Stochastic Control of Anti-Sm Autoantibodies in MRL/Mp-*lpr/lpr* Mice

Robert A. Eisenberg, Sylvia Y. Craven, Robert W. Warren, and Philip L. Cohen
Departments of Medicine, Pediatrics, and Microbiology/Immunology, University of North Carolina, Chapel Hill, North Carolina 27514

Abstract

MRL/Mp-*lpr/lpr* autoimmune mice consistently show an ~ 25% incidence of the systemic lupus eyrthematosus marker autoantibody anti-Sm. In the present report, we show that the failure to find anti-Sm antibodies in three-quarters of 5-mo-old MRL/*lpr* mice was not an artifact of an insensitive assay, but rather that the mice fell into two populations as regards their anti-Sm positivity. Based on an extensive analysis of the incidence of anti-Sm positivity in 5-mo-old mice according to their cage of residence, we found no evidence for genetic, environmental, or parental influences on the propensity of an individual animal to become anti-Sm positive. Also, the gender of the mouse, its Sm antigen level, or its length of survival were not related to anti-Sm antibody, nor was the anti-Sm antibody status of either parent. Some animals became anti-Sm positive after 5 mo of age, but this was less likely than becoming positive before 5 mo of age. Finally, a survey of 205 autoimmune C57BL/6-*lpr/lpr* mice confirmed the uniqueness of the MRL background for this autoantibody response. These results together indicate that the possibility of making anti-Sm antibodies is under genetic control, but that the expression of this capability in an individual animal is governed by stochastic events. We hypothesize further that such random processes may involve the expression of particular immunoglobulin variable-region genes combined with mechanisms of extensive somatic mutation or positive feedback amplification, which would transmute an initial monoclonal response into an eventual polyclonal one.

Introduction

MRL/Mp-*lpr/lpr* (MRL/*lpr*)[1] mice are highly inbred and reproducibly develop an autoimmune syndrome resembling human systemic lupus erythematosus (SLE) (1). This strain and its congenic partner MRL/Mp-+/+ (MRL/+), which develops a more indolent autoimmune disease, are particularly interesting because they produce high titers of autoantibodies to the nuclear ribonucleoprotein Sm (2). The spontaneous production of anti-Sm antibodies is a highly specific marker for the diagnosis of SLE in mice, humans, and dogs (3, 4, 5).

We have been studying the immunoregulation of the anti-Sm response in MRL/*lpr* mice as a model of a significant SLE autoantibody. In our initial studies, we reported that ~ 25% of the MRL/*lpr* animals became anti-Sm positive (3). This proportion was similar to that found in outbred populations of humans and dogs with SLE (4, 5). In humans, some studies have suggested that genetics might determine whether an individual potentially becomes anti-Sm positive (6). However, the MRL mice have been inbred since the early 1970s, and, thus, the individuals of the strain should be near complete genetic homogeneity, unless there is selective reason for enforced heterozygosity. It is, thus, of considerable interest to determine why only a minority of MRL/*lpr* mice develop anti-Sm antibodies.

In this study, we have investigated several potential parameters that could control whether or not an individual MRL/*lpr* mouse becomes anti-Sm positive. We have also compared the MRL/*lpr* mice with the C57BL/6-*lpr*/*lpr*(B6-*lpr*) mice to distinguish between the contributions of the *lpr* gene and those of the MRL background. Our results indicate that although the predisposition to make anti-Sm antibodies in autoimmune *lpr* homozygous mice is under genetic control, the expression of this potential in an individual MRL/*lpr* animal is governed by stochastic events with a defined probability.

Methods

Mice. The MRL/*lpr*, MRL/+, and B6-*lpr* mice were originally obtained from the Jackson Laboratory, Bar Harbor, ME, and have been bred in our colony at the University of North Carolina. The epidemiology experiments were performed on cohorts of mice from 1979 to 1980 and from 1982 to 1983. The mice were weaned at age 3–4 wk and then caged such that siblings of the same sex were housed together. Mice were bled at the indicated ages by retroorbital puncture, and sera were stored for short periods at 4°C or for longer periods at −20°C. Normal mice were C57BL/6 and bm12 from our colony.

