

DISTRIBUTION OF LYMPHOCYTES IDENTIFIED BY
SURFACE MARKERS IN MURINE STRAINS WITH
SYSTEMIC LUPUS ERYTHEMATOSUS-LIKE SYNDROMES*

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The pathogenesis of autoimmune disease in certain inbred mouse strains has been a subject of thorough study for many years, due to its possible relationship to similar syndromes in man. Despite such scrutiny, however, no common etiologic denominator that may signal the onset of these disease processes has yet been found. In recent years, much attention has been given to the analysis of abnormal function among various immunocompetent cell types in such mice. The separation of murine lymphocytes into T and B cells and their subsets is based on the expression of certain surface markers which, in turn, have been linked to distinct biological functions. Thus, B cells interact with antigens via surface immunoglobulin (Ig) molecules and communicate with other cells via Fc and complement (C) receptors (1). Moreover, subpopulations of B cells of different Ig isotypes can, in certain instances modulate the immune response of one another so that a coordinated response is achieved. Certain subsets of T lymphocytes act, respectively, to facilitate or to diminish immune responses (2). These subsets express on their surfaces different Ly alloantigens and *I*-region-associated (Ia) antigens (3, 4). In addition, overall internal self-regulation of the immune system may also involve the functional effects of anti-idiotypic reactions at both T- and B-cell surfaces (5).

This complex interplay of distinct cell subsets and molecular products of such cells must obviously retain a critical integrity for the system to function normally. Perturbations among the cellular components may, therefore, be associated with the onset of autoimmune reactivity in experimental animals as well as man. In order to examine the relationship of such potential perturbations to autoimmune manifestations, we have conducted a thorough analysis of the phenotypic expression of various surface markers on lymphocytes from lymphoid organs of New Zealand mice (NZB, [NZB × NZW]F₁) and of the newly described MRL/l, MRL/n, and BXSB strains (6-8). Particular attention has been paid to the development of alterations in the T- and B-

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cell compositions of these mice as their genetically characteristic autoimmune disease appears. Furthermore, we assessed whether phenotypic abnormalities, if any, of T and B cells were identical or different among the five autoimmune murine strains.

In this report, we describe the frequency and number of cells bearing surface Ig, C receptors, IgG Fc receptors, θ -antigen, Ly alloantigens, and suppressor cell Ia antigens in these mice. We also determined the various isotypes of Ig expressed on B cells in newborn and adult animals. The results indicate that all five autoimmune mouse strains manifest alterations in their T- and B-cell compositions with age and the appearance of disease. However, these alterations are not the same in all five strains.

Materials and Methods

Mice. C3H/St ($H-2^b$), C57BL/6 ($H-2^b$), BALB/c ($H-2^d$), NZB ($H-2^d$), (NZB \times NZW)F₁, (NZB \times W) ($H-2^{d/z}$) mice are bred and maintained in our colony. The inbred strains BXSB ($H-2^b$), MRL/l ($H-2^k$), and MRL/n ($H-2^k$) were developed by Dr. E. M. Murphy and originally obtained from The Jackson Laboratories, Bar Harbor, Maine, but were bred and maintained thereafter in our colony. The age and sex of the animals employed is indicated in the results section.

Cells. All cell suspensions were prepared at room temperature. Cells from spleens, mesenteric lymph nodes, and thymuses were prepared by passage through a stainless steel screen in minimum essential medium buffered with 25 mM Hepes (Grand Island Biological Co., Grand Island, N. Y.) and containing 5% fetal bovine serum (thereafter referred to as medium). Bone marrow cells were extracted from the femurs by cutting one end and flushing the cells out the other end with a syringe. The cell clumps were then dissociated by being pipetted up and down several times. Peripheral blood lymphocytes were obtained from heparinized blood after dextran sedimentation and Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) centrifugation. All cell preparations were allowed to settle to remove large aggregates. Erythrocytes were lysed with 0.17 M NH₄Cl (4°C, 10 min), and nucleated cells were washed twice with medium. Cell suspensions were then kept at 37°C for 1 h to allow for the release of any cytophilic antibodies absorbed *in vivo* and washed again. Cells were counted, and viability was determined by trypan blue dye exclusion. Viabilities ranged from 75 to 90% (spleen), from 85 to 95% (lymph node), and >90% (all others). Newborn mice were killed by decapitation, their spleens obtained for preparation of cell suspensions as above, and the peritoneal cavity was carefully examined to determine the sex of each individual.

Antisera. A fluorescein isothiocyanate (FITC)¹-conjugated polyvalent rabbit antiserum to mouse Ig and a rhodamine (Rö)-conjugated rabbit antiserum to mouse brain were kindly provided by Dr. S. Russell (Scripps Clinic and Research Foundation). The Fab'2 fractions of both antisera were employed (9).

The preparation and use of Ly 1.2, Ly 2.1, Ly 3.2 and Thy 1.2 antisera (generously supplied by Dr. F. W. Shen, Memorial Sloan-Kettering Cancer Center, N. Y.) has been described (10, 11). These antisera were absorbed with spleen cells from congenic strains of mice and extensively tested to verify their specificity on target cells. The Ly 2.1 antiserum was additionally absorbed with BALB/c spleen cells before use.

FITC goat anti-mouse IgG₁ and IgG₂ (IgG_{2a} and IgG_{2b} combined) antibodies were obtained from Meloy Laboratories, Inc., Springfield, Va. Anti-mouse IgA serum was raised in rabbits immunized with TEPC 15 myeloma protein (IgA,k). This antiserum was absorbed on Sepharose (Pharmacia-Fine Chemicals, Div. of Pharmacia Inc.)-bound MOPC 315 myeloma protein (IgA, λ) and conjugated with FITC by a dialysis procedure (12). Antiserum to IgM was raised in rabbits injected with MOPC 104E myeloma protein (IgM, λ). The antiserum was affinity purified on Sepharose-bound ABPC22 myeloma protein (IgM, κ). This antiserum was further absorbed with Sepharose-bound IgG and IgA to render it specific and conjugated with FITC. Rabbit anti-mouse IgD (13), a gift from Dr. R. L. Coffman (Stanford University), was absorbed

¹ *Abbreviations used in this paper:* BSA, bovine serum albumin; DNP, dinitrophenyl; FITC, fluorescein isothiocyanate conjugated; lpr, lymphoproliferation; Rö, rhodamine; SLE, systemic lupus erythematosus.

twice with an equal volume of MRL/l lymph node cells (>95% of T origin). F(ab')₂ fragments were prepared from the IgG fraction of this antiserum by pepsin digestion and Sephadex G-200 chromatography. Antibody made in sheep against rabbit IgG was affinity purified on Sepharose-bound F(ab')₂ of rabbit IgG, and then digested with pepsin and chromatographed on a Sephadex G-200 column. This F(ab')₂ anti F(ab')₂ antibody was fluoresceinated as above.

