PHYSIOLOGY OF B CELLS IN MICE WITH X-LINKED IMMUNODEFICIENCY (*xid*)

III. Disappearance of *xid* B Cells in Double Bone Marrow Chimeras

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The CBA/N mouse strain has a well-characterized syndrome of X-linked immunodeficiency (*xid*)[1] that principally affects B lymphocytes (1). This strain has proved particularly useful in defining the two subsets of B cells found in normal mice. Unlike CBA/N mice, normal mice contain a population of mature B cells that develops late in ontogeny, expresses Lyb 3,5 surface alloantigens and a low density of IgM (reviewed in references 1–3). Normal mice also contain immature B cells exhibiting a high density of surface IgM and a lack of Lyb 3,5 alloantigens. These immature B cells are said to account for the vast majority of the B cells in CBA/N mice.

Current concepts of the properties of the two B cell subsets rest heavily on the assumption that CBA/N B cells are indeed the counterpart of the immature B cells of normal mice. Although CBA/N B cells do share many of the features of normal immature B cells, it is equally conceivable that CBA/N B cells are intrinsically abnormal and do not have an exact equivalent in normal mice. In support of this possibility, the present paper demonstrates that when irradiated mice are reconstituted with a mixture of *xid* and normal stem cells, the *xid* B cells differentiating in the chimeras eventually become totally replaced by B cells of the nondefective strain. By this parameter, *xid* B cells are a unique population not represented in normal mice.

Materials and Methods

Mice. (C57Bl/6 (B6) × CBA/J)F_1, (AKR/J × DBA/2)F_1, CBA/Ca, BALB/c, and B6 mice were obtained from The Jackson Laboratory, Bar Harbor, ME. CBA/N and (CBA/N × DBA/2)F_1 mice were kindly provided by Dr. D. Mosier, Institute for Cancer Research, Philadelphia, PA. Male mice were used unless stated otherwise.

Preparation of Chimeras. Mice were irradiated and reconstituted with anti-Thy-1.2 (monoclonal) and complement (C′)-treated marrow as described elsewhere (4). Chimeras were maintained on neomycin and polymyxin B in the drinking water for 3–5 wk postreconstitution.

Irradiation. Mice were exposed to ^{137}Cs γ-irradiation using a Gamma cell irradiator (Atomic Energy of Canada) at a dose rate of 92 rads/min.

This work was supported by U. S. Public Health Service grants AI 21687 and CA 38355.

[1] *Abbreviations used in this paper:* C′, complement; LN, lymph nodes; LPS, lipopolysaccharide; NMS, normal mouse serum; PFC, plaque-forming cells; TNP, trinitrophenyl; *xid*, X-linked immunodeficiency.

Antibodies and Antisera. The following antibodies were used: monoclonal anti-Thy-1.2 (J1j) antibody (5) (ascites fluid diluted to 1:20); affinity-purified rabbit anti-mouse IgG (RαMig) antiserum, diluted 1:10, obtained from Cappel Laboratories, Westchester, PA (see reference 6 for details on the specificity of this reagent); monoclonal antibody J11d (culture supernatant diluted 1:5), a reagent specific for >95% of B cells, most "null" cells and early hematopoietic cells but not mature T cells (5); monoclonal anti-I-A^k (11-5.2) antibody (ascites fluid diluted 1:10) from hybridoma cells kindly provided by the Herzenberg group, Stanford; monoclonal antibody BP107 specific for I-$A^{b,d}$ but not I-A^k (7) (ascites fluid diluted 1:10); monoclonal anti-K^bD^b (28-8-6s) antibody (culture supernatant diluted 1:5) (8); monoclonal anti-D^d (34-4-21s) antibody (culture supernatant diluted 1:5) (8); and CBA/J anti-B6 and B6 anti-CBA/J H-2 alloantisera (9). For detecting H-2^b-bearing cells, CBA/J anti-B6 antiserum was used in some experiments and 28-8-6s monoclonal antibody was used in others.

Cytotoxicity Assays. A one-step assay was employed, using Ficoll-gradient-separated cells (5). A mixture of mouse-spleen-absorbed guinea pig serum (final concentration 1:6) and rabbit serum (final concentration 1:60) was used as a source of C′. Cell viability was measured by phase contrast microscopy.

Plaque-forming Cells (PFC) to Trinitrophenyl (TNP). Direct anti-TNP PFC were assayed according to Rittenburg and Pratt (10). Anti-TNP PFC in the spleen were measured after intraperitoneal immunization of mice with 20 μg TNP_{54} aminoethylcarbamylmethyl Ficoll (TNP-Ficoll) or 20 μg TNP-lipopolysaccharide (TNP-LPS) kindly provided by Dr. D. Mosier, Institute for Cancer Research, Philadelphia, PA.

