X-LINKED B-LYMPHOCYTE DEFECT IN CBA/N MICE

III. Abnormal Development of B-Lymphocyte Populations Defined by Their Density of Surface Immunoglobulin*

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A substantial heterogeneity exists in the amount of immunoglobulin (Ig) on the surface of bone marrow-derived (B) lymphocytes as measured by labeling these cells with fluorescein-conjugated (FITC) anti-Ig and analyzing them by the technique of rapid flow microfluorometry (RFMF). Ig-bearing lymphocytes from neonatal mouse spleen and from adult mouse bone marrow are very diverse populations when studied in this way. However, fluorescence profiles of adult mouse spleen cells analyzed by RFMF display a distinct peak indicating the presence of a large subpopulation of cells with a low-to-intermediate density of surface Ig. By analysis of spleen cells from mice 1-4 wk of age, we have shown that this subpopulation of B lymphocytes develops with the maturation of the animal (1).

In order to further define the functional significance of this population of Ig-bearing lymphocytes, we have studied the amount of Ig on the surface of individual lymphocytes from a strain of mice with an X-linked defect in B-lymphocyte function. These mice, which are members of the CBA/N strain, fail to respond to certain thymus-independent antigens (2-4), display diminished responses to thymus-dependent antigens, and their lymphocytes have a markedly reduced potential to lyse antibody-coated EL-4 target cells (5). In addition, CBA/N mice have diminished numbers of Ig-bearing lymphocytes and these cells have an abnormally high ratio of surface IgM to putative IgD homolog (5, 6). In this communication, we report that adult lymphoid cells from the spleens of CBA/N mice labeled with FITC anti-Ig have a fluorescence profile which is characterized by a marked diminution in the population of B lymphocytes with a...
low-to-intermediate density of surface Ig. This is associated with an increase in
the relative frequency of B lymphocytes with a high density of surface Ig. The
fluorescence profile of Fl anti-μ-labeled CBA/N spleen cells demonstrates a
relative decrease in B lymphocytes with a low density of surface IgM and a
relative increase in cells with a high density of surface IgM when compared to
the profile of similarly labeled normal spleen cells. These results support the
concept that the immune defect of CBA/N mice is due to an arrest in normal B-
lymphocyte development.

Materials and Methods

Animals. CBA/N, BALB/cAnN, DBA/2N, (CBA/N δ × DBA/2 strain)F1, (CBA/N δ × BALB/c
δ)F1, and (BALB/c γ × CBA/N δ)F1, mice were obtained from the Rodent and Rabbit Production
Section, Division of Research Services, National Institutes of Health, Bethesda, Md., or bred in
our laboratory. CBA/J mice were purchased from The Jackson Laboratory, Bar Harbor, Maine.
Adult mice were from 8 to 12 wk of age at the time of sacrifice. In all instances, lymphoid organs
from individual mice were studied except when newborn to 3-wk-old mice were analyzed, when the
lymphoid organs of four to five individual mice were pooled.

Lethally irradiated mice (1,000 rads, cobalt-60 source, dose rate 40 rads/min) were reconstituted
on the day after irradiation with 10⁷ bone marrow cells administered intravenously. Recipient
mice were sacrificed and their spleen cells examined 8 wk after reconstitution.

Anti-Ig Reagents. The source, methods of preparation, and the results of the specificity
analysis of the Fl anti-Ig, Fl anti-κ, and Fl anti-μ used in these studies have been described
previously (1).

Lymphocyte Labeling and RFMF. Lymphocytes were labeled as previously described (1). The
principles of RFMF have recently been presented by Loken and Herzenberg (7). Our studies were
performed with a Fluorescence Activated Cell Sorter (FACS; Becton, Dickinson Electronics
Laboratory, Mountain View, Calif.).