Anti-*I-J^k* serum (3R anti-5R) was kindly provided by Dr. J. Silver (Scripps Clinic and Research Foundation), and anti-*I-J^b* (5R anti-3R) serum was from Doctors M. E. Dorf and B. Benacerraf (Harvard Medical School, Boston, Mass.).

Enumeration of Surface Ig- and θ -Antigen-Bearing Cells. The frequency of Ig- and θ -positive cells in the various cell suspensions was ascertained simultaneously by using a mixture of FITC-F(ab')₂ anti-mouse Ig antibody and R \ddot{o} -F(ab')₂ anti-mouse brain antibody. The staining was done as follows: 2×10^6 nucleated cells were incubated (4°C, 30 min) with 50 μ l total volume of equal parts of a 1:200 final dilution of FITC F(ab')₂ anti mouse Ig antibody and a 1:8 final dilution of R \ddot{o} F(ab')₂ anti-mouse brain antibody. Antisera were diluted in medium containing 0.1% NaN₃. Subsequently, the cells were washed three times with cold medium, centrifuged, and the pellets were resuspended in 10 μ l phosphate-buffered saline containing 10% Patho-o-cyte (Miles Laboratories Inc., Miles Research Products, Elkhart, Ind.). Drops were placed on a slide under a coverslip, sealed, and kept on ice until viewed. These cells were examined under a Zeiss microscope (Carl Zeiss, Inc., N. Y.) using a vertical illuminator equipped with a combination of excitation and barrier filters for the selective visualization of either FITC or R \ddot{o} stains. Approximately 300 cells were counted, and the percent of FITC-(B cells) and R \ddot{o} -(T cells) positive cells was recorded.

Enumeration of C3- and IgG Fc Receptor-Bearing Cells. C3 receptor-bearing cells were assessed as described (14) using sheep erythrocytes sensitized with IgM rabbit hemolysin and reacted with C5-deficient serum obtained from B10.D2/old mice (EAC). These intermediates are immune adherence negative (14) and, therefore carry C3d but not C3b.

Receptors for IgG Fc, which are present on subsets of both T and B cells as well as on other unclassified lymphocytes (15), were assessed two ways: (a) rosetting of ox erythrocytes sensitized with an optimum amount of the IgG fraction of a rabbit anti-ox erythrocyte antibody (16) (EA); and (b) uptake of soluble rabbit anti-dinitrophenyl (DNP)-bovine serum albumin (BSA) plus DNP-human gamma globulin (HGG) complexes at a fourfold antigen excess and staining with FITC sheep anti-rabbit Ig (15).

Analysis of Cell-Surface Ig Isotypes on B Cells. The percentages of splenic B cells from adult animals bearing most isotypes of murine Ig (IgG₁, IgG₂, IgM, IgA, and IgD) were determined using direct or indirect immunofluorescence followed by processing in a cell sorter. No attempts were made to determine cells bearing combinations of isotypes on their surface.

IgG₁, IgG₂, IgA, and IgM-bearing cells were detected by direct immunofluorescence. In brief, spleen cells were subjected to Ficoll-Hypaque centrifugation to remove dead cells; then aliquots of 2×10^6 cells were incubated (4°C, 30 min) with optimum amounts of the isotype specific FITC-conjugated antisera. Cells were then washed three times in medium containing 0.1% NaN₃ and brought to 2×10^6 cells/ml before being subjected to cell sorting. IgD-bearing cells were detected by indirect immunofluorescence. 4 million Ficoll-Hypaque-separated cells were incubated (4°C, 30 min) with an optimum dilution of F(ab')₂ rabbit anti-IgD antibody, washed three times, stained with FITC-conjugated F(ab')₂ sheep anti-rabbit F(ab')₂ antibody (4°C, 30 min), washed again, and brought to 2×10^6 cells/ml.

FITC-stained cell suspensions were counted and sorted on the basis of bright fluorescence using the Becton-Dickinson FACS-III cell sorter (BioQuest, BBL, & Falcon Products, Becton, Dickinson, & Co., Cockeysville, Md.) as previously described (17). Bright cells were contaminated with up to 1% of dull cells, and dull cells were contaminated with up to 0.2% of bright cells as judged by repeat analysis of sorted cells in the regular fluorescence microscope. The frequencies of neonatal splenic B cells bearing surface IgM and/or surface IgD were determined as above using direct and indirect immunofluorescence techniques, respectively, but employing the regular fluorescence microscope instead of the cell sorter.

Analysis of Ly-Subclass Distribution. The phenotype of T-cell surface allo-antigens is (a) *H-2^k*, Ly 1.2, Ly 2.1, Ly 3.1, Thy 1.2, and Tla^b for MRL/l; and (b) *H-2^b*, Ly 1.2, Ly 2.2, Ly 3.2, Thy 1.2, and Tla^b for BXSb mice (6). The percentage of θ -positive cells in lymph nodes from MRL/l and C57BL/6 mice were estimated using a Thy 1.2 antiserum and selected rabbit serum as a

source of complement in a two-stage cytotoxicity assay (11). Cell viability was determined by trypan blue exclusion. The proportions of lymph node cells expressing one or more Ly components were similarly estimated after exposure to various Ly antisera as well as combinations of two of these antisera, according to a protocol detailed previously (18). θ -positive cells that did not express Ly alloantigens (Results) were designated as Ly null T cells.

