ASSOCIATION OF IMMUNITY AND TOLERANCE TO HOST
H-2 DETERMINANTS IN IRRADIATED F₁ HYBRID
MICE RECONSTITUTED WITH BONE MARROW CELLS FROM
ONE PARENTAL STRAIN

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Radiation chimeras—irradiated animals injected with allogeneic hemopoietic cells—often succumb eventually to graft-vs.-host (GVH)[1] disease (1). The etiology of this condition, which is considered to be mediated by T lymphocytes, is not clearly understood (2). In many situations it is probably caused by immunocompetent T cells contaminating the donor cell population used. Alternatively, the disease might reflect emergence of host-reactive T cells differentiating from donor stem cells via the host thymus. In order to assess this second possibility it is essential to know whether or not host-reactive T cells do differentiate under such circumstances. The present study has examined this question by studying the reactivity of lymphocytes from irradiated F₁ hybrid mice injected several months previously with parental strain bone marrow cells depleted of T cells. The results indicate that lymphocytes from such mice show "split tolerance" towards host-type determinants, the cells responding to these determinants in mixed leukocyte culture but failing to differentiate into detectable cytotoxic lymphocytes.

Materials and Methods

Mice. CBA/J (*H-2ᵏ*, Mlsᵈ), CBA/H (*H-2ᵏ*, Mlsᵇ), C3HJ (*H-2ᵏ*, Mlsᶜ), DBA/2 (*H-2ᵈ*, Mlsᵃ), C57BL/6 (*H-2ᵇ*, Mlsᵇ) (C57BL), SJL (*H-2ˢ*), (CBA/J × DBA/2)F₁, (CBA/J × C57BL)F₁ and (C3H/J × SJL)F₁ mice were used and kept under conventional circumstances.

Cells. Thoracic duct lymphocytes (TDL) and cells from spleen, mesenteric lymph nodes and bone marrow were obtained by standard techniques (3, 4).

Preparation of Chimeras. Semiallogeneic radiation chimeras were prepared by injecting 2 × 10⁷ viable (usually CBA/J) bone marrow cells intravenously into X-irradiated (900 R) 2-mo old (CBA × DBA/2)F₁ or (CBA × C57BL)F₁ mice; to prevent a GVH reaction the marrow cells were treated with a 1:5 dilution of anti-θ C3H antiserum and complement to remove T cells (5). With this regime it was hoped to obtain healthy chimeras in which the hemopoietic system was derived entirely from the donor stem cells. The mice received 100 mg/l Neomycin (Médial, Geneva, Switzerland) and 10 mg/l Polymyxin B (Nova, Copenhagen, Denmark) in their drinking water for 2 wk after irradiation. The identity and properties of lymphocytes from the chimeras were investigated 6 mo after irradiation, i.e. at a stage when it was hoped the lymphoid system would be fully repopulated. For simplicity,

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[1] *Abbreviations used in this paper:* CML, cell-mediated lympholysis; GVH, graft-vs.-host; MLR, mixed leukocyte reaction: TDL, thoracic duct lymphocytes.

chimeras prepared by injecting CBA/J marrow cells into irradiated (CBA/J × DBA/2)F₁ mice will be referred to as "CBA/J→DBA/2 F₁" chimeras etc.

Alloantisera. Anti-CBA serum was obtained from DBA/2 mice hyperimmunized with CBA/J spleen cells, anti-DBA/2 serum from C3H mice injected with BALB/c spleen cells and anti-θ serum from AKR mice given C3H thymus cells as described elsewhere (5).

Testing Chimerism. An antihost-*H-2* alloantiserum was used to detect the presence of host cells in the chimeras. Cells were incubated with antiserum (1:4 dilution) at 37°C followed by guinea pig complement and cytotoxic indices calculated by dye exclusion, using the method of Boyse et al. (6).

