SYSTEMIC AUTOIMMUNE DISEASE ARISES FROM POLYCLONAL B CELL ACTIVATION

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Autoimmune diseases such as systemic lupus erythematosus are characterized by antibodies reactive with a wide variety of autoantigens including DNA, cell surface molecules, and intracellular matrix proteins (1, 2). Some investigators attribute the diversity of autoantibodies in these diseases to generalized (polyclonal) activation of Ig-producing B cells (1, 3). Others believe that autoreactive clones are specifically and preferentially stimulated but that B cells producing antibodies of conventional specificity remain quiescent (4). This second view is supported by several findings: (a) autoreactive clones proliferate preferentially in organ-specific autoimmune diseases (5), (b) there is evidence that autoreactive B cells arise from a physiologically distinct subpopulation of Ly-1+ lymphocytes, which may be abnormally responsive to immune stimulatory signals (6), (c) B cells capable of autoantibody production expand more rapidly than other lymphocytes when transferred into B cell-depleted recipients (7), and (d) adult autoimmune mice respond poorly (or not at all) to immunization with exogenous antigens (8).

The analysis of systemic autoimmunity has been facilitated by the availability of animal models of human SLE, such as mice that spontaneously develop hypergammaglobulinemia and elevated levels of serum autoantibodies (1, 9). In this report, Ig secreting cells and serum from autoimmune and congenic non-autoimmune mice were studied for reactivity with a panel of seven autoantigens and two conventional antigens. Our results suggest that systemic autoimmunity results from generalized polyclonal activation and not from specific stimulation of autoreactive clones.

Materials and Methods

Animals. NZB, BXSB, and congenic xid mice were bred and raised in our animal colony. MRL and DBA/2 mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice used in these experiments were 4-8-mo-old females unless otherwise stated.

ELISA. Flat-bottomed Immunlon I microtiter plates (Dynatech Labs, Alexandria, VA) were coated with soluble protein (10) or glutaraldehyde-fixed cell surface antigens (11) and then blocked with 1% BSA in PBS as previously described (7). Dilutions of sera derived from blood clotted at 37°C for 90 min were incubated on antigen-coupled plates for 2 h. Unbound Ig was washed away with PBS/0.05% Tween 20. Alkaline phosphatase-conjugated anti-mouse Ig (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) was added for 2 h, and the plates were again washed. The concentration of specific antibody bound to the plate was determined by comparison to a standard curve generated using known dilutions of high-titered antisera, as previously described (7).
FIGURE 1. The amount of antigen-specific antibody in the sera of seven mice from each strain was measured by ELISA. The relative increase in antibody titer of autoimmune mice relative to their congenic non-autoimmune counterparts was assessed by comparison to serially diluted standard antisera, as previously described (7). Antibodies against conventional antigens (narrow crosshatched bars) and against autoantigens (wide crosshatched bars) contributed equally to the increase in total IgM and IgG levels in autoimmune mice.

**Results and Discussion**

*Serum Antibody Levels in Autoimmune Mice Reflect Polyclonal Activation.* Sera from mice of three autoimmune strains: NZB, MRL lpr/lpr, and BXSB were analyzed for antibodies reactive with a panel of six autoantigens and two conventional antigens, and for total IgM and IgG content. Sera from congenic counterparts of each strain that had not developed autoimmune disease were used as standards to determine whether autoimmune mice produced antibodies preferentially against one or more of these antigens (1, 9). We found that comparable increases in the concentration of antibodies reactive with both self antigens and conventional antigens contributed to the hypergammaglobulinemia of autoimmune mice (Fig. 1).

*Ig-secreting Cells from Autoimmune Mice Reflect Polyclonal Activation.* It is difficult to accurately determine the rate of synthesis of individual antibody specificities compared to the total Ig pool (13). A precise way of analyzing Ig production...
is to identify and count individual B cells secreting antibodies against known antigens. This can be accomplished using a spot ELISA, in which freshly isolated splenic lymphocytes are cultured in plastic dishes to which specific antigens are adsorbed. Only antibodies binding to the adsorbed antigen remain on the dish when it is washed free of B cells and nonspecific Ig. These antigen-bound antibodies mark the position of the B cells that produced them and can be detected as discrete blue spots in a colorimetric spot-developing assay (12).