Detection of I-J Alloantigen-Bearing Cells. Spleen cells from MRL-l, MRL-n, and BXSB mice and histocompatible normal C3H and C57BL/6 mice of various ages were examined. 50 million spleen cells were obtained after Ficoll-Hypaque centrifugation and incubated with an optimum dilution of FITC-conjugated rabbit anti-mouse Ig. Cells were washed three times with medium containing NaN₃, brought to 2×10^6 cells/ml and subjected to cell sorting. Dull cells (>95% were T cells as judged by positive stainings with R \ddot{o} -conjugated anti-mouse brain antibody) were collected. 2 million isolated T cells from each of the above strains were incubated (4°C, 30 min) with an optimum amount of mouse anti-I-J^h antiserum (MRL, C3H) or anti-I-J^b antiserum (BXSB, C57BL/6) and washed three times with medium containing NaN₃. Control suspensions were similarly treated with medium or normal mouse serum. Then the cells were stained (4°C, 30 min) with an optimum dilution of FITC-rabbit anti-mouse Ig antibody and washed three times. FITC-positive cells were determined under the fluorescence microscope as above. Percentages of I-J positive cells were corrected for surface Ig-bearing cells contamination by subtracting the percentage positive cells in control suspensions (<1%).

Results

Characteristics of the Autoimmune Syndromes. The age-sex correlations of disease manifest in the various autoimmune strains can be briefly summarized as follows (7, 8): NZB mice of both sexes have a 50% mortality at 21–23 mo old. In NZB \times W mice, the disease appears in the female around the 6th mo with 50% mortality at 8.5 mo and a 90% mortality at 12.8 mo. The male hybrid develops his disease after yr of age with a 50% mortality at \approx 15 mo of age. The MRL mice have been subdivided into two similar strains sharing 89% of their genomes. The MRL/l strain with the lpr (lpr = lymphoproliferation) gene is characterized by massive lymphoproliferation and early disease (50% mortality in both sexes at a little over 5 mo and 90% mortality by 6.8 mo in the female and 8.8 mo in the male). The MRL/n strain, without the lpr gene, is characterized by late disease with 50% mortality at 17 and 23 mo for female and male, respectively, and 90% mortality at 23 and 27 mo. The male BXSB mouse begins to show signs of his disease by the 3rd–4th mo, with 50% mortality at 5 mo and 90% mortality at 8 mo. In this strain, the female first appears ill shortly after 1 yr of age with 50% mortality at 15 months and 90% mortality at 2 yr.

Enumeration of B Cells. The frequencies of nucleated cells bearing surface Ig and C3d receptors in the various lymphoid organs of young and old mice of strains with the systemic lupus erythematosus (SLE)-like syndromes and one normal strain are shown in Figs. 1 and 2. The absolute numbers of these cells in spleens and mesenteric lymph nodes are presented in Table I. Generally, except for the old BXSB male mice, all strains examined had normal organ distributions of surface Ig-bearing cells, being higher in the spleens than in the lymph nodes and peripheral blood. In the normal C57BL/6 mice, the frequencies and absolute numbers of these cells remained stable with age. NZB and NZB \times W mice had reduced frequencies and numbers of Ig-bearing cells in spleens and lymph nodes with advanced age and disease. In MRL/l mice there was a similar substantial reduction in frequencies, but not absolute numbers, of these cells, especially in lymph nodes, with advancing disease. MRL/n mice equaled the normal C57BL/6 on all counts. In contrast, BXSB male mice, but not females (data not shown), had a substantial increase in frequencies and absolute

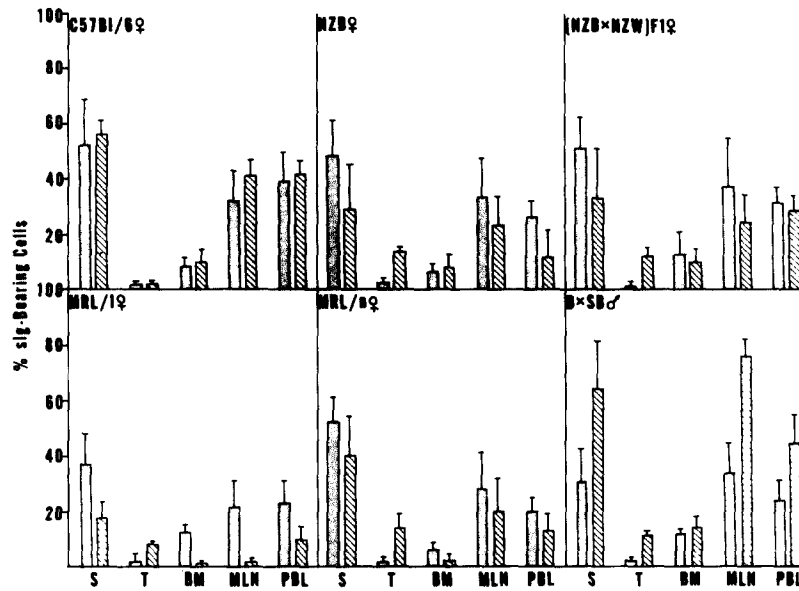


FIG. 1. Surface Ig-bearing cells in lymphoid organs of normal and autoimmune mice. Each column represents the mean \pm SD obtained from three animals. Dotted columns depict animals 1 mo old and hatched columns depict animals of 8 mo old, except MRL/l and BXSB mice where animals of 1 and 4½-5 mo were examined. S = spleen, T = thymus, BM = bone marrow, MLN = mesenteric lymph nodes, PBL = peripheral blood lymphocytes.

numbers of Ig-bearing cells associated with advanced disease. As all the mice with autoimmune manifestations aged, the frequency of Ig-bearing cells in the thymus increased. In general, reductions or increases in frequencies of Ig-bearing cells appeared to be reflected equally in spleens and lymph nodes.

The frequencies of the C3d receptor-bearing cells in lymphoid organs, except bone marrows, were lower than those of the Ig-bearing cells in all mice tested (Fig. 2). However, the general trends seen with the surface Ig-bearing cells were similarly reflected in the C receptor-bearing cell populations, with the notable exception of the BXSB male mice. There was no significant difference between young and old normal C57BL/6 mice. In NZ mice, and more prominently in male BXSB mice, the frequency of C3d receptor-bearing cells was higher during youth than in normal mice, but declined with advanced age and disease. MRL/l, but not MRL/n, mice also exhibited a reduced frequency of C3d receptor-bearing cells with advanced disease, consistent with the reduced frequency of Ig-bearing cells.

