Brief Definitive Report

PRESENCE OF ANTI-Sm REACTIVITY IN AUTOIMMUNE MOUSE STRAINS*

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The investigation of the fine specificities of antinuclear antibodies (ANAs) has been fruitful in terms of the nosology and immunopathogenesis of human autoimmune syndromes. Particular reactivities serve as "markers," in that patients with certain syndromes have a much higher incidence of such ANAs than do patients with other diseases. In this category is the almost exclusive presence in certain systemic lupus erythematosus (SLE) patients of reactivity against the nuclear acidic protein Sm. Reactivity to Sm can be detected by precipitation in agar, complement fixation, or passive hemagglutination (1, 2).

Autoimmune mouse strains have also provided a fertile field for the investigation of the basic phenomena of self-reactivity. In particular, the NZB strain and its hybrid NZB × NZW have been considered excellent models for human SLE and have therefore been studied in great detail (3, 4).

In addition, Murphy et al at The Jackson Laboratory, Bar Harbor, Maine, have developed several new inbred mouse strains that spontaneously develop SLE-like syndromes (5, 6). These are the BXSB strain, which has a male dominant disease characterized by little antinative DNA antibody; the MRL/l, which develops massive, nonmalignant lymphadenopathy, associated with enormous increases in serum immunoglobulin levels and fulminant renal disease; and the MRL/n, which does not develop SLE-like disease until well into the 2nd yr of life, but like the MRL/l develops high titers of ANA and fatal glomerulonephritis. The MRL/l differs from MRL/n in only about 10% of its genome, including the gene responsible for the MRL/l's lymphoproliferation.

In the current study, we have used the technique of double immunodiffusion (ID) in agarose with standard human reference sera (of known ANA specificity) to survey a large number of mice from the NZB, NZB × NZW, MRL/l, MRL/n, BXSB, and other strains. We report here the finding of the anti-Sm "marker" antibody almost uniquely in the MRL/l and MRL/n animals. These two related strains may serve as experimental models to explore the mechanism stimulating the production of this unique autoantibody in SLE.

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Materials and Methods

Mice. NZB and NZW, originally obtained from Dr. M. Bielschowsky (University of Otago Medical School, Dunedin, New Zealand), have been bred at our institution since 1965. BXSB, MRL/l, and MRL/n mice were originally obtained from The Jackson Laboratory, and have been bred at our institution since 1976. A compendium of our initial experience with these new strains, in comparison with the New Zealand strains, will be published separately. Mice were bled either by retro-orbital puncture under ether anesthesia, or were exsanguinated through the axillary vessels. Sera were frozen no more than once before assay. In early testing, pools of sera from two to five animals were used and tabulated as single animal experiments. The great majority of tests were done on sera from individual animals.

Double Immunodiffusion Assay. Preliminary studies were done with 0.4% agarose in phosphate- (0.01 M) buffered saline, pH 7.2, with 0.1% NaN_3, in 100-mm glass Petri dishes. Sera of human origin containing precipitating antibodies were used to identify the Sm, nuclear ribonucleoprotein, Sjogren's B, and DNA antigens, and had been characterized according to methods described previously (1, 7-9). Because of the limited amount of individual mouse sera available, we mostly used wells 4 mm in diameter, 3 mm apart, and holding 25 μl.

Antigens. A soluble phosphate buffer extract of a commercial rabbit thymus extract (RTE, Pel-Freeze Bio-Animals, Inc., Rogers, Ark.) was used as a source of nuclear antigen as previously described (10). Sera were also screened against an extract of a human lymphoblastoid cell line (Wil2) (8), but no strong lines were seen that were not also formed against RTE.

Precipitin Curves. Quantitative precipitin reactions were carried out with 25 or 50 μl of serum and equal volumes of serial two-fold dilutions of RTE that had been predigested with RNAse (10). Samples were incubated at 37°C for 15 min and then at 4°C for 4 days. After washing three times in cold phosphate-buffered saline, precipitates were redissolved in 1 ml of 1 N NaOH and the optical density at 280 nm determined. Appropriate corrections were made for antigen-only and serum-only controls. Results obtained were standardized to 1 ml serum.

Results

Strain Survey. The most prominent reactivity found against RTE was anti-Sm (Fig. 1). With the sole exception of one old NZB × NZW female pool, this specificity was found only in the MRL/l and MRL/n strains (Table I). All of the NZB, BXSB or animals of normal strains, and 36/37 NZB × NZW showed no evidence of anti-Sm. The patterns of reactivity regarding age and sex in the two positive strains were of interest (Fig. 2). The MRL/l strain develops rapidly progressive disease beginning about 4 mo of age, characterized by lymphadenopathy, hypergammaglobulinemia, antibodies to DNA, ANA, and fatal glomerulonephritis. The females show a slightly more severe disease. In our immunodiffusion assay, none of the animals of either sex showed any anti-Sm reactivity before the age of 4 mo. Of the 4- and 5-mo animals, many of them moribund, the males gave a higher percentage of reactivity than the females (37% vs. 10%). The combined percentage positivity (males plus females, all greater than 3 mo of age) for the sick animals of this strain is 24%. This number falls in the range of positivity for human SLE patients (20-30%, 2).

