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An analysis of apoptosis in lymphoid organs and lupus disease in murine systemic lupus erythematosus (SLE)

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SUMMARY

Apoptosis is a programmed cell death process that helps to regulate both T cell and B cell development. In this study, we have investigated the levels of apoptotic death in cells of the thymuses and spleens (white matter) of autoimmune MRL-*lpr/lpr* mice with progressive lymphadenopathy and SLE disease activity; we also examined the renal pathology in these animals. Fas is a cell surface receptor, which when activated initiates the sequence of events that lead to apoptosis. In MRL-*lpr/lpr* mice Fas is defective, so the competency for apoptosis may be reduced. In young animals of advancing age the thymuses enlarged until in 5-month-old females the average weight was three times that at 1 month, and spleen and kidney weights also increased in size disproportionately. At light microscope level apoptotic cells in tissue sections were counted using both routine eosin and haematoxylin staining (to identify them by their morphology) and *in situ* end-labelling of cells with DNA strand breaks; their presence was further confirmed by electron microscopy. As the mice aged, the numbers of apoptotic cells in thymic cortex, thymic medulla and spleen white pulp areas reduced significantly ($P < 0{\cdot}01–0{\cdot}001$), whereas in BALB/c normal controls they increased significantly ($P < 0{\cdot}05$). These changes were coincident with the development of severe lupus, whose activity was assessed by measuring serum anti-ssDNA and anti-dsDNA antibody titres and urinary protein (albumin) level which were elevated significantly by 5 months of age ($P < 0{\cdot}001$ for both ssDNA and dsDNA and $P < 0{\cdot}01$ for urine albumin) compared with their younger counterparts. Thus, lymphoid organ enlargement, decrease in apoptotic indices, elevated serum anti-ssDNA and anti-dsDNA antibody levels, and impaired renal function coincided with the onset and severity of lupus disease in lpr mice. It seems likely that there is a causal relationship between defective deletion of autoreactive lymphoid cells, imperfect Fas-mediated apoptosis and development of murine SLE.

Keywords apoptosis thymus spleen autoimmunity systemic lupus erythematosus

INTRODUCTION

SLE is an autoimmune rheumatic disease characterized by the production of a variety of autoantibodies against a number of antigens. Mice homozygous for the *lpr* (lymphoproliferation) gene develop a remarkable degree of lymphoid organ enlargement and progressive SLE-like autoantibody formation [1,2]. The enlargement in this strain is due to the massive accumulation of non-malignant $CD4^{-}CD8^{-}$ T lymphocytes [3] and an expansion of non-malignant B cells [4]. Dramatic abnormalities in T cells, B cells and other cells of haematopoietic origin have also been described in MRL-*lpr/lpr* (lpr) animals [5]. Apoptosis is an internally programmed cell death pathway (frequently initiated by extracellular signals), that regulates both T cell and B cell development [6,7]. Accumulating evidence suggests that in the normal situation, immature self-reactive thymocytes undergo apoptotic death (negative selection) upon stimulation via the T cell receptors [3,8,9]. Both positive and negative selection events are involved in determining which T cell clones mature in the thymus.

Subsequent to the discovery that the *lpr* gene codes for a defective configuration of the Fas antigen [10], the normal form of which mediates a signalling pathway that initiates apoptosis [11,12], we investigated whether there may be a new insight into the relationship between defective Fas-mediated apoptosis and autoimmunity in the lpr mouse. In lpr mice the functions of the immune system are perturbed, leading to a typical lupus-like syndrome over a period of several months as young animals achieve maturity. In this study we have examined the levels of apoptosis in the thymic cortex, thymic medulla and spleen white pulp areas of growing lpr mice of both sexes with progressing lupus disease activity. This is the first investigation in which

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numbers of apoptotic cells have been counted and compared between lpr and appropriate control animals.

MATERIALS AND METHODS

Animals

Three-week-old lpr mice of both sexes, and female BALB/c mice were purchased from Tuck & Sons Ltd (London, UK). Animals were housed in groups in cages and maintained in the department's animal house under routine conditions. Female BALB/c mice were chosen as controls since the development of the lupus-like syndrome is more prevalent in female lpr mice.

Experimental procedures

One-month-old cohort. Protein excretion in the urine of groups of four, 1-month-old female lpr, male lpr, and female BALB/c animals was measured using Albustix (Bayer Diagnostics, Basingstoke, UK). When the animals were killed their bodies were weighed and blood samples were taken. A full autopsy examination of the internal organs was performed after exsanguination. Thymuses, spleens and kidneys were removed and weighed and values were expressed as a percentage of body weight (relative weights) in all categories. Tissues were taken for light microscopy and electron microscopy. Specimens of kidney were also snap frozen for immunohistochemical studies by immunofluorescence and were coded and screened blind for evidence of glomerular pathology.