Ig Isotypes on the Surfaces of Ig-Bearing Cells. The frequencies of IgG1-bearing splenic cells were 5% or less in all strains tested at all ages (Table II). From 5-19% of the spleen cells in the various murine strains had IgG2 on their surfaces. In general, mice with autoimmune manifestations had a higher frequency of these cells than the normal mice, especially in later life. Similarly, autoimmune mice had many fold higher percentages of IgA-positive cells, although the frequencies were <10% in all cases. Many of the cells, as expected, were positive when stained with anti-IgM and anti-IgD sera. In all young mice, the proportions of IgM-positive cells and of IgD-positive cells were similar except in the MRL/l where IgD cells were twice as numerous as IgM. However, in NZB and NZB \times W females and BXSB male mice,

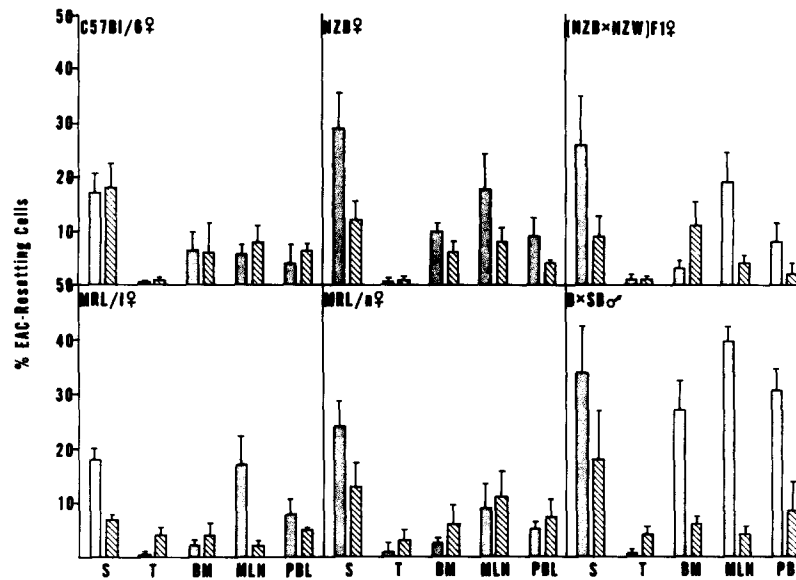


Fig. 2. C3d receptor-bearing cells in lymphoid organs of normal and autoimmune mice. See legend to Fig. 1.

the ratio of IgM- over IgD-bearing cells increased with age. In contrast, in MRL/l mice, there was a decrease in ratio of IgM- over IgD-bearing cells with increasing age.

Sequential Expression of Ig Isotypes on Developing B Lymphocytes. Spleen cells of newborn mice were examined sequentially from the 1st to 7th d after birth for expression of IgD and IgM. The results are presented in Table III. The time of appearance and the frequencies of cells bearing IgM or IgD in the autoimmune mice were similar to those observed in two normal strains. IgM-bearing cells were present on spleen cells obtained immediately after birth, whereas IgD-bearing cells appeared for the first time 3 d after birth.

Enumeration of IgG Fc Receptor-Bearing Cells. The proportion of these cells as shown in Fig. 3 and Table IV were similar in a normal strain and the autoimmune murine strains while young. However, in all mice with SLE-like syndromes, but not the one normal strain, the frequencies of these cells decreased with advanced age and disease.

Enumeration of T Cells. The proportions and absolute numbers of nucleated cells in the various lymphoid organs expressing θ -antigen are presented in Fig. 4 and Table I. In general, there were normal organ distributions of these cells, being higher in the lymph nodes than the spleens of all animals tested. As the normal C57BL/6 mice aged, their T-cell values remained stable. However, there was a decline in numbers of these cells in the lymphoid organs of NZB and NZB \times W females and BXSB male mice with advanced age and disease. MRL/n mice showed a modest increase in frequencies and absolute numbers of these cells with age. In MRL/l mice, a clear increase in T-cell frequencies and massive increase in absolute numbers was observed with advanced disease. More than 90% of the cells in the enlarged lymph nodes of these mice were positive for θ -antigen, indicating that the lymphadenopathy seen in these mice was largely due to T-cell proliferation.

Enumeration of Ly Alloantigen-Bearing Cells. The frequency of Ly1⁺, Ly23⁺, and

TABLE I
Enumeration of B and T cells in Lymphoid Organs of Normal and Autoimmune Mice

Strain	Age	Spleen			MLN*		
		Total No. ($\times 10^{-6}$)	No. sIg ⁺ cells ($\times 10^{-6}$)	No. θ^+ cells ($\times 10^{-6}$)	Total No. ($\times 10^{-6}$)	No. sIg ⁺ cells ($\times 10^{-6}$)	No. θ^+ cells ($\times 10^{-6}$)
	<i>mo</i>						
C57BL/6 ♀	1	90‡	47	33	11	4	6
	8	105	59	36	14	6	7
NZB ♀	1	83	39	36	15	5	9
	8	90	25	23	17	4	8
(NZB \times NZW)F ₁ ♀	1	100	51	41	15	5	9
	8	110	35	28	22	5	9
BXS _B ♂	1	65	19	39	17	4	10
	5	215	135	38	32	19	7
MRL/n ♀	1	50	26	16	30	8	19
	8	70	27	31	43	8	33
MRL/l ♀	1	52	19	21	30	7	7
	4.5	310	52	226	610	12	579

* MLN, mesenteric lymph nodes.

‡ Numbers represent the mean of three animals.

Ly123⁺ T-cell subclasses in the spleen cell populations of female NZB and BALB/c mice of increasing ages have been reported by Cantor et al. (19). We have determined the various Ly subsets in lymph node cells of MRL/l females at early and advanced ages and compared them to similar data from 12-wk-old normal C57BL/6 females. The results shown in Fig. 5 are expressed as a percent of total cells recovered from the nodes and as a percent of the θ -antigen-bearing cells in that population expressing the various Ly phenotypes. In the C57BL/6 females and young MRL/l female mice, distributions of the two large subsets, the Ly1⁺ and the Ly123⁺, and the much smaller Ly23⁺ subset were quite similar. The sum of Ly1⁺, Ly23⁺, and Ly123⁺ cells was similar to that of θ -antigen-bearing cells in C57BL/6 and young MRL/l mice. However, an unexpected and striking observation in MRL/l mice of advanced age and disease was the extreme reduction in numbers of Ly123⁺ cells and Ly23⁺ cells compared to the young animals and a concomitant increase in the percentage of θ^+ , Ly⁻, or null cells. The accumulation of Ly null cells in these mice followed a straight line with age (Fig. 6).