Mixed Leukocyte Reaction

MEASUREMENT IN VIVO. The system described by Sprent and Miller (7) was used. This method involves transferring lymphoid cell populations lacking stem cells into irradiated (800 R) allogeneic or semiallogeneic mice; the proliferative response (MLR) of the donor-strain T cells against the host histocompatibility antigens at a required time is then assessed by measuring radioactivity in the spleen within 1 h of an intravenous injection of tritiated thymidine ([³H]TdR). In most experiments this system was selected in favor of the more commonly used in vitro assay for three reasons: (*a*) The in vivo assay was considered to closely approximate the environment encountered in the chimeras by donor-derived T cells processed by the host thymus. (*b*) The in vivo assay is quantitative, [³H]TdR incorporation with limited numbers of cells being directly proportional to the number of T cells injected (see Table II). (*c*) "Backstimulation" (8) of the donor cells by the host does not occur; in this respect, whereas parental strain cells can cause marked stimulation of F₁ cells in vitro (8) this does not occur with the present system (see last line of Table II).

MEASUREMENT IN VITRO. In some experiments MLR were measured in vitro using a standard technique (9) in which 10⁶ lymph node cells were cultured with 5 × 10⁵ mitomycin-C-treated stimulator cells in 1 ml tissue culture medium. Tritiated thymidine (Amersham, England [spec act 5 Ci/mmol]) was added after 96 h to the culture at 2 µCi/ml for 24 h before harvest. Levels of radiolabeled DNA in the cells were then measured.

Cell-Mediated Lympholysis

WITH DBA/2 MASTOCYTOMA CELLS AS TARGETS. CBA/J lymphocytes were activated in vivo by transferring them intravenously to irradiated (800 R) (CBA/J × DBA/2)F₁ mice. 6 days later spleen cells from the mice were incubated at 37°C in vitro at various ratios with 5 × 10⁴ ⁵¹Cr-labeled DBA/2 mastocytoma cells according to the method of Brunner et al. (10). Specific ⁵¹Cr release was determined 6 h later.

WITH PHYTOHAEMAGGLUTIN-STIMULATED BLASTS AS TARGETS. Lymphocytes were activated in vitro and assayed for cell-mediated lympholysis (CML) according to the method of Nabholz et al. (11). Briefly, 1.5–2 × 10⁷ viable spleen cells (responders) were cultured for 5 days in vitro with 8 × 10⁶ mitomycin C-treated allogeneic spleen cells as stimulator cells. The cells were then resuspended to 1 ml and threefold serial dilutions were made. 100 µl of the suspensions were incubated with 2 × 10⁴ ⁵¹Cr-labeled, phytohemagglutin (PHA)-stimulated lymph node cells for 4 h at 37°C. The ⁵¹Cr released into the supernate was then counted. Total releasable ⁵¹Cr was determined by adding Zaponin (Coulter Electronics Inc., Hialeah, Fla.) to the targets. For each incubation the % specific release was calculated as:

$$\frac{100 \times (\% \text{ experimental release } (ABm/C_T) - \% \text{ spontaneous release } (C_T))}{100 - \% \text{ spontaneous release } (C_T)}$$

where A = responder, B_m = stimulator and C_T = target cells.

Results

Health of Chimeras. The following chimeras were prepared: CBA/J→ DBA/2 F₁, CBA/J→ C57BL F₁, CBA/H→ C57BL F₁, and C3H→ SJL F₁. With each strain combination survival was excellent, 80–100% of the mice remaining in good health for at least 6 mo. Random autopsies on mice killed between 1 and 6 mo

revealed no signs of a GVH reaction in 18/20 mice. Two mice killed at 3 mo postirradiation showed grossly enlarged spleens (three–four times normal size). In over 25 mice killed at 6 mo postirradiation there was no evidence of a GVH reaction.

