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Relationship of age and sex to autoantibody expression in MRL−+/+ and MRL-lpr/lpr mice: demonstration of an association between the expression of antibodies to histones, denatured DNA and Sm in MRL−+/+ mice

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SUMMARY

Despite the protean nature of the clinical characteristics of systemic lupus erythematosus (SLE), autoantibodies represent an almost constant feature. Furthermore, they are common to both human SLE and murine lupus. Nonetheless, the mechanism by which they arise has not been established. Amongst the several processes that have been proposed, evidence has emerged supporting specific antigen drive as a significant mechanism. We have documented the age- and sex-related differences in the prevalance of antibodies to both chromatin-related (histone and DNA) and non-chromatin-related (Sm) antigens in MRL mice. Our finding of an association between antihistone antibodies and anti-denatured DNA antibodies is consistent with chromatin being the putative antigen. Additionally, antibodies to the individual histones H1 and H2B, the most exposed histones in chromatin, were more prevalent than antibodies to the remaining histones (H2A, H3, H4). This, again, supports specific antigen drive as a mechanism for autoantibody production. However, associations were also found between antibodies to histone and DNA and antibodies to Sm. As Sm is a non-chromatin protein antigen, the associations between antibodies to Sm and those to histone and DNA suggest that mechanisms in addition to specific antigen drive are important in autoantibody production.

Keywords antihistone antibodies anti-DNA murine lupus

INTRODUCTION

Although autoantibodies are a hallmark of autoimmune disease, the mechanism(s) by which they arise remains obscure. Studies in both human systemic lupus erythematosus (SLE) (Portanova *et al.*, 1987; Gohill *et al.*, 1985) and the murine graft-versus-host disease (GVHD) model of SLE (Portanova, Claman & Kotzin, 1985) have suggested an important role for antigen in the antibody response to the chromatin related antigens. If chromatin were the putative antigen driving the production of antoantibody, then a close association should exist between antibodies to the chromatin components, histone and DNA. However, it might be expected that antibodies to non-chromatin-related antigens, such as Sm, should occur independently of these chromatin-related antibodies.

We have sought to determine whether such a relationship exists by observing the age- and sex-related changes in antihistone antibodies (AHA) and anti-denatured DNA (dnDNA) antibodies in the spontaneous murine SLE strains, MRL-lpr/lpr and MRL−+/+. These changes were compared with the changes in the occurrence of antibodies to the non-chromatin-related nuclear antigen, Sm. Our study has found that the association between autoantibodies is not restricted to dnDNA and histone and extends to Sm. Significant age and sex related differences were noted in the frequency of AHA and anti-Sm antibodies but were less evident with anti-dnDNA antibodies. Panspecific antihistone fraction antibodies (AHFA) were a common occurrence in mice positive for AHA. Our findings suggest that specific antigen driven responses are not solely responsible for autoantibody production in murine SLE.

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MATERIALS AND METHODS

Mice and sera

MRL−+/+ and MRL-lpr/lpr mice obtained from Jackson Laboratories (Bar Harbor, ME, USA) were bred and maintained at the Gore Hill Animal Unit, RNSH. Two hundred and three MRL−+/+ mice aged 5 to 82 weeks were tested for AHA, anti-dnDNA and anti-Sm while 42 MRL-lpr/lpr mice

