Murine Models of Sjögren’s Syndrome

Immunohistologic Analysis of Different Strains

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Lacrimal gland inflammation develops in several strains of autoimmune mice, including MRL/Mp-lpr/lpr (MRL/lpr), MRL/Mp-+/+ (MRL/+), and NZBxNZW F1 hybrids (NZB/W). These mice all develop an autoimmune disease characterized by glomerulonephritis and autoantibody formation, but each strain has unique clinical features and immunologic abnormalities. Previous studies have suggested that the intrinsic immunologic defect in MRL/lpr mice may be at the level of T cells, while in NZB/W mice it appears to be B cell-mediated. Immunohistologic analysis of the lacrimal gland lesions was performed on all three strains. Although T cells predominated (MRL/lpr 85%, MRL/+ 78%, and NZB/W 57%), differences in the immunohistologic profiles did exist. NZB/W mice had a significantly higher percentage of B cells (33% vs. 10% for MRL/lpr and 13% for MRL/+), and a correspondingly lower percentage of T cells. MRL/lpr mice differed from MRL/+ mice in that they exhibited a significantly higher percentage of helper T cells (63% vs. 49%) and a lower percentage of suppressor/cytotoxic T cells (14% vs. 30%). Class II antigen expression could be detected on the mononuclear cells at inflammatory sites within the lacrimal glands of all three strains, suggesting T cell activation and an active autoimmune immunologic event occurring in the lacrimal gland. Invest Ophthalmol Vis Sci 29:1437-1443, 1988

Multiple murine models of autoimmune have been described, including the NZB/NZW F1 hybrid (NZB/W), MRL/Mp-Ipr/Ipr (MRL/lpr), MRL/Mp-+/+ (MRL/+), BXSB, and PN strains.1-4 While these different murine strains share many common features, including polyclonal B cell activation, hypergammaglobulinemia, autoantibodies, and glomerulonephritis, there are differences in both their clinical and immunologic abnormalities. Thus, MRL/lpr mice develop a massive lymphadenopathy not seen in the congenic substrain MRL/+ or in NZB/W mice. Studies using neonatal thymectomy or therapeutic anti-T cell monoclonal antibodies have suggested different intrinsic immunologic defects in MRL/lpr and NZB/W mice.5-7 Specifically, the intrinsic immunologic defect appears to be at the level of the B cell in NZB/W mice, with polyclonal B cell activation a characteristic of the underlying immunologic problem. Conversely, MRL/lpr mice appear to have a defect at the level of the T cell, with polyclonal B cell activation due to T cell secretion of a B cell differentiation factor.8,9 MRL/lpr and MRL/+ mice differ by a single autosomal recessive gene, the Ipr gene. Possession of this gene leads to massive lymphadenopathy in the MRL/lpr mice and markedly accelerates the autoimmune disease present in MRL/+ mice. MRL/lpr mice die at age 6 months with an acute glomerulonephritis, arthritis, and vasculitis.1-4,10,11 MRL/+ mice survive to 2 years, and develop a chronic glomerulonephritis with a less fulminant multisystem autoimmune disease.1-4

Among the many autoimmune lesions present in MRL/lpr, MRL/+, and NZB/W mice is lacrimal gland inflammation consisting of multiple foci of mononuclear inflammatory cells.12-14 These lesions are not seen in the lacrimal glands of control strains such as BALB/c or C3H/HeJ, and it has been suggested that these lacrimal gland inflammatory lesions may be a model for the human disorder, Sjögren’s syndrome.12-14 We undertook an immunohistologic analysis of the lacrimal gland lesions in MRL/lpr, MRL/+, and NZB/W mice, in order to compare the different strains and to determine the effect of the Ipr gene on the composition of these lesions.
Materials and Methods

Mice

Four-week-old MRL/lpr, MRL/+, and NZB/W mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and kept under standard conditions in the animal facilities of the Woods Research Building of The Johns Hopkins Hospital. Animals were sacrificed by exsanguination and exorbital lacrimal glands and cervical lymph nodes removed. One of the lacrimal gland specimens was fixed in 4% buffered formaldehyde, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin. The other lacrimal gland and the lymph node were embedded in OCT, frozen in liquid nitrogen, sectioned at 6 μm on a cryostat, and stained as outlined below. MRL/lpr mice were sacrificed at ages 20–26 weeks, at a time when they have fully developed autoimmune disease. MRL/+ mice were sacrificed at two age groups: nine mice were sacrificed at age 26 weeks and an additional five mice at age 52 weeks. NZB/W mice were similarly sacrificed at two different ages. Fourteen NZB/W mice were sacrificed at 38 weeks, a time when all NZB/W female mice have fully developed autoimmune disease and males have earlier lesions, and an additional four male mice were sacrificed at 52 weeks of age, a time by which almost all female mice have died but males are developing full-blown autoimmune disease. All investigations adhered to the ARVO Resolution on the Use of Animals in Research.