Enumeration of Ia Antigens Coded by the I-J Subregion in Splenic Lymphocytes. The percentages of I-J positive T cells isolated from the spleens of four immunologically normal (C3H, MRL/n, C57BL/6, and BXS_B females) and the two autoimmune strains (MRL/l, BXS_B males) of young and advanced age are presented in Table V. No significant reduction of cells bearing Ia antigens coded by the I-J subregion of the H-2 gene complex was observed in any of the mice tested. However, MRL/l mice of advanced age showed a high frequency and absolute number of these cells.

Discussion

Many speculations have been raised concerning the extent and types of functional lymphoid cell abnormalities that might play important pathogenic roles in the development of autoimmune disorders in both experimental animals and man.

TABLE II
Surface Ig Isotypes of Splenic B Cells from Normal and Autoimmune Mice

Antiserum	C57BL/6 ♀		NZB ♀		NZB × W ♀			
	1	8	1	8	1	8		
	<i>mo</i>	<i>mo</i>	<i>mo</i>	<i>mo</i>	<i>mo</i>	<i>mo</i>		
Anti-IgG1	1.7*	1.6	3.8	3.8	2.7	5.8		
Anti-IgG2	5.6	7.8	3.4	8.3	5.5	12.0		
Anti-IgA	0.7	0.9	2.5	6.7	3.2	5.2		
Anti-IgM	38.0	31.7	34.2	23.8	36.2	31.8		
Anti-IgD	42.5	29.0	37.1	10.0	37.6	13.0		

Antiserum	BXS B ♀		BXS B ♂		MRL/n ♀		MRL/l ♀	
	1	8	1	8	1	8	1	4.5
	<i>mo</i>	<i>mo</i>	<i>mo</i>	<i>mo</i>	<i>mo</i>	<i>mo</i>	<i>mo</i>	<i>mo</i>
Anti-IgG1	1.2	2.8	2.3	4.6	2.1	4.1	2.0	1.7
Anti-IgG2	7.0	12.0	11.5	18.7	6.1	12.1	5.3	11.5
Anti-IgA	3.3	7.2	2.8	ND§	9.5	2.5	2.0	3.1
Anti-IgM	23.4	27.2	22.4	48.0	32.8	30.3	9.4	2.4
Anti-IgD	24.2	23.9	19.4	10.2	34.3	29.0	22.5	18.0

* Numbers represent the mean percent cells positive for three animals.

§ ND, not determined.

Because the predominant phenotypic expression of autoimmunity shows clear overlap from one individual (or inbred strains in the case of experimental animals) to another, one might logically expect the existence of certain basic common pathways to the clinically relevant end point. To date, however, this has not been borne out despite considerable evaluation over many years.

In this study, we have analyzed, in varying aspects, the distribution, frequency relationships, ontogenic changes, and defined genetic markers of lymphoid cells in five different kinds of mice manifesting autoimmune syndromes and normal nonautoimmune counterparts. Although varying types of distribution and frequency abnormalities were detected in certain of these strains, particularly in association with advanced age and disease onset, the most striking message derived from these analyses is the diversity of such abnormalities among the different experimental mouse strains. This makes it clear, therefore, that despite similarities in autoimmune manifestations that may exist from one individual to the next, the pathogenesis of such disorders most probably includes a spectrum of primary or secondary lymphoid cell abnormalities.

Thus, frequencies and absolute numbers of B cells decrease substantially as non-Ig, non- θ -bearing cells increase in spleens and lymph nodes of aging, sick NZB and NZB × W mice. Stobo et al. (20) demonstrated a similar low frequency of B cells in NZB and NZB × W mice of advanced age; this is not due to alterations in the cell surface that renders Ig determinants less accessible for binding by antibody. They postulated that the decrease in B cells indicated either (a) an abnormality in the capacity of Ig negative progenitor cells (possibly through a defect in the T-cell compartment) to progress along normal pathways which involve the acquisition of surface Ig determinants, or (b) unusual numbers of B cells in transition between the precursor state and the state of active secretion of Ig and, therefore, low in quantities of surface Ig (21).

TABLE III
Ontogeny of Splenic B Cells Bearing Surface IgM or Surface IgD in Normal and Autoimmune Mice

Strain*	Age	Cells bearing	
		sIgM	sIgD
	<i>d</i>	%	
C57BL/6 ♀	1	3.4	<0.01
	2	4.1	<0.01
	3	9.8	1.9
	5	10.7	5.3
	7	12.2	7.9
BALB/c ♀	1	2.8	<0.01
	2	6.4	<0.01
	3	7.2	0.8
	5	9.8	2.7
	7	10.9	5.6
NZB ♀	1	5.2	<0.01
	2	5.7	<0.01
	3	7.8	2.4
	5	8.4	3.5
	7	11.0	7.0
(NZB × NZW) _{F1} ♀	1	3.1	<0.01
	2	4.7	<0.01
	3	7.2	2.0
	5	7.8	5.1
	7	7.9	7.2
BXSB ♀	1	6.8	<0.01
	2	7.0	<0.01
	3	7.2	1.8
	5	9.0	4.6
	7	12.7	5.5
BXSB ♂	1	3.2	<0.01
	2	6.3	<0.01
	3	7.4	0.8
	5	12.1	2.6
	7	18.2	5.5
MRL/l ♀	1	4.7	<0.01
	2	8.3	<0.01
	3	8.8	2.6
	5	12.0	5.1
	7	13.1	8.1

* A pool of nucleated cells from three spleens at the various time intervals was examined.

In view of the recent findings of Izui et al. (22), Moutsopoulos et al. (23) and our own unpublished observations² indicating high production of Ig and large numbers of Ig-

² Theofilopoulos, A. N., R. A. Eisenberg, and F. J. Dixon. Ig-secreting cells in mice with SLE-like syndromes. Manuscript in preparation.

