Brief Definitive Report

INDUCTION OF PARTIAL CHIMERISM IN NONIRRADIATED B-LYMPHOCYTE-DEFICIENT CBA/N MICE*

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CBA/N mice, a mutant subline of the CBA/Ca strain, have a marked abnormality in bone marrow-derived, B-lymphocyte function, which is inherited as an X-linked recessive trait (1, 2). CBA/N mice and immunologically abnormal F_1 male mice derived from this strain fail to produce specific antibody after immunization with certain "thymus-independent" antigens (1–4). Moreover, in vitro analysis of spleen cells derived from these mice demonstrates diminished proliferative responses to B-cell mitogens (5, 6); reduced killing in antibody-dependent, cell-mediated cytotoxicity assays (5); and an inability to form B-lymphocyte colonies in agar cultures after stimulation with bacterial lipopolysaccharide (LPS) (7). Analysis of the surface membrane characteristics of B lymphocytes derived from immune defective CBA/N and from normal mice indicated that the functional abnormality of CBA/N mice was associated with a deficiency in a subpopulation of splenic B lymphocytes (8–11). This subpopulation is first detected in the spleens of normal mice at 2–3 wk after birth and has been characterized as (*a*) bearing both surface IgM and the putative mouse homologue of human IgD (12); (*b*) functionally expressing minor lymphocyte-activating determinants (13–15); and (*c*) bearing Lyb5 antigens (16).

The apparent deficiency of a B-lymphocyte subset in the CBA/N mice in the absence of any evidence of T-lymphocyte functional abnormalities (5, 17) provides an interesting model to study the mechanisms involved in the acceptance or rejection of engrafted lymphoid tissue in a nonirradiated recipient. Earlier functional studies suggested that stable chimerism occurred when lymphoid cells of immunologically normal (CBA/N × DBA/2)F_1 female mice were given to nonirradiated, immunologically deficient (CBA/N × DBA/2)F_1

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male mice (3). Thus, F_1 male mice who received F_1 female cells were shown to be responsive to antigens to which they were normally unresponsive; and in vitro experiments suggested that such reconstitution was not due to the development of responsiveness in F_1 male cells secondary to the influence of F_1 female cells (18).

To study this phenomenon further, immunologically abnormal CBA/N and normal CBA/CaHN mice, who are known to reciprocally accept skin grafts, (A. K. Berning and I. Scher, unpublished observations) were given spleen cells from CBA/CaHN-T6 mice without preparative radiation or other immunosuppression; and the distribution of the transplanted cells and their responsiveness to phytohemagglutinin (PHA) or LPS was determined.

Materials and Methods

Mice. CBA/CaHN, chromosomally-marked CBA/CaHN-T6, and immune defective CBA/N mice were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md.

Analysis for the Presence of T6 Cells. At various time intervals after receiving 50×10^6 viable nucleated CBA/CaHN-T6 spleen cells, groups of recipient mice were injected with colchicine (CLC) intraperitoneally (100 μg of CLC dissolved in 0.5 ml of distilled water). 3 h later, the mice were killed by cervical dislocation and bone marrow, thymus lymph node, Peyer's patches, and spleen cells were harvested and processed for chromosome studies by the modified squash method described elsewhere (19). Briefly, 5×10^6 cells were suspended in 2 ml of RPMI-1640 (Grand Island Biological Co., Grand Island, N.Y.) medium and 2 ml of distilled water. After 5 min, the cells were recovered by centrifugation and fixed by adding 2 ml of glacial acetic acid:distilled water (1 vol:1 vol) for 10 min. After a second centrifugation, the fixed cells were transferred to a clean slide and squashed for chromosome studies. The slides were stained with Wright's stain and analyzed with light microscopy. Utilizing this method of preparation, one can distinguish between the donor CBA/CaHN-T6 mitotic cells, bearing two easily recognizable tiny chromosomes (T6T6), and the host mitotic cells, which lack these chromosome "markers" in their karyotype. In a second series of experiments, single-cell suspensions of 5×10^6 cells were prepared in RPMI-1640 (supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin, 2 mM 1-glutamine, 25 mM Hepes, and 5% heat-inactivated, pooled human serum). These suspensions were cultured in duplicate with either the T-cell mitogen PHA (2 μg/culture; Wellcome Research Laboratories, Beckenham, England), the B-cell mitogen LPS (100 μg/culture; 0111:B4, Difco Laboratories, Detroit, Mich.), or media as a control as previously described (20).