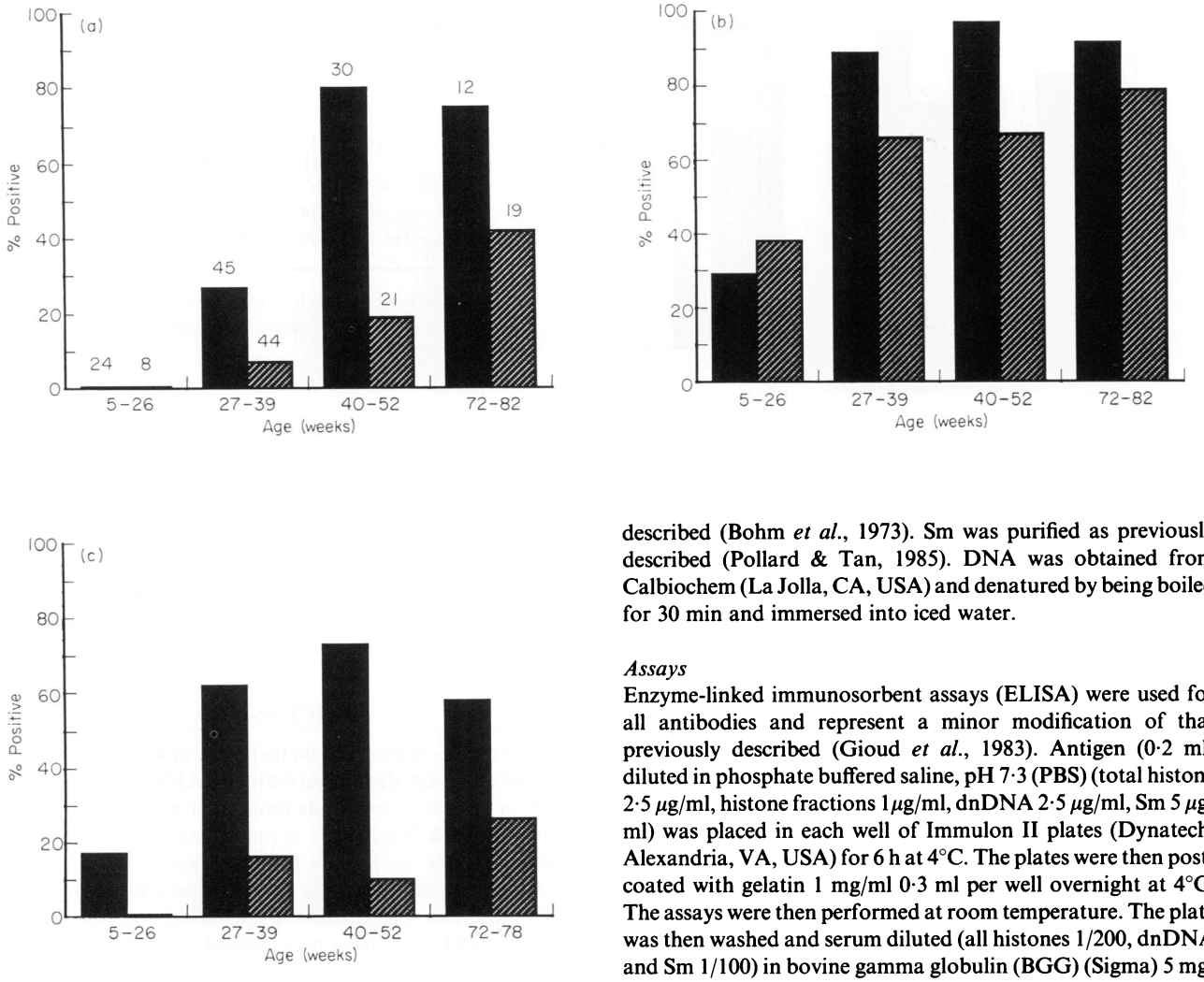

Fig. 1. (a) The prevalance of antihistone antibodies in MRL−+/+ mice. The number of mice in each group are indicated at the top of each column. (b) The prevalance of anti-denatured DNA antibodies in MRL−+/+ mice. Numbers of mice in each group are the same as in (a). (c) The prevalance of anti-Sm antibodies in MRL-+/+ mice. The numbers of mice in each group are the same as for (a) (■) Female; (■) male.

aged 6 to 31 weeks were tested for AHA. Sera from non-autoimmune CBA mice, obtained from the Department of Pathology, University of Sydney, were used to define the normal range for all assays. All mice were killed by cervical dislocation and bled by cardiac puncture. The blood was immediately centrifuged and serum stored at −20°C until assayed. Sera from individual mice were tested for antibodies to histones, dnDNA and Sm excepting four MRL-lpr/lpr mice that were tested for AHA only because of insufficient quantities of serum. Only mice that were positive for AHA were screened for AHFA.

