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MONOCYTOSIS IN THE BXSB MODEL FOR SYSTEMIC
LUPUS ERYTHEMATOSUS

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BXSB mice spontaneously develop an autoimmune disease characterized by Coombs-positive hemolytic anemia, antibodies to double-stranded DNA, and fatal immune-complex glomerulonephritis (1). These mice are unique among murine models for systemic lupus erythematosus (SLE) in that males are much more severely affected than females. The male-predominant pattern of disease has its genetic basis in the Y-chromosome (2), but the cellular basis for abnormal immunoregulation in BXSB mice is unclear. As the disease progresses, there is B cell proliferation in the spleen and lymph nodes of BXSB males (3). There is also spontaneous polyclonal activation of B cells (4) and impaired interleukin 2 production by cultured T cells (5), but B and T cell function in vivo appears to be normal (6). To further examine the cellular basis for autoimmunity in BXSB mice, we systematically characterized peripheral blood mononuclear cells (PBMC) from BXSB males and females at monthly intervals. We found a dramatic, progressive increase in monocytes in BXSB males, beginning as early as 2 mo of age. By 6 mo, monocytes accounted for >50% of PBMC and, in some mice, comprised >90% of PBMC. Monocytosis does not occur in other murine models for SLE, suggesting that it may be a fundamental aspect of the disease of BXSB mice, not simply a secondary phenomenon associated with autoimmunity in general.

Materials and Methods

Mice. BXSB mice were bred from pairs obtained from The Jackson Laboratory, Bar Harbor, ME. In contrast to the initial description of a 5-mo median survival for BXSB males (1), median survival in our colony and in other BXSB colonies is 8 mo (7).

Cell Preparation. Peripheral blood (400 μ l) was obtained from individual mice by retroorbital bleeding into heparinized pipettes. Cells were separated from plasma by centrifugation, and PBMC were collected by centrifugation over Lympholyte-M (Cedarlane, Ontario, Canada) and quantified using a Coulter ZBI cell counter (Coulter Electronics, Hialeah, FL).

Fluorescence Analysis of PBMC Subpopulations. PBMC were analyzed using fluorescein-conjugated monoclonal antibodies (MAb) and a fluorescence-activated cell sorter (FACS) (B-D FACS Systems, Sunnyvale, CA) as previously described (8). T and B cells were quantified by staining with rat MAb to Thy-1.2 (30-H12) and ThB (59-9.2), respectively (8). In some experiments, cells also were stained with rat MAb to Lyt-1 (53-7.1), Lyt-2

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(53-6.7), and T200 (30-F11), and, for detection of surface immunoglobulin, with rabbit antiserum against mouse Fab. These reagents, described previously (8), were generously provided by Dr. Jeffrey A. Ledbetter, Genetic Systems, Seattle, WA. To detect Mac-1, PBMC (10^6 cells) were incubated with 0.5 μ g of biotin-conjugated MAb to Mac-1 (from hybridoma TIB 128, American Type Culture Collection, Rockville, MD) and then stained with 0.5 μ g of avidin-fluorescein (Becton, Dickinson & Co., Mountain View, CA). Staining could be inhibited by unconjugated MAb to Mac-1 but not by another rat MAb of the same subclass (30-H12).

Detection of I-A and NK-1.1 Antigens. I-A and NK-1.1 antigens were detected by complement-mediated cytotoxicity using MAb to I-A^b (Litton Bionetics, Kensington, MD) and anti-NK-1.1 antiserum as described previously (9). Anti-H-2^b antiserum (C57BL/10.D2 anti-C57BL/10) was used as a positive control. In concomitant studies, we showed that BXSb splenic NK activity was specifically abrogated by anti-NK-1.1 plus complement, demonstrating that BXSb NK cells bear NK-1.1.

Detection of Fc Receptors. Fc receptors were assessed by rosette formation with IgG-coated sheep erythrocytes (SE) (10). Cells binding >4 SE were counted as positive.