Results

When immunologically normal (CBA/CaHN) or abnormal (CBA/N) recipients were given 50×10^6 CBA/CaHN-T6 spleen cells, no donor T6T6 mitotic cells were detected in the bone marrow or the thymus 2–5 wk after transplantation (Table I). Small numbers of T6 mitoses were detected in the spleen, lymph node, and Peyer's patches of 4 of 8 CBA/CaHN recipients, although their frequency was no greater than 1 of 47 (T6T6 mitoses/total mitoses). In contrast, donor T6 mitotic cells were detected in the Peyer's patches, spleen, and lymph nodes of 8 of 8 CBA/N recipients, with an incidence which ranged from 2 of 85 (2%) in Peyer's patches at 2 wk to 9 of 17 (53%) in lymph nodes at 3 and 4 wk. The level of chimerism within the different organs of engrafted CBA/N mice appeared to be stable during the time interval studied, except in the case of Peyer's patches, where the frequency increased from 2% at 2 wk to $\geq$18% thereafter.

To study the functional characteristics of engrafted CBA/CaHN-T6 cells, spleen cells from recipient mice who had received 50×10^6 CBA/CaHN-T6 cells

TABLE I

*Frequency of CBA/CaHN-T6 Mitotic Cells in the Lymphohematopoietic Organs of Normal Adult (CBA/Ca) or B-Lymphocyte-Deficient (CBA/N) Mice Given 50 × 10^6 CBA/CaHN-T6 Spleen Cells Intravenously**

Recipient strain	Organ	No. of T6T6 mitoses/total mitoses scored (Time in weeks after the injection of T6T6 spleen cells)			
		2	3	4	5
CBA/Ca	Bone marrow	0/115	0/97	0/120	0/103
	Thymus	0/25	0/9	0/35	0/19
	Spleen	1/66	0/53	0/77	0/45
	Lymph node	0/12	1/52	0/16	1/47
	Peyer's patches	0/53	1/72	0/50	0/8
CBA/N	Bone marrow	0/115	0/119	0/278	0/326
	Thymus	0/19	0/29	0/26	0/11
	Spleen	24/108	5/43	5/47	3/20
	Lymph node	6/36	9/17	9/17	9/24
	Peyer's patches	2/85	25/93	6/34	5/18

* These data represent the number of T6T6 mitoses of total mitoses scored for two animals at each time interval.

2–5 wk previously were cultured in vitro with the T- or B-cell mitogens, PHA, or LPS. Under these conditions, the frequency of CBA/CaHN-T6 mitoses in engrafted CBA/CaHN mice was similar to those observed in the experiments described above; and no differences were observed between the PHA- or LPS-stimulated cultures (PHA stimulation ≤6 of 280 or 2.1%; LPS stimulation ≤4 of 140 or 2.8%; eight individual mice examined with at least 140 mitoses counted). By contrast, the results obtained after stimulation of engrafted CBA/N spleen cells with PHA or LPS were distinct from each other and different from the experiments detailing organ distribution. The incidence of CBA/CaHN-T6 mitoses in PHA-stimulated cultures was ≤38 of 478 or 7.9%, a lower figure than that observed in both the unstimulated in vitro cultures (Table II), and in the organ distribution analysis (Table I). However, stimulation of the engrafted CBA/N spleen cells with LPS induced frequencies of CBA/CaHN-T6 mitoses that were higher 194 of 377 or 51.4%; 312 of 445, or 70.1%) than observed with unstimulated spleen cells derived from CBA/N mice in all experiments (Tables I and II).