Identity of PFC. The identity of PFC in the spleens of double chimeras was established according to the technique of Miller and Mitchell (9). In brief, aliquots of spleen cells containing a known number of PFC were incubated in the presence of C′ with anti-H-2 antibody (either alloantisera or monoclonal antibody) or normal mouse serum (NMS). After this treatment the cells were then assayed for PFC in Cunningham chambers (9).

Purification of T and B Cells. B cell–enriched populations were prepared by treating spleen or lymph node (LN) cells (pooled cervical, inguinal, axillary, and mesenteric nodes) with anti-Thy-1.2 antibody plus C′. T cell-enriched populations were obtained either by passing cells over nylon wool columns (5) or by treating the cells with a cocktail of J11d and anti-I-A antibodies. Dead cells were removed by Ficoll gradient separation before using the cells for cytotoxicity assays (5).

Results

Experimental Approach. In addition to B cells, the T cells and macrophages of CBA/N mice are reported to show subtle abnormalities (1). In this respect, the initial aim of the experiments described below was to investigate whether the B cell defect in CBA/N mice was secondary to a defect in other cell types. If this were the case, the addition of normal (nondefective) lymphohematopoietic cells during early CBA/N B cell differentiation would be expected to abrogate the B cell defect. To investigate this question, double ("tetraparental") bone marrow chimeras were prepared, using a mixture of *xid* and normal stem cells. Irradiated (1,000 rads) (B6 × CBA/J)F_1 mice were reconstituted with a mixture of *xid* CBA/N (H-2^k) and nondefective B6 (H-2^b) T-depleted marrow cells (CBA/N + B6 → F_1 chimeras) (Materials and Methods); controls were reconstituted with a mixture of nondefective CBA/Ca (H-2^k) (closely related to CBA/N) and B6 marrow cells (CBA/Ca + B6 → F_1 chimeras). The origin of the B cells differentiating in the chimeras was established with the aid of RαMig and specific anti-H-2 and anti-I-A reagents.

Response of Double Chimeras to TNP-Ficoll and TNP-LPS: Origin of PFC. CBA/N mice characteristically respond poorly to thymus-independent Type II (TI-2)

antigens, e.g. TNP-Ficoll, but react well to TI-1 antigens, e.g. TNP-LPS (1). To examine whether CBA/N B cells would gain responsiveness to TNP-Ficoll as the result of differentiation in the presence of normal lymphohematopoietic cells, the above two groups of double chimeras were immunized with TNP-Ficoll at 6 months postreconstitution.

Both groups of chimeras gave high anti-TNP responses when splenic PFC were measured on day 6 (Table I). To establish the identity of the PFC, aliquots of spleen cells pooled from each group were treated in the presence of C′ with anti-H-2^k antibody, anti-H-2^b antibody, or NMS, and then assayed for numbers of PFC. In the case of the CBA/Ca + B6 → F_1 chimeras, PFC numbers relative to the NMS controls were reduced by 75% with anti-H-2^k antibody and by 12% with anti-H-2^b antibody. This finding implied that although the chimeras were reconstituted with equal numbers of CBA/Ca and B6 marrow cells, the majority of the PFC were of CBA/Ca origin. [Possible reasons for this imbalance will be discussed later.] Very different findings were observed with the CBA/N +B6 → F_1 chimeras. In these chimeras, virtually all of the PFC were of B6 origin (Table I). Another experiment gave identical results.

At face value, the above data clearly implied that CBA/N-derived B cells in

TABLE I

Identity of PFC in Spleens of CBA/N + B6 → F_1 Double Bone Marrow Chimeras Immunized 6 d Before with TNP-Ficoll

Chimeras*	No. of mice/ group	Total numbers of direct (IgM) PFC/ spleen‡	No. of PFC after treating aliquots of spleen cells with anti-H-2 antibody + C′¶		
			Antibody	No. of PFC	% reduction
CBA/Ca + B6 → F_1 chimeras	3	128,622(1.11)§	NMS	657	—
			α H-2^b	576	12
			α H-2^k	164	75
CBA/N + B6 → F_1 chimeras	3	80,910(1.11)	NMS	675	—
			α H-2^b	13	98
			α H-2^k	682	0
Normal B6	1	183,620	NMS	351	—
			α H-2^b	36	90
			α H-2^k	297	15
Normal CBA/J	1	34,000	NMS	522	—
			α H-2^b	540	0
			α H-2^k	13	98

* (B6 × CBA/J)F_1 mice were exposed to 1,000 rads and injected with a 1:1 mixture (2×10^6 of each) of anti-Thy-1.2 + C′-treated marrow cells taken from B6 and CBA/Ca or CBA/N mice. Chimeras were tested at 6 months post-reconstitution.