Animals in the 3 month and 5 month cohorts were similarly killed, weighed and samples taken.

Routine light microscopy

Tissues were fixed in neutral buffered formalin before embedding in paraffin wax: 4-μm sections were then cut and lightly stained with haematoxylin and eosin.

In situ *end labelling*

The procedure used was based on that described by Wijsman *et al.* [13]; formalin-fixed 4-μm sections were cut from the paraffin wax tissue blocks, dewaxed, rehydrated and treated with 0·1% hydrogen peroxide for 15 min to block endogenous peroxidase activity. Tissues were then incubated with 50 μl of 0·5% w/v DNAse-free pepsin in 0·1 M HCl, in a humidity chamber at 37°C for 15 min. After thorough washing sections were incubated with nick translation reaction buffer (50 mM Tris pH 7·5, 5 mM $MgCl_2$, 10 mM β-mercaptoethanol, 0·005% DNAse-free bovine serum albumin (BSA)—fraction V) for 5 min at room temperature to equilibrate. This buffer was tapped off and 50 μl of reaction buffer containing 10 μl each of dATP, dGTP, dCTP, 0·3 μM biotin-16-dUTP (Boehringer, Lewes, UK) and 2·5 U/ml of Klenow fragment of DNA polymerase I (Pharmacia) were pipetted on to each section and incubated for 1 h at 37°C in the humidity chamber. Sections were rinsed well in water, incubated in PBS for 5 min at room temperature, then incorporated biotinylated nucleotide was detected using StrepABC-HRP developed with DAB. Sections were then lightly counterstained with haematoxylin before routine mounting. To provide negative controls, DNA polymerase was omitted at the appropriate stage.

In thymic cortex, thymic medulla and spleen white matter, sections were scanned at light microscope level using the $\times 40$ objective lens. Using an eye-piece graticule, at least 1000 consecutive cells (nuclei) were counted from randomly chosen appropriate areas. The number of apoptotic nuclei encountered was noted in each case (apoptotic lymphocytes rarely fragment, so each apoptotic body was interpreted as a single apoptotic cell) and apoptotic indices were expressed as a percentage of the total number of cells scored.

Electron microscopy

Tissue samples, not exceeding 1 mm^3 in volume, were fixed in 2% glutaraldehyde for 2 h. After washing in phosphate buffer, blocks were osmicated and dehydrated in acidified 2,2 dimethoxypropane (DMP) before routine embedding in TAAB resin. Sections (1 μm) were cut and stained with toluidine blue for observation at light microscope level and selection of relevant blocks, followed by ultrathin sections of $\approx$100 nm, collected on nickel grids and stained with uranyl acetate and lead citrate, for observation on a Philips CM-10 electron microscope.

Kidney immunofluorescent staining

Deposition of immunoglobulin in the renal glomeruli was analysed by a direct immunofluorescence assay. Sections of kidney fixed in cold acetone (−20°C) were exposed to fluoresceinated goat anti-mouse IgG (Sigma) diluted 1:50 in PBS containing 10% normal goat serum for 45 min. Sections were washed three times in PBS, mounted in 50% Glycerol/PBS (Cityflour) and examined using a Zeiss Universal microscope equipped with epi-fluorescence illumination. All sections were interpreted without knowledge of their category (single blind) and graded for development of lupus disease acitvity according to the staining.

Detection of serum reactivity to ssDNA and dsDNA

Reactivity of sera to single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) was determined using direct binding ELISAs as described previously [14]. Briefly, ssDNA was coated onto ELISA plates at a concentration of 10 μg/ml (Sigma) and dsDNA at 5 μg/ml. Sera were diluted to 1:200 in PBS containing 0·1% Tween. The plates were washed three times with PBS, goat anti-mouse IgG/alkaline phosphatase conjugate (Sigma) at a dilution of 1:1000 was applied to the plates, followed by addition of substrate (paranitrophenyl phosphate (Sigma, Poole, UK) at 1 μg/ml in carbonate buffer pH 9·6). Plates were read at 405 nm using a Dynatech MR4000 ELISA plate reader. Serum dilutions were checked for non-specific binding to control wells lacking antigen, on the same plate as the antigen-coated wells. Final optical density (OD) values were determined by the mean of triplicate readings obtained in the uncoated half of the plate being subtracted from the corresponding mean values obtained from the plates coated with antigen.