TABLE IV
*IgG Fc-Receptor-Bearing Cells in Lymphoid Organs of Mice with SLE-Like
 Syndromes as Detected by Uptake of Soluble Immune Complexes*

Strain	Age	IgG Fc-receptor-bearing cells	
		Spleen	MLN*
	<i>mo</i>		<i>%</i>
NZB ♀	1	48‡	33
	8	32	23
(NZB × NZW)F ₁ ♀	1	73	41
	8	39	30
BXSB ♂	1	48	59
	5	34	22
MRL/l ♀	1	33	34
	4.5	21	12

* MLN, mesenteric lymph nodes.

‡ Numbers represent the mean of three animals.

secreting cells in spleens of older mice, the loss of surface Ig due to advanced maturation is the more likely explanation.

Unlike NZ mice, BXSB male mice with advanced disease exhibit an increased frequency and number of surface Ig-bearing cells. As reported (6-8), BXSB male mice develop spontaneous autoimmune disease characterized by moderate lymphadenopathy and splenomegaly, hypergammaglobulinemia, immune complex glomerulonephritis, and various kinds of autoantibodies. Histologically, the lymphoproliferation shows a predominance of lymphocytes with admixed plasma cells and histiocytes and appears from the present studies to be of B-cell origin. As Murphy and Roths (24) noted, this syndrome closely resembles the nonneoplastic hyperimmune proliferation of B cells recently described in man as immunoblastic lymphadenopathy (25). The fact that BXSB males, contrary to NZ mice, have a high frequency of Ig-bearing cells even late in life may be related to their tendency toward lymphoproliferation and/or to produce less mature B cells. In fact, BXSB male mice have fewer Ig-secreting cells in their spleens than NZ mice,² as tested by a reverse hemolytic plaque assay. In contrast to BXSB mice, as MRL/l mice advanced in age and disease, low frequencies, but not absolute numbers of B cells, characteristically accompanied massive T-cell proliferation.

The results of our experiments indicate that Ig isotypes appear on the surfaces of spleen cells from mice with autoimmune disease in the same sequence as in normal mice (26); IgM is present at birth and IgD appears 3 d later. Therefore, autoimmunity is probably not caused by some irregularity in the generation of B-cell isotype diversity associated with resistance to induction of self-tolerance. This latter consideration arises from work by other investigators who showed that murine B cells from bone marrow (27) and neonatal spleens (28, 29) are more readily tolerized to thymus-dependent antigens than splenic B cells from adults, and that maturational decrease in susceptibility to tolerance induction is determined by the acquisition of surface IgD determinants (30-32).

We studied the expression of surface Ig isotypes in spleen cells of aging autoimmune mice without regard to combinations on a single cell and found a high frequency of IgG-bearing cells. This finding may indicate advanced maturation of B cells in these

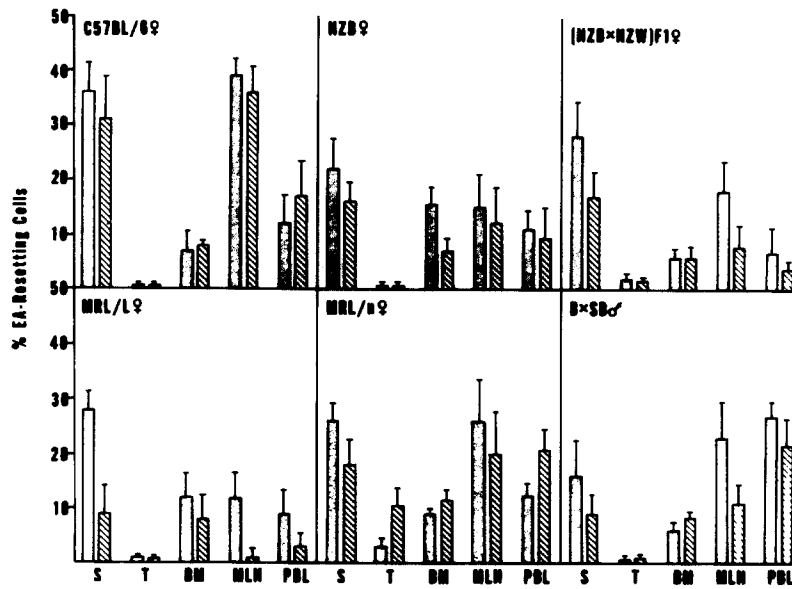


FIG. 3. IgG Fc receptor-bearing cells in lymphoid organs of normal and autoimmune mice. See legend to Fig. 1.

mice. Furthermore, because IgG-bearing cells differentiate to IgG-secreting cells and can depress IgM production (33, 34), our finding is in accord with the observed switch of anti-DNA antibodies in New Zealand mice from IgM to IgG late in life (35) and with the switch by cells of all autoimmune strains from IgM to IgG secretion as the host ages and manifests disease.² The frequencies of IgG-bearing cells were not artificially elevated by the binding of rabbit antiserum via Fc receptors, because $F(ab')_2$ reagents produced similar results. Nor was the mouse IgG passively absorbed, because incubation at 37°C did not reduce numbers of IgG-positive cells (36). Additionally, NZ and BXSB mice of advanced age and disease have a high ratio of IgM/IgD. Because >90% of B cells in normal adult animals bear both IgM and IgD (37, 38), the cells we examined in the sick, older animals are probably driven by antigenic stimulation to lose their surface IgD (39). Apparently, surface IgD was also lost preferentially during LPS-induced activation of B lymphocytes from immunologically normal mice (40) and during aging of NZB mice (41). Cohen et al. (41) concluded that the increased ratio of surface IgM to IgD in NZB mice indicates advanced maturation possibly due to polyclonal activation and is not due to a maturational arrest nor acquisition of IgM anti-thymocyte antibodies from serum. Compared to NZ and BXSB mice, MRL/l mice manifested a higher percentage of IgD-bearing than IgM-bearing cells and, in general, have fewer mature B cells as indicated by their lower frequencies of splenic Ig secreting cells.² These IgD positive cells may represent immature IgG memory cells that give rise to secretion of IgG antibodies of low avidity (39). However, the Ig-secreting cells appear to be exceptionally productive as shown by the exceedingly high levels of IgG in sera of old MRL/l mice (7).