‡ Mice immunized with 20 μg TNP-Ficoll given intraperitoneally. Normal CBA/N mice, not tested in this particular experiment, were invariably totally unresponsive to TNP-Ficoll.

§ Geometric mean; number in parentheses is the value by which the mean is multiplied or divided to give upper and lower limits of the SE.

¶ See Materials and Methods for details. Pooled spleen cells from each group were used for PFC identification.

the chimeras remained totally unresponsive to TNP-Ficoll. Nevertheless, it was important to show that CBA/N-derived B cells were indeed present in the chimeras. The chimeras were therefore immunized against TNP-LPS, a known immunogen for CBA/N B cells (see above). Surprisingly, virtually all of the anti-TNP PFC in the CBA/N + B6 → F_1 chimeras given TNP-LPS were of B6 origin, i.e., 100% of the PFC were killed with anti-H-2^b antibody plus C′ (Table II); this treatment lysed only ~30% of PFC in spleens of the CBA/Ca + B6 → F_1 chimeras, implying that most of the PFC in these chimeras were of CBA/Ca origin. In a further experiment, the two groups of double chimeras were immunized against horse erythrocytes (HRC), a T-dependent antigen. As for responses to TNP-Ficoll and TNP-LPS, virtually all of the anti-HRC PFC in the CBA/N + B6 → F_1 chimeras were of B6 origin (compared with <30% B6 PFC in CBA/Ca + B6 → F_1 chimeras) (data not shown).

Two possibilities could account for the above findings: (*a*) The CBA/N-derived B cells in the chimeras were unresponsive to all three antigens tested, or (*b*) CBA/N-derived B cells failed to differentiate in the chimeras. To assess this second possibility, the identity of total T and B lymphocytes in the chimeras was tested.

Identity of T and B Cells in Double Chimeras. Spleen and LN cells from the two groups of double chimeras were passed over nylon wool columns or treated with anti-Thy-1 antibody and C′ to yield populations enriched for T or B lymphocytes, respectively. The identity of the T and B cells was assessed by cytotoxicity, using anti-H-2 and anti-I-A antibodies (Table III). In the case of the CBA/Ca + B6 → F_1 chimeras, approximately two-thirds of the B (Thy-1^-, I-A^+) cells were of CBA/Ca origin and one-third of B6 origin. These data correlated closely with the origin of the antigen-specific PFC in these chimeras (Tables I and II). B cells in the CBA/N + B6 → F_1 chimeras, by contrast, were almost entirely of B6 origin. The proportion of CBA/N-derived B cells was very small

TABLE II

Identity of PFC in Spleens of CBA/N + B6 → F_1 Double Bone Marrow Chimeras Immunized 5 d Before with TNP-LPS

Chimeras*	No. of mice/ group	Total no. of direct (IgM) PFC/spleen‡	No. of PFC after treating aliquots of spleen cells with anti-H-2 antibody + C′§		
			Antibody	No. of PFC	% reduction
CBA/Ca + B6 → F_1 chimeras	5	58,940(1.18)§	—	620	—
			α H-2^b	440	29
CBA/N + B6 → F_1 chimeras	4	20,290(1.21)	—	490	—
			α H-2^b	0	100
Normal (B6 × CBA/J)F_1	1	84,600	—	212	—
			α H-2^b	3	99

* As for Table I.

‡ Mice were immunized with 20 μg TNP-LPS given intraperitoneally.

§ As for Table I.

TABLE III

Paucity of CBA/N B Cells but Not T Cells in CBA/N + B6 → F_1 Double Bone Marrow Chimeras

Chimeras tested*	Time after marrow reconstitution	Cells tested‡	Cytotoxic indices with§:					
			αThy-1.2	RαMig	αI-A^k	αI-A^b	αH-2^k	αH-2^b
CBA/Ca → F_1	6 months	Unsep. spleen	25	43	48	0	97	2
CBA/N → F_1	6 months	Unsep. spleen	28	38	31	0	96	1
CBA/Ca + B6 → F_1	6 months	Spleen B	6	76	64	35	70	34
		LN B	5	99	—	—	68	44
		LN T	91	2	—	—	55	58
CBA/N + B6 → F_1	6 months	Spleen B	2	77	7	82	8	91
		LN B	2	96	—	—	9	97
		LN T	90	7	—	—	54	54
CBA/Ca + B6 → F_1	9 months	Spleen B	0	65	60	26	57	29
		LN T	96	0	0	2	70	43
CBA/N + B6 → F_1	9 months	Spleen B	0	74	2	88	0	88
		LN T	95	0	0	0	72	42

* As for Table I.

‡ Spleen or LN B, cells pretreated with anti-Thy-1.2 antibody + C′; LN T, nylon wool-purified LN cells or LN cells pretreated with J11d (anti-B) antibody plus C′ (see Materials and Methods).