Number and Identity of Recirculating Lymphocytes. Thoracic duct fistulas were established in seven of the CBA/J→ DBA/2 F_1 chimeras. TDL outputs collected over 14–16 h are shown in Table I. With the exception of one mouse (chimera 4) the outputs were similar to those found in normal CBA/J or (CBA/J × DBA/2)F_1 mice. With specific antialloantisera no TDL of host origin could be detected. Lymph node cells from two mice showed similar results (data not shown). The proportion of θ-positive cells studied in three mice (74–81%) was comparable to that in normal CBA/J mice. Collectively, these data indicated that

TABLE I

Number and Identity of TDL From Heavily Irradiated (CBA/J × DBA/2)F_1 Mice Injected 6 Mo Previously with CBA/J Bone Marrow Cells Treated with Anti-θ Serum

Mice	TDL outputs	Period of collection	Cytotoxic index* with:		
			Anti-CBA/J serum	Anti-DBA/2 serum	Anti-θC3H serum
	$\times 10^{-6}$	h			
Chimera 1	110	16	100	2	—
Chimera 2	90	16	98	2	—
Chimera 3	67	14	100	0	74
Chimera 4	35	14	99	2	81
Chimera 5	110	14	100	1	79
Chimera 6	58	14	100	0	—
Chimera 7	75	14	99	0	—
Normal CBA/J	72‡	14	100	1	77
Normal (CBA/J × DBA/2)F_1	90‡	14	100	99	—

* Established by dye exclusion (see Materials and Methods).

‡ Mean of results of five–six mice per group.

the recirculating pool of T lymphocytes in the chimeras was fully repopulated with cells of donor origin.

Response of Chimera Lymphocytes to Host-Type Determinants

MIXED-LEUKOCYTE REACTION. MLR were measured both in vivo and in vitro (see Materials and Methods). In the in vivo assay, when TDL from CBA/J→ DBA/2 F_1 chimeras were transferred to irradiated donor strain (CBA/J) mice, [³H]TdR in corporation measured in the spleen at 5 days was not above the "background" levels found in controls given [³H]TdR but no lymphocytes (Table II). When the cells were exposed to the host antigens, however, i.e. by transfer to DBA/2 or (CBA/J × DBA/2)F_1 mice, significant responses were obtained. These responses were directly proportional to the number of cells injected (see data on TDL from chimeras 1 and 5). In all cases, however, they were less than the

TABLE II

Proliferative Response of Chimera TDL Transferred to Irradiated Mice of Various Strains

Exp.	TDL transferred	No. of cells	[³H]TdR incorporation: cpm* $(\times 10^{-3})$ in spleen			
			CBA/J	DBA/2	(CBA/J × DBA/2)F₁	(CBA/J × C57BL)F₁
I						
	Normal CBA	4 × 10⁵	0.4	21.8	—	—
		2 × 10⁵	—	12.0	—	—
	Chimera 1	4 × 10⁵	0.8	14.4	—	—
		2 × 10⁵	—	6.8	—	—
	Normal CBA/J ⎱ + chimera 1 ⎰	2 × 10⁵ 2 × 10⁵	—	19.6	—	—
	Chimera 2	4 × 10⁵	1.1	11.8	—	—
II						
	Normal CBA/J	8 × 10⁵	0.3	37.6	31.2	28.4
		4 × 10⁵	0.4	23.5	22.4	16.9
		2 × 10⁵	—	—	12.6	9.3
		1 × 10⁵	—	—	5.0	4.2
	Chimera 3	8 × 10⁵	0.2	8.6	6.0	26.1
	Chimera 4	8 × 10⁵	0.1	4.0	3.7	31.3
	Chimera 5	8 × 10⁵	0.3	13.2	7.8	23.6
		4 × 10⁵	—	—	4.4	11.4
		2 × 10⁵	—	—	2.2	5.8
	Normal DBA/2	8 × 10⁵	—	0.9	—	—
	Normal (CBA/J × DBA/2)F₁	8 × 10⁵	—	—	0.3	—
	Normal (CBA/J × C57BL)F₁	8 × 10⁵	0.4	—	—	0.2