Assessment of Adherence and Phagocytic Capacity. Adherent PBMC were quantified by overnight culture in Falcon 3047 multiwell tissue culture plates (Becton Dickinson Labware, Oxnard, CA). The phagocytic capacity of the adherent cells was assessed by their ability to ingest antibody-coated SE (11). Cells containing one or more SE were counted as positive.

Electron Microscopy. For transmission electron microscopy, cells were fixed in 2.5% glutaraldehyde, postfixed in 1% OsO₄, dehydrated in graded ethanol, and imbedded in Polybed 812 (Polysciences, Inc., Warrington, PA). Thin sections were stained with uranyl acetate and lead citrate and examined in a JOEL 100S transmission electron microscope. For scanning electron microscopy, cells were placed on coverslips coated with 1 μ g/ml poly-L-lysine (Sigma Chemical Co., St. Louis, MO), fixed in 3% glutaraldehyde, postfixed in 0.5% OsO₄, and examined in a Cambridge Stereoscan S-150 scanning electron microscope.

Cytochemistry. Esterase activity was assessed by staining with 5% alpha naphthyl butyrate and 1% hexazotized pararosanilin, followed by counterstaining with Harris hematoxylin (12). Peroxidase activity was assessed by staining with benzidine dihydrochloride, sodium nitroprusside, and hydrogen peroxide, followed by counterstaining with Diff-Quik solution (Harleco, Gibbstown, NJ) (12, 13). Normal mouse spleen cells and human peripheral blood leukocytes were used to provide positive controls for these stains.

Results

Fluorescence Analysis of PBMC Using Antilymphocyte Antibodies. PBMC from 22 BXSb males and 6 age-matched BXSb females were analyzed at monthly intervals beginning at 2 mo of age, using MAb to ThB, Thy-1.2, and Lyt-2. These mice were representative of the BXSb strain, in that males developed proteinuria (>100 mg/dl) at 4 mo of age and reached 50% mortality by 8 mo of age, while all of the females were alive without significant proteinuria at 9 mo. In males, but not in females, there was a marked rise in the number of PBMC beginning at 4–5 mo of age (Fig. 1 A) so that, by 8 mo, PBMC had increased 3-fold ($P < 0.01$). The increase in PBMC was due solely to an increase in cells that lacked both T and B cell markers (Fig. 1 D). These non-T non-B cells were significantly increased in males relative to females as early as 2 mo of age (399 ± 108 cells/mm³ vs. 63 ± 24 cells/mm³), rose dramatically at 4 mo of age, and by 8 mo were increased 16-fold in males relative to females ($P < 0.001$). B cells did not increase (Fig. 1 B) and there was a statistically significant, although numerically small, reduction in circulating T cells after 3 mo of age (Fig. 1 C). Lyt-2⁺ and Lyt-2⁻ T cells were comparably reduced (not shown).

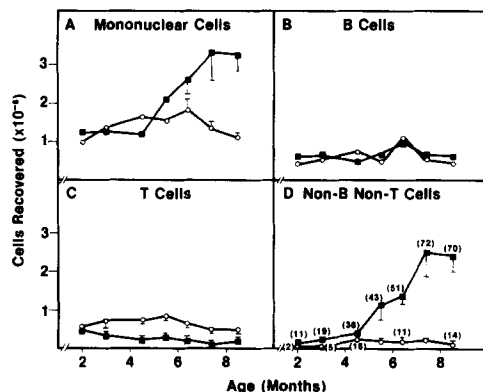


FIGURE 1. Total cells recovered from 400 μ l of blood in BXSB males (■) and females (○). Each point represents the mean of individual surviving mice. (A) Total mononuclear cells, measured by Coulter counter. (B) Total B cells, assessed by staining with fluoresceinated anti-ThB. (C) Total T cells, assessed by staining with fluoresceinated anti-Thy-1.2. (D) Total cells lacking B and T cell markers. The frequency of these cells, as a percent of PBMC, is shown in parentheses.