Anti-Sm assays. The detection of anti-Sm antibodies by double immunodiffusion was performed, as previously described, using a human SLE anti-Sm reference serum (7). The anti-Sm enzyme-linked immunoabsorbent assay (ELISA) utilized an affinity-purified preparation of Sm from rabbit thymus extract (8, 9). Sera were tested in dilutions of 10^{-3}–10^{-6}, and antibodies were detected by a biotinylated affinity-purified goat anti-mouse IgG (pFc' specific) followed by avidin-alkaline phosphatase and paranitrophenyl phosphate. In assays comparing positive and negative mice, each sample was tested in the presence and absence of 100 μg/ml of the Fab fragments of human anti-Sm IgG to ensure specificity in each case. Data analysis in these assays utilized the difference between the optical densities (ODs) obtained with and without the Fab fragments. To quantitate the level of anti-Sm antibodies in individual sera in some experiments, each serum was tested at dilutions, and a titer was determined as the point where a graph of the dilution vs. OD for that serum crossed a standard OD of 0.100.

Sm antigen was detected by ELISA utilizing $F(ab')_2$ fragments of an affinity-purified anti-Sm antibody from a human source. Mouse tis-

This work has been partially presented as an abstract (1986. *Arthritis Rheum.* 29:S34).

Address all correspondence to Dr. Eisenberg, 932 FLOB 231H, University of North Carolina, Chapel Hill, NC 27514.

Received for publication 16 January 1987 and in revised form 1 May 1987.

1. *Abbreviations used in this paper:* B6-*lpr*, C57BL/6-*lpr*/*lpr*; MRL/*lpr*, MRL/Mp-*lpr*/*lpr*; MRL/+, MRL/Mp-+/+; OD, optical density; SLE, systemic lupus erythematosus.

J. Clin. Invest.

0021-9738/87/09/0691/07 $2.00
Volume 80, September 1987, 691–697

sues were harvested from freshly killed animals and homogenized in borate-buffered saline with 0.1% NP-40 as described (10).

Statistical analysis. Data analysis was performed by Dr. Sandra Stinnett at the Biometric Consulting Laboratory, Department of Biostatistics, University of North Carolina. For the cage analysis, the expected distribution of trait (i.e., anti-Sm)-positive animals in cages containing varying numbers of mice was calculated using the binomial distribution formula $E(x) = \binom{n}{x} P^x(1-P)^{n-x}$, where n is the number of cages of each cage size; x, the number of mice with the trait; and P, the overall probability of developing the trait in the population. In the pooled population from two experiments, 75 out of 336 mice were anti-Sm positive at age 5 mo for an overall probability of 0.223. Similarly, the distribution of the trait by litter size was determined in the second experiment, with 142 mice, in which the litter size was definitely known. All comparisons were tested for goodness of fit by chi-square analysis.

Results

Genetics of anti-Sm. We have previously reported that anti-Sm antibodies are rarely found in any unmanipulated mice other than those of the MRL strains, both MRL/*lpr* and MRL/+ (3). These observations indicated that the MRL background was sufficient for anti-Sm production. Since the *lpr* gene on other backgrounds causes the production of various autoantibodies, it was possible that it could also induce anti-Sm. We have therefore utilized the anti-Sm ELISA assay to compare levels of anti-Sm antibodies in B6-*lpr* mice with those in MRL/*lpr* mice. 205 B6-*lpr* sera from male and female mice aged 2–14 mo were tested at a 1:1,000 dilution. One mouse showed an OD of 0.302. No other mouse had an OD of > 0.200, and only 11 additional sera had ODs > 0.100 (Table I). In contrast, all eight anti-Sm positive MRL/*lpr* sera gave corrected ODs of > 0.270, even when tested at a 1:10,000 dilution. These results indicate that the B6 strain lacks the genetic ability to make anti-Sm antibodies spontaneously, even in the presence of a homozygous *lpr* gene. In an additional set of experiments, we tested MRL/+ mice from the colony at the University of North Carolina. From a group of 50 animals aged 11–21 mo, 15 (30%) were typed as anti-Sm positive by double immunodiffusion. No obvious age effect was seen, as 5 out of 18 (28%) animals aged 11–13 mo and 10 out of 32 (31%) animals aged 16–21 mo were positive. Therefore, in contrast to our earlier findings on a small number of mice (3), the prevalence of positivity in the MRL/+ mice was similar to that seen in MRL/*lpr* mice.