Discussion

These data support previous studies (21) which demonstrate little, if any, engraftment of donor cells after their administration to syngeneic, unprepared normal recipients. The mechanisms responsible for the nonacceptance of the donor cells under these circumstances are unknown, but it has been postulated that in order for successful engraftment to occur, recipients of bone marrow must provide a suitable microenvironment (22). This microenvironment must allow for the physical expansion of the donor cells (space) and provide for the necessary humoral and/or cellular factor(s) necessary for cell engraftment. Further insight into this problem has been provided by two experimental systems. The first such system described involved mice that are homozygous for mutant genes at the W locus, resulting in a stem-cell abnormality which leads to severe macrocytic anemia (23). Recent data reported from our laboratory suggest that these animals also have a defective or absent T-lymphocyte

TABLE II

Frequency of CBA/CaHN-T6 Mitotic Cells in Mitogen-Induced Spleen Cultures From B-Lymphocyte-Deficient (CBA/N) Mice Given 50 × 10^6 CBA/CaHN-T6T6 Spleen Cells Intravenously

Months after injection of T6T6 spleen cells	Number of animals	No. of T6T6 Mitoses/total mitoses scored (%)		
		Media control	PHA	LPS
1	1	3/14 (21.4%)	6/256 (2.3%)	9/19 (64.2%)
2	3	4/27 (14.8%)	13/370 (3.5%)	194/377 (51.4%)
3	2	7/22 (31.8%)	18/350 (5.1%)	202/345 (58.5%)
4	3	7/23 (30.4%)	38/478 (7.9%)	312/445 (70.1%)

* The spleens of the CBA/N host mice were removed, single cell suspensions prepared, and cells cultured in the presence of PHA, LPS, or media as a control. After 3 days in culture, the dividing cells were arrested in metaphase by the addition of CLC and chromosome analysis was performed.

subpopulation (24). When such mice are given bone marrow or spleen grafts from histocompatible, normal T6 donors, a stable chimera develops with a large frequency of donor cells apparent in all hematopoietic and lymphoid organs (25). In the second system, after CBA/H mice were made T-lymphocyte-deficient by neonatal thymectomy, they accepted engraftment of normal T6T6 spleen cells, although the engrafted donor cells were only found in lymph nodes (20). Presumably, in both of these systems, the absence of a population of cells in the recipient animals (as a result of a stem-cell defect or by depletion of T cells) allowed for engraftment because of the availability of space in the setting of an appropriate humoral and cellular milieu.

CBA/N mice demonstrate engrafted CBA/CaHN-T6 cells in their Peyer's patches, spleens, and lymph nodes, but not bone marrow or thymus glands. This organ distribution is exactly that of a subpopulation of late-developing B lymphocytes, which can be identified in normal mice, but which are deficient in CBA/N mice (8–16). These findings suggest that there is a selective engraftment of T6 cells in CBA/N mice and that the cells which are successful in this engraftment may represent a distinct B-lymphocyte subpopulation. This view is supported by the increase in frequency of T6 cells obtained from the spleens of engrafted CBA/N mice after stimulation in vitro with LPS in the absence of a PHA-induced T6 proliferative response. The selectivity of engrafted lymphoid cells seen in these experiments is similar to that observed in the T-cell-depleted CBA/H system, where engrafted cells can only be found in recipient lymph nodes (21). By contrast, normal donor cells are found in all lymphoid organs but in varying amounts of engrafted W/W^v mice who have a defective multipotential stem cell (25). Studies of these three experimental systems demonstrate that engraftment of donor lymphoid tissues in unprepared hosts can occur if the host has a deficiency in a lymphoid-cell population. Moreover, the current study suggests that engraftment occurs in a specific manner and provides a model in which to study the mechanisms by which a host rejects or accepts syngeneic donor cells.

Summary

Nonirradiated B-lymphocyte-deficient CBA/N mice given T6T6 chromosome-marked normal CBA/CaHN spleen cells became lymphoid chimeras exhibiting donor-type mitoses. Normal CBA/CaHN recipients did not exhibit significant

numbers of donor-type mitoses. The lymphoid cell chimerism in the CBA/N host appeared in spleen, lymph nodes, and Peyer's patches, but not in bone marrow or thymus. Stimulation of CBA/N-recipient spleen cells in vitro suggested that the chimerism involved donor T6T6 cells which were responsive to the B-lymphocyte mitogen, lipopolysaccharide, but not to the T-lymphocyte mitogen, phytohemagglutinin. These data indicate that stable, long-term chimerism of a specific class of lymphocytes is possible in nonirradiated, B-lymphocyte-deficient CBA/N mice.

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