Antigens

Histones were obtained from Sigma Chemicals (St Louis, MO, USA) and confirmed by SDS-polyacrylamide gel electrophoresis, and column chromatography to contain each of the histone classes. Purification of individual histones was as previously described (Bohm *et al.*, 1973). Sm was purified as previously described (Pollard & Tan, 1985). DNA was obtained from Calbiochem (La Jolla, CA, USA) and denatured by being boiled for 30 min and immersed into iced water.

Assays

Enzyme-linked immunosorbent assays (ELISA) were used for all antibodies and represent a minor modification of that previously described (Gioud *et al.*, 1983). Antigen (0·2 ml) diluted in phosphate buffered saline, pH 7·3 (PBS) (total histone 2·5 μg/ml, histone fractions 1μg/ml, dnDNA 2·5 μg/ml, Sm 5 μg/ml) was placed in each well of Immulon II plates (Dynatech, Alexandria, VA, USA) for 6 h at 4°C. The plates were then post-coated with gelatin 1 mg/ml 0·3 ml per well overnight at 4°C. The assays were then performed at room temperature. The plate was then washed and serum diluted (all histones 1/200, dnDNA and Sm 1/100) in bovine gamma globulin (BGG) (Sigma) 5 mg/ml, bovine serum albumin (BSA) (Sigma) 1 mg/ml, gelatin 1 mg/ml, and PBS with Tween-20 0·05% (PBST) was added. Incubations were 1·5 h for all histone-coated plates and 2 h for the other assays. Again the plate was washed and then 0·2 ml of peroxidase-labelled antibodies to mouse IgG and IgM (Tago, Burlingame, CA, USA) (diluted 1/2000 in BGG 1 mg/ml, BSA 5 mg/ml, PBST) was added. Finally the plate was washed and 0·2 ml of substrate (1 mg/ml 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) (Sigma) in 0·1 M McIlvaine's buffer, pH 4·6, and 0·005% H_2O_2) was added. Plates were read at 414 nm by means of an automated spectrophotometer (Flow Labs, Rickmansworth, Herts, UK). The mean of duplicates was obtained.

Statistical methods

The effect of age and sex on the prevalance of autoantibodies was assessed by logistic regression. The Mantel-Haenszel summary Chi square test was used to assess any association between the different autoantibodies in order to remove the effect of age.

RESULTS

Normal ranges for autoantibodies by ELISA

The upper limit of normal was defined as the mean plus two standard deviations of the absorbance values obtained using

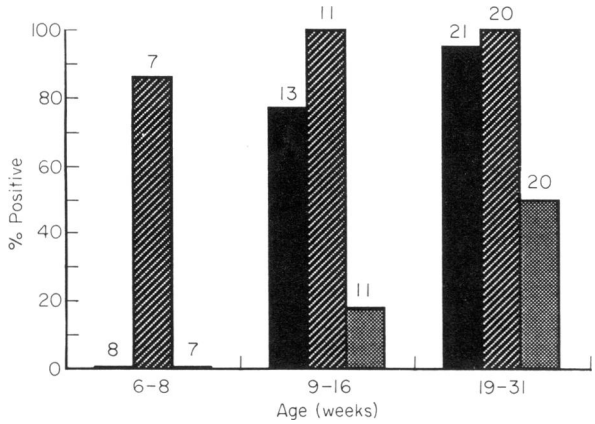

Fig. 2. The prevalence of antibodies to histones, denatured DNA and Sm in MRL-1pr/1pr mice. The number of mice in each group are indicated at the top of each column. (■) AHA; (▨) anti-DNA; (▩) ANTI-Sm.

sera obtained from male and female CBA mice. All subsequent assays were run with a positive MRL-1pr/1pr control and CBA controls to verify the normal range.