Results

Fluorescence Profiles of CBA/N and CBA/J Adult Spleen Cells Labeled with
Fl Anti-Ig or Fl Anti-κ. Fig. 1a compares the fluorescence profile of spleen
cells from adult CBA/J mice, a normal strain, with that of spleen cells from
immune defective CBA/N mice. Both cell populations have been labeled with Fl
anti-Ig. The profile of CBA/J cells is characterized by a distinct fluorescence
peak in the low-to-intermediate intensity range (channels 200–400), which we
have previously described for adult spleen cells of a variety of normal strains (1).

Spleen cells from CBA/N mice fail to display this characteristic peak. The
fluorescence profiles of splenic B lymphocytes from CBA/J and CBA/N mice
were further compared by plotting the data from the CBA/N strain (39.1% Ig-
positive cells) so that the area underlying channels 80–1,000 (Ig-positive cells)
was equivalent to that of the CBA/J profile (49.8% Ig-positive cells) (Fig. 1 b).

When analyzed in either way, the fluorescence profile of Fl anti-Ig-labeled
spleen cells from adult CBA/N mice is characterized by a decrease in the
population of B lymphocytes with a very-low-to-intermediate (channels 80–400)
density of surface Ig. Thus, the relative frequency of cells with a very-low-to-
intermediate density of surface Ig among the Ig-positive cells from adult CBA/J
mice and adult CBA/N mice was 70.7% ± 2.8 (mean ± SEM, n = 3), and 44.7% ±
2.6 (n = 3), respectively.

Fluorescence Profiles of (CBA/N × DBA/2)F1, (BALB/c × CBA/N)F1, and
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Fig. 1. Fluorescence profiles of (a) CBA/J spleen cells (---) or CBA/N spleen cells (—) labeled with FI anti-Ig, and (b) CBA/J spleen cells (—) or CBA/N spleen (---) labeled with FI anti-Ig with the CBA/N profile plotted so that the area under the Ig-positive portion of the curve (channels 80-1,000) is equivalent to that of the CBA/J profile.

Fig. 2. Fluorescence profiles of (a) (CBA/N × DBA/2)F1 female (—) or male (---) spleen cells labeled with FI anti-Ig, and (b) (BALB/c × CBA/N)F1 male (—) or (CBA/N × BALB/c)F1 male (---) spleen cells labeled with FI anti-Ig.

(CBA/N × BALB/c)F1 Adult Spleen Cells Labeled with FI Anti-Ig. Since the immune defect of CBA/N mice is an X-linked trait, the association between the abnormal fluorescence profile and the immune defect can be further supported by examining fluorescence profiles of FI anti-Ig-labeled spleen cells derived from (CBA/N × DBA/2)F1 male and female mice (Fig. 2a) and from (BALB/c × CBA/N)F1 and (CBA/N × BALB/c)F1 male mice (Fig. 2b). Male mice which have inherited a CBA/N X chromosome [(CBA/N × BALB/c)F1 and (CBA/N × DBA/2)F1], display the characteristic CBA/N immune defect (2-4) and have splenic fluorescence profiles that are similar to each other and to the FI anti-Ig profile of CBA/N spleen cells. By contrast, the fluorescence profiles of spleen cells from the immunologically normal F1 mice which have inherited an X chromosome from either the DBA/2 [(CBA/N × DBA/2)F1 female], or BALB/c parent [(BALB/c × CBA/N)F1 male], resembled those of normal strains (Figs. 2a and b).