Immunohistologic Analysis

Analysis of frozen sections of lacrimal gland and lymph node was performed as previously described, using a panel of monoclonal antibody to cell surface markers and the avidin-biotin-peroxidase complex (ABC) technique. The monoclonal antibodies used for purposes of identification were the following: rat anti-Thy 1.2 (Becton Dickinson, Mountain View, CA) for T cells; rat anti-L3T4 (GK 1.5, ATCC, Rockville, MD or Becton Dickinson) for helper T cells (Th)20; rat anti-Lyt 2 (Becton Dickinson) for suppressor/cytotoxic T cells (Ts)21,22; rat anti-Mac 3 (M3/84.6.34, ATCC) for macrophages22; goat F(ab')2 anti-mouse IgG + IgM (Tago, Inc., Burlingame, CA) for surface immunoglobulin on B cells (slg), or a pan-B cell (pre-B, B cells, and plasma cells) monoclonal antibody (RA3-LC2/1, ATCC), for B cells23,24; and rat anti-la antigens, I-A<sup>b</sup>-d<sup>e</sup>, and I-E<sup>d,k</sup> (M5/114.15.2, ATCC), for class II antigen expression.25,26

Briefly, frozen sections were fixed in chilled (4°C) acetone, air-dried, rehydrated in phosphate buffered saline (PBS) and incubated for the appropriate blocking agent (Vector Laboratories, Burlingame, CA) for 20 min. The primary monoclonal antibody was applied and the slides incubated for 60 min. Slides were washed in PBS and then incubated with a biotinylated secondary antibody for 30 min, rinsed in PBS, incubated with the ABC reagent for 45 min, washed again in PBS, developed with a hydrogen peroxide (0.1%) and 3-amino-9-ethyl-carbazole containing acetate buffer, and counterstained with Harris' hematoxylin. Lymph node sections from each animal were simultaneously stained to compare with the lacrimal gland results. The percentage of mononuclear inflammatory cells staining positively with a given monoclonal antibody was enumerated using a 10 x 10 grid net micrometer disc, covering an area of 0.16 mm<sup>2</sup> with a x25 objective.

Data analysis was carried out on a VAX computer using the Statistical Package for the Social Sciences (SPSS-X). Group means were compared using a one-way analysis of variance, tested using the student-Newman-Keuls procedure which controls for multiple comparisons. Differences were considered significant at the P < 0.05 level.27

Results

All animals used for immunohistologic staining had advanced lacrimal gland disease, with multiple foci of mononuclear inflammatory cells present in the gland; these lesions were all grade III or IV using the previously described semiquantitative scale.14 Lacrimal glands of the 13 MRL/lpr, 14 MRL/+, and 18 NZB/W mice were analyzed, and results are summarized in Table 1.