Analysis of anti-Sm positivity by ELISA. It was possible that the failure to detect anti-Sm antibodies in a majority of the MRL/*lpr* mice at age 5 mo was due either to the insensitivity of the double immunodiffusion assay or to the delayed development of positivity in certain animals. To analyze the first issue, we performed quantitative ELISA assays on serial dilutions of sera from 5-mo-old MRL/*lpr* mice identified as positive by double immunodiffusion. We compared these ELISA titers with those of age- and sex-matched immunodiffusion negative mice. Fig. 1 shows results with male mice. The ELISA titers of the immunodiffusion positive and negative sera clearly fell into two distinct populations, which differed by two to three orders of magnitude. Fig. 2 shows a similar comparison between 5-mo-old MRL/*lpr* male mice that were negative for anti-Sm by immunodiffusion and normal mice that had no known autoimmunity. The MRL/*lpr* mice showed no indication of a response to the Sm antigen beyond that of the "normal background." (Whether this normal background is specific or nonspecific we do not know, although the fact that it is poorly inhibitable by anti-Sm Fab fragments suggested the latter.) Therefore, the division of MRL/*lpr* mice into anti-Sm positive and anti-Sm negative groups is not an artifact of an insensitive assay; rather, the mice are distributed in a bimodal population with regard to this trait. Similar results were obtained with female mice and in a separate repeat experiment (data not shown).

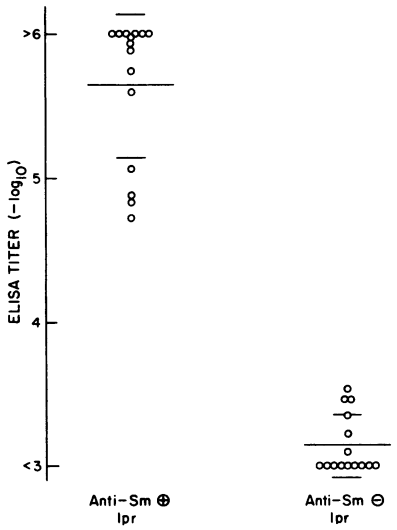

Figure 1. Bimodal distribution of anti-Sm antibodies in 5-mo-old male MRL/*lpr* mice. 15 anti-Sm immunodiffusion-positive and 15 anti-Sm negative mice were tested for anti-Sm antibodies by ELISA at dilutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} in the presence and absence of human Fab anti-Sm inhibitor. Resultant ODs were graphed and the dilution giving a standard OD of 0.100 was interpolated as the ELISA titer.

The second issue is whether the 0.25 incidence of anti-Sm positivity is related to the age at which we sampled the mice. To analyze this question, we determined the incidence density of the development of anti-Sm positivity in a group of mice bled biweekly from the age of 3 mo. Levels of anti-Sm anti-

Table I. Anti-Sm Antibodies by ELISA in lpr Mice

Strain	*n*	Immunodiffusion	Dilution	Corrected OD 405			
				>0.300	0.200–0.299	0.100–0.199	0–0.099
C57BL/6-*lpr*	205	ND	10^{-3}	1	0	11	193
MRL/*lpr*	8	+	10^{-4}	6	2	0	0
			10^{-5}	4	1	2	1
MRL/*lpr*	2	–	10^{-4}	0	0	0	2

* Anti-Sm antibodies by immunodiffusion.

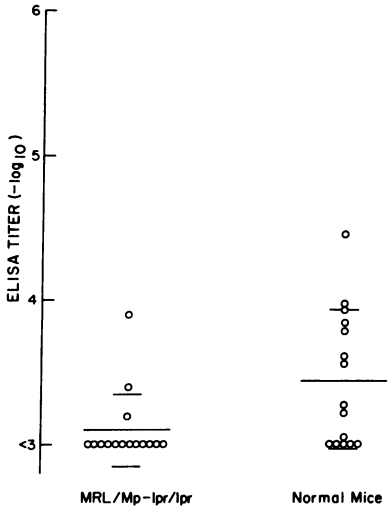

Figure 2. Anti-Sm antibodies in immunodiffusion-negative 5-mo-old male mice. 15 MRL/*lpr* anti-Sm negative and 15 age-matched controls from C57BL/6 congenic strains were tested for anti-Sm antibodies as described in the legend to Fig. 1.

bodies of the IgM, IgG1, IgG2a, IgG2b, and IgG3 isotypes were determined in all sera. The details of this isotype analysis will be presented elsewhere (11). For the current analysis we looked at the onset of anti-Sm positivity as the attainment of a certain OD value for any isotype in the anti-Sm ELISA. As this is an arbitrary criterion, several values were studied. We then determined the fractional probability of a mouse becoming positive in a given month before the age of 5 mo, compared with after the age 5 mo. Mice were initially bled at age 3 mo and, if positive at that time, were considered to have become positive in the previous month (i.e., ages before 2 mo were not counted). As shown in Table II, the chance of becoming positive for anti-Sm antibodies was greater for younger than for older mice, particularly using the more stringent ELISA criteria. For a cutoff value of 0.700, for example, a mouse had a 0.09 chance of becoming positive in a month before age 5 mo vs. a 0.03 chance after age 5 mo ($P = 0.03$). In this entire group of mice, the overall incidence of anti-Sm positivity by ELISA ranged from 0.28 to 0.4 depending on the criterion. Therefore, the 0.25 incidence at age 5 mo is not absolute. After 5 mo, mice continue to become anti-Sm positive, but their chances of becoming so are diminished. Furthermore, many of the mice die soon after 5 mo. The assignment of anti-Sm positivity at 5 mo, then, although arbitrary, represents a reasonable qualitative estimation of the population. On the other hand, the development of anti-Sm positivity occurs over time.