RESULTS

As expected, changes due to the disease were seen to be more severe in female lpr mice compared with males of the same age. Total body weights of lupus-prone lpr mice and normal mice increased with age (Fig. 1). In BALB/c animals the weight of thymuses decreased with increasing age so that by 5 months they had involuted to less than half their 1 month values; the absolute weights of spleens and kidneys increased proportionally with the body weights in BALB/c animals as they matured. In lpr females the thymuses and spleens enlarged and had achieved three times their weight at 1 month by the age of 3 months ($P<0·001$); these organs remained large until 5 months of age. In male lpr mice the

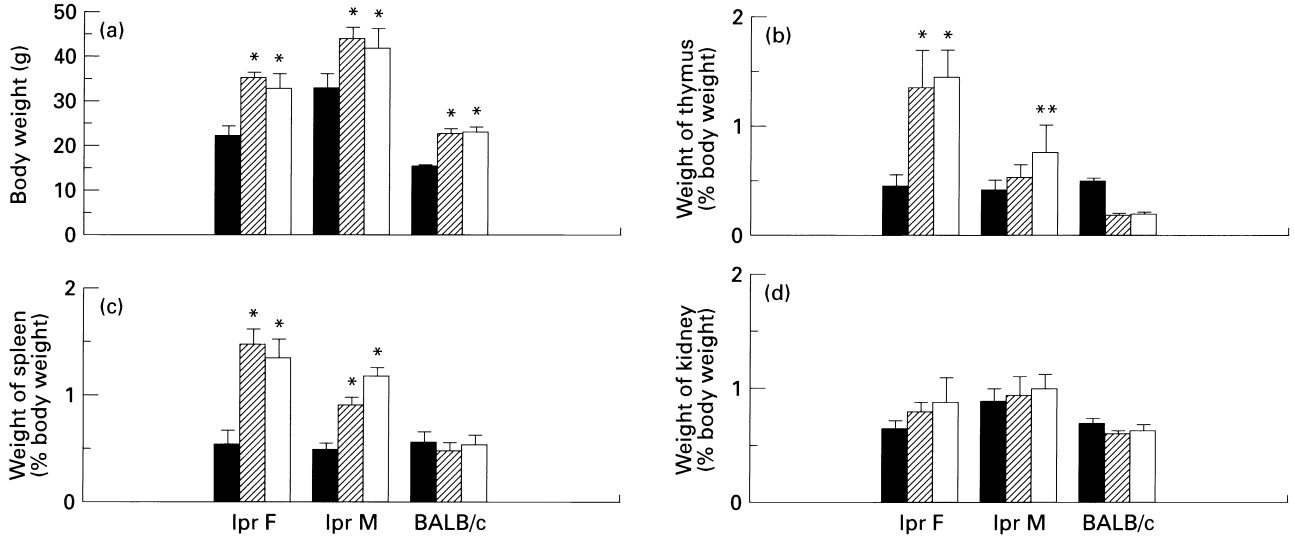

Fig. 1. (a) Body weights of lpr females (lpr F), lpr males (lpr M) and BALB/c mice at 1 month (■), 3 months (▨) and 5 months of age (□). (b) Relative weights of thymuses of all categories. (c) Relative weights of spleens of all categories. (d) Relative weights of kidneys of all categories; ± s.d. Weights of thymuses and spleens increase significantly with age in lpr F and lpr M animals (Student's *t*-test; **P*<0·001; ***P*<0·01).

thymuses showed no regression at 3 months of age and by 5 months of age they had enlarged significantly ($P<0{\cdot}01$).

The gross pathology of lupus disease in lpr mice has already been reported [4], and all of the characteristic changes were readily identifiable in our routinely processed tissues. Large numbers of aberrant B cells were typical components of the thymuses of lpr mice (Fig. 2) of both sexes, and by 5 months of age significant glomerular basement membrane, epithelial and mesangial cell deposits of immunoglobulin were observed in the kidneys by immunofluorescence and by electron microscopy (Fig. 3); lymphocytic infiltrates and apoptotic lymphocytes were also apparent. Apoptotic cells in the thymic cortex, thymic medulla and spleen white pulp areas were readily recognizable with routine haematoxylin and eosin (H–E) staining by their characteristic morphology under the light microscope (Fig. 4). Their identity was confirmed by *in situ* end labelling (Fig. 5) and by electron microscopy (Fig. 6).