* Arithmetic mean of radioactivity in spleen measured at 5 days after intravenous transfer of TDL and 45 min after intravenous injection of 25 μCi [³H]TdR: four-five mice per group. Levels of radioactivity obtained in irradiated mice given [³H]TdR but no lymphocytes have been subtracted from the values shown; the values $(\times 10^{-3})$ were 3.4 in CBA/J, 4.0 in DBA/2, 2.6 in (CBA/J × DBA/2)F₁ and 3.6 in (CBA/J × C57BL)F₁. The standard error of each group was within 5–10% of the mean.

responses obtained with comparable numbers of TDL from normal CBA/J mice. When a mixture of normal CBA/J TDL and TDL from chimera 1 were injected, levels of [³H]TdR incorporation were similar to the sum of the values obtained when the cells were injected separately. This suggested that the reduced response of the chimera TDL was not explainable in terms of "suppressor" cells (12). Transfer of the chimera TDL to (CBA/J × C57BL)F₁ mice showed that the cells expressed normal or near normal reactivity to third party antigens (Table II).

TDL or lymph node cells were used to measure MLR's in vitro. An experiment with a CBA/J→ DBA/2 F₁ chimera is illustrated in Table III. As when assayed in

TABLE III

Proliferative Response of Chimera Lymphnode Cells in the In Vitro Mixed Leukocyte Reaction to Various Stimulator Cells

Responder cells 1 × 10⁶	Stimulator cells (5 × 10⁵, mitomycin-C-treated)			
	DBA/2	(CBA/J × DBA/2)F₁	(CBA/J × C57BL)F₁	CBA/J
Chimera	101.4 ± 8	76.7 ± 4	376.4 ± 7	9.5 ± 1
CBA/J	572.2 ± 10	560.7 ± 32	459.0 ± 55	16.8 ± 0

Numbers represent arithmetic means of cpm × 10^{-3} of triplicate cultures ± standard deviation. Counts for stimulator cells cultured alone were $< 5 \times 10^{-3}$.

vivo it is evident that the chimera lymphocytes mounted a definite, though reduced response against host-type determinants while responding normally to third party determinants. Similar results were found with other CBA/J→ DBA/2 F₁ chimeras and also with CBA/J→ C57BL F₁ and C3H→ SJL F₁ chimeras (data not shown).

CELL-MEDIATED LYMPHOLYSIS. To investigate whether the antihost MLR was associated with the production of cytotoxic lymphocytes directed against CML determinants, 10^7 TDL pooled from CBA/J→ DBA/2 F₁ chimeras 3–5 were injected intravenously into each of five irradiated (CBA/J × DBA/2)F₁ mice. Spleen cells from the recipients were pooled 6 days later and incubated in vitro with ^{51}Cr-labeled DBA/2 mastocytoma cells. ^{51}Cr release measured 6 h later was no higher than when the target cells were incubated alone, even when high lymphocyte: target cell ratios were used (Table IV). This contrasted with control experiments in which normal CBA/J TDL activated in vivo produced marked target cell destruction. Table IV also shows that, compared to normal CBA/J serum, serum from the chimeras did not significantly impair lysis.

The choice of DBA/2 mastocytoma cells as targets in the above experiment did not allow study of CML to third party determinants. Therefore, to investigate the specificity of the unresponsiveness in CML, additional experiments were performed in which PHA-stimulated lymphocytes were used as targets. Spleen cells from the chimeras were either tested directly on PHA-target cells or exposed for 5 days to DBA/2 and C57BL determinants in vitro (see Materials and Methods and legend to Fig. 1) and then incubated with ^{51}Cr-labeled PHA-stimulated cells derived from these strains. Fig. 1 shows the results of an experiment with a CBA/J→ DBA/2 F₁ chimera. It is evident that the chimera cells failed to lyse target cells of host (DBA/2) origin but were effective against third party (C57BL) determinants after 5 days of culture. Spleen cells from normal CBA/J mice, by contrast, lysed both DBA/2 and C57BL targets though not syngeneic cells. Five other experiments with these chimeras and also with CBA/J→C57BL F₁ and C3H→SJL F₁ chimeras gave similar results: in three of these experiments lymph node cells from the chimeras were tested in MLR and shown to give a positive response against host-type determinants. Unresponsiveness to host-type determinants was also observed when (a) the in vitro exposure to these determinants was reduced to 3 days and (b) with chimera cells tested before culture (data not shown).