In each case, the majority of cells in the lacrimal gland lesions were positive when stained for Thy 1.2 and were thus identified as T cells. While MRL/lpr (mean 85%) (Fig. 1) and MRL/+ (mean 78%) were not significantly different, NZB/W mice had significantly fewer T cells present (mean 57%, P < 0.05). For each animal, L3T4+ T<sub>s</sub> cells predominated. However, MRL/lpr (mean 63%) (Fig. 2) had significantly more L3T4+ cells than did MRL/+ (mean 49%, P < 0.05) or NZB/W (mean 47%, P < 0.05). MRL/+ had a significantly higher percentage of Lyt 2+ T<sub>s</sub> cells (mean 30%) (Fig. 3) than did MRL/lpr (mean 14%, P < 0.05) or NZB/W (mean 6%, P < 0.05). The difference in percentage of Lyt 2+ cells between MRL/lpr and NZB/W was also significant (P < 0.05). B cells were significantly more common in NZB/W mice (mean 33%) (Fig. 4) than in either MRL/lpr (mean 10%, P < 0.05) or MRL/+ (mean 13%, P < 0.05). While NZB/W mice also had a significantly greater percentage of macrophages (mean 7%) than did either MRL/lpr (mean 3%, P < 0.05) or...
Table 1. Immunohistologic staining of lacrimal gland lesions in autoimmune mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Number (M:F)*</th>
<th>Mean percent (±SD) of mononuclear cells recognized by monoclonal antibody against:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Thy 1.2</td>
</tr>
<tr>
<td>MRL/lpr</td>
<td>13 (8:5)</td>
<td>85 ± 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(75-92)†</td>
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<tr>
<td>MRL/+</td>
<td>14 (8:6)</td>
<td>78 ± 10</td>
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<tr>
<td></td>
<td></td>
<td>(60-91)</td>
</tr>
<tr>
<td>NZB/W</td>
<td>18 (10:8)</td>
<td>57 ± 6</td>
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<tr>
<td></td>
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<td>(47-70)</td>
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</table>

* Male to female. † Range.

MRL/+ mice (mean 2%, P < 0.05), these differences were small. T cell activation with class II antigen expression (Fig. 5) could be detected in all three strains; the mean percentage of mononuclear cells staining with M5/114 was 55% for MRL/lpr, 59% for MRL/+, and 89% for NZB/W mice.

Significant differences were looked for within each group of mice. No significant difference between male and female mice could be detected for MRL/lpr or MRL/+ mice. Within the NZB/W mice, the only significant sex difference was that female NZB/W mice had fewer Lyt 2+ staining cells (mean 3%) than did male NZB/W mice (mean 8%, P < 0.0001). The only significant age difference detected was that MRL/+ mice had greater staining with M5/114 at 52 weeks (mean 71%) than they did at 26 weeks (mean 53%, P < 0.05), suggesting increasing class II antigen expression with advancing age.

As a control on our technique, simultaneous lymph node sections from each animal were stained for each antibody. Lymph nodes from MRL/+ and NZB/W mice had relatively normal architecture with B cells present in follicles and T cells (Thy 1.2+) seen primarily in the interfollicular cortex. T cells stained for either L3T4 or Lyt 2. In contrast, lymph nodes from mature MRL/lpr mice were massively enlarged with loss of normal architecture. While some B cells were present, follicles were usually not seen, and the nodes were comprised almost entirely of Thy 1.2+ T cells. These lymph node lymphocytes were generally “double-negative” T cells, as only a minority stained for L3T4 (approximately 10-15%) or Lyt 2 (generally less than 10%).

Discussion

The two substrains of MRL (MRL/lpr and MRL+/) and the F1 hybrid NZB/W mice all develop
lacrimal gland inflammation. The immunocytologic profiles of the mononuclear inflammatory cells involved in the lacrimal gland lesions differ among all three. In MRL/lpr mice there is a predominant T cell infiltrate composed primarily of Th cells, with a much lesser number of Ts cells. B cells and macrophages are infrequent, and generally present at the periphery of the lesion. Class II antigen expression can be detected, indicating T cell activation. The MRL/+ mice, which do not possess the lpr gene and have a much less aggressive autoimmune disease, present a somewhat different profile. T cells are again the predominant mononuclear inflammatory cell, but Th cells are less and Ts cells more frequent than in MRL/lpr mice. B cells, macrophages, and class II antigen expression are similar in MRL/+ and MRL/lpr
mice. While NZB/W mice have a slight T cell predominance, they have a significantly greater number of B cells present throughout the lesion than is seen in MRL mice. Almost all the T cells present in the NZB/W lacrimal gland lesions are T\(_h\) cells, with a very small number of T\(_s\) cells. Macrophages are present, and class II antigen expression is seen on almost all of the cells present in the lesion.