Fluorescence Profile of CBA/N and BALB/c Neonatal and Immature Spleen.
Cells Labeled with Fl Anti-Ig. Spleen cells from 3-, 10-, 17-, and 30-day-old and adult CBA/N and BALB/c mice were labeled with Fl anti-Ig and their fluorescence profiles determined. For ease of comparison, profiles of the BALB/c spleen cells were plotted so that the area underlying the Ig-positive portion of the curve (channels 80–1,000) was equivalent to that of the CBA/N spleen cell profile. A fluorescence peak is not seen when spleen cells from either 3-day-old CBA/N or BALB/c mice are studied (Fig. 3a). Moreover, the fluorescence profiles and distribution of Fl anti-Ig-labeled fluorescence-positive cells in these two strains at this age is similar (Fig. 3; Table I). Thereafter, the developing BALB/c spleen cells are characterized by a gradual increase in the percent of cells with a low-to-
Table I
Frequency of Ig-Positive Lymphocytes Among Populations of Neonatal Spleen Cells Labeled with Fl Anti-Ig

| Figure | Strain  | Age | Ig-positive cells | Frequency of cells, among the Ig-positive population, with a fluorescence intensity of: |
|--------|---------|-----|-------------------|-----------------------------------------------------------------------------------|
| 3a     | CBA/N   | 3   |                   | 80-200* | 200-400* | 400-1,000* |
|        | BALB/c  | 3   |                   | 34.5    | 40.8     | 24.7       |
| 3b     | CBA/N   | 10  |                   | 19.5    | 30.1     | 39.7       |
|        | BALB/c  | 10  |                   | 34.8    | 42.8     | 40.7       |
| 3c     | CBA/N   | 17  |                   | 43.6    | 27.9     | 35.5       |
|        | BALB/c  | 17  |                   | 54.0    | 44.2     | 42.1       |
| 3d     | CBA/N   | 30  |                   | 41.9    | 18.5     | 32.8       |
|        | BALB/c  | 30  |                   | 49.5    | 36.5     | 43.0       |
| 3e     | CBA/N   | Adult |                 | 44.7    | 12.3     | 32.4       |
|        | BALB/c  | Adult |                 | 43.8    | 23.2     | 50.8       |

* Cells in channels 80-200, 200-400, and 400-1,000 are classed as having very-low-to-low, low-to-intermediate, and high density of surface Ig, respectively.

The developing CBA/N fluorescence profile is quite different. There is a progressive increase in the percent of cells with relatively large amounts of surface Ig (channels 400-1,000) which leads, finally, to the pattern typical of adult CBA/N mice (Fig. 3; Table I).

**Fluorescence Profiles of Adult CBA/N Bone Marrow, Lymph Node, and Peyer's Cells Labeled with Fl Anti-Ig.** The fluorescence profiles of CBA/N bone marrow, lymph node, and Peyer's patch cells labeled with Fl anti-Ig are shown in Figs. 4a, b, and c, respectively, and the frequency of Ig-positive cells in these lymphoid organs was 10.5, 5.3, and 36.2%, respectively. The profile of CBA/N bone marrow labeled with Fl anti-Ig was similar to that obtained with CBA/J bone marrow. By contrast, the characteristic low-to-intermediate density peak seen after labeling CBA/J lymph node cells with Fl anti-Ig was absent from the fluorescence profile of lymph node cells from CBA/N mice. Moreover, the relative frequency of cells with a high density of surface Ig (channels 400-1,000) among the Ig-positive cells is considerably greater in both CBA/N Peyer's patch and lymph node cells (21.3 and 47.2%, respectively) than in CBA/J Peyer's patches and lymph nodes (13.4 and 30.3%, respectively) (1).

