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In a radiation chimaera, host H–2 antigens determine immune responsiveness of donor cytotoxic cells

CELL membrane structures controlled by genes in the major histocompatibility complex (H–2 in mice) are involved in most immune interactions between T lymphocytes and other cells[1]. Cytotoxic T lymphocytes (CTL) immunised against viruses[2], haptens[3], minor histocompatibility antigens[4] or tumour antigens[5], are specific for self H–2 antigens as well as for the foreign antigen. But CTL are not restricted to recognising antigens in combination with only self H–2. H–2^d homozygous CTL which have matured in an irradiated H–2^d/H–2^k host can respond to antigen plus H–2^k in addition to antigen plus H–2^d (refs 6–8). It is not known whether the H–2 environment in which T cells mature influences their range of specificity, that is, whether CTL from a normal mouse can respond quantitatively as well to antigen plus foreign H–2 as they do to antigen plus self H–2. These experiments were designed to test this influence. The results suggest that host H–2 antigens do exert an effect on the specificity of T-cell responses.

A single suspension of bone marrow cells from F_1(BALB/c × BALB.B) (F_1(C × C.B), H–2^d/H–2^b) mice was used to reconstitute groups of lethally irradiated parental mice, C(H–2^d) ([F_1→C] chimaeras) and C.B(H–2^b) ([F_1→C.B] chimaeras). Eight weeks later these chimaeric mice and normal F_1(C × C.B) mice were primed against minor H antigens by injecting 8×10^6 F_1(B10 × B10.D2) (H–2^b/H–2^d) spleen cells. The B10 background offers more than 30 minor histocompatibility antigenic differences that can be recognised by BALB mice[9,10]. Some weeks later the primed spleen cells were boosted in culture with irradiated F_1(B10 × B10.D2) stimulator cells and assayed for cytotoxicity 5 d later.

Following this immunisation procedure, cells from normal F_1(C × C.B) mice lysed B10 targets and B10.D2 targets almost equally (Table 1). (The two activities are mediated by separate pools of CTL[2–4]) The chimaeras responded differently. In the same conditions of immunisation with F_1(B10 × B10.D2) cells, they responded preferentially to the minor antigens in association with the H–2 antigens of the host. CTL from the [F_1→C] chimaeras killed B10.D2 targets better than B10 targets, whereas CTL from [F_1→C.B] chimaeras lysed B10 targets better than B10.D2 targets (Table 1).

Spleen cells from five chimaeras were assayed for their content of host and donor cells at time of killing. Complement-mediated lysis with H–2^b anti-H–2^d serum and with H–2^d anti-H–2^b serum indicated that in all cases at least 85% of the cells were of F_1 (donor) origin. The cytotoxic effector cells were also lysed with anti-H–2 serum and complement just before the ^{51}Cr-release assay (Table 2). Here, the [F_1→C] chimaera cells lysed B10.D2 targets ninefold more efficiently than they lysed B10 targets (data not shown). The killer cells were treated with antiserum plus complement, washed, and assayed for lysis of labelled B10.D2. Table 2 shows, most importantly, that BALB/c anti-C57BL/6 (anti-H–2^b) serum reduced the cytotoxic activity 86% compared with controls. This antiserum does not lyse BALB/c effector cells. Therefore, at least 86% of the CTL were of F_1 bone marrow origin.

These experiments show that H–2^d/H–2^b cytotoxic cells which mature in an irradiated H–2^d host respond preferentially to antigens plus H–2^d, whereas H–2^d/H–2^b cells which mature in an irradiated H–2^b host respond preferentially to the same antigens in conjunction with H–2^b gene products. The experiments were designed to test the 1971 Jerne hypothesis[14], or a modified version of it[4,15]. The hypothesis accepts that a somatic theory of generation of receptor diversity is correct and proposes that self-H–2 antigens drive the diversity. Immature T cells first express an anti-self-H–2 receptor, leading to proliferation and to accumulation of V gene mutations until there is no significant reaction with self-H–2. According to this hypothesis, the receptor repertoire of A strain T cells which had matured in an A environment would be quite different from that of A strain T cells which had matured in a B environment. The results presented here are compatible with this hypothesis and with another theory of 'adaptive differentiation'[1].