Young BXSB and NZ mice have more C3d receptor-bearing cells than normal mice, again suggesting hastened maturation because the C3d receptor appears late in

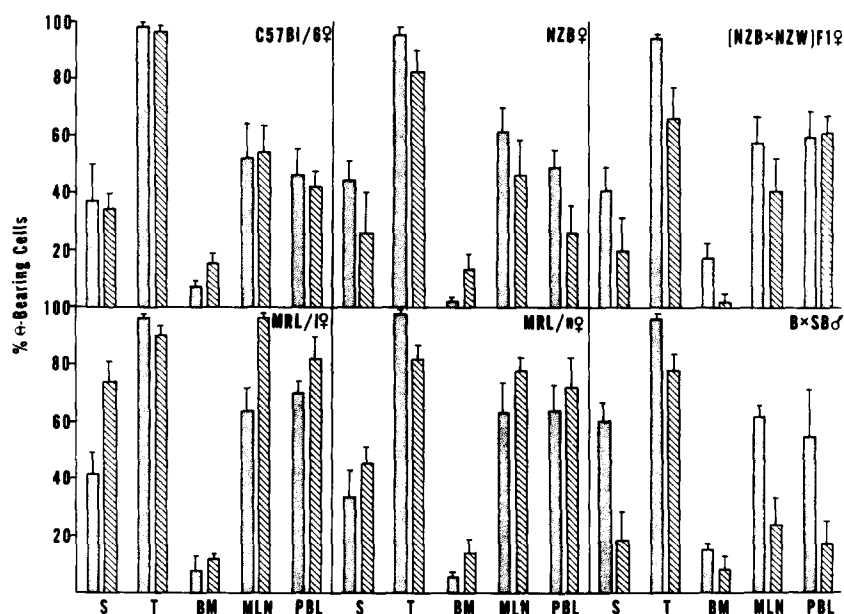


FIG. 4. θ antigen-bearing cells in lymphoid organs of normal and autoimmune mice. See legend to Fig. 1.

B-cell development (42, 43). However, the frequency of these cells fall markedly with advanced age and disease. This decreased frequency of the EAC-rosetting cells as disease advanced may suggest occupation of C3d receptors by C-fixing immune complexes, shown by ourselves to be present in the sera of these mice late in life (7). We (44) and Eden et al. (45) have also shown that soluble C-fixing immune complexes bind to C3b and C3d receptors on B cells, and that bound complexes are not easily removed from these receptors even after prolonged incubation. In addition, C receptor-bearing cells may be eliminated from the circulation when bound to soluble complexes.

Autoimmune mice lose IgG Fc receptor-bearing cells as they age. This was demonstrated by two techniques, namely rosetting with particulate complexes and binding of soluble complexes. The results were parallel, but not quantitatively equivalent, possibly due to the different accessibilities of the Fc receptors by soluble and particulate complexes. Although the IgG Fc receptor appears on B and T cells as well as some unclassified lymphocytes, its role has not been fully elucidated. Fc receptor-bearing cells can be mediators of antibody-dependent cytotoxicity (15) or may function as regulators of antibody production at the B-cell level (46, 47). Moreover, Stout et al. (48) found that murine Fc receptor-positive T cells responded to concanavalin A, contained the differentiated cytotoxic effector cells in cell-mediated lympholysis and included amplifier T cells but not helper T cells. The decline of IgG Fc-receptor positive cells in autoimmune mice with advanced disease may result because immune complexes occupy the receptors or because these cells leave the circulation after binding immune complexes.

In terms of T cells, there is a moderate decrease in frequency and numbers of T cells in NZ mice with age and onset of disease. Other workers obtained similar results (49, 50). Shirai et al. (51) and Klassen et al. (52) attributed the reduction of T cells to

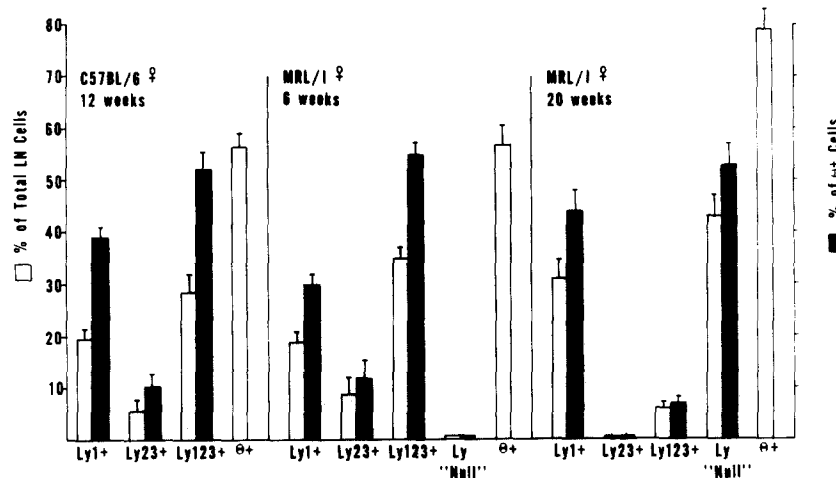


FIG. 5. Ly subsets in MRL/l mice. Columns represent the mean of three animals.

selective loss of suppressor T cells mediated by natural thymocytotoxic antibodies. In BXSB mice there was also a decrease in numbers of T cells with age. In contrast, old MRL/l mice of both sexes manifest extremely high frequencies and numbers of T cells. MRL/l mice, which have the genotype *lpr/lpr*, exhibit massive lymphadenopathy beginning at 8 wk old and progressing by 16 wk old to over 100 times the weight of control lymph nodes (6, 7). Attempts by Murphy et al. (6, 24) and ourselves (unpublished) to transplant enlarged lymph nodes to find evidence of malignancy have failed. However, these proliferating T cells may contribute to the autoimmune disease process, because the *lpr* minus congenic MRL/n mice develop the disease much later in life than the MRL/l mice.