§ In the case of unseparated (Unsep.) spleen cells or spleen B cells, cell suspensions from individual mice (3 mice/group) were tested. For these cells the data shown in the table represent the arithmetic mean of the values obtained. For convenience SD are not shown; SD were generally within 10–20% of the mean. In the case of LN B cells and LN T cells, cells from 3 mice/group were pooled to prepare the purified cells. Cytotoxic indices were then established on the pooled cells.

(≃8%). Indeed, when further chimeras of the same batch were left for another 3 months (9 months post-reconstitution), virtually no CBA/N-derived B cells were detected (Table III). It should be mentioned that CBA/N-derived B cells were readily detected in single chimeras, i.e., irradiated (B6 × CBA/J)F_1 mice reconstituted with CBA/N marrow alone (Table III).

In marked contrast to B cells, a high proportion (~50%) of the T cells in CBA/N + B6 → F_1 chimeras were of CBA/N (H-2^k) origin. Indeed, the ratio of CBA:B6 T cells was indistinguishable in the two groups of double chimeras.

[Note that the sum of the cytotoxic indices observed with anti-H-2^k and anti-H-2^b antibodies sometimes exceeded 100%, particularly in the case of LN T cells. Such a finding indicates that a small proportion of the cells were of F_1 host origin. On this point, previous studies have shown that although very few stem cells or B cells withstand heavy irradiation (1,000 rads), small numbers of T cells can survive for several months post-irradiation (6).]

Time Course of Survival of CBA/N-derived B Cells in Double Chimeras. Since the double chimeras in the above experiments were typed at ≥6 months post-reconstitution, it was important to determine whether CBA/N-derived B cells were detectable at an earlier stage post-reconstitution. To study this question, new batches of CBA/Ca + B6 → F_1 and CBA/N + B6 → F_1 double chimeras

were prepared. The chimeras were then typed for the origin of their lymphoid cells at 1.5, 3.5, 7.5, and 20 wk post-reconstitution. The data shown in Table IV can be summarized as follows.

At 1.5 wk post-reconstitution, the spleens of both types of chimeras consisted largely of $Ig^-I\text{-}A^-$ "null" cells, the vast majority of which were of CBA origin (see below).

At 3.5 wk, Thy-1^+, Ig^+, and I-A^+ cells were detectable in the spleen, but only in small numbers. Typing with anti-I-A antibodies indicated that I-A^k cells were more common than I-A^b cells in both groups of chimeras.

At 7.5 wk, typical T and B cells were conspicuous in both spleen and LN. In the case of the CBA/Ca + B6 → F_1 chimeras, B (Thy-1^-, I-A^+) cells were derived from both parental strains, with only a slight preponderance of CBA/Ca-derived cells (compare with the origin of the null cells at 1.5 wk). In the CBA/N + B6 → F_1 chimeras, B6-derived B cells clearly predominated. Significantly, however, in contrast to the long-term reconstituted chimeras considered earlier (Table III), CBA/N-derived B cells were present in appreciable numbers, both in spleen and LN (≃20%). The T cells in both groups of chimeras were predominantly of CBA origin (≃2:1 ratio of CBA:B6).

By 20 wk post-reconstitution, the proportion of CBA/N-derived B cells in

TABLE IV

Appearance, Then Disappearance of CBA/N-derived B Cells in CBA/N + B6 → F_1 Double Chimeras: Time Course

Chimeras tested*	Time after marrow reconstitution	Cells tested‡	Cytotoxic indices with§:						
			αThy-1.2	RαMig	αI-A^k	αI-A^b	J11d	αH-2^k	αH-2^b
CBA/Ca + B6 → F_1 chimeras	1.5 wk	Unsep. spleen	2	3	5	6	59	71	9
	3.5 wk	Unsep. spleen	10	16	21	14	78	65	38
	7.5 wk	Spleen B	4	87	61	37	93	64	42
		LN B	0	93	70	47	90	68	46
		LN T	88	7	0	0	0	83	36
	20 wk	Spleen B	0	—	50	50	—	51	54
		LN B	0	91	46	56	—	42	62
		LN T	94	0	0	0	—	86	41
CBA/N + B6 → F_1 chimeras	1.5 wk	Unsep. spleen	1	3	3	4	50	62	9
	3.5 wk	Unsep. spleen	11	10	8	3	71	64	32
	7.5 wk	Spleen B	2	63	18	64	84	32	64
		LN B	0	96	20	87	91	29	78
		LN T	91	7	3	3	0	75	39
	20 wk	Spleen B	1	—	4	86	—	13	79
		LN B	1	88	4	87	—	6	87
		LN T	96	2	1	6	—	90	17
CBA/Ca → F_1 chimeras	20 wk	Unsep. spleen	24	62	78	2	—	—	5
CBA/N → F_1 chimeras	20 wk	Unsep. spleen	49	31	49	0	—	—	2

*‡§ As for Table III.