There is an alternative explanation of the results. It may be that the host haplotype preference seen at the level of effector CTL does not reflect a bias in specificity at the level of precursor CTL. The H–2^d/H–2^b precursor CTL in the H–2^d host may have exactly the same range of reactivity as those in the H–2^b host. The haplotype preference of the effector CTL would then be due to the way antigen is presented to CTL precursors. Even though the immunogen (B10 minor antigens) was introduced on H–2 heterozygous cells, the antigen which was responsible for priming CTL may have been processed antigen presented on radiation resistant host cells[4]. In the [F_1→C] chimaera such radiation-resistant antigen-present-

Table 1 Specificity of H–2^d/H–2^b cytotoxic cells from normal and chimaeric mice

Responder*	Immunised with†	% Specific lysis of targets‡ B10 H–2^b	B10.D2 H–2^d	B10.BR H–2^k	F_1(C × C.B) H–2^d/H–2^b	Ratio of lytic activity on§ B10/B10.D2
Experiment 1						
Normal F_1 (C × C.B)(H–2^d/H–2^b)	F_1(B10 × B10.D2)(H–2^b/H–2^d)	56.2	62.2	1.5	ND	0.7
Chimaera [F_1→C]	F_1(B10 × B10.D2)(H–2^b/H–2^d)	19.3	72.7	2.9	ND	0.02
Chimaera [F_1→C.B]	F_1(B10 × B10.D2)(H–2^b/H–2^d)	51.8	29.1	0.1	ND	5.2
Normal F_1(C × C.B)	C3H(H–2^k)	4.5	3.8	71.5	ND	—
Experiment 2						
Normal F_1(C × C.B)	F_1(B10 × B10.D2)	54.8	68.4	ND	0.9	0.5
Chimaera [F_1→C]	F_1(B10 × B10.D2)	4.8	80.5	ND	1.5	<0.02
Chimaera [F_1→C.B]	F_1(B10 × B10.D2)	61.3	8.8	ND	0.1	>43.0

*Chimaeras were prepared as follows: BALB/c(C(H–2^d)) and BALB.B(C.B(H–2^b)) mice were irradiated with 850 R and reconstituted on the same day with 13.4×10^6 anti-Thy-1 plus complement (C′)-treated F_1(C × C.B) bone marrow cells. Eight weeks later they were immunised.

†Primed against minor H antigens by injecting 8×10^6 viable F_1(B10 × B10.D2) spleen cells intraperitoneally. Spleen cell suspensions were prepared 4 weeks later (experiment 1) or 6 weeks later (experiment 2), and boosted for 5 d in culture with an equal number of 1,000 R irradiated F_1(B10 × B10.D2) or C3HebFe/J spleen cells as described previously[11].

‡2-d con A (conavalin A) blasts were labelled with ^{51}Cr-sodium chromate and used as targets as described previously[11]. Serial dilutions of the killers were assayed against a constant number of targets, and the figures for specific lysis presented here are for a killer : target ratio of 100 : 1. Spontaneous release of ^{51}Cr varied from 18.1–22% in experiment 1 and from 9.6–13.7% in experiment 2.

§Calculated from the titrations of killers : targets[12,13]. For example, in experiment 1, the [F_1→C] cells lysed B10.D2 targets 50 times better than they lysed B10 since a 100 : 1 ratio caused 19.3% specific release from ^{51}Cr-B10, whereas the same amount of specific lysis of ^{51}Cr-B10.D2 was obtained at a ratio of 2 : 1.

ND, Not determined.

Table 2 Sensitivity of chimaera cytotoxic cells to anti-H-2 serum plus C'

Cytotoxic cells*	Treatment of effector cells†	% Specific lysis of ^{51}Cr-B10.D2‡	% Reduction in lytic activity§
[$F_1 \rightarrow C$] anti-F_1(B10 × B10.D2)	Medium	34.0	—
	Normal B10 serum + C'	34.3	0
	Anti-H-2^b + C'	9.8	86
	Anti-H-2^d + C'	5.1	94

*[$F_1 \rightarrow C$] chimaeras were primed *in vivo* 8 weeks after reconstitution, their spleen cells boosted in culture 20 weeks later and assayed for cytotoxicity on day 5 of culture.

†BALB/c anti-C57BL/6 and C57BL/6 anti-BALB/c sera were prepared by hyperimmunisation with spleen cells. Effector cells were incubated with mouse sera 1:2, washed, and incubated in guinea pig serum 1:9 as a source of complement (C'). Cells were re-suspended to the same volume and assayed.

‡Con A blasts from B10.D2 mice were labelled with ^{51}Cr and used as target. Data presented are for original number of responder spleen cells:target cells of 7:1. Spontaneous release of ^{51}Cr was 20.6%.

§Calculated from the titrations of killer:targets as in Table 1.

ing cells would be homozygous H-2^d and would naturally stimulate only anti-B10.D2(H-2^d) CTL, not anti-B10(H-2^b) CTL. Experiments to decide between these interpretations are in progress.

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