In female lpr mice the apoptotic indices reduced significantly ($P<0{\cdot}001$) in the thymic cortex, thymic medulla, and spleen white pulp at 3 months and 5 months of age compared with the 1 month age group (Fig. 7). In lpr male thymuses and spleens, although

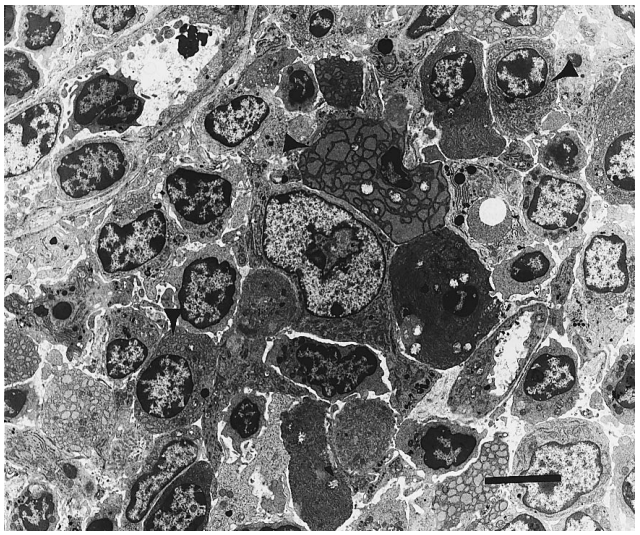

Fig. 2. Electron micrograph of the thymic cortex of a 5-month-old female lpr mouse. Many B cells are present (examples arrowed) in a variety of activation states. Bar = 6 μm.

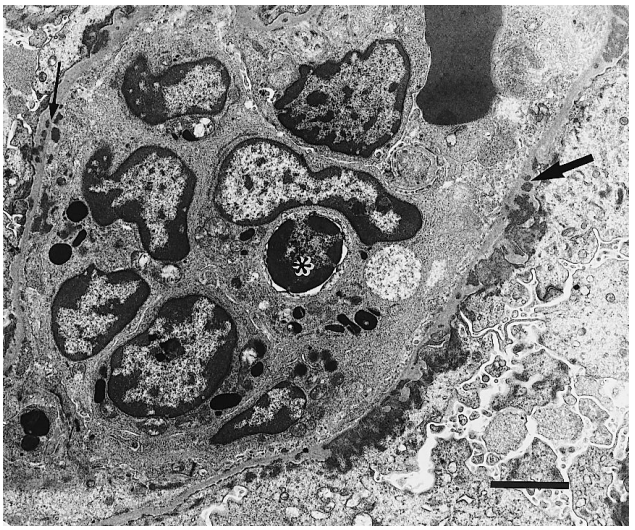

Fig. 3. Electron micrograph of a capillary tuft of a renal glomerulus of a 5-month-old female lpr mouse. There are immunoglobulin deposits in subepithelial locations (thick arrow) and subendothelial locations (thin arrow) of the basement membrane, as well as within foot processes of epithelial cells. There is an apoptotic lymphocyte (*) in the lumen of the vessel, illustrating that apoptosis is able to occur simultaneously with the development of the lupus syndrome, i.e. Fas mediation is not the only mechanism effecting apoptosis in these animals. Bar = 2·5 μm.

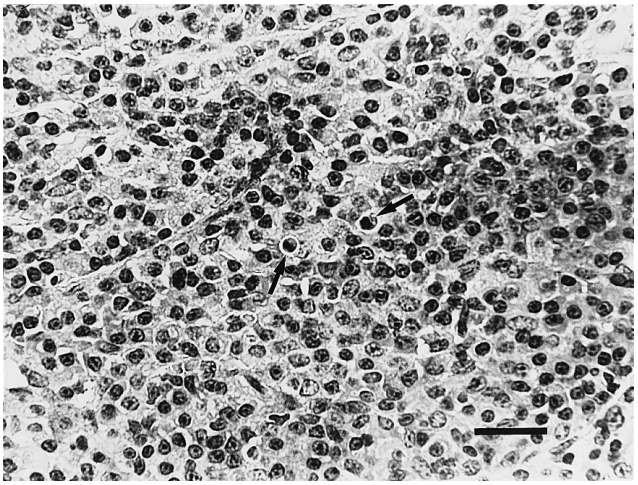

Fig. 4. Light micrograph of thymic cortex of a 5-month-old female lpr mouse stained with haematoxylin and eosin. Note that apoptotic figures (arrows) are discernible by their characteristic morphology. Bar = 65 μm.

there was a significant reduction in the apoptotic level at 3 months of age, by 5 months the indices had recovered somewhat compared with 3-month-old animals. In contrast, in thymic cortex of BALB/c controls the apoptotic indices consistently increased with the age of the animals (P<0·05) to 5 months, as the normal organs involuted. Apoptotic levels were consistent in the thymic medullas, but also increased in spleen white pulp of these animals.