Cage analysis of anti-Sm positivity. Mice from our colony are caged after weaning according to their litter and gender. Therefore, animals from a given cage would share a single set of parents; they would be siblings of the same sex; and they would be living in a similar environment. By analyzing the incidence of anti-Sm positivity in mice from a large set of different cages, it was possible to determine whether the probability of a mouse becoming anti-Sm positive depended on the cage it lived in. If it did, that would suggest the influence of genetic, parental, or environmental effects. On the other hand, if the anti-Sm positive mice were distributed randomly in various cages, then genetic, parental or environmental factors could not play a major role. Two groups of mice were analyzed. The first group of 194 mice was examined retrospectively. These mice were bled at ~ age 5 mo and their parents and litter of origin were not known precisely. Therefore, a second prospective study was undertaken in which this information was known for 142 mice, and all mice were bled at exactly age 5 mo. In the first study, 46 (23.7%) of the mice were anti-Sm positive, and in the second study, 29 of 142 mice (21.4%) were positive for an overall incidence of 22.3%. Anti-Sm positivity was determined by immunodiffusion.

Table III shows the observed distribution of anti-Sm positivity in 94 cages containing 1 to 10 mice per cage. For example, of the 24 cages that housed 4 mice, 12 had 0 mice with anti-Sm antibodies; 7, 1 such mouse; 3, 2 mice; 1, 3 mice; and 1, 4 mice. Table IV shows the expected distribution of positive mice based on the overall probability of 0.223 and the binomial distribution (see Methods). For example, in the same 24 cages housing 4 mice, 8.6 cages would be expected to have no anti-Sm positive animals; 10.1 cages, 1 positive animal; and so on. The expected and observed values were compared by chi-square analysis in those cages with two to six mice. None of the chi-squared values approached statistical significance. Also, the chi-square values were summed over all the cages containing from two to six mice to give a total P value of 0.580, which indicates no significant departure of the observed values from the expected distribution based on random assignment of positive mice.

In the prospective study of 142 mice, it was possible to be certain of the litter of origin and parental anti-Sm status. The distribution of anti-Sm positivity by litters was no different than random ($P = 0.598$ by chi-square analysis). Similarly, the parental anti-Sm status (determined at age 5 mo for both parents) had no significant influence on the eventual development of anti-Sm antibodies in offspring, although the small

Table II. Incidence Density of the Development of Anti-Sm Antibody Positivity as Related to Age

ELISA criterion*	Age group ≤5 mo			>5 mo			
	No. positive	Mouse months‡	Incidence density§	No. positive	Mouse months	Incidence density	P‖
0.400	17	159	0.11	7	84	0.08	0.29
0.500	16	160	0.10	5	90	0.06	0.12
0.700	14	163	0.09	3	105	0.03	0.03

* A mouse was counted as positive if it gave an anti-Sm ELISA value for any isotype greater than or equal to the criterion value. The ELISA positive mice in the ≤5 mo group were all immunodiffusion positive at 5 mo. ‡ A mouse month is one mouse at risk to become anti-Sm positive for 1 mo. A mouse is no longer at risk if it dies or becomes positive. § Incidence density is No. of mice becoming positive per mouse months. ‖ Incidence density compared before and after age 5 mo. (12).

*Table III. Observed Distribution of Mice with Trait by Cage Size**

No. mice with trait per cage	Observed values (No. of mice per cage)										
	1	2	3	4	5	6	7	8	9	10	Total
0	5	13	5	12	8	1	1	0	0	0	45
1	4	6	6	7	6	3	0	0	0	0	32
2		2	2	3	2	2	0	0	0	1	12
3			0	1	1	0	0	0	0	0	2
4				1	1	0	0	0	0	0	2
5					0	0	1	0	0	0	1
6						0	0	0	0	0	0
7							0	0	0	0	0
8								0	0	0	0
9									0	0	0
10										0	0
Total cages	9	21	13	24	18	6	2	0	0	1	94

* 94 cages containing 336 mice were typed for anti-Sm at age 5 mo. The table shows cages of different Nos. of mice per cage, and indicates the numbers of such cages with a given No. of mice with the anti-Sm positive trait.

number of anti-Sm positive parents could have masked a minor effect (2 anti-Sm positive mice among 15 offspring with 1 positive parent vs. 27 positive mice among 127 offspring of negative parents, $P = 0.76$ by the Fisher exact test). Finally, the males and females of these mice showed no difference in anti-Sm positivity (16 of 70 males vs. 13 of 72 females, $P = 0.536$ by the Fisher exact test).