Age and sex distribution of serum autoantibodies in MRL−+/+ mice

Serum AHA were not detected above control levels, for MRL−+/+ mice under 26 weeks of age (Fig. 1a). Of the older mice, 35% were found to have AHA. There was an age related increase in their prevalence until 40–52 weeks ($P<0.001$) and AHA were more common in females than males ($P<0.001$).

In contrast, dnDNA antibodies were common in both sexes by 26 weeks of age (Fig. 1b) and were more common in the 27–39 week old age group ($P<0.001$) but there was no statistically significant increase in prevalance beyond that age. Again dnDNA antibodies were more common in female than in male mice ($P<0.001$).

Anti-Sm antibodies, observed in 37% of the mice, were present in 17% of female MRL−+/+ mice by 26 weeks of age (Fig. 1c). As with dnDNA antibodies, anti-Sm were more prevalent in the 27–39 week old age group without any significant increase in the older mice. Similar to the other autoantibodies, anti-Sm were more prevalent in female than male mice.

Age distribution of serum autoantibodies in MRL-lpr/lr mice

Both AHA and anti-Sm were first detected at 9 weeks of age in these mice whereas anti-dnDNA was common in the 6 to 8 week age group (Fig. 2). AHA were present in 71% of all mice, anti-dnDNA in 97% and anti-Sm in 32%.

Association between serum AHA, anti-Sm and anti-dnDNA antibodies

An association between the presence of AHA and anti-dnDNA antibodies in MRL−+/+ mice was observed ($P<0.01$). However, associations were also observed between the presence of AHA and anti-Sm antibodies ($P<0.001$) and between anti-dnDNA and anti-Sm antibodies ($P<0.001$). Because of the very high prevalance of AHA and anti-dnDNA antibodies in the MRL-1pr/1pr strain, associations between the various autoantibodies could not be assessed.

Table 1. Prevalence of antibodies to the histone fractions

	Histone fraction				
	H1	H2A	H2B	H3	H4
MRL−+/+	84%	58	69	48	61
MRL−1pr/1pr	100	100	100	100	93

Only sera in which AHA were detected were further tested for antibodies to individual histones.

Antibodies to individual histones in MRL mice

The prevalence of antibodies to each individual histone is detailed in Table 1. Considerable diversity was seen in the profiles of AHFA and the MRL−+/+ strain with antibodies directed against all five histone fractions in 33%, four fractions in 18%, three fractions in 15%, two fractions in 13%, one fraction in 10% and no fractions in 10%. Antibodies to all histone fractions were present in all MRL-1pr/1pr mice having AHA excepting two mice lacking only anti-H4 antibodies.

DISCUSSION

Autoantibodies are a common feature of human systemic lupus erythematosus (SLE) and murine lupus. AHA and anti-dnDNA occur in several strains while anti-Sm, a marker antibody for SLE (Pollard & Tan, 1985), is found only in the MRL strain (Theofilopoulos & Dixon, 1985). Whereas the age-related changes of autoantibodies to histone (Costa & Monier, 1986), DNA (Andrews *et al.*, 1978) and Sm (Eisenberg, Tan & Dixon, 1978) in MRL mice have been separately reported, there has been no description of all three of these antibodies being measured simultaneously.

The age related increase in the incidence of autoantibodies in both MRL strains and our observation of their more frequent occurrence in MRL−+/+ female mice is consistent with findings in other murine lupus strains (Andrews *et al.*, 1978).

The finding of anti-Sm in 32% of MRL-1pr/1pr mice is consistent with the report of Eisenberg *et al.* (1978) but greater than the 17% prevalence described by Williams *et al.* (1986). Additionally, the prevalence of anti-Sm in MRL−+/+ that we have observed, is comparable with previous findings (Eisenberg *et al.*, 1978). We consider it unusual that there was a similar prevalence of anti-Sm antibodies in both MRL substrains as the autoimmune disease in the MRL−+/+ mice is milder than that of the MRL-1pr/1pr mice. This contrasts with both AHA and anti-dnDNA which, in our study, were more common in MRL-1pr/1pr mice.