Significantly elevated levels of serum antibody against both ssDNA and dsDNA were found in both sexes of lpr animal at 3 and 5 months of age (P<0·001), illustrating lupus disease activity (Fig. 8). Anti-dsDNA antibody levels were significantly higher in lpr females than in males of the same age groups (P<0·001): in BALB/c animals antibodies to both ssDNA and dsDNA were low and did not increase as the animals got older. Urine protein excretion also progressively increased in lpr mice of both sexes (0–1 + at 1 month, 1–3 + at 3 months and 3–4 + at 5 months).

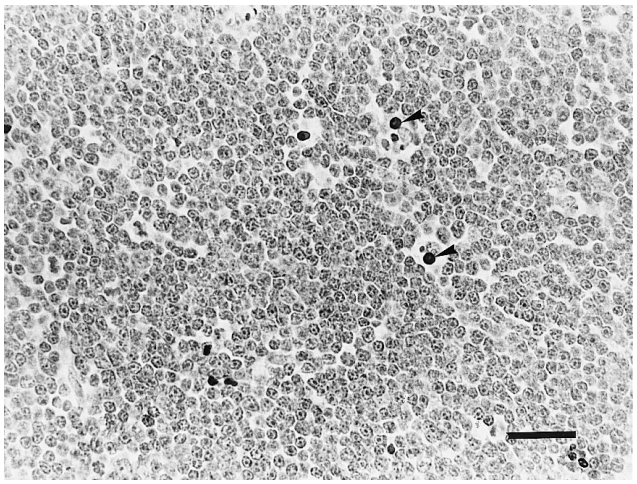

Fig. 5. *In situ* end-labelling of apoptotic figures in the thymic cortex of a 5-month-old female lpr mouse. The labelled cells are very readily identifiable, but apoptotic morphology is masked by the label. Arrows indicate apoptotic bodies phagocytosed by thymic macrophages. Bar = 80 μm.

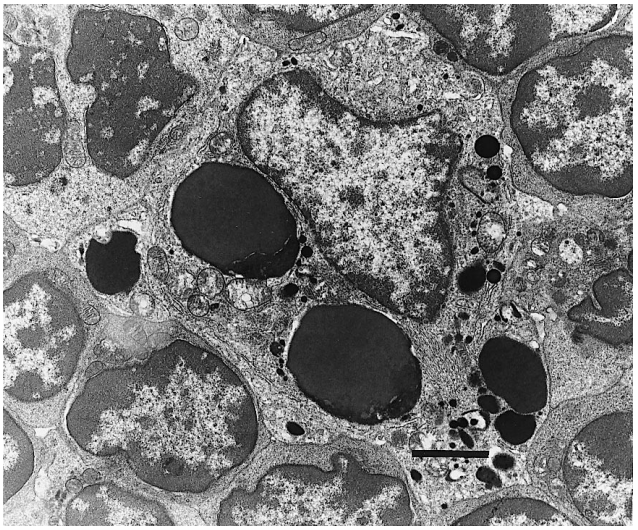

Fig. 6. Electron micrograph of a thymic macrophage from a 1-month-old lpr mouse, containing two condensed phagocytosed apoptotic cells. Bar = 1·8 μm.

DISCUSSION

The *fas* gene on chromosome 19 [15] of the normal mouse codes for a cell surface receptor Fas which when activated modulates cell death by apoptosis [11]. It is a transmembrane protein, a member of the tumour necrosis factor receptor (TNFR) superfamily, and is most closely related to TNFR I. The *lpr* gene is a mutated form at the same location on chromosome 19 of the lpr mouse, and includes the insertion of an early transposable element (ETn) into its second intron. This insertion carries two poly(A) adenylation sites, which lead to early termination of the gene and thus a truncated protein-defective Fas [16,17].

In the normal situation, complete/intact Fas is expressed by lymphoid cells and its expression can be induced in mature peripheral T cells [18–21]. This tissue distribution of Fas and defects in the Fas-mediated apoptosis pathway in autoimmune-prone lpr mice suggests that defective deletion of autoreactive T cells [6] and autoantibody-producing B cells [7] may be responsible in part for the pathogenesis of the autoimmune syndrome in these animals.

Mice homozygous for the mutation in the *lpr* gene develop lupus-like autoimmunity and lymphoproliferative disorders characterized by the accumulation of double-negative T cells ($CD4^-CD8^-$ TCR $\alpha\beta^+B220^+$), and non-malignant autoantibody-producing B cells [1,2,4]. Although Watanabe-Fukunaga *et al.* [10] wrote in 1992 that abnormalities of thymus, liver, heart and ovary had not been described in these animals, in fact the gross pathology of lpr mice has been well documented since the 1970s [4], when doubling of the thymus weight in animals of 22 weeks of age was observed.