Overall, then, none of the traits identified in this study could be shown to influence anti-Sm positivity. Additional mice that were anti-Sm positive were killed, and the Sm content of their spleens, lymph nodes, and livers were compared with those of age-matched anti-Sm negative mice. No differences were seen (Table V). Finally, among those animals living at least 5 mo the survival of anti-Sm positive mice was no different from that of anti-Sm negative animals (mean survival±SD: 18 anti-Sm positive mice, 7.9±2.0 mo; 31 anti-Sm negative mice, 8.4±2.8 mo).

Discussion

In the current report, we have attempted to explain the epidemiology of the anti-Sm response in SLE mice. It is clear that the potential to generate anti-Sm antibodies in the context of autoimmunity is under genetic control. The MRL/*lpr* and MRL/+ mice, because of genes in the MRL background, spontaneously make anti-Sm autoantibodies. B6 mice, even under the influence of the *lpr* gene, which induces several autoantibodies, including antichromatin and anti-DNA, fail to make anti-Sm antibodies. It is possible that the genetic elements that permit the anti-Sm response in the MRL strains

Table IV. Expected Distribution of Mice with Trait by Cage Size

No. mice with trait per cage	Expected values (No. of mice per cage)*									
	1	2	3	4	5	6	7	8	9	10
0	7.0	12.6	6.1	8.6	5.0	1.3	0.3	0	0	0.1
1	2.0	7.4	5.2	10.1	7.4	2.3	0.7	0	0	0.3
2		1.0	1.6	4.3	4.1	1.6	0.7	0	0	0.3
3			0.1	1.0	1.3	0.6	0.3	0	0	0.2
4				0	0.2	0.1	0.1	0	0	0.1
5					0	0	0	0	0	0
6						0	0	0	0	0
7							0	0	0	0
8									0	0
9										0
10										0
Total cages	9	21	13	24	18	6	2	0	0	1

* The expected distribution of numbers of positive mice in cages of varying sizes can be computed by the binomial distribution formula, $E(x) = \binom{n}{x}P^x(1-P)^{n-x}$, where n is the number of cages of each cage size (last row of Table III); x, the number of mice with the trait; and P, the overall probability of developing the trait in the population (0.223).

Table V. Sm Antigen in 5-Mo-Old MRL/Mp-lpr/lpr Mice

		Tissue lysate*		
	n	Lymph node	Spleen	Liver
Anti-Sm ⊕	10	221 (195)‡	54 (58)	122 (95)
Anti-Sm ⊖	10	208 (160)	54 (25)	185 (70)

* Tissues were obtained from 5-mo-old animals established to be anti-Sm positive or anti-Sm negative. They were processed as previously described (10), and Sm antigen levels were determined by an ELISA assay.

‡ Nanograms Sm per microgram DNA (SD).

also favor the markedly more severe autoimmune syndrome in MRL/*lpr* mice as compared with B6-*lpr* mice. This prediction would be consistent with the high specificity of the anti-Sm response for the diagnosis of SLE in several species. We are currently investigating this genetic aspect by a classical breeding analysis.

The other aspect of the epidemiology of the Sm response that we have explored in this paper is the tendency for an individual animal with a permissive genotype (i.e., an MRL mouse) to become anti-Sm positive. This consideration is prompted by the repeated finding that ~ 25% of MRL/*lpr* mice become anti-Sm positive by age 5 mo. The ELISA data presented in the current report clearly indicate that the MRL/*lpr* mice fall into two populations regarding anti-Sm antibodies: (*i*) responders, which make high titers of anti-Sm antibodies as detected by ELISA; and (*ii*) nonresponders, which show no more anti-Sm antibodies in their sera than do normal mice. Therefore, the 25% positivity rate in the MRL mice is a reflection of fundamental processes in the development of disease in individual animals and is not an artifact of an insensitive assay.