TABLE IV

Failure to Detect Differentiation of Chimera TDL Into Host-Reactive Cytotoxic Cells

Test lymphocytes	Lymphocyte: target cell ratio*	Serum added (dilution 1:6)	^{51}Cr released by target cells (DBA/2 masto-cytoma)‡
			%
Spleen cells from irradiated (CBA/J ×	30:1	Normal CBA	70.1¶
DBA/2)F₁ mice injected with normal	30:1	Chimera‖	67.0
CBA/J TDL§	10:1	—	81.2
Spleen cells from irradiated (CBA/J ×	60:1	Normal CBA	16.4
DBA/2)F₁ mice injected with chimera	60:1	Chimera‖	17.3
TDL§	30:1	—	16.5
	10:1	—	17.5
	Target cells alone	—	17.8

* Viable cells.

‡ Assayed essentially according to the method of Brunner et al. (14). Incubation was over 6 h.

§ 10⁷ TDL were transferred intravenously to irradiated (800 R) mice and the spleens removed 6 days later.

‖ Pooled from chimeras 3 and 5.

¶ Each figure represents arithmetic mean of three–four cultures. The standard errors were within 3% of the mean.
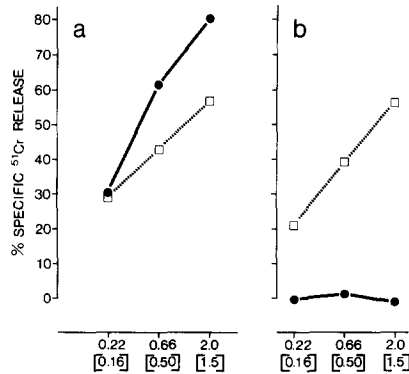

FIG. 1. Lysis of (a) C57BL targets and (b) DBA/2 targets by spleen cells from a CBA/J → DBA/2 F₁ chimera (●——●) and a normal CBA/J mouse (□---□) activated in vitro by (C57BL × DBA/2)F₁ cells. On the abscissae, the doses of killer cells incubated with the (constant) number of target cells are given in terms of the numbers of responder cells initially cultured, i.e. with respect to preactivation cell counts. Aliquots of chimera cells given in brackets, normal CBA/J cells not in brackets. These ratios differ because the numbers of cells initially cultured (preactivation counts) were 2 × 10⁷ for the chimera cells and 1.5 × 10⁷ for the normal CBA cells. Each point given in the figure represents the arithmetic mean of three killer-target mixtures. Standard errors were within 2-3% of the mean. Lysis of syngeneic (CBA/J) targets was nil with both populations (data not shown). All targets were PHA blasts.