In the present experiments, as animals aged toward maturity the thymuses of the control mice involuted while weights of spleens and kidneys increased proportionally to their body weights [22]. In the lpr animals, however, thymuses increased in size and spleen and kidney weights increased disproportionately due to the massive lymphocyte accumulation; this is in agreement with other workers [23]. The non-malignant T and B cells are non-proliferative [24], and the T cells in particular, being

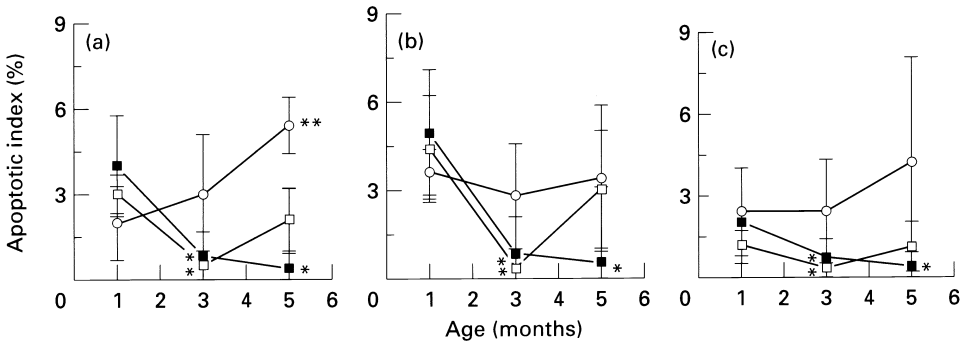

Fig. 7. Apoptotic indices in thymic cortex (a), thymic medulla (b) and spleens (c) in lpr female (■), lpr male (□) and BALB/c mice (○) at 1, 3 and 5 months of age. Apoptotic cells were counted under the light microscope and indices were calculated as described; results were analysed using Student's *t*-test. The indices decreased significantly (**P*<0·01–0·001) in lpr animals of both sexes and increased significantly (***P*<0·05) in BALB/c mice, with increasing age. Each point represents the mean value ± s.d.

double-negative, are virtually inert [25]. In MRL-*lpr/lpr* mice, failure of apoptosis of $CD4^+$ cells in the periphery of the thymus allows self-reactive T cells to persist and ultimately drive autoantibody production by B cells [26]; despite their abnormality lpr B cells require the presence of lpr T cells in order to produce antibodies [27]. Accumulation of B cells related to an apoptosis defect is critical in the $CD23^{low}$ B cell population, and this may have a fundamental importance in autoimmunity [28]. In this study we have not reported on mitotic indices in the tissues under investigation, nor data on the recruitment/retention/phenotype of lymphoid cells—this work is currently in progress.

Lupus disease develops in nine times more women than men [29,30], and this trend is echoed in mice [31]. Sex hormones can substantially affect development of the disease in mouse, with androgens protecting males resulting in prevalence of the syndrome in females [23]. In the normal mouse prostate after castration, cell death by apoptosis is intimately linked to the expression of testosterone-repressed prostate message-2 gene (TRPM-2) [32], so it is not inconceivable that similar gene expression might be involved in an apoptosis-governed (reduced apoptosis) autoimmunity in this disease.

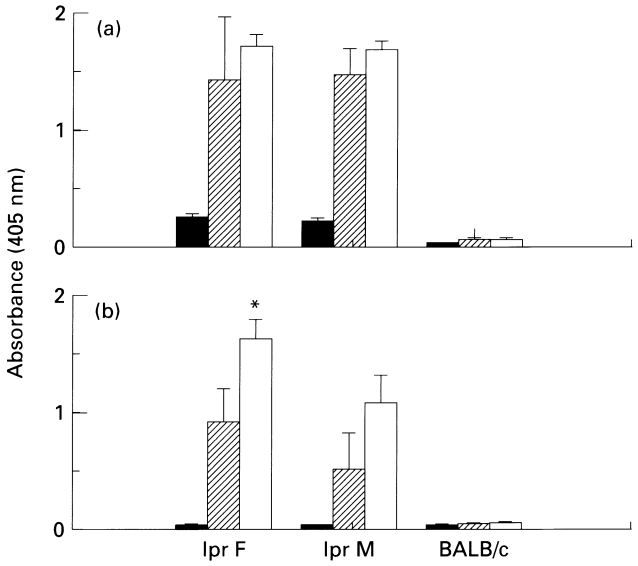

Fig. 8. Serum anti-ssDNA (a) and anti-dsDNA antibody levels (b) in the sera of lpr females (lpr F), lpr males (lpr M) and BALB/c mice measured using ELISA. dsDNA antibody levels in 5-month-old lpr female mice were significantly higher (**P*<0·001) than in 5-month-old lpr males. ■, 1 month; ▨, 3 months, □, 5 months.