The recurrent finding of 25% positivity in the MRL/*lpr* mice is even more striking in that the same level of positivity occurs in both human and canine lupus and in our current survey of the MRL/+ mice. On the other hand, the concordance of these proportions, although highly provocative, needs to be examined critically. The MRL mice are highly inbred. As discussed below, the differentiation between anti-Sm positive and anti-Sm negative individuals of this strain is not on a genetic basis. In comparison, the human and canine populations that have been tested are genetically heterogeneous, and it would not be surprising that one of the factors that play a role in determining anti-Sm positivity among such individuals with SLE is genetic. For example, some surveys have shown that blacks with SLE have a much higher percentage of anti-Sm positivity than whites (6). Also, there is some arbitrariness of the 25% figure in the MRL strains. We have chosen to consider mice at age 5 mo, as that is a time at which disease is fully developed, but that does not exclude the majority of animals due to premature death. However, as we point out, some mice do become anti-Sm positive after age 5 mo, although the tendency to become positive, as measured by incidence density, decreases with age. Therefore, the processes that determine anti-Sm positivity in individual MRL mice continue to operate over time.

In this study, then, we have attempted to determine by a simple cage analysis which factors could influence the development of anti-Sm antibodies in individual mice. As the environment of an individual mouse is determined by its cage, and since we cage our mice by sex and litter, mice from a single cage would have similar environment, parental influence, and genetics (if there should be any genetic heterogeneity within our population). Therefore, if any of these factors should play an important role in determining anti-Sm positivity, then the distribution of anti-Sm positive mice over a large number of cages would not be random, but, rather, positive mice would be clustered in certain cages and negative mice in others. The presented results failed to reveal any nonrandom clustering of anti-Sm reactivity by cage and therefore indicate that none of these factors plays a major role in determining anti-Sm positivity. Also, separate consideration of litter, gender, or parental anti-Sm status also failed to show any significant effect on an individual mouse's anti-Sm response. Although we of course cannot rule out minor influences, as we have surveyed a limited number of mice, the development of anti-Sm by a consistent proportion of animals cannot be explained by genetic, parental, or environmental influences.

The results of the cage analysis prompted us to label the factors that determine anti-Sm positivity in individuals as "stochastic." This term to some degree reflects our ignorance; yet, it is difficult to imagine a preprogrammed mechanism in our genetically and environmentally homogeneous population that could result in the 25% positivity rate without the essential effect of a truly random, i.e., unpredictable process. On the other hand, this does not mean that we cannot investigate this process further. For example, the finding that the mortality of anti-Sm positive mice is no different from that of anti-Sm negative mice suggests that the random process that determines anti-Sm positivity in individual MRL mice is not one that has a major influence on the course of disease. This is consistent with the failure to identify definitive subsets of human SLE with relation to anti-Sm positivity (13–15). Furthermore, the similarity of tissue Sm antigenic levels in anti-Sm positive MRL/*lpr* mice as compared with age-matched anti-Sm negative animals suggests that the random processes are not operating through the quantitative expression of antigen (qualitative differences cannot be ruled out). All things considered, a potentially fruitful direction to investigate for processes with major random influences that could determine anti-Sm positivity appears to be the rearrangement, expression, and mutation of variable region genes for immunoglobulins or for T cell receptors. This approach is attractive since it would provide an obvious explanation for the specificity of the random process for anti-Sm reactivity. In a sense, this is reviving the "forbidden clone" hypothesis of MacFarlane Burnet (16), a theory that is currently in disfavor due to the frequent finding of autoantibodies in a variety of situations (17). The Sm antibody response, however, is unusual in that the precursors of the response are not detectable in normal or anti-Sm negative MRL mice (18), even though some must arise, at least periodically, as MRL mice can uniformly respond to immunization with exogenous Sm (19). An additional complication for a forbidden clone hypothesis is the fact that the fully developed anti-Sm response, both in humans and in mice, is not monoclonal (11, 20).

The polyclonality of the anti-Sm response can be reconciled with the random development of an unusual specificity in two ways. First, appropriate T cell receptor variable-region expression could be postulated to occur in only 25% of ani-

mals, and this monoclonal T cell response might be necessary for development of a polyclonal B cell response. We have already demonstrated a T cell proliferative response to the Sm antigen, which is genetically restricted to the MRL mice and to certain normal strains (21). However, all MRL/+ mice are potential responders, and there is no difference between anti-Sm positive and anti-Sm negative individuals (22). Although the connection between the T cell proliferative response to Sm that we have investigated and a possible role for anti-Sm-specific T cells in vivo is not clear, we feel that the current evidence is against T cell receptor specificity being the determining factor between anti-Sm positive and anti-Sm negative MRL mice. This is further substantiated by our failure to demonstrate either spontaneous anti-Sm-specific help or suppression in the MRL mice (23).