**Fluorescence Profiles of CBA/N, CBA/J, (CBA/N × DBA/2)F1, (BALB/c × CBA/N)F1, and (CBA/N × BALB/c)F1 Adult Spleen Cells Labeled with Fl Anti-μ.** We have previously shown that fluorescence profiles of normal adult spleen cells labeled with Fl anti-μ are quite different from patterns obtained after labeling Fl anti-Ig. Fluorescence profiles of CBA/J and CBA/N spleen cells labeled with Fl anti-μ are shown in Fig. 5a. The fluorescence profile of Fl anti-μ-labeled CBA/N spleen cells was characterized by an extreme heterogeneity,
with no dominant fluorescence intensity; thus these cell populations possessed a substantial frequency of cells with a high density (channels 400–1,000) of surface IgM (56.1%) among the Ig-positive cells. This is in contrast to the fluorescence profile of Fl anti-μ-labeled CBA/J spleen cells where there is a predominance of B lymphocytes with a very-low-to-intermediate (channels 80–100) density of surface IgM and the frequency of cells with a high density of surface IgM among the Ig-positive cells was 24.8% (Fig. 5a). The Fl anti-μ-labeled fluorescence profiles of immune defective (CBA/N × DBA/2)F1 male and (CBA/N × BALB/c)F1 male spleen cells and of phenotypically normal (CBA/N × DBA/2)F1 female and (BALB/c × CBA/N)F1 male adult spleen cells (Figs. 5b and c) demonstrate profiles which are similar to those of Fl anti-μ-labeled splenic cells from CBA/N and CBA/J mice, respectively.

Fluorescence Profiles of CBA/N and BALB/c Neonatal and Immature Spleen Cells Labeled with Fl Anti-μ. The development of the Fl anti-μ-labeled fluorescence profiles of 3-, 10-, 17-, and 30-day-old BALB/c and CBA/N spleen cells is shown in Figs. 6a–d. The fluorescence profiles of the BALB/c spleen cells are plotted so that the area under the Ig-positive portion of the curves (channels 100–1,000) is equivalent to that of the CBA/N profiles. BALB/c spleen cells show a clear pattern of maturation with the fraction of cells in the very-low-to-intermediate intensity range increasing progressively with age (Table II). Fl anti-μ-labeled CBA/N spleen cells are distinct from BALB/c cells even at 3 days
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Fig. 5. Fluorescence profiles of (a) CBA/J spleen cells (---) and CBA/N spleen cells (----), (b) (CBA/N × DBA/2)F₁, female (-----) and male (-----) spleen cells, and (c) (BALB/c × CBA/N)F₁, male (-----) and (CBA/N × BALB/c)F₁, male (-----) spleen cells labeled with F1 anti-μ.

of age, although the difference among these neonatal cells is quite small. By contrast, CBA/N spleen cells at each age studied show a relatively similar fluorescence profile when labeled with F1 anti-μ. Thus, no significant changes in the distribution of cells with different densities of surface IgM are seen when B lymphocytes from maturing CBA/N mice are studied and the extreme heterogeneity noted with F1 anti-μ-labeled adult CBA/N spleen cells is also seen with CBA/N neonatal spleen cells.

Fluorescence Profiles of Lethally Irradiated (CBA/N × DBA/2)F₁ Males and Females Reconstituted with F₁ Male or Female Bone Marrow. In order to determine the relative roles of the lymphocyte precursor populations and of the developmental microenvironment in the failure of CBA/N cells to develop normally, (CBA/N × DBA/2)F₁, male and female mice were lethally irradiated and reconstituted with bone marrow cells from either (CBA/N × DBA/2)F₁, males or females. 8 wk later, spleens from all four groups were obtained, labeled with F1 anti-Ig, and their fluorescence profiles determined. F₁, male or female recipients of F₁, male bone marrow displayed the abnormal fluorescence profile (Figs. 7a and b) characteristic of CBA/N spleen cells. By contrast, F₁, male or female recipients of F₁, female bone marrow displayed a normal fluorescence profile (Figs. 7c and d). This strongly argues that the defect in immunoglobulin profile is intrinsic to the CBA/N lymphocyte line rather than to the microenvironment in which such cells develop.
Discussion