In this study, apoptotic cells were identified by their typical morphology (shrunken cells with condensed nuclear chromatin, in a 'halo'), for the purpose of counting them at light microscope level. Care needs to be taken to examine sections very closely for correct identification of all apoptotic cells in H–E-stained sections. *In situ* end-labelled cells are much more readily visible, but this technique labels all broken DNA fragments, including any that might be present as a result of necrotic degradation. Thus, it cannot be used alone to estimate numbers of apoptotic cells. Together on serial sections, each technique can be used to verify the identity of apoptoses evident in the other.

Levels of apoptosis in the outer cortex of the thymus (where immature proliferating T cells are found), in the medulla of the thymus (where mature T cells are assembled) and the white pulp areas of spleen (where mature T cells are found around the B cell-populated germinal centres) were analysed. There was no significant difference in the extent of apoptosis in these organs of lpr and normal BALB/c mice at 1 month old. At this time lpr animals had low levels of serum anti-ssDNA and anti-dsDNA antibody and showed no detectable impairment in renal function—confirming that at 1 month, when the apoptotic distribution was normal, there was no detectable lupus disease activity in lpr mice. Initially in lpr mice there is no global defect in negative selection of the T cell repertoire, and positive selection of $CD4^+$ and $CD8^+$ subsets appears to be normal [33]. Failure of apoptosis of self-reactive $CD8^+$ cells can then lead to down-regulation of CD8 and persistence as $CD4^-CD8^-$ T cells which contribute to the lymphadenopathy [23].

Over the 5 months of this investigation, apoptotic indices decreased in thymuses and spleens of lpr animals of both sexes. Quantitative DNA flow cytometry has shown that lpr spleen cells *in vitro* behave similarly [28]. In females the decrease was consistent, but in males the levels recovered as the animals reached sexual maturity, reinforcing the notion that androgens might play a role [23]. Even in the most severely affected mice, however, examples of apoptotic cell death could be found. It is notable that even in renal glomeruli in which there was widespread deposition of immunoglobulin (indicating development of the autoimmune syndrome), there was also apoptosis in adjacent

lymphoid cells; this indicates that although a defect in Fas might link faulty ability for apoptosis with lymphoid cell accumulation, apoptosis is still able to occur by other mechanisms. Also, whereas the normal *fas* gene plays an essential role in activation-induced death in mature lymphocytes of peripheral lymph nodes, it may be less crucial in the negative selection of immature cells in the thymus [34], and the apoptosis-inducing activity of Fas antigen is up-regulated on mature peripheral T cells by stimulation through the T cell receptor [35].

Normally, B lymphocytes that produce autoantibodies and self-reactive T lymphocyte clones are deleted by apoptosis early in their development [6,7]. Thus, a decrease in number of apoptotic cells in the lymphoid tissues of lpr animals coinciding with the development of lupus disease could be explained by persistence of autoreactive T and B cell clones. The predecessors of these cells have been unable to 'self-destruct' as they bear the defective form of Fas coded by the *lpr* gene, and have not received/transduced the signal. Self-reactive B cells which are sequestered in compartments free of self-antigens may survive, and be activated for generation of pathogenic autoantibodies in autoimmune diseases [36].

This study has shown that lymphoid organ enlargement, decrease in apoptotic indices, elevated serum anti-ssDNA and anti-dsDNA antibody levels, and impaired renal function coincided with the onset and severity of lupus disease in lpr mice, particularly the females. It seems likely that there is a causal relationship between imperfect deletion of autoreactive lymphoid cells, imperfect Fas-mediated apoptosis and development of murine SLE. However, the possible effect of native androgens and ability of apoptosis to occur, initiated and executed through other pathways, indicates that the relationship between these events is complex.

ACKNOWLEDGMENTS

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REFERENCES

1 Cohen PL, Eisenberg RA. lpr and gld: single gene models of systemic autoimmuniy and lymphoproliferative disease. Annu Rev Immunol 1991; **9**:243–69.

2 Bhandoola A, Yui K, Siegel RM, Zerva L, Greene MI. gld and lpr mice: single gene mutant models for failed self tolerance. Int Rev Immunol 1994; **11**:231–44.