CBA/N + B6 → F_1 chimeras had fallen to very low levels (<5% I-A^{k+}). The proportion of CBA/N-derived T cells in these chimeras, by contrast, was very high, in fact higher than at 7.5 wk. In the case of the CBA/Ca + B6 → F_1 chimeras, the origin of the T and B cells at 20 wk was similar to 7.5 wk except for a relative increase in B6-derived B cells.

The significant finding in the above experiment was that CBA/N-derived B cells did make an appearance in CBA/N + B6 → F_1 chimeras, but that this appearance was only transient. It should be mentioned that results virtually identical to those shown in Table IV were obtained in an additional experiment with a quite separate batch of chimeras (data not shown).

Before proceeding, comment should be on the conspicuous paucity of B6-derived null cells in the double chimeras assayed at 1.5 wk post-reconstitution (Table IV). In all probability, this was a manifestation of Hh resistance, B6 (D^b) cells being particularly susceptible to this reaction (11). To seek support for this explanation, a 50:50 mixture of CBA/N and B6 marrow cells (2×10^6 of each) were transferred to F_1 mice conditioned by split dose irradiation, i.e., 600 rads followed 2 wk later by 1,000 rads. This procedure is known to abolish Hh resistance (11). When spleens from the chimeras were removed 1 wk later, the ratio of B6:CBA cells was now close to 1:1 (data not shown). This compared with a ratio of about 1:15 when control mice received a single dose of 1,000 rads. Although these data strongly suggest that Hh resistance was responsible for the early paucity of B6-derived cells in the spleen, it might be asked why these cells rebounded in proportion at later stages (see Table IV). The likely answer is that the effector cells for Hh resistance are short-lived cells that reside largely in the spleen, but not in other organs (11). Donor B6 marrow cells seeding the marrow cavity of the recipients fail to encounter Hh resistance: the descendants of these cells then appear in the spleen once Hh resistance in this organ has waned.

The irradiated hosts in the preceeding experiments were all (B6 × CBA/J)F_1 mice. To determine whether CBA/N-derived B cells would fail to thrive in a different F_1 combination, (AKR × DBA/2)F_1 (H-2^k × H-2^d) mice were used as recipients of a mixture of BALB/c (H-2^d) and CBA/N or CBA/Ca (H-2^k) marrow. As shown in Table V, control single chimeras prepared by injecting either CBA/Ca or CBA/N marrow cells alone were near totally repopulated with donor-derived T and B cells when the chimeras were typed 4 months later. The splenic B cells from CBA/Ca + BALB/c → F_1 chimeras were derived from both parental strains with an unexpected preponderance of BALB/c (I-A^d)-derived cells. ~20% of the B cells were CBA/Ca (I-A^k)-derived. In CBA/N + BALB/c → F_1 chimeras, by contrast, CBA/N (I-A^k)-derived B cells were completely undetectable. CBA/N-derived T cells, however, were conspicuous and indeed outnumbered the BALB/c-derived T cells.

Double Chimeras Prepared with Sublethal Irradiation. A dose of irradiation in the range of 750 rads destroys the vast majority of mature T and B cells, but spares small numbers of CFU/s. Transfer of parental strain marrow cells to these mice thus produces a form of double chimera in which the lymphohematopoietic system is derived from both donor and host (12). Table VI shows the effects of transferring CBA/Ca vs. CBA/N marrow cells to sublethally irradiated (CBA/N × DBA/2)F_1 mice; the recipients were either female (normal, nondefective) or

TABLE V

Paucity of CBA/N-derived B Cells but Not T Cells in Irradiated (AKR/J × DBA/2)F_1 Mice Reconstituted with CBA/N Plus BALB/C Marrow Cells 4 Months Previously

Chimeras tested*	Cells tested‡	No. of Mice	Cytotoxic indices with§:					
			αThy-1.2	RαMig	αI-A^d	αI-A^k	αH-2^d	αH-2^k
CBA/Ca + BALB/c → F_1 chimeras	Spleen B	6	0	63(±4)	65(±3)	21(±2)	—	—
	LN T		97	0	3	4	66	45
CBA/N + BALB/c → F_1 chimeras	Spleen B	8	0	62(±2)	92(±4)	0	—	—
	LN T		96	4	0	0	44	64
CBA/Ca → F_1 chimeras	Unsep. spleen	3	24(±6)	58(±1)	4(±4)	75(±4)	4(±4)	—
CBA/N → F_1 chimeras	Unsep. spleen	3	53(±6)	31(±10)	4(±3)	46(±7)	4(±4)	—

* F_1 mice were exposed to 1,000 rads and then, 4 h later, injected intravenously with anti-Thy-1-treated marrow cells in a dose of 2 × 10^6 cells (2 × 10^6 of each population for double chimeras).
‡§ As for Table III; SD are shown in parentheses (except for LN T where pooled cells were used—see Table III).