The distribution of cells with a given density of total Ig and of IgM on the surface of neonatal splenic and adult bone marrow CBA/N B lymphocytes was similar to that found on the surface of similar populations of B lymphocytes derived from normal mice. However, cells derived from normal adult spleen, lymph node, or Peyer's patches, lymphoid organs which are presumably the residence of more mature B lymphocytes, had fluorescence profiles of total surface Ig which were considerably different from those of comparable CBA/N
TABLE II
Frequency of Ig-Positive Lymphocytes Among Populations of Neonatal Spleen Cells Labeled with Fl Anti-μ

| Figure | Strain | Age | IgM-positive cells | Frequency of cells, among the IgM-positive population, with a fluorescence intensity of: |
|--------|--------|-----|-------------------|-----------------------------------|
|        |        |     |                   | 100-400* | 400-1,000* |
| 6a     | CBA/N  | 3   | 45.7              | 40.6     | 59.4       |
|        | BALB/c | 3   | 33.6              | 51.3     | 48.7       |
| 6b     | CBA/N  | 10  | 20.9              | 41.5     | 58.5       |
|        | BALB/c | 10  | 34.8              | 59.2     | 40.8       |
| 6c     | CBA/N  | 17  | 48.1              | 37.8     | 62.2       |
|        | BALB/c | 17  | 53.9              | 67.2     | 32.8       |
| 6d     | CBA/N  | 30  | 43.7              | 36.1     | 63.9       |
|        | BALB/c | 30  | 46.1              | 72.4     | 28.6       |
| 6e     | CBA/N  | Adult | 46.4         | 37.1     | 62.9       |
|        | BALB/c | Adult | 39.2         | 75.7     | 24.2       |

* Cells in channels 100-400 and 400-1,000 are classed as having a low-to-intermediate and high density of surface IgM, respectively.

The immature distribution of total surface Ig and IgM on B lymphocytes derived from the CBA/N strain is most likely due to an abnormality in the development of mature B lymphocytes from their immature precursors. The increase in the frequency of cells with a high mean density of total surface Ig and surface IgM in the spleens of adult CBA/N mice would therefore be the result of an inability of an immature subclass of CBA/N lymphocytes to develop
F1 anti-Ig fluorescence profiles of lethally irradiated (a) male or (b) female (CBA/N × DBA/2)F1 mice which had been reconstituted with F1 male bone marrow cells. Fluorescence profiles of lethally irradiated (c) male or (d) female (CBA/N × DBA/2)F1 mice that were reconstituted with F1 female bone marrow cells labeled with F1 anti-Ig.

further. Alternatively, an independent B-lymphocyte subline, which appears relatively late in the maturation of the mouse and has a low mean density of total surface Ig and of IgM, may fail to develop in the CBA/N strain. In recent observations reported from our laboratories by Finkelman et al. (6), the ratio of IgM to the putative homolog of human IgD on the surface of adult CBA/N splenic B lymphocytes was shown to be approximately three times greater than the ratio of IgM to IgD on the surface of B lymphocytes derived from spleens of normal adult mice. Since the acquisition of IgD on the surface of murine B lymphocytes occurs during the first 4 wk of life (8), the high ratio of surface IgM to IgD on CBA/N B lymphocytes is consistent with the hypothesis that the development of mature B lymphocytes or a late maturing B-lymphocyte subline in the CBA/N strain is abnormal. Moreover, these studies, combined with the data derived in this paper, suggest that this defect occurs during a phase of CBA/N B-lymphocyte development when IgD is first appearing on the surface of these cells, or that the defect involves the deletion of a subline of B lymphocytes that have a low ratio of surface IgM to IgD (or perhaps which bear only IgD).

The demonstration that (CBA/N × DBA/2)F1 and (CBA/N × BALB/c)F1 male mice have abnormal distributions of Ig on the surface of their B lymphocytes shows that this phenomenon, like the functional B-lymphocyte defect in these mice, is an X-linked characteristic. Furthermore, the dependence of the fluorescence profile of spleen cells from lethally irradiated (CBA/N × DBA/2)F1 mice
on the origin of the donor bone marrow implies that the microenvironment of the recipient host is not a critical factor in the development of the characteristic adult splenic F1 anti-Ig fluorescence profile.