Specific blocking factors, produced by the chimera cells in culture, or suppressor T cells might have accounted for the above results. To investigate this possibility varying numbers of spleen cells from CBA/J→ DBA/2 F_1 chimeras or from normal (CBA/J × DBA/2)F_1 mice were cultured together with a constant number (10^7) of normal CBA/J spleen cells and stimulated with (C57BL × DBA/2)F_1 cells. After 5 days the killer activity of the various cultures on DBA/2 PHA blasts was tested. As indicated in Figs. 2 and 3 neither chimera cells nor normal (CBA/J × DBA/2)F_1 cells inhibited the differentiation of normal CBA/J cells into cells cytotoxic for the DBA/2 targets. Analogous experiments with CBA/J→ C57BL F_1 chimeras and C57BL targets gave similar results, irrespective of whether the period of cocultivation was for 3 or 5 days (data not shown).
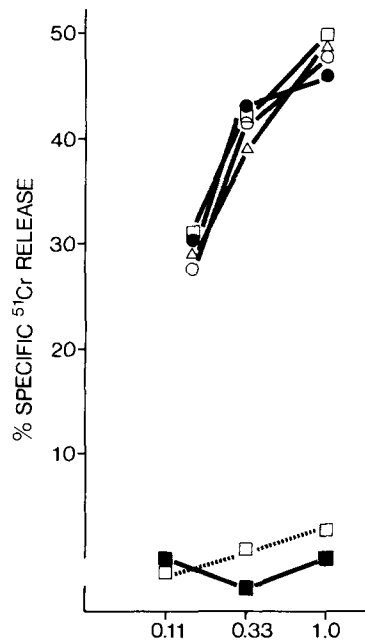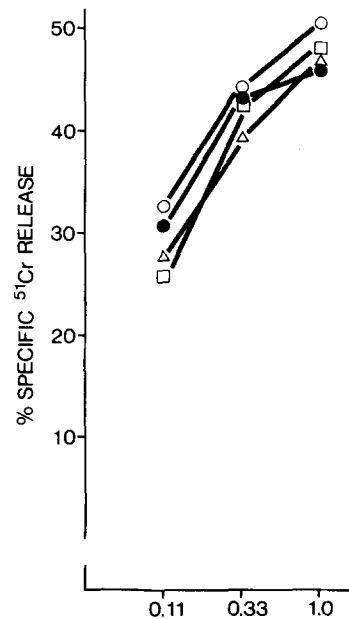

Fig. 2.   Lysis of DBA/2 PHA targets by normal CBA/J cells cocultivated in vitro with varying numbers of CBA/J → DBA/2 F_1 chimera cells and stimulated by (C57BL × DBA/2)F_1 cells. On the abscissae the doses of CBA killer cells incubated with the (constant) number of target cells are given in terms of the numbers of CBA/J responder cells initially cultured. Each point given in the figure represents the arithmetic mean of three cultures. (■——■), 15 × 10^6 chimera spleen cells not cocultivated (i.e. cultured with stimulator cells only). (□---□), 15 × 10^6 CBA/J × DBA/2)F_1 cells not cocultivated; (●——●), 10 × 10^6 normal CBA/J cells not cocultivated; (O——O), 10 × 10^6 normal CBA/J + 5 × 10^6 chimera cells; (□——□), 10 × 10^6 normal CBA/J + 10 × 10^6 chimera cells; (△——△), 10 × 10^6 normal CBA/J + 15 × 10^6 chimera cells.

Fig. 3.   Lysis of DBA/2 PHA targets by normal CBA/J cells cocultivated in vitro with varying numbers of normal (CBA/J × DBA/2)F_1 cells and stimulated by (C57BL × DBA/2)F_1 cells. On the abscissae the doses of CBA killer cells incubated with the (constant) number of target cells are given in terms of numbers of CBA responder cells initially cultured. Each point given in the figure represents the arithmetic mean of three cultures. (●——●), 10 × 10^6 normal CBA/J cells not cocultivated; (O——O), 10 × 10^6 normal CBA/J cells + 5 × 10^6 (CBA/J × DBA/2)F_1 cells; (□——□), 10 × 10^6 normal CBA/J cells + 10 × 10^6 (CBA/J × DBA/2)F_1 cells; (△——△), 10 × 10^6 normal CBA/J cells + 15 × 10^6 (CBA/J × DBA/2)F_1 cells.

