ABNORMAL RATIO OF MEMBRANE IMMUNOGLOBULIN CLASSES IN MICE WITH AN X-LINKED B-LYMPHOCYTE DEFECT*

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CBA/N mice are an inbred line of CBA mice with an X-linked immune defect characterized by very low serum IgM concentrations and a failure to respond to a series of thymus-independent antigens (1-5). Scher et al. (3) have shown that the X-linked defect of CBA/N mice is associated with a diminished number of nucleated cells per spleen and a diminished fraction of immunoglobulin (Ig)-bearing lymphocytes in spleen cell suspensions. This diminution in number of Ig-bearing lymphocytes in CBA/N mice does not appear to be sufficient to account for their almost total lack of T-independent antibody responses. Because of this, we further investigated the Ig-bearing lymphocytes of CBA/N mice and of mice hemizygous for the CBA/N X chromosome for qualitative or quantitative abnormalities. In this report we demonstrate that Ig-bearing lymphocytes of CBA/N mice and of mice hemizygous for the CBA/N X chromosome for qualitative or quantitative abnormalities. In this report we demonstrate that Ig-bearing lymphocytes of CBA/N mice and phenotypically abnormal (CBA/N × DBA/2)F1, male mice show a marked abnormality in the ratio of membrane μ-chain to the recently described cell membrane heavy (H) Ig chain which has been proposed as the analog of human δ-chain (6, 7). For simplicity, we will refer to this H chain as δ-analog. However, it has not yet been demonstrated to be chemically or serologically analogous to human δ-chain, and this designation must be regarded as tentative.

Materials and Methods

Adult mice (6 wk–3 mo in age) of the CBA/N, DBA/2N, BALB/c, and (CBA/N♀ × DBA/2♂)F1 strains were obtained from the Divisions of Research Services, National Institutes of Health. F1 mice derived from matings of DBA/2N♀ and CBA/N♂ mice were bred in our animal quarters. Spleens, mesenteric lymph nodes, and Peyer’s patches were obtained from mice homozygous or hemizygous for the CBA/N X chromosome and from age-matched controls. Suspensions of 1–4 × 10⁶ nucleated cells were surface labeled with ³²P by the lactoperoxidase technique (8, 9). After labeled cells were washed, their plasma membranes were dissolved in 0.5% Nonidet P-40 (Shell"

* This work was supported in part by the Bureau of Medicine and Surgery, Navy Department, Work Unit No. MR041.02.01.0020 B2GI. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. The animals used in this study were handled in accordance with the provisions of Public Law 89-54 as amended by Public Law 91-579, "Animal Welfare Act of 1970," and the principles outlined in "Guide for the care and use of laboratory animals," U.S. Department of Health, Education, and Welfare Publication No. (NIH) 73-23.

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Chemical Co., New York), and the mixture was centrifuged to remove nuclei. The serine esterase inhibitor diisofluorophosphate was added to retard enzymatic degradation of cell membrane molecules, and the lysates were dialyzed against phosphate-buffered saline (PBS) for 4 h. Anti-Ig or control precipitates were then made from the dialysates by adding 25 μl of rabbit antimouse κ-chain antiserum (a gift of Dr. Rose Mage) or normal rabbit IgG, followed by equivalence amounts of sheep antirabbit Ig. Precipitates were washed in PBS, dissolved in 2% sodium dodecyl sulfate (SDS) solution, reduced with 2-mercaptoethanol, and electrophoresed on SDS 11% acrylamide gels for 12 h at 1.5 mA/gel (10). After electrophoresis was terminated, gels were sliced into 1- or 2-mm sections, the radioactivity in each section quantitated with a Searle gamma counter (G. D. Searle & Co., Chicago, Ill.), and the counts per minute per section plotted against distance of the section from the gel origin. Peaks on these plots indicate the positions of Ig H and light (L) chains. The position of μ-chain in these gels was determined by electrophoresing reduced serum IgM on parallel gels, staining for protein with Coomassie Blue, and destaining with 7% acetic acid. All procedures after 125I-surface labeling and before the dissolving of immune precipitates were carried out at 0–4°C.

**Results and Discussion**

The results obtained by the electrophoresis of control and anti-κ-precipitates of extracts from 125I-surface-labeled spleen cells of 9 wk old F1 male and female (CBA/N × DBA/2) littermates are illustrated in Fig. 1. SDS-polyacrylamide gel electrophoresis (PAGE) of specific precipitates of extracts derived from phenotypically normal F1 female mice (heterozygous for the CBA/N X chromosome), reveals two peaks in the H-chain region, one with the mobility of μ-chain and one that has the mobility of the δ-analog. These two H-chain peaks are similar in magnitude, as was the experience of Melcher et al. in studies of adult BALB/c mice (6). On the other hand, SDS-PAGE of specific precipitates prepared from extracts derived from phenotypically abnormal F1 male cells revealed that the peak identified as the δ-analog was substantially smaller than the μ-chain peak. It should be noted that the absolute number of counts per minute in either peak is less important than the ratio of the peaks, as the efficiency of labeling and the amount of radioactivity applied to the gels varies from experiment to experiment.