The MRL/n mice have a median survival for the females of 17 mo and for the males of 23 mo. We have not yet had the opportunity to investigate in detail the parameters of their autoimmune syndrome, but based on our preliminary data and that of Murphy (unpublished observations), it appears that the disease in the MRL/n is very similar to that of the MRL/l except for its slower

1 B. S. Andrews, R. A. Eisenberg, C. B. Wilson, P. J. McConahey, E. D. Murphy, J. B. Roths, A. N. Theofilopoulos, and F. J. Dixon. Spontaneous murine lupus like syndrome. I. Clinical and immunopathological comparisons in several kinds of mice. Manuscript in preparation.
time-course and the lack of the massive lymphadenopathy. A few of the MRL/n showed Sm reactivity before the age of 4 mo (Table I and Fig. 2). The degree of positivity in the MRL/n males and females at 4- to 5-mo of age is strikingly similar to that of the MRL/l. After that age, it was not possible to determine incidence of anti-Sm in the MRL/l since most of the animals die from their disease. The anti-Sm positivity of the MRL/n continues to rise, with the females eventually showing greater reactivity than the males (at 9–12 mo of age, females are 83% positive, while males are 57% positive). We have not yet had the opportunity to examine mice of greater than 1 yr of age.

Other Specificities. One NZB × NZW 11-mo female pool reacted with a clear line of identity with our anti-RNP reference serum. Otherwise, although
TABLE I

Presence of Anti-Sm in Various Mouse Strains

| Strain   | Sex | Age   | Number tested | Positive |
|----------|-----|-------|---------------|----------|
| MRL/I   | M + F | 1-3   | 24            | 0        |
| MRL/I   | M    | 4-5   | 27            | 37       |
| MRL/I   | F    | 4-5   | 21            | 10       |
| MRL/n   | M    | 1-3   | 49            | 3        |
| MRL/n   | F    | 1-3   | 38            | 5        |
| MRL/n   | M    | 4-12  | 43            | 35       |
| MRL/n   | F    | 4-12  | 62            | 45       |
| NZB     | M + F | 4-12  | 77            | 0        |
| NZB x NZW | M + F | 1-17  | 37            | 3        |
| BXSB    | M + F | 2-12  | 44            | 0        |
| Others  | M + F | 1-12  | 41            | 0        |

![Figure 2](image)

**Fig. 2.** Age relationship of anti-Sm reactivity in MRL mice. Open symbols represent MRL/n mice. Numbers next to points indicate size of each age group tested. MRL/I mice are shown by the closed symbols at 4-5 mo only, as all younger MRL/I tested were negative. Circles represent females, triangles represent males.

we occasionally saw strong lines which were not Sm, no other lines of identity were seen. This means that precipitating antibodies to Sjögren's B antigen and DNA are probably not prominent in our mice. Additional strong lines were seen particularly with the MRL/n sera. Preliminary investigations show that these lines are not directed against soluble DNA-histone and that the antigen involved is sensitive to pepsin, but not to RNase or DNase.

**Further Studies.** Six ID-positive MRL/I sera were subjected to immunoelectrophoresis. Five of the sera were strong enough reactors to give precipitin lines against the nuclear antigen (Fig. 3). In all cases the line observed had a gamma mobility somewhat more restricted than that of the whole serum IgG. This is similar to the experience with human anti-Sm sera processed the same way (7). In quantitative precipitin studies we found that no serum tested contained more than 0.4 mg/ml of anti-Sm antibody protein.

**Discussion**

We have surveyed autoimmune mouse strains for the specificities of their anti-nuclear antibodies by double immunodiffusion in agarose, an approach
which has proven to be very productive as applied to human connective tissue diseases. The most prominent of our findings was a high percentage of reactivity with the Sm antigen in the MRL/l and MRL/n mice. Outside of these two strains, we have thus far found only a single reactive sample, a pool of 11 mo old NZB × NZW females. We feel that the presence of this marker antibody in the MRL mice make them interesting for study as models of human SLE. On the other hand, we do not understand why anti-Sm reactivity is not commonly found in the NZB × NZW mice, as their disease in many other respects resembles that of the MRL. The NZB and BXSB mice, on the other hand, have somewhat different syndromes, with lower levels of ANA and anti-DNA antibodies.

Beyond its status as a marker antibody, the immunopathological significance of anti-Sm antibody both in man and mice is unclear at this time. Although we have yet to study this question systematically, we have not noticed a peculiarity of the disease pattern of the anti-Sm positive animals, as has been suggested in humans (11). In the MRL/l mice, the appearance of anti-Sm is detected at an age (4 mo) when the animals' disease is already rampant. In the MRL/n, on the other hand, anti-Sm reactivity begins to appear at a very early age (Fig. 2), and is found in a majority of the animals by the time they become seriously ill. We have not yet been able to study this strain in detail, so that we cannot make any statements regarding the relationship of appearance of anti-Sm to other parameters of autoimmune disease.

We realize, of course, that failure to detect anti-Sm reactivity in particular specimens may be the result of the insensitivity of the methods employed. We are currently attempting to develop a radioimmune assay that would permit us to look at the "negative" sera more closely. In this regard, it is interesting that in human SLE no low titer positives were found: sera were either very positive or completely negative (2). We have the impression, based on the strength of the precipitin lines observed, that with the MRL/l all positive animals were
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strongly positive. We have not seen a single case of a faint ID line with these mice. In the case of the MRL/n mice, on the other hand, particularly in the very young animals, we have frequently noted weak, though clear, immunoprecipitin lines. A radioimmune assay would permit us to look at this situation in a more quantitative way.

Our data at this point do not permit us to speculate on the nature of the immunological aberration which causes production of anti-Sm antibody in the MRL/l and MRL/n strains, nor on the pathogenetic role of this antibody. The observation that anti-Sm antibody appears to be somewhat restricted to certain strains of mice is interesting, in view of published reports that anti-Sm antibody is highly restricted to human SLE and rarely found in other human autoimmune diseases. The availability of animal models may make it possible to explore approaches to some of these problems.

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