The working hypothesis we favor at present is that the initial anti-Sm response depends upon the effective expression of anti-Sm recognizing immunoglobulin variable regions. This event would occur with a 0.25 probability by the age 5 mo. The generation of these variable regions may depend on any of the mechanisms that are now known to determine immunoglobulin antigen binding sites (24). The eventual polyclonality of the response would result from somatic diversification of the initial clone (25). This mechanism would explain the isoelectric focusing heterogeneity we have observed for both human and mouse anti-Sm antibodies, but would not be compatible with our light chain results in human sera (20). Another mechanism that may play a role is antibody-mediated positive-feedback enhancement, since the injection of a monoclonal anti-Sm antibody stimulates the production of endogenous anti-Sm antibodies in both MRL/+ and MRL/*lpr* mice (26, 27).

The stochastic nature of the anti-Sm response in MRL mice is probably not unique to this particular autoantibody. Whereas the phenomenon is especially striking in this case, perhaps secondary to the feedback amplification by passive antibody, it is highly likely that multiple aspects of the autoimmune disease in these mice and in other species are governed by random processes. Therefore, it is not surprising that there is a spectrum of findings regarding any disease parameter one chooses to investigate, e.g., mortality, glomerulonephritis, rheumatoid factor, and etc. Although historically the individual variation in genetically inbred mice has merely presented difficulties regarding the analysis of data, from the present point of view it represents an opportunity to study mechanisms that are neither environmental nor genetic, but that may have a major influence on important disease manifestations. Similar considerations should be kept in mind in the analysis of human data for identical twins (28). Although the failure to find 100% concordance for the presence of SLE between identical twins has generally been interpreted to indicate the importance of environmental influences, it is also possible that stochastic processes are the major determinants in this situation. In fact, Burnet has already applied such reasoning to SLE (16). Assuming a background of genetic susceptibility and the influence of stochastic processes over time, he was able to develop mathematically incidence density curves that are consistent with the known epidemiology of human SLE. Our findings for the anti-Sm response in MRL/*lpr* mice represent a qualitative validation of this approach.

Acknowledgments

Excellent secretarial assistance was provided by Ms. Penny Ward and Ms. Alice Kennedy.

This work was supported by National Institutes of Health grants AM-26574, AM-34156, AM-33887, AM-34253, and AM-25733. Drs. Eisenberg and Cohen were Senior Investigators of the Arthritis Foundation. Dr. Warren was a postdoctoral Fellow of the Arthritis Foundation.

References

1. Andrews, B. S., R. A. Eisenberg, A. N. Theofilopoulos, S. Izui, C. B. Wilson, P. J. McConahey, E. D. Murphy, J. B. Roths, and F. J. Dixon. 1978. Spontaneous murine lupus-like syndromes: clinical and immunopathological manifestations in several strains. *J. Exp. Med.* 148:1198–1215.

2. Pettersson, I., M. Hinterberger, T. Mimori, E. Gottlieb, and J. A. Steitz. 1984. The structure of mammalian small nuclear ribonucleoproteins: identification of multiple protein components reactive with anti-(U1)ribonucleoprotein and anti-Sm autoantibodies. *J. Biol. Chem.* 259:5907–5914.

3. Eisenberg, R. A., E. M. Tan, and F. J. Dixon. 1978. Presence of anti-Sm reactivity in autoimmune mouse strains. *J. Exp. Med.* 147:582–587.

4. Notman, D. D., N. Kurata, and E. M. Tan. 1975. Profiles of antinuclear antibodies in systemic rheumatic diseases. *Ann. Intern. Med.* 83:464–469.

5. Costa, O., C. Fournel, E. Lotchouang, J. C. Monier, and M. Fontaine. 1984. Specificities of antinuclear antibodies detected in dogs with systemic lupus erythematosus. *Vet. Immunol. Immunopathol.* 7:369–382.

6. Hochberg, M. C., R. E. Boyd, J. M. Ahearn, F. C. Arnett, W. B. Bias, T. T. Provost, and M. B. Stevens. 1985. Systemic lupus erythematosus: a review of clinico-laboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets. *Medicine* (Baltimore). 64:285–295.

7. Tan, E. M., and H. G. Kunkel. 1966. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J. Immunol.* 96:464–471.

8. Eisenberg, R. A., D. G. Klapper, and P. L. Cohen. 1983. The polypeptide structure of the Sm and RNP nuclear antigens. *Molec. Immunol.* 20:187–195.

9. Eisenberg, R. A., J. B. Winfield, and P. L. Cohen. 1982. Subclass restriction of anti-Sm antibody in MRL mice. *J. Immunol.* 129:2146–2149.

10. Boyer, C. M., R. A. Eisenberg, and P. L. Cohen. 1985. Quantitation of the Sm nuclear antigen in activated lymphocytes. *Arthritis Rheum.* 28:294–299.