We also studied the distribution of the Ly-bearing cells in young and old MRL/l mice. T cells expressing Ly1 are inducers or helpers; T cells expressing Ly23 are killer and suppressor cells, and T cells expressing Ly123 represent an intermediate pool of T cells that is uncommitted to helper or suppressor function but express regulating signals on both Ly1 and Ly23 subsets (19). Gershon (53) and Cantor et al. (19) showed that Ly123 regulatory T cells decline in aging NZB mice and suggested its relationship to autoimmunity. Our MRL/l mice display a striking drop of the large Ly123⁺ subset of T cells with age concomitant with massive lymphoproliferation and acquisition of Ly null cells which represent the bulk of the θ^+ cells. As the inability to lyse cells in a direct cytotoxicity assay may be a reflection of low antigen expression (18), our observations do not imply that these null cells are necessarily devoid of Ly antigens. The data do indicate, however, a significant difference in the lymph node cells obtained from young and old MRL/l mice. Because Ly1⁺ cells are $\cong 40\%$ of the θ^+ -cells in C57BL/6, and in the old MRL/l the Ly1⁺ cells are 44% of the θ^+ -cells, one may suggest that the Ly null cells descend from the Ly123⁺ subset. This extremely interesting hypothesis suggests that in T-cell ontogeny, at least some of the Ly123⁺ cells eventually differentiate into null cells. Therefore, lymphoproliferation in these mice may stem from the fact that precursor (probably θ^+ , Ly123⁺) cells are released to proliferate and differentiate to θ^+ , Ly null cell.

Finally, we examined the expression of Ia antigens coded by the *I-J* subregion of

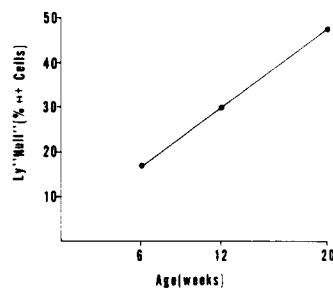


FIG. 6. Ly null cells in MRL/l mice. Each point represents the mean of three animals.

the *H-2* gene complex on isolated splenic T cells from BXSB and MRL/l mice and histocompatible normal mice. Apparently Ia antigens coded by the *I-J* subregion are expressed on suppressor T cells (6) and concanavalin A-reactive T cells (54), whereas the Ia antigens controlled by the other subregions appear predominately on B lymphocytes and on helper T cells (54, 55). Furthermore, the *I-J* subregion codes for determinants on soluble factors that suppress antibody responses (56). Functional studies with NZB \times W lymphocytes have suggested a decline with age of suppressor T cells (57). Unfortunately, no antiserum with specificity for *I-J^d*-coded determinants was available for phenotypic studies in NZB mice. Nevertheless, our results showed no differences between normal and two autoimmune strains (MRL/l and BXSB). These mice have percentages of *I-J⁺* cells similar to that described by Parish (58) in normal mice using a rosette procedure. This finding does not exclude functional abnormalities of suppressor T cells in mice with SLE-like syndromes, but perhaps, contrary to NZB mice (51, 52), suggest that natural thymocytotoxic antibodies found in BXSB male mice and to much lesser extent in MRL/l mice (7) may not have specificity for suppressor T cells. In MRL/l mice, as noted, we found a decline of the *Ly23⁺* subset with age. However, with anti-*I-J^b* sera we found an increased frequency and number of cells bearing Ia antigens controlled by the *I-J* subregion of the *H-2* complex. These results, taken together, suggest that *Ly23⁺* cells may also proliferate in aged MRL/l mice and lose their Ly but retain their *I-J*-controlled surface alloantigens.

If a parallel can be drawn between our study in mice with SLE-like syndromes and the studies of others (36, 59-61) in humans with SLE, then indeed a spectrum of abnormalities in T and B cells and their subsets may be associated with this disease process. Now the role and the contribution of these defects must be established by using well defined *in vitro* systems with various subpopulations of T and B cells as well as *in vivo* cell transfer systems in normal and autoimmune mice.

Summary

The frequencies and absolute numbers of B and T cells in the lymphoid organs of five murine strains (NZB, (NZB \times NZW)_{F1}, BXSB, MRL/l, and MRL/n) with SLE-like syndromes were examined. We assessed the frequencies of cells bearing surface Ig, C3d and IgG Fc receptors, and θ -antigen. The sequential expression of Ig isotopes on developing B cells and the Ig isotypes expressed on adult B cells were ascertained. In addition, the Ly subsets and the expression of Ia antigens coded for by the *I-J* subregion of the mouse *H-2* complex were examined.

TABLE V

Cells Bearing I-J Subregion-Controlled Antigenic Determinants in Spleens of Normal and Autoimmune Mice

Strain*	Age	No. nucleated cells	θ^+ cells	No. θ^+ -cells	Cells bearing I-J-controlled determinants	
					θ^+	Total
	<i>mo</i>	$\times 10^{-6}$	%	$\times 10^{-6}$	%	$\times 10^{-6}$
C3H ♀	1	48*	42	20	17.7	3.5
	8	140	38	53	7.0	3.7
MRL/l ♀	1	52	41	20	23.2	4.6
	4.5	310	73	226	30.4	68.7
MRL/n ♀	1	50	33	16	12.0	1.9
	8	160	45	72	19.0	13.6
C57BL/6 ♀	1	90	37	33	13.4	4.4
	9	105	34	36	16.7	5.9
BXSB ♂	1	65	60	39	15.7	6.1
	5	215	18	39	16.6	6.4
BXSB ♀	1	58	53	31	20.3	6.2
	8	120	39	47	12.5	5.8

* Each number represents results obtained with a single pool of three spleens.

Compared to normal, older mice, New Zealand mice had low frequencies and absolute numbers of B cells, BXSB mice had a moderate B-cell proliferation, and MRL/l mice had normal absolute numbers of B cells but a reduced frequency concomitant with a massive T-cell proliferation. Old New Zealand mice and BXSB mice had reduced frequencies and absolute numbers of T cells compared to old controls. The developmental Ig-isotype diversity during the 1st wk of age was similar in normal mice and those with autoimmune manifestations. Mature B cells were present in lymphoid organs of New Zealand mice and BXSB mice as evidenced by the high frequency of C3d receptor-bearing cells and Ig-isotype expression (high ratio of IgM- to IgD-bearing cells) in adult spleen cells. Numbers of IgG Fc receptor-bearing cells were reduced in autoimmune mice with advanced age and disease. The proliferating T cells in MRL/l mice were found to be θ -antigen positive but Ly null. These θ^+ , Ly null cells may have arisen from Ly123⁺ T cells. MRL/l and BXSB mice seemed normal in their content of T cells bearing Ia antigens coded for by the I-J subregion of H-2. Overall, mice with autoimmune manifestations appear to express perturbations in T and B cells with development of disease, and their patterns of change vary from one strain to another.

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