These differences in immunohistology seem to reflect the apparent different intrinsic immunologic defect between MRL/lpr and NZB/W mice. MRL/lpr mice have an intrinsic T cell defect, as evidenced by the amelioration of their disease by neonatal thymectomy or after whole-mouse treatment with monoclonal antibody to T cells.4-7 Conversely, the autoimmune disease in NZB/W mice shows little effect of
treatment with either neonatal thymectomy or a monoclonal anti-T cell antibody. These results have led to the suggestion that the intrinsic immunologic defect in these mice may be at the B cell level. The results in the immunohistologic profile of the lacrimal gland lesions in this study are commensurate with the immunologic features in these mice. MRL/lpr mice have an overwhelming T cell lesion, with a small amount of B cells and plasma cells at the periphery of the lesion. NZB/W mice have a slightly greater percentage of T cells than B cells, but a significantly larger percentage of B cells than either MRL/lpr or MRL/+ mice. Previous studies have demonstrated antibody-dependent cytotoxicity to lacrimal gland epithelial cells in NZB/W mice, but no cell-mediated cytotoxicity to lacrimal gland epithelial cells, a result in keeping with the theme of an intrinsic B cell abnormality in these mice. Studies are currently in progress in our laboratory to determine the mechanism of lacrimal gland damage in MRL/lpr mice.

MRL/lpr and MRL/+ mice differ by a single autosomal recessive gene, the lpr gene. This gene appears to both accelerate the autoimmune disease present in MRL/Mp mice and induce lymphoproliferation with lymph nodes up to 100 times normal size. While both congenic substrains develop lacrimal gland inflammatory infiltrates characterized by the overwhelming predominance of T cells, differences do exist between them. Specifically, MRL/lpr mice have a significantly greater percentage of $T_s$ and lower percentage of $T_c$ cells, with a $T_s/T_c$ ratio of 4.5:1 in the lesion. MRL/+ mice have a $T_s/T_c$ ratio of only 1.6:1. It is conceivable that the significantly greater percentage of $T_s$ cells in the lacrimal gland lesions of MRL/+ mice reflects the less aggressive autoimmune disease present in these mice.

The enlarged lymph nodes in MRL/lpr mice contain an unusual "double-negative" T cell. This cell is Thy 1.2+, weakly Lyt 1+, but negative for both L3T4 and Lyt 2.24-31 In addition, these double-negative T cells possess some B cell surface markers. Since T cells in other murine strains are either L3T4+ or Lyt 2+, the precise nature and function of these double-negative T cells have been unclear. Because of the possibility of finding non-immunoglobulin B cell antigens on double-negative T cells, MRL/lpr mice were analyzed for B cells using slg staining. A monoclonal antibody to B cell surface antigens was used for MRL/+ and NZB/W mice. Adjacent sections of MRL/+ lacrimal gland lesions were analyzed using both slg and the pan-B cell monoclonal reagents. The results were found to be very similar using either staining technique (data not shown). Thus, we believe that our results are relatively comparable among MRL/lpr, MRL/+, and NZB/W mice. The finding of L3T4 expression on MRL/lpr lacrimal gland lesions was surprising and quite different than anticipated from the profile in lymph nodes and spleen, where the double-negative T cells predominate in mature MRL/lpr mice and both L3T4+ and Lyt 2+ T cells are comparatively rare. We simultaneously stained lymph nodes with our technique to control for this phenomenon, and found similar results in our lymph nodes to those published by other authors using fluorescein-activated cell sorter analysis. Specifically, the great majority (over 75%) of the lymphocytes in MRL/lpr nodes were T cells (Th 1.2+), approximately 10-15% L3T4+, and generally less than 10% Lyt 2+. The finding of L3T4 expression in the lacrimal gland lesions of MRL/lpr mice suggests that the process occurring in the target organ lesion is fundamentally different from that seen in the lymph node.

The presence of mononuclear inflammatory lesions in the lacrimal glands of MRL/lpr, MRL/+, and NZB/W mice has suggested that they may be models for the human disorder Sjögren's syndrome.12-14 This syndrome is characterized by dry eyes and xerostomia caused by a lymphocytic infiltrate into the lacrimal and salivary glands, with attendant glandular damage. Although biopsy tissue of human lacrimal glands is generally not available for analysis, immunohistologic analysis of minor salivary gland biopsy specimens from patients with Sjögren's syndrome has demonstrated that the majority of cells (50-75%) are T cells of the $T_s$ phenotype and that less than 25% of the lymphocytes are of the $T_c$ phenotype.32,33 Lesser numbers of B cells and macrophages are present. The similarity of these results in humans to our findings in MRL/lpr mice suggests that the MRL/lpr mouse may be an appropriate experimental model for Sjögren's syndrome.

Key words: Sjögren's syndrome, lacrimal gland, autoimmunity, immunohistology

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References