TABLE VI

Identity of T and B Cells in Sublethally Irradiated (750 rads) (CBA/N × DBA/2)F_1 Female vs. Male Mice Reconstituted with CBA/Ca vs. CBA/N Marrow Cells

Group	Chimeras*	Immune status of donor/recipient	Cells examined‡	Cytotoxic indices with§:		
				αThy-1.2	RαMig	αH-2^d (anti-host)
1	CBA/Ca → F_1 ♀	Normal → normal	Spleen B	0	78(±4)	34(±2)
			LN T	96	0	20
2	CBA/N → F_1 ♂	*xid* → *xid*	Spleen B	0	64(±7)	25(±2)
			LN T	99	0	50
3	CBA/Ca → F_1 ♂	Normal → *xid*	Spleen B	0	78(±4)	1(±1)
			LN T	98	0	25
4	CBA/N → F_1 ♀	*xid* → normal	Spleen B	0	68(±10)	74(±7)
			LN T	97	0	29

* F_1 mice were exposed to 750 rads and then injected intravenously with 2 × 10^6 T cell–depleted CBA/Ca or CBA/N male marrow cells. Chimeras were assayed at 4 months post-reconstitution.
‡§ As for Tables III, V.

male (*xid*). T and B cells were typed at 4 months post-reconstitution. The results can be summarized as follows.

In the case of B cells, the proportion of host (H-2^d-bearing) cells in CBA/Ca → F_1 ♀ chimeras (Group 1) and CBA/N → F_1 ♂ chimeras (Group 2) was quite similar (34% vs. 25%). Note that in these two situations, the donor and host were either *both* nondefective (normal) (Group 1) or *both* defective (*xid*) (Group 2). Very different results were seen in the other two situations. With transfer of nondefective marrow to *xid* recipients (CBA/Ca → F_1 ♂, Group 3), host cells were virtually undetectable (1%). In marked contrast, host cells reached 74% in the reciprocal situation, i.e., with transfer of *xid* marrow to nondefective recipi-

ents (CBA/N → F_1 ♀, Group 4). In the case of T cells, there was no marked variation in the proportion of host cells among the four groups (20–50%).

These results indicate that normal B cells displace *xid* B cells, irrespective of whether the *xid* B cells originate from the donor marrow or from the F_1 host. These data with sublethally irradiated chimeras thus corroborate the earlier findings with *xid* B cell growth in double chimeras given lethal irradiation (Tables III–V).

Pluripotential Stem Cells in Long-term CBA/N + B6 → F_1 Double Chimeras. The finding that CBA/N-marrow-derived B cells were virtually absent in long-term reconstituted double chimeras given 1,000 rads (Table III) raised the question of whether CBA/N-derived CFU/s would survive in these chimeras. To examine this question, marrow cells were taken from CBA/N + B6 → (B6 × CBA/J)F_1 chimeras at 5 months post-reconstitution; the spleens of these chimeras contained ≲5% I-A^k-bearing cells. As controls, marrow cells were also taken from CBA/Ca + B6 → F_1 chimeras; these chimeras contained >60% I-A^k-bearing cells in the spleen. Marrow cells (4×10^6 anti-Thy-1-treated cells) from these two groups of chimeras (3 donors/group) were transferred to normal (B6 × CBA/J)F_1 mice, pretreated with split dose irradiation (600 rads plus 1,000 rads) to overcome Hh resistance. Spleens were removed from the recipients 7 d later and typed with anti-H-2 antibody; the spleens at this stage were large (≳10^8 cells) and consisted mostly of primitive hematopoietic cells. As shown in Table VII, the proportion of B6-derived cells, i.e., cells lysed by anti-H-2^b antibody, was very similar in the two groups (≃40%). As controls, the anti-H-2^b antibody lysed <2% of spleen cells from irradiated F_1 mice given normal (nonchimeric) CBA/N marrow 7 d

TABLE VII

Survival of CBA/N-Derived Pluripotential Stem Cells in Long-term Double Chimeras: Identity of Spleen Cells in Lethally Irradiated (B6 × CBA/J)F_1 Mice Injected 7 d Before with Marrow Cells from CBA/N + B6 → F_1 Chimeras