Characteristics of the Predominant PBMC Subpopulation in Adult BXSB Males. We examined PBMC from six selected 8-mo-old BXSB males in which non-T non-B cells accounted for at least 80% of PBMC. In these mice, 69–80% of PBMC expressed Mac-1. The Mac-1⁺ cells were larger than the Thy-1.2⁺ and ThB⁺ cells, as assessed by FACS analysis. Light and electron microscopy confirmed that the predominant cells were larger than lymphocytes (7–9 μ m diam). On Wright's stain, these cells had indented nuclei, coarse linear chromatin, and pale blue cytoplasm (Fig. 2A). Transmission electron microscopy demonstrated convoluted nuclei, abundant cytoplasmic granules, prominent Golgi apparatus, and filopodia (Fig. 2B). Scanning electron microscopy revealed a ruffled surface with lamellapodia (Fig. 2C). These features all are typical of monocytes. Other characteristics of the predominant PBMC subpopulation are summarized in Table I. In addition to Mac-1, the cells expressed the common leukocyte antigen, T200, but they lacked antigens expressed on T cells (Thy-1, Lyt-1, Lyt-2), B cells (ThB, Ig), and NK cells (NK-1.1). They also lacked I-A^b. In the selected mice, 49–66% of PBMC bore Fc receptors, compared with 24–32% in age-matched females. There was a threefold increase in adherent cells in males relative to females, with a corresponding increase in the frequency of such cells that were capable of erythrophagocytosis (70% in males compared with 38% in females). Finally, the predominant PBMC subpopulation lacked nonspecific esterase and myeloperoxidase.

Examination of bone marrow from 14 BXSB males and 9 BXSB females revealed a slight increase in large Mac-1⁺ cells in males beyond 6 mo of age. However, all marrow elements appeared morphologically normal, and there was no evidence of any infiltrative or malignant process.

Discussion

Our studies demonstrate that autoimmunity in BXSB mice is associated with early, and progressively dramatic, monocytosis. There is no such increase in

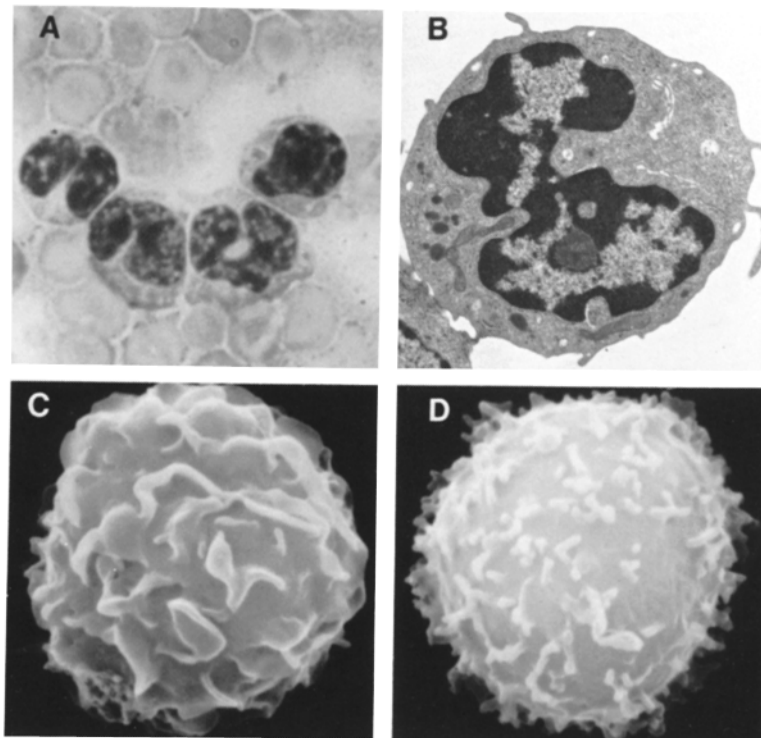


FIGURE 2. Morphology of peripheral blood leukocytes from an 8-mo-old BXSB male. (A) Wright's stain ($\times 2,000$) of a blood smear showing monocytes with indented nuclei and coarse chromatin. (B) Transmission electron micrograph ($\times 7,500$) of the predominant cell type, demonstrating a convoluted nucleus, abundant cytoplasmic granules, prominent Golgi apparatus, and filopodia. (C) Scanning electron micrograph ($\times 7,500$) of the predominant cell type, demonstrating a ruffled surface with lamellapodia. For comparison, a lymphocyte from the same mouse is shown in (D).