The experiment illustrated in Fig. 4 represents a more thorough attempt than that in Table IV to investigate whether chimera serum contains "blocking" activity. The serum was taken from a C3H→ SJL F_1 chimera whose lymphocytes had produced an antihost response in MLR but not in CML; the serum and lymphocytes were taken at 6 mo postirradiation. It is evident from Fig. 4 that when normal C3H cells were activated against SJL and DBA/2 determinants in vitro, their capacity to lyse either SJL or DBA/2 targets was only slightly inhibited when incubated in the presence of ⅕ (vol/vol) chimera serum. Normal (C3H × SJL)F_1 serum, by contrast, caused marked inhibition.
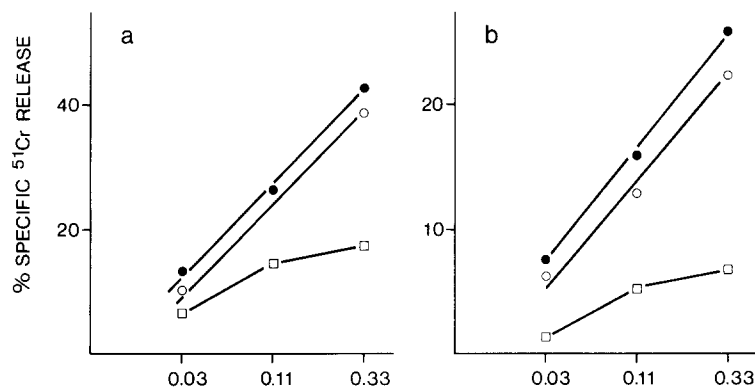

Fig. 4. Lysis of DBA/2 (a) and SJL (b) PHA targets by C3H spleen cells stimulated in vitro for 5 days with both DBA/2 and SJL cells. During the killer assay the cells were incubated with either no serum (●——●), ⅕ (vol/vol) of serum from a C3H → SJL F_1 chimera (O——O), or ⅕ (vol/vol) of normal (C3H × SJL)F_1 serum (□——□). Each point given in the culture represents the arithmetic mean of three cultures.

Discussion

Attempts to obtain long-term survival of radiation chimeras made between H-2-incompatible strains have usually (1, 2), though not invariably (18), been unsuccessful, most mice dying within a few weeks. The studies presented here indicate that, under the strict experimental conditions used, semiallogeneic radiation chimeras totally repopulated with parental strain bone marrow cells survive for a least 6 mo in apparent good health.

TDL outputs from the chimeras indicated that at 6 mo postirradiation the recirculating pool of T lymphocytes was of normal size. Thus, quantitatively speaking, there was no evidence that T-cell differentiation from stem cells was impaired in a semiallogeneic environment.

Studies on the reactivity of lymphocytes from the chimeras showed that the cells were responsive to host-type determinants in MLR, but not in CML; against third party antigens, by contrast, the cells responded well in both MLR and CML. How can these findings be explained? Answering this question requires consideration of the genetic control of the determinants involved in these reactions. In the mouse, the CML determinants detected on PHA blasts are considered to be coded for predominantly by the K and D regions of the H-2

complex (13, 14). Stimuli for the MLR and GVH reaction, by contrast, are believed to be controlled largely by the I region of the H-2 complex (14, 15); MLR can also be elicited by (non-H-2)M locus differences (16). MLR determinants have been referred to as "lymphocyte-defined" (13) since they seem to be expressed predominantly by lymphohemopoietic cells. If so, then radiation chimeras entirely repopulated with cells of donor origin would lack host-type MLR determinants, and thus fail to stimulate an MLR by these cells; this is supported by evidence that T cells of donor origin, unlike those of other origins, fail to proliferate when transferred to reirradiated radiation chimeras (7). The lack of MLR determinants would thus fail to eliminate (tolerize) cells reactive to these determinants. It would follow, therefore, that donor-derived lymphocytes differentiating in radiation chimeras would have the *potential* to respond to host-type MLR determinants. Host-type CML determinants, by contrast, not being restricted to lymphoid cells would exist in the chimeras and therefore be available to confront CML-reactive cells. If induction of tolerance, e.g. by deletion, followed such confrontation, lymphocytes reactive to host determinants in CML, would be absent.