Associations between AHA and antibodies to native DNA (nDNA), have been described in SLE (Gioud, Ait-Kaci & Monier, 1982; Krippner *et al.*, 1984), and between AHA and anti-dnDNA in DILE (Rubin *et al.*, 1985). However, previous reports have failed to demonstrate any relationship between the levels of antibodies to nDNA, Sm and ribonucleoprotein (RNP) in humans with SLE (McCarty *et al.*, 1982), or between the levels of antibodies to either nDNA or dnDNA and Sm in the MRL−+/+ and the MRL-1pr/1pr strains (Pisetsky,

McCarthy & Peters, 1980). Our study is similar in finding an association between AHA and anti-dnDNA but differs in demonstrating that the association extends to anti-Sm. This difference may be due to our assessing the presence or absence of the autoantibodies whereas the other studies have examined the changing *levels* of autoantibodies which were already present.

Chromatin more readily absorbs antibodies to the histones H1 and H2B, the histones most accessible in chromatin, than those directed against other histones (Goldblatt & Bustin, 1975). Thus, antibodies to these histones would be expected to predominate (Hardin & Thomas, 1983). This has been well described in human SLE (Gioud, Ait-Kaci & Monier, 1982; Hardin & Thomas, 1983; Gohill *et al.*, 1985; Bernstein *et al.*, 1985). Costa & Monier (1986) made similar observations in MRL−+/+ and PN mice, but found that although anti-H1 was the commonest AHFA in the MRL-lpr/lpr strain, antibodies to H3 and H4 were the most prevalent of the core histone antibodies. In NZB/NZW mice, antibodies to H1 and H2B were only slightly more prevalent than other AHFA. Portanova, Claman & Kotzin (1985) have also shown inter-strain differences with the highest levels of AHFA (in order) being to H2B, H2A and H1 and in the GVHD model of autoimmunity; to H1 in the MRL-lpr/lpr; and to H2B, H3 and H2A in the NZB/NZW strain. Although these represent the highest levels of AHA, it would appear from the data, that antibodies to all the histone fractions were commonly present.

Our study confirms the high prevalence of anti-H1 and anti-H2B in the MRL +/+ mice but also shows that antibodies to the other fractions are common. In 10% of MRL−+/+ mice having AHA detected, no AHFA were found. This is most likely due to antibodies to conformational determinants on the total histone preparation or antibodies to histone complexes (Rubin *et al.*, 1985). Additionally, with only two exceptions, we have observed that antibodies to all the histone fractions are present in MRL-lpr/lpr mice having AHA detected with the total histone preparation.

The association between AHA and anti-dnDNA in addition to the AHFA profiles that have been observed, supports antigen drive as being germane to the spectrum of autoantibodies occurring in SLE and murine lupus. The association between anti-Sm and the other autoantibodies is less readily explained. It is unlikely that they arise due to the simultaneous presentation of antigens, possibly from dying cells, because of the different ages at which the antibodies first become apparent. It may be relevant that Sm has been shown to be capable of binding to DNA, although this is of uncertain significance. Alternatively, the appearance of anti-Sm antibodies maybe due to somatic mutation of genes encoding for anti-dnDNA (Migliorini *et al.*, 1987). However, six of the 75 MRL−+/+ mice that had anti-Sm antibodies, did not have detectable anti-dnDNA; thus confirming that anti-Sm can occur independently from anti-dnDNA antibody expression (Pisetsky, McCarty & Peters, 1980).

We postulate that there is sequential expression of autoreactive B cells, with antigen presentation exerting a selective pressure upon the potential autoreactive B cells. Whether such autoreactive B cells arise via somatic mutation, or are part of the naturally occurring B cell repertoire, remains to be elucidated as both mechanisms can produce antibodies with similar specificities (Davidson *et al.*, 1987).

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