9	23.8 ± 2.3	0
		3	39.8 ± 1.2	3.3 ± 0.6	5.4 ± 1.1	6.4 ± 1.4	6.3 ± 1.0	0
4. (CBA × C57BL/6)F ₁ → C57BL/6	Secondary	9	49.9 ± 3.1	12.5 ± 0.6	73.8 ± 2.1	13.0 ± 1.9	26.1 ± 0.4	26.0 ± 2.6
		3	23.1 ± 3.0	6.9 ± 1.1	67.1 ± 4.9	9.8 ± 4.9	10.5 ± 1.3	12.9 ± 0.7
		1	6.1 ± 2.2	2.2 ± 0.7	57.4 ± 3.0	0	4.5 ± 2.0	8.4 ± 3.2
5. (CBA × C57BL/6)F ₁ → CBA	Secondary	9	62.3 ± 2.4	9.0 ± 2.1	24.0 ± 4.1	3.6 ± 1.2	21.2 ± 4.8	12.3 ± 1.9
		3	54.6 ± 2.2	1.6 ± 0.7	16.6 ± 2.6	0	10.3 ± 2.6	11.3 ± 2.5
		1	46.9 ± 3.9	0	2.3 ± 1.1	0	7.7 ± 1.8	2.6 ± 1.5
6. (CBA × C57BL/6)F ₁ → CBA	Secondary	9	60.0 ± 4.8	3.6 ± 1.0	11.6 ± 1.9	11.3 ± 2.1	11.7 ± 2.0	3.2 ± 1.7
		3	61.6 ± 3.4	3.6 ± 0.8	5.4 ± 1.0	6.2 ± 0.5	6.0 ± 1.3	4.3 ± 2.2
		1	45.8 ± 1.8	0	0	0	12.1 ± 3.4	5.8 ± 2.5

* Primary responses in the spleen were assayed 5 d after infection; secondary responses were assayed after 5 d of culture as detailed in Materials and Methods.

‡ Data are means of percent ⁵¹Cr release in triplicate ± SE of mean and have had spontaneous release subtracted.

reconstitution. With 41 of these mice, spleen cells were assayed for cytotoxicity 5 d after injection, the peak of the primary response, on infected and uninfected macrophage targets of A, B, and C type (Table I). If virus challenge was given at 4, 5, or 6 wk postreconstitution, some animals gave detectable primary responses and others did not, but by 7 wk or more postreconstitution, all mice responded well. Of 33 successful responders, 26 gave significant lysis of both A or B type infected targets (above uninfected control targets), though more lysis of host type targets was always observed (host bias); the other 7 responders gave lysis significantly above uninfected control targets only on infected targets of host type (absolute restriction). Low levels of lysis of both uninfected and infected third party (C) targets was often observed as reported previously (10). The three examples of primary responders given in Table I contain one extreme case (chimera 1) in which specific lysis of infected CBA targets (nonhost) was very prominent, a second case from the same group of fetal liver recipients in which there was much less lysis of infected CBA targets (chimera 2), and a third case (chimera 3) which demonstrates that host bias rather than absolute restriction occurs independently of the genotype of host and donor.

The remaining 12 chimeras were used for secondary responses in vitro. Spleen cells from nine of these mice showed virus-immune T_c-cell responses with host bias and three showed absolute restriction. The examples given in Table 1 show that the secondary T_c-cell populations are at least 10-fold more active than the primary cells as would be expected (8), but their characteristics are otherwise similar. There are two examples given with host bias to different extents (chimeras 4 and 5) and one with absolute restriction (chimera 6).

Taken together, the results revealed no factors enabling a prediction as to whether an individual chimera would show host bias or absolute restriction. The specificity pattern of target lysis obtained seemed to be unrelated to whether the response was primary or secondary, to the time after irradiation and reconstitution, to strains of mice involved, or to the batch of stem cells used. This last point is important, because otherwise it might be argued with force that some fetal liver stem cell preparations (and not others) were contaminated with mature T cells. However, such contamination seems unlikely, because it would require that the thymus exports significant numbers of mature T cells early in the 3rd wk of fetal life, a suggestion which seems unlikely in view of the known time scale of murine thymic development (11) and the profound T-cell depletion caused by neonatal thymectomy in mice (12).

The data reported here agree with the results of Bevan (1) and Matzinger and Mirkwood (2) who found host bias, but differ somewhat from those of Zinkernagel et al. (4) who found absolute restriction. This discrepancy is likely to be attributed to differences in target cell sensitivity and assay time. We used macrophage targets in 16 h assays, whereas Zinkernagel et al. (4) used fibroblast cell lines often in 6-h assays. In previous comparisons we noted that macrophages were considerably more sensitive targets than fibroblasts (13).

It seems probable, therefore, that most (AXB) $F_1 \rightarrow$ A chimeras possess T_c cells that recognize foreign antigen together with H-2 antigens of type B, though from the data in Table I these are usually at least 10-fold less numerous than T_c cells restricted to H-2 type A. Limiting dilution assays should provide definitive information on the relative precursor frequencies.

These results have implications for current models of T cell maturation in the thymic environment. Positive selection (3, 4, 7) models involve recognition by primordial T cells of self H-2 antigens displayed on radioresistant thymic elements, presumably epithelial cells (4), as an essential step in maturation towards antigen-sensitive precursors, which are then exported to secondary lymphoid tissues. The present results suggest that recognition of thymic epithelium is not essential, though it remains possible that it is the most efficient and common pathway. Alternative negative selection models (5, 7) are compatible with the findings reported here.

Summary

Chimeras produced by reconstitution of 950 rads irradiated type A or type B host mice with (AXB) F_1 fetal liver stem cells were examined in primary (in vivo) and secondary (in vitro) T_c -cell responses to ectromelia virus infection. Of 33 individual chimeras which gave primary responses, 26 produced significant specific lysis of infected targets of both A and B type, though host type targets were invariably lysed more efficiently (host bias). The other 7 chimeras gave lysis of infected host type targets only (absolute restriction). 12 individual chimeras were used in secondary responses. Nine showed host bias, and three showed absolute restriction. Whether an individual chimera showed host bias or absolute restriction seemed to be unrelated to whether the response was primary or secondary, to the time after reconstitution (ranging from 4 to 22 wk), to strain of mouse, or to the batch of fetal liver stem cells used.

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