3 Schwartz RH. Acquisition of immunologic self-tolerance. Cell 1989; **57**:1079–81.

4 Murphy ED, Roths JB. Autoimmunity and lymphoproliferation: induction by mutant gene *lpr*, and acceleration by male-associated factor in strain BXSB mice. In: Rose NR, Bigazzi PE, Warner NL, eds. Genetic control of autoimmune disease. New York: Elsevier, 1978:207–21.

5 Cohen PL, Eisenberg RA. The *lpr* and *gld* genes in systemic autoimmunity: life and death in the Fas lane. Immunol Today 1992; **13**:427–8.

6 Smith CA, Williams GT, Kingston R, Jenkinson EJ, Owen JJ. Antibodies to CD3/T cell receptor complex induce death by apoptosis in immature T cells in thymic culture. Nature 1989; **337**:181–4.

7 Liu Y-J, Joshua DE, Williams GT, Smith CA, Girdon J, MacLennon ICM. Mechanism of antigen-driven selection in germinal centres. Nature 1989; **342**:929–31.

8 Kabelitz D, Phol T, Pechhold K. Activation-induced cell death (apoptosis) of mature peripheral T lymphocytes. Immunol Today 1993; **14**:338–9.

9 Von Boehmer HA. The developmental biology of T lymphocytes. Annu Rev Immunol 1988; **6**:309–26.

10 Watanabe-Fukunaga R, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 1992; **356**:314–7.

11 Itoh N, Yonehara S, Ishii A *et al.* The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 1991; **66**:233–43.

12 Nagata S, Suda T. Fas and Fas ligand: lpr and gld mutations. Immunol Today 1995; **16**:39–43.

13 Wijsman JH, Jonker RR, Keijzer R, van de Velde CJ, Cornelisse CJ, van Dierendonck JH. A new method to detect apoptosis in paraffin sections: *in situ* end labelling of fragmented DNA. J Histochem Cytochem 1993; **41**:7–12.

14 Ravirajan CT, Staines NA. Involvement in lupus disease of idiotypes Id.F423 and Id.IV-228 defined, respectively, upon foetal and adult MRL/Mp-lpr/lpr DNA-binding monoclonal autoantibodies. Immunology 1991; **74**:342–7.

15 Watanabe T, Sakai Y, Miyawaki S, Shimizu A, Koiwai O, Ohno K. A molecular genetic linkage map of mouse chromosome 19, inducing the *lpr, Ly-44, Tdt* genes. Biochem Genet 1991; **29**:325–36.

16 Adachi M, Watanabe-Fukunaga R, Nagata S. Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. Proc Natl Acad Sci USA 1993; **90**:1756–60.

17 Wu J, Zhou T, He J, Mountz JD. Autoimmune disease in mice due to integration of an endogenous retrovirus in an apoptosis gene. J Exp Med 1993; **178**:461–8.

18 Watanabe-Fukunaga R, Brannan CI, Itoh N, Yonehara S, Copeland NG, Jenkins NA, Nagata S. The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. J Immunol 1992; **148**:1274–9.

19 Drappa J, Brot N, Elkon KB. Fas protein is expressed at high levels on $CD4^+CD8^+$ thymocytes and activated mature lymphocytes in normal mice but not in the lupus-prone strain, MRL-*lpr/lpr*. Proc Natl Acad Sci USA 1993; **90**:10340–4.

20 Miyawaki T, Uehara T, Nibu R, Tsuji T, Yachie A, Yonehara S, Taniguchi N. Differential expression of apoptosis-related Fas antigen on lymphocyte subpopulations in human peripheral blood. J Immunol 1992; **149**:3753–8.

21 Owen-Schaub LB, Yonehara S, Crump III WL, Grim EA. DNA fragmentation and cell death is selectively triggered in activated human lymphocytes by Fas antigen engagement. Cell Immunol 1992; **140**:197–205.

22 Hinsull SM, Bellamy D. Tissue homeostasis and cell death. In: Bowen ID, Lockshin RA, eds. Cell death in biology and pathology. New York: Chapman and Hall, 1981:123–40.

23 Steinberg AD. Concepts of pathogenesis of systemic lupus erythematosus. Clin Immunol Immunopathol 1992; **63**:19–.

24 Sobel ES, Kakkanaiah VN, Rapoport RG, Eisenberg RA, Cohen PL. The abnormal lpr double negative T cell fails to proliferate *in vivo*. Clin Immunol Immunopathol 1995; **74**:177–84.

25 van Houten N, Budd RC. Lessons from the *lpr mouse*—T lymphocyte development. Semin Immunol 1994; **6**:1–2.

26 Steinberg AD. MRL-*lpr*/*lpr* disease: theories meet Fas. Semin Immunol 1