11. Eisenberg, R. A., S. Y. Craven, and P. L. Cohen. 1987. Isotype progression and clonality of anti-Sm autoantibodies in MRL/Mp-lpr/lpr mice. *J. Immunol.* 139:In press.

12. Kleinbaum, D. G., L. L. Kupper, and H. Morgenstern. 1982. Epidemiologic Research: Principles and Quantitative Methods. Van Nostrand Reinhold Co., New York. 529 pp.

13. Winn, D. M., J. F. Wolfe, D. A. Lindberg, F. H. Fristoe, L. Kingsland, and G. C. Sharp. 1979. Identification of a clinical subset of systemic lupus erythematosus by antibodies to the Sm antigen. *Arthritis Rheum.* 22:1334–1337.

14. Winfield, J. B., C. M. Brunner, and D. Koffler. 1978. Serologic studies in patients with systemic lupus erythematosus and central nervous system dysfunction. *Arthritis Rheum.* 21:289–294.

15. Munves, E. F., and P. H. Schur. 1983. Antibodies to Sm and RNP: prognosticators of disease involvement. *Arthritis Rheum.* 26:848–853.

16. Burnet, Sir M. 1972. Auto-Immunity and Auto-Immune Disease: A Survey for Physician or Biologist. F. A. Davis Co., Philadelphia. 243 pp.

17. Portnoi, D., A. Freitas, D. Holmberg, A. Bandeira, and A. Coutinho. 1986. Immunocompetent autoreactive B lymphocytes are activated cycling cells in normal mice. *J. Exp. Med.* 164:25–35.

18. Cohen, P. L., E. W. Shores, R. A. Eisenberg, and D. S. Pisetsky. 1985. Anti-Sm autoantibodies in MRL mice: analysis of precursor frequency. *Cell. Immunol.* 96:448–454.

19. Shores, E. W., R. A. Eisenberg, and P. L. Cohen. 1986. Role of the Sm antigen in the generation of anti-Sm autoantibodies in the SLE-prone MRL mouse. *J. Immunol.* 136:3662–3667.

20. Eisenberg, R. A., K. Dyer, S. Y. Craven, C. R. Fuller, and W. J. Yount. 1985. Subclass restriction and polyclonality of the systemic lupus erythematosus marker antibody anti-Sm. *J. Clin. Invest.* 75:1270–1277.

21. Bernard, N. F., R. A. Eisenberg, and P. L. Cohen. 1985. H-2 linked Ir gene control of T cell recognition of the Sm nuclear autoantigen and the aberrant response of autoimmune MRL/Mp-+/+ mice. *J. Immunol.* 134:3812–3818.

22. Cohen, P. L., and R. A. Eisenberg. 1982. T-cell recognition of the Sm nuclear antigen: induction of T-cell proliferative responses in MRL/Mp-+/+ mice. *J. Immunol.* 129:2142–2145.

23. Shores, E. W., P. L. Cohen, and R. A. Eisenberg. 1984. Nature of the T cell requirements for spontaneous production of autoantibodies to the nuclear protein, Sm in MRL/Mp-lpr/lpr mice. *Arthritis Rheum.* 27:S80. (Abstr.)

24. Perlmutter, R. M., S. T. Crews, R. Douglas, G. Sorensen, N. Johnson, N. Nivera, P. J. Gearhart, and L. Hood. 1984. The generation of diversity in phosphorylcholine-binding antibodies. *Adv. Immunol.* 35:1–37.

25. Clarke, S. H., K. Huppi, D. Ruezinsky, L. Staudt, W. Gerhard, and M. Weigert. 1985. Inter- and intraclonal diversity in the antibody response to influenza hemagglutinin. *J. Exp. Med.* 161:687–704.

26. Brennan, F. M., D. G. Williams, D. Bovill, M. R. Stocks, and R. N. Maini. 1986. Administration of monoclonal anti-Sm antibody prolongs the survival and renal function of MRL-*lpr/lpr* mice. *Clin. Exp. Immunol.* 65:42–50.

27. Eisenberg, R. A., S. Y. Craven, D. S. Pisetsky, E. W. Shores, and P. L. Cohen. 1987. Anti-Sm autoantibodies enhance their own production by a positive feedback mechanism. *Arthritis Rheum.* 30:521. (Abstr.)

28. Block, S. R., M. D. Lockshin, J. B. Winfield, M. E. Weksler, M. Imamura, R. J. Winchester, R. C. Mellors, and C. L. Christian. 1976. Immunologic observations on 9 sets of twins either concordant or discordant for SLE. *Arthritis Rheum.* 19:545–554.