Donors of marrow cells*	Recipients of marrow cells pretreated with split-dose irradiation‡	Total no. of cells/spleen ($\times 10^{-8}$)	Identity of spleen cells removed at 7 days: cytotoxic indices with anti-H-2^b + C′
CBA/Ca + B6 → F_1 chimeras	(B6 × CBA/J)F_1	98(±12)	36(±12)§
CBA/N + B6 → F_1 chimeras	(B6 × CBA/J)F_1	130(±22)	38(±3)
Normal CBA/N	(B6 × CBA/J)F_1	115(±5)	1(±3)
Normal B6	(B6 × CBA/J)F_1	105(±25)	84(±8)

* Marrow cells were taken from the chimeras at 6 months post-reconstitution; the chimeras were from the same batch used in Table IV. Marrow cells pooled from 3 chimeras/group were treated with anti-Thy-1 antibody + C′ and transferred intravenously in a dose of 4×10^6 viable cells/mouse.

‡ Mice were first exposed to 600 rads, left for 10 d and then exposed to 1,000 rads; marrow cells were transferred 4 h after the second dose of irradiation.

§ Arithmetic mean of 3 mice/group (± SD).

before, and 84% of spleen cells from F_1 mice given normal B6 marrow cells. These data imply that the early hematopoietic cells developing in the spleens of both groups of irradiated mice given chimera marrow cells were derived in approximately equal proportions from CBA and B6 stem cells in the donor marrow. In contrast to B cells, there was thus no evidence that CBA/N-derived CFU/s become outnumbered by normal CFU/s during long-term residence in the chimeras.

Discussion

The results in this paper demonstrate that *xid* B cells differentiating from stem cells in bone marrow chimeras gradually disappear when the chimeras harbor stem cells/B cells from nondefective mice. *xid* B cells are detectable within the first 1–2 months of marrow reconstitution, but then eventually become almost totally replaced by normal B cells, both in spleen and LN. This replacement of *xid* cells by normal cells does not apply to T cells or CFU/s.

On *a priori* grounds, one might argue that the gradual disappearance of *xid* B cells in chimeras is a reflection of chronic alloaggression, mediated either by the irradiated host or by cells differentiating from the nondefective marrow inoculum. Rejection by the host would seem to be ruled out by the finding that *xid* B cells differentiate effectively in CBA/N → F_1 single chimeras (mice reconstituted with CBA/N marrow alone, Tables III and V). Alloaggression mediated by cells derived from the nondefective marrow also seems most unlikely. To sustain this argument one would have to postulate a novel form of alloaggression that (*a*) escapes tolerance induction (the marrow cells were rigorously T-depleted before transfer), (*b*) is unidirectional (anti-*xid* but not vice versa), and (*c*) is directed selectively to *xid* B cells and not other *xid*-marrow-derived cells, i.e., T cells or CFU/s. Perhaps the most convincing evidence against alloaggression is provided by the data on the growth of *xid* B cells in sublethally irradiated CBA/N (male) → (CBA/N × DBA/2)F_1 male vs. female mice (Table VI). Here it was found that CBA/N-derived B cells differentiated well in the male F_1 recipients (*xid* → *xid*) but not in the female recipients (*xid* → normal). Interpreting this finding in terms of alloaggression is virtually impossible. [A response to a Y-chromosome controlled antigen is ruled out by the fact that CBA/Ca male B cells grew well in F_1 female recipients.]

If alloaggression can be disregarded, the simplest interpretation of the data is that the rate of differentiation of *xid* B cells is slightly slower than for normal B cells. A precedent for this notion stems from findings of Kincade et al. (13). This group has reported that, unlike normal B cells, *xid* B cells are unable to form colonies in agar. These workers observed that young heterozygous female mice raised between *xid* and normal mice contained about one-half the number of colony-forming B cells as homozygous normal mice, implying that the heterozygotes are X-chromosome mosaics containing a roughly 1:1 ratio of defective and normal B cells. Interestingly, the reduction in colony-forming B cells was less apparent in older heterozygotes, suggesting that the *xid* B cells in these mice gradually become replaced by normal B cells. More recently, Nahm et al. (14) used X-chromosome linked alloenzymes to study mosaicism in *xid*-heterozygous females. These workers observed that, in marked contrast to other cell types,

spleen cells enriched for B cells were "nearly devoid" of the isoenzyme encoded by the X-chromosome bearing the *xid* gene. This suggested a virtual absence of *xid* B cells in the heterozygotes. Unfortunately, the age of the mice examined in this elegant study was not stated. If old mice were used, the data would be fully consistent with the data of Kincade et al. (13) mentioned above and also with the findings in the present paper.