TABLE I
Characteristics of the Predominant Circulating Cell Type in Adult BXSB Males

Present	Absent
Surface antigens: T200, Mac-1	Surface antigens: Thy-1, Lyt-1, Lyt-2, ThB, Ig, NK-1.1, I-A
Fc receptors	Nonspecific esterase
Phagocytic capacity	Myeloperoxidase

monocytes in autoimmune 10-mo-old NZB/NZW (B/W) females or 5-mo-old MRL/lpr females (unpublished data), indicating that monocytosis does not reflect murine lupus in general, but is unique to BXSB mice. These findings suggest that monocytes may contribute fundamentally to the pathogenesis of autoimmune disease in BXSB mice.

Despite the importance of lymphocyte/macrophage interactions in normal immunity, little is known about the role of the mononuclear phagocyte system in the development of autoimmunity. In B/W mice, acquired T helper cell deficiency has been linked to the presence of splenic macrophage suppressor cells (14). In humans with SLE and other autoimmune diseases, clearance of antibody-coated red cells by the mononuclear phagocyte system is impaired (15).

A similar defect develops in B/W and MRL/lpr mice (16, 17). The current report is the first investigation of the mononuclear phagocyte system in BXSB mice.

Theofilopoulos et al. (3) examined the distribution of lymphocyte subpopulations in 1-mo-old and 5-mo-old BXSB mice. They demonstrated proliferation of B cells rather than monocytes in the spleen and lymph nodes of adult BXSB males. Preliminary studies in our laboratory confirm these observations, although we find a marked increase in splenic monocytes in 8-mo-old BXSB males (unpublished data). Interestingly, Theofilopoulos et al. reported that 20% of PBMC in 1-mo-old BXSB males, and 40% of PBMC in 5-mo-old BXSB males, lacked T cell and B cell markers. Our findings indicate that these cells were monocytes and that monocytosis is an early event in the course of BXSB disease.

In normal mice, ~90% of circulating monocytes produce peroxidase (13). Although we found an increase in monocytes in BXSB males, we were unable to detect peroxidase in these cells. Either the monocytes in BXSB mice are defective in peroxidase production or they reflect an increase in the monocyte subset that normally lacks this enzyme. BXSB monocytes also lacked nonspecific esterase. In mice, resident macrophages produce nonspecific esterase, but circulating monocytes do not (12).

Studies of murine lupus have identified numerous immunoregulatory abnormalities that could contribute to autoimmunity (1-5). Some of these are common to all murine models for SLE (4, 5). However, other abnormalities are associated with particular strains. For example, in MRL/lpr mice, T cell proliferation appears to be causally related to the development of autoimmunity (18). It is appealing to speculate that, in BXSB mice, monocyte proliferation might lead to autoimmune disease. This hypothesis is supported by the early occurrence of monocytosis in BXSB males and by the lack of monocytosis in other murine models for SLE. Nonetheless, it is also possible that monocytosis in BXSB mice is a secondary phenomenon, either incidental or compensatory to autoimmunity. Studies designed to selectively deplete or transfer the monocytes will help clarify these issues.

Summary

Autoimmunity in BXSB mice is associated with a progressive increase in the number of peripheral blood mononuclear cells (PBMC). This is due to a marked rise in circulating monocytes, identified by: (a) their appearance on light and electron microscopy; (b) their surface antigenic characteristics; (c) their expression of Fc receptors; and (d) their capacity for phagocytosis. Among murine models for systemic lupus erythematosus, only the BXSB strain is characterized by monocytosis, suggesting that cells of monocytic lineage may contribute significantly to the pathogenesis of autoimmune disease in BXSB mice.

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