The sensitivity and specificity of this assay have been documented (11, 12, 14) and confirmed for the antigens studied in this report in several ways: (a) ELISA spots were competitively inhibited by the addition of homologous but not heterologous antigen (Fig. 2), (b) hybridoma cells producing antibodies of known specificity could be individually counted on plates coated with their target antigen, but not other antigens, (data not shown), and (c) antigenic stimulation in vivo led to a specific increase in the frequency of B cells producing antibodies of the corresponding specificity but not of other specificities (data not shown).

The absolute number of Ig-secreting B cells and the number of B cells producing antibodies reactive with seven autoantigens and two conventional antigens were independently quantitated in 10 mice from each strain. The frequency of B cells reactive with many of these antigens had not previously been described. However, estimates from other laboratories of the total number of Ig-secreting lymphocytes and the frequency of anti-DNA and anti-bromelain-treated mouse red blood cell (BrMRBC)–producing B cells were consistent with our own (14). Such estimates, derived using the spot ELISA, were generally several fold higher than those described when less sensitive hemolytic plaque assays were used (15).

As has been described previously, the absolute number of anti-DNA–secreting B cells was significantly greater in spleens from autoimmune as opposed to...
FIGURE 3. Splenic lymphocytes from 5-mo-old female MRL lpr/lpr (crosshatched bars) and MRL +/+ (solid bars) mice were analyzed by the ELISA spot technique. The absolute number of Ig secreting B cells and the number producing antibodies against DNA, TNP-KLH, and myosin is shown for four mice from each strain in the top panel. Note the significant difference between MRL lpr/lpr (crosshatched bars) and congenic non-autoimmune mice (solid bars) in terms of the absolute number of antibody-producing B cells. The number of lymphocytes producing antibodies of each specificity was divided by the total number of Ig secreting lymphocytes from that animal (bottom panels). Note the now-identifiable similarity in the repertoires of these congenic strains of mice.

congenic non-autoimmune mice (Fig. 3, top). So too were the number of anti-myosin, anti-TNP-KLH (a conventional antigen) and total Ig-secreting cells (Fig. 3, top). Whereas other investigators have reported that autoantibody-secreting B cells are quantitatively increased in autoimmune mice, they could not determine whether polyclonal activation or specific stimulation caused the expansion of autoreactive lymphocytes (1, 9, 15, 16). By simultaneously quantitating both antigen-specific and total Ig-secreting B cells, we could calculate the frequency of antigen-specific lymphocytes as a percentage of the total expressed repertoire in each animal. Analyzing the data in this manner permitted the novel observation that autoimmune and congenic non-autoimmune mice devoted a very similar proportion of their B cell repertoires to the production of anti-DNA antibodies (Fig. 3, bottom).

A compilation of the data from all mice tested supported this conclusion: the proportion of B cells producing antibodies of each antigenic specificity, as a percentage of the total expressed repertoire, was virtually identical among autoimmune and congenic non-autoimmune mice (Table I). Furthermore, B cells producing antibodies against conventional antigens increased at a frequency commensurate with that of autoantibody-secreting lymphocytes. For example, anti-DNA–producing B cells (considered the sine qua non of autoimmunity in NZB mice) were present with equal frequency in the repertoires of NZB, NZB.xid and normal DBA/2 mice. Moreover, the relative number of B cells secreting antibodies against conventional antigens increased in NZB mice to the same extent as those producing anti-DNA.
Summary

The number of B cells producing antibodies reactive with any of seven autoantigens or two conventional antigens was compared at the single-cell level to the total number of Ig-secreting B cells present in the spleens of NZB, MRL lpr/lpr, and BXSB autoimmune mice. The proportion of lymphocytes producing antibodies of each specificity, expressed as a percentage of the total B cell repertoire, was virtually identical among autoimmune and congenic nonautoimmune animals. Furthermore, B cells and serum antibodies reactive with conventional antigens increased commensurately with those reactive with autoantigens.
These results indicate that systemic autoimmune diseases arise from polyclonal B cell activation.

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References