Although the life history of B cells is still not fully understood, in mice it appears to involve production in the marrow (15), selection and differentiation in the spleen (16, 17), followed by entry of a portion of the cells into the recirculating lymphocyte pool (18). Some B cells are presumed to die rapidly, whereas others survive for many months in interphase. Precisely where in this life history normal B cells have a growth/survival advantage over *xid* B cells is unclear. The finding that *xid* B cells do appear within the first few weeks of reconstitution and then disappear progressively suggests that a reduced rate of production of *xid* B cells in the marrow is probably not the explanation.[2] Likewise, the kinetics of the appearance and disappearance of *xid* B cells is against the possibility that *xid* B cells die more rapidly than normal B cells (see below). Although neither of these possibilities can be ruled out, we lean to the view that *xid* B cells might have a reduced capacity to turn over (expand) in the secondary lymphoid tissues, perhaps in response to environmental antigens or mitogens (see reference 13). In this respect, it is well documented that *xid* B cells respond poorly in vitro to certain antigens and mitogens, including anti-Ig and particular extracts of LPS (1, 19). Recent studies from this laboratory have shown that most *xid* B cells do turn over (incorporate tritiated thymidine) at a slow rate in vivo.[3] Whether the rate of turnover of *xid* B cells is actually slower than normal B cells, however, is not clear from the available data.

The finding that *xid* B cells tend to disappear in the presence of normal B cells adds to the accumulating evidence that *xid* B cells probably cannot be equated with the Lyb 5^- subset of immature B cells found in normal mice (see below, and reference 19). In our view, *xid* B cells are a unique population that is not represented in normal mice. *xid* B cells certainly resemble normal Lyb 5^- B cells in several respects, notably in the capacity to respond only to TI-1 and not TI-2 antigens (1). In other respects, however, *xid* B cells share some of the properties attributed to mature Lyb 5^+ B cells, e.g. a slow turnover (for LN and most splenic B cells),[3] capacity to recirculate[3] and the expression of strong Mls determinants (20). The retarded differentiation of *xid* B cells (this paper) and the odd T-dependency of *xid* B cell differentiation (6, 21, 22), however, sets these cells apart from both the Lyb 5^- and Lyb 5^+ B subsets of normal mice. Although it is difficult to provide a simple explanation for how the *xid* gene might control these various departures from normal B cell physiology, a subtle

[2] Assuming that *xid* mice have ~30% of the number of total B cells found in normal mice (reference 1), double chimeras constructed with a 1:1 mixture of *xid* and normal B cells would be expected to generate *xid* and normal B cells in a ratio of 1:3–4. In this respect it is of interest that early after marrow reconstitution, i.e., at 7 wk, the ratio of CBA:B6 ($I\text{-}A^k$:$I\text{-}A^b$) B cells in the spleen was 1:3.6 in CBA/N + B6 → F_1 chimeras vs. 1.6:1 in CBA/Ca + B6 → F_1 chimeras (calculated from the data in Table IV). Comparison of these two ratios gives a relative ratio of 1:6, quite close to the ratio of 1:3–4 considered above.

[3] Sprent, J., and J. Bruce. 1984. Physiology of B cells in mice with X-linked immunodeficiency. I. Size, migratory properties and turnover of the B cell pool. Manuscript in preparation.

alteration in the biochemistry of *xid* cells is a logical working hypothesis. Searching for such alterations might be informative.

Summary

Evidence is presented that B cells from mice with X-linked immunodeficiency (*xid*) differentiate at a slower rate than normal B cells. This conclusion stems from studies in which (B6 × CBA/J)F_1 mice were heavily irradiated (1,000 rads) and reconstituted with a mixture of T-depleted marrow cells taken from (*a*) nondefective B6 mice (H-2^b) and (*b*) *xid* CBA/N or nondefective CBA/Ca mice (both H-2^k). With transfer of CBA/Ca plus B6 marrow cells, the irradiated recipients become repopulated with B cells derived from both parental marrow sources; except for an early imbalance (probably reflecting Hh resistance), the degree of chimerism remained relatively stable over a period of more than 6 months. Very different results occurred with transfer of a mixture of *xid* CBA/N and normal B6 marrow. Within the first 2 months after marrow reconstitution, a low but significant proportion of the B cells in both spleen and lymph nodes were of CBA/N origin. Thereafter the proportion of these cells fell progressively, and by 6–9 months virtually all of the B cells were of B6 origin. This gradual decline in CBA/N-derived cells did not apply to other cell types, i.e., T cells or pluripotential stem cells. Analogous results were obtained with transfer of CBA/N vs. CBA/Ca marrow cells into sublethally irradiated (750 rads) (CBA/N × DBA/2)F_1 male vs. female mice. For example, CBA/N-marrow derived B cells differentiated effectively and survived for long periods in F_1 male mice (*xid* → *xid*) but not in F_1 female mice (*xid* → normal).

The finding that *xid* B cells eventually disappear in the presence of normal B cells strengthens the view that *xid* B cells are an abnormal population not represented in normal mice.

Received for publication 7 May 1984.

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