This decreased ratio of δ-analog to μ-chain peak was observed in three experiments comparing (CBA/N × DBA/2)F1 male and female littermate spleen cells, in one experiment comparing spleen cells from CBA/N and BALB/c females of the same age, and in one experiment comparing spleen cells from a (CBA/N × DBA/2N)F1 male (which has a CBA/N X chromosome) with spleen cells of an age-matched (DBA/2N × CBA/N)F1 male (which has a DBA/2 X chromosome) (Table I). The latter two comparisons are particularly important as they rule out male-female differences as the explanation for the altered δ-analog:μ-ratio. Furthermore, the reciprocal F1 experiment provides a definitive association of the abnormal ratio of δ-analog:μ with the CBA/N X chromosome. The mean ratio of δ-analog to μ of extracts of normal spleen cells in these five experiments was 1.01±0.09, while the ratio for extracts of spleen cells homozygous or hemizygous for the CBA/N X chromosome was 0.37±0.09. The P value for a t test comparison of these means was <0.001.

An abnormally low δ-analog to μ-ratio was also observed in studies of extracts of lymph node cells and of Peyer's patch cells from male (CBA/N × DBA/2N)F1 donors. The δ-analog:μ-ratio of extracts of lymph node and Peyer's patch cells
FIG. 1. SDS acrylamide gel electrophoresis of cell membrane H chains from 9-wk-old (CBA/N x DBA/2) F1 female and male mice. Rabbit antimouse κ-chain and control precipitates were prepared from NP-40 extracts of enzymatically radioiodinated (CBA/N x DBA/2) F1 female and male mouse spleen cells. Dissolved, reduced precipitates were electrophoresed on parallel SDS 11% acrylamide gels. The electropherogram of the anti-κ-precipitate of the female spleen cell extract is shown in the upper panel (solid line) and that of the male in the lower panel (solid line). No κ-chain peaks are seen as these were allowed to migrate out of the gel before electrophoresis was terminated. Control precipitates are shown with dotted lines. Arrows mark the position of a serum μ-chain standard electrophoresed on a parallel gel.
from normal donors is greater than that for spleen cells from normal donors, as has been reported by others (11).

Recent studies of Scher et al. (12, 13) quantitatively the relative amount of surface Ig among spleen cell populations of CBA/N and normal mice provide an interesting analogy to this finding. Using the fluorescence-activated cell sorter to analyze relative intensity of staining, a population of cells bearing low to intermediate amounts of Ig was identified in spleens of normal mice with a fluorescein-anti-α-reagent. This population is diminished or absent in mice homozygous or hemizygous for the CBA/N X chromosome. When fluorescein anti-µ is used, the fluorescence patterns of the normal and defective cells are not distinguishable. It is possible that the population absent in CBA/N spleen represents cells that have relatively large amounts of surface δ-analog and little or no surface µ.

Three possible explanations exist for the association of the immune defect of the CBA/N mouse with an altered δ-analog:µ-ratio. First, B-cell activation by T-
independent antigens may normally require an antigen interaction with IgD molecules or a cross-linking of IgD and IgM which does not occur efficiently on CBA/N cells because of the abnormal H-chain ratio. A second possibility is that the $\delta$-analog:$\mu$-ratio is strongly dependent upon lymphocyte population changes occurring as a result of responses to T-independent antigens, and the abnormal ratio of $\delta$-analog to $\mu$ on plasma membranes of CBA/N B lymphocytes is a result, rather than a cause, of their immune defect. Observations by Vitetta et al. (11) that the development of surface $\delta$-analog occurs normally in germ-free mice suggest that antigen stimulation is not critical to the appearance of membrane $\delta$-analog and thus render this possibility less attractive. A final possibility is that the critical defect of the CBA/N mouse is the failure to develop a population of B lymphocytes responsive to T-independent antigens, although the development of B lymphocytes responsive to T-dependent antigens proceeds relatively normally. To explain the abnormal $\delta$-analog:$\mu$-ratio observed in CBA/N lymphoid populations one would postulate that the "T-independent" B cells would have a very high $\delta$-analog:$\mu$-ratio, or perhaps express only $\delta$-analog.

Our studies provide a lymphocyte membrane correlate of the immune defect of CBA/N mice. Although we cannot, as yet, determine the relationships of the altered $\delta$-analog:$\mu$-ratio to defective function of CBA/N B lymphocytes, this system should provide an important tool in the understanding of B lymphocyte ontogeny, differentiation, and activation and the functional role of membrane-bound IgD.

Summary

CBA/N mice have an X-linked genetic defect in B-lymphocyte function manifested by inability to make antibody responses to T-independent antigens. Plasma membrane immunoglobulin (Ig) on spleen, lymph node, and Peyer's patch cells was analyzed by lactoperoxidase-catalyzed iodination, NP-40 extraction, specific immunoprecipitation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. These studies indicated that the X-linked immune defect was associated, in all three cell types, with a decrease in the ratio of cell membrane IgD analog to cell membrane IgM. This suggests either that IgD analog may be important in initiation of T-independent antibody responses or that CBA/N mice lack a subpopulation of B cells specialized to respond to T-independent antigens, and that these cells are relatively rich in plasma membrane IgD analog.

We thank Dr. Rose Mage and Dr. Kenneth W. Sell for their help and advice in the course of these studies.

Received for publication 7 July 1975.

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