In interpreting the present findings in terms of this argument, three points should be considered: (a) According to the above explanation, the antihost MLR of the chimera lymphocytes should have been similar in magnitude to that of normal donor-type T cells. The fact that it was considerably lower might be explained in two ways. Firstly it could be argued that MLR determinants are not restricted to lymphoid cells but are expressed to some extent by other cell types. If so, host-type MLR determinants would be reduced in the chimeras but not absent. The second possibility is that, in spite of the large dose of irradiation used, a few host-type lymphohemopoietic cells survived, i.e. in numbers sufficient to stimulate (and tolerize) the donor cells but too small to be detected by the dye exclusion technique used for cell identification. At present, it is impossible to distinguish between these two possibilities. (b) An obvious prediction is that in chimeras containing a mixture of donor-derived and host-derived (or host-type) lymphohemopoietic cells, the donor-derived lymphocytes would lack the potential to mount an anti-host MLR. This has been found to be the case in two situations, firstly in chimeras prepared after sublethal irradiation (unpublished data) and secondly in lethally irradiated mice injected with hemopoietic cells from both parent—tetraparental chimeras (17). (c) Other workers have reported that radiation chimeras contain blocking serum factors demonstrable in a microcytotoxicity assay (18). Such factors, or suppressor T cells, might have accounted for the unresponsiveness in CML observed in the present study. This possibility was investigated by determining (i) whether serum from the chimeras could specifically inhibit CML of normal donor-strain cytotoxic lymphocytes and (ii) if addition of chimera lymphocytes to normal donor-type lymphocytes would prevent the latter from differentiating into cytotoxic lymphocytes. In neither situation was there any evidence of specific inhibition. It would seem likely therefore that the unresponsiveness observed in CML reflected deletion of the reactive cells rather than suppression.

Whatever the interpretation, the present findings, together with those on tetraparental radiation chimeras [which live for prolonged periods in good health

(17)], indicate that the differentiation of stem cells to competent T cells in a semiallogeneic environment does not have a deleterious effect on the host. Thus it would seem that whereas harmful side effects follow confrontation of competent T cells with alloantigens, T cells encountering such antigens during differentiation from stem cells are eliminated with no injurious sequelae. The problem of preparing healthy chimeras would therefore seem to lie mainly with effective removal of competent T cells from the stem cell population used.

As a whole, the present findings provide direct support for the proposal of Bach et al. (19) that lymphocytes reactive in MLR and in CML have different specificities. Whether this also applies to cells involved in graft rejection, GVH reactions and alloantibody production is currently being investigated with the system described here.

Summary

Semiallogeneic radiation chimeras were prepared by injecting heavily irradiated F_1 hybrid mice with bone marrow cells from one parental strain; the bone marrow cells were treated with anti-θ serum and complement to remove T cells and injected in large numbers (2×10^7 cells). The mice survived in excellent health until sacrifice 6 mo later. Thoracic duct cannulation at this stage showed that the mice possessed normal numbers of recirculating lymphocytes. Close to 100% of thoracic duct lymphocytes and lymph node cells were shown to be of donor strain origin.

The capacity of lymphocytes from the chimeras to respond to host-type determinants was tested in mixed leukocyte culture and in an assay for cell-mediated lympholysis (CML). Mixed leukocyte reactions (MLR) were measured both in vitro and in vivo; tumor cells and phytohemagglutinin-stimulated blast cells were used as target cells for measuring CML. While responding normally to third party determinants, cells from the chimeras gave a definite, though reduced MLR when exposed to host-type determinants. However, this proliferative response to host-type determinants, unlike that to third party determinants, was not associated with differentiation into cytotoxic lymphocytes. No evidence could be found that unresponsiveness in this situation was due to blocking serum factors or suppressor T cells. It is argued that the results support the concept that lymphocytes responsive in mixed leukocyte culture have a different specificity to those exerting cell-mediated lympholysis.

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