The abnormalities in the distribution of adult splenic CBA/N cells with a given density of total surface Ig and IgM are associated with a number of defects in B-lymphocyte function. It is tempting to conclude that the cells which are markedly diminished in the strain, the low-to-intermediate surface Ig-bearing and the very low surface IgM-bearing cells are those which in normal mouse strains are responsible for the functions which are deficient in the CBA/N strain. Alternatively, the cells with relatively large amounts of total surface Ig and of IgM, which are in relative excess among CBA/N B-lymphocyte populations, may be defective in both their immune responsiveness and in their capacity to develop into cells characteristic of mature B lymphocytes.

Summary

CBA/N mice have an X-linked defect in B-lymphocyte function characterized by a failure to respond to certain thymus-independent antigens. When studied by rapid flow microfluorometry, adult CBA/N splenic B lymphocytes labeled with either fluorescein-conjugated (F1) anti-Ig or F1 anti-μ had fluorescence profiles which were considerably different from those of B lymphocytes derived from normal mice. By studying progeny of crosses of CBA/N and normal mice, it was shown that the abnormal fluorescence profiles of CBA/N B cells were determined by an X-linked gene. The fluorescence profile of adult CBA/N splenic B lymphocytes labeled with anti-μ were very similar to the patterns of neonatal normal and of neonatal CBA/N splenic B lymphocytes suggesting that the defect of CBA/N mice is due to a failure in the development of a mature B-lymphocyte population. The fluorescence profiles of adult CBA/N splenic B lymphocytes labeled with F1 anti-Ig also had immature characteristics in that the frequency of cells with large amounts of surface immunoglobulin was increased in comparison to that of normal strains and the population of cells with low-to-intermediate density of total surface immunoglobulin, which appear characteristic of normal adult splenic B lymphocytes, was markedly diminished.

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References

1. Scher, I., S. O. Sharrow, R. Wistar, Jr., R. Asofsky, and W. E. Paul. 1976. B-lymphocyte heterogeneity: ontogenetic development and organ distribution of B-lymphocyte populations defined by their density of surface immunoglobulin. J. Exp. Med. 144:494.

2. Amsbaugh, D. F., C. T. Hansen, B. Prescott, P. W. Stashak, D. R. Barthold, and P. J. Baker. 1972. Genetic control of the antibody response to Type III pneumococcal polysaccharide in mice. I. Evidence that an X-linked gene plays a decisive role in determining responsiveness. J. Exp. Med. 136:931.

3. Scher, I., M. M. Frantz, and A. D. Steinberg. 1973. The genetics of the immune
response to a synthetic double-stranded RNA in a mutant CBA mouse strain. J. Immunol. 110:1396.

4. Scher, I., A. D. Steinberg, A. K. Berning, and W. E. Paul. 1975. X-linked B-lymphocyte immune defect in CBA/N mice. II. Studies of the mechanisms underlying the immune defect. J. Exp. Med. 142:637.

5. Scher, I., A. Ahmed, D. M. Strong, A. D. Steinberg, and W. E. Paul. 1975. X-linked B-lymphocyte immune defect in CBA/HN mice. I. Studies of the function and composition of spleen cells. J. Exp. Med. 141:788.

6. Finkelman, F. D., A. H. Smith, I. Scher, and W. E. Paul. 1975. Abnormal ratio of membrane immunoglobulin classes in mice with an X-linked B-lymphocyte defect. J. Exp. Med. 142:1316.

7. Loken, M. R., and L. A. Herzenberg. 1975. Analysis of cell populations with a fluorescence-activated cell sorter. Ann. N. Y. Acad. Sci. 254:163.

8. Vitetta, E. S., U. Melcher, M. McWilliams, M. E. Lamm, J. M. Phillips-Quagliata, and J. W. Uhr. 1975. Cell surface immunoglobulin. XI. The appearance of an IgD-like molecule on murine lymphoid cells during ontogeny. J. Exp. Med. 141:206.