INFLUENCE OF THE SEX-LINKED DEFECT
IN CBA/N MICE ON AUTOIMMUNE
RESPONSES TO ISOLOGOUS ERYTHROCYTES
Ability to Overcome the Defect with Age

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The spleens of normal mice, both conventional and germ free, contain many plaque-forming cells (PFC) secreting antibody specific for determinants on isologous erythrocytes; such PFC are revealed using indicator cells treated with the proteolytic enzyme bromelain (bromelain-treated mouse erythrocytes [BrMRBC]) (1). The levels of these autoimmune PFC vary considerably among different strains (1) with the highest numbers occurring in NZB mice, which spontaneously develop haemolytic anemia (2). Although injection of BrMRBC does not result in any significant increase in the levels of background splenic PFC, the numbers may be greatly increased by injections of lipopolysaccharide (LPS) or anti-lymphocyte serum (3). In addition, a specific T-cell-dependent increase can be seen after lethal malaria infections (4).

CBA/N mice have a sex-linked defect conferring an inability to respond to several polysaccharide antigens, generally called TI-2 antigens, such as type III pneumococcal polysaccharide (5), dextran, levan (6), trinitrophenyl-Ficoll (7), and phosphoryl choline on any carrier (8); and resulting in several changes in the phenotypic and functional patterns of the B-cell population (9–12). Because the nature of the immune response to BrMRBC bears certain similarities to the above antigens in that the former is usually T-cell independent, is predominantly an IgM response (1), and, based on a very homogeneous plaque morphology, may also be of restricted heterogeneity (Y. Rosenberg. Unpublished observation.), it was of interest to investigate whether CBA/N mice were also defective in their antibody responses to modified self erythrocytes.

The results show that although the B-cell response to the BrMRBC antigen is indeed under sex-linked control, low levels of PFC can be induced by either specific or nonspecific mechanisms. This defect, observed in both homozygous and hemizygous mice, may be largely overcome with age, probably as a result of environmental stimulation, thus indicating the presence of at least small numbers of functional B lymphocytes specific for this antigen in nonresponder mice.

Materials and Methods

Mice. CBA/N, NZB, (CBA/N × NZB)F1, (NZB × CBA/N)F1, (CBA/N × DBA/2)F1, (CBA/N × BALB/c)F1, and (BALB/c × CBA/N)F1 of either sex were obtained from the Animal Production Unit of the National Institutes of Health, Bethesda, Md. In most experiments CBA/N female F1 or reciprocal F1 male mice (CBA/N-fathered F1 mice) were used as controls. In one case, CBA/J mice (The Jackson Laboratory, Bar Harbor, Maine) served as controls. In one case, CBA/J mice (The Jackson Laboratory, Bar Harbor, Maine) served as controls.
Table I

Levels of Background Anti-BrMRBC PFC in the Spleens of CBA/N Mice and Their F1 Hybrids*

| Mice                                      | Sex       | PFC/spleen‡ |
|-------------------------------------------|-----------|-------------|
| CBA/N                                    | Male (10)§ | 0           |
|                                           | Female (10)| 0          |
|                                           | Female (5)§ | 0          |
| NZB (CBA/N × NZB)F1                       | Male (5)  | 17 (0-150)  |
|                                           | Female (2) | 3,817 (2,750-4,450) |
| (NZB × CBA/N)F1                           | Male (13) | 6,050 (1,200-11,600) |
| (CBA/N × BALB/c)F1                        | Male (8)  | 12 (0-100)  |
|                                           | Male (8)  | 2,536 (1,000-5,400) |
| (CBA/N × DBA/2)F1                         | Male (8)  | 0           |
|                                           | Female (8) | 3,012 (1,600-5,500) |

* Mice were 7-12 wk of age.
‡ Arithmetic mean and range (in parentheses).
§ Number of mice assayed.
¶ 35-wk-old mice.

controls for homozygous CBA/N. Mice ranging from 5 to 35 wk of age were studied.

Preparation of Cells. Cell suspensions were prepared in balanced salt solution plus 5% fetal calf serum (Microbiological Associates, Walkersville, Md.) by mincing spleens through stainless steel grids. Clumps were removed by passage through glass wool.

Injections. Mice were given 50 μg of the B-cell mitogen LPS (from Escherichia coli 0128:B12, Difco Laboratories, Detroit, Mich.) i.p. or infected with 10⁶ 17XL Plasmodium yoelii i.p., a parasite causing lethal malaria in mice.

PFC Assay. Direct PFC were detected with the Cunningham and Szenberg modification (13) of the hemolytic plaque assay with BrMRBC as indicators and rabbit serum as a source of complement (1).

Results and Discussion

The spontaneous levels of PFC directed against self erythrocytes in the spleens of male and female CBA/N mice and their F1 hybrids are shown in Table I. In contrast to the number found in CBA/N-mothered female F1 hybrids—which vary according to the paternal strain, and, as a result of the Lyon effect (14), are usually fewer than the responding parent—background BrMRBC PFC are virtually lacking in the spleens of homozygous CBA/N and their male F1 hybrids. The sex-linked defect that determines the level of such PFC is most evident in the crosses between CBA/N and NZB mice. Despite the abnormally high levels of anti-erythrocyte PFC usually found in NZB mice, increasing with the degree of autoimmunity, such male (CBA/N × NZB)F1 mice fully express the CBA/N phenotype in that anti-BrMRBC PFC are absent.

The failure of the CBA/N mice to respond to certain antigens, in this study a self antigen, could reflect (a) the lack of a mature B-cell population either by a maturation arrest of B-cell development or the absence of an entire B-cell lineage (15, 16); (b) the inability of B lymphocytes specific for the antigen to be triggered (12) or to correctly assemble its immunoglobulin molecules for secretion after triggering (17); or (c) low frequencies of precursors specific for the particular antigen. In an attempt to distinguish among these possibilities, defective mice of various ages were either stimulated with the mitogen LPS, a potent nonspecific activator of B lymphocytes, or infected with lethal malaria, a good T- and B-cell inducer known to specifically stimulate anti-BrMRBC precursors via a T-cell-dependent mechanism.

In Fig. 1 A-F the LPS-induced anti-BrMRBC responses of aged-matched nonresponder (CBA/N male and female, (CBA/N × BALB/c)F1 male, (CBA/N × DBA/
Fig. 1. Anti-BrMRBC PFC response of 5- to 35-wk-old defective (○) and normal control (●) mice after injection of 50 μg LPS i.p. Data (expressed as geometric means ± SE) represent the responses of three mice at each time point. The defective and responder strains are shown for each experiment.

A shortage of older mice in some experiments precluded more extensive kinetic studies.

2) F1 male] and control responder mice [(CBA/N × DBA/2)F1 female, (BALB/c × CBA/N)F1 male] are compared. Although at 5–7 wk, the anti-BrMRBC PFC levels in control mice were usually 10⁵ per spleen, 100-fold greater than the defective mice, the latter nevertheless gave small but consistent responses (10³/spleen). The differences were, however, less dramatic if older mice were used. Fig. 1 D–F show that the responses of 16- or 35-wk-old homozygous CBA/N or 12-wk-old (CBA/N × BALB/c)F1 male mice were around 10-fold higher than that observed in the younger defective mice and, in the case of the 35-wk-old group, were <10-fold lower than control CBA/J. It should be noted that although responses usually decreased after day 4, several mice continued to generate high levels of anti-BrMRBC PFC (data not shown). Indeed, variations were often seen in the responses of nonresponder mice, in particular the older animals, resulting in occasional large standard errors. It is also of interest that in old defective mice, either unstimulated or stimulated, abnormally high levels of splenic IgA-secreting cells were seen (Y. Rosenberg. Unpublished observation.).

Whereas the above experiments demonstrate the existence of functional B cells specific for BrMRBC in defective mice, they indicated only that these cells could be triggered by a nonspecific mitogenic signal and did not address the question of specific induction via the immunoglobulin receptors. To test this, 8-wk-old (CBA/N × BALB/c)F1 male and (BALB/c × CBA/N)F1 male mice were infected with malarious MRBC to stimulate anti-BrMRBC B-cell precursors specifically. Fig. 2 indicates that nonresponder F1 mice can respond in a specific fashion and that the levels of PFC, 10³/spleen, are similar to those induced in the same young mice given LPS (Fig. 1).

Of the possibilities listed above, it is generally held that CBA/N mice do not respond to the common TI-2 antigens because they lack a subpopulation of mature or unique B cells (12, 15, 16). These findings that a sex-linked response may increase with age suggests that although a population may be absent, functional specific
precursors must be present in low frequencies, and that they can be triggered and expanded as a function of age as a result, most likely, of stimulation by environmental antigens or hormonal effects of aging. Similar age-related increases in the responses of CBA/N mice to group A streptococcal vaccine (M. Nahm. Personal communication.) and the finding that immunized CBA/N spleen cells can generate hybridomas that secrete specific anti-SSS-III antibodies after fusion (17) also support the idea that B cells responsive to TI-2 antigens are present in mice expressing the sex-linked defect.

Finally, although the defect in CBA/N mice is not as extreme in the case of T-dependent antigens, such responses are nevertheless greatly reduced compared with normal mice. IgM and IgG PFC responses to the T-cell-dependent antigen sheep erythrocytes (SRBC) are, for example, more than 20-fold decreased in defective mice (Y. Rosenberg. Unpublished observation.). In this context it is of interest to note that because SRBC and BrMRBC cross-react at both the B- and T-cell level (18, 19), an absence in young CBA/N mice, of any response to the BrMRBC antigen, apparently first expressed on gut tissue (20) may, in part, account for the lower anti-SRBC responses. Similarly, TI-2 antigens, many of which are derived from bacterial antigens,
may possibly play a role in the expansion of the B-cell pool in general (21). The observations that spleens of young CBA/N mice contain 10-fold fewer IgM-secreting cells and the low IgG3 PFC levels (22, 23) are consistent with such a view. Whether the age-related increases reported here correlate with phenotypic changes in the B cells of CBA/N mice, for example the acquisition of Lyb5 antigens (11), is currently being examined.

Summary

Normal mice spontaneously develop plaque-forming cells (PFC) specific for antigens on modified self erythrocytes (bromelain-treated mouse erythrocytes [BrMRBC] antigens). Our study demonstrates that the sex-linked defect that results in the inability of CBA/N mice to respond to several T-independent antigens (TI-2 antigens) also regulates the autoantibody response to BrMRBC antigens. Thus, in CBA/N homozygous mice and male F1 offspring of CBA/N-mothered crosses, e.g., (CBA/N × NZB)F1 males, such PFC are absent. To examine whether specific autoreactive B cells are present in defective mice, the latter were stimulated either nonspecifically with the mitogen LPS or by infection with lethal malaria (17XL Plasmodium yoelii) known to induce anti-BrMRBC PFC specifically. The results indicate that modest antibody responses to self antigens could be induced in young (5- to 7-wk old) defective mice and that these responses increased as a function of age. The data is consistent with the view that the defect in CBA/N mice does not result from an absence of functional anti-BrMRBC B cells but rather from low frequencies of the specific precursors, which can be triggered and expanded with age probably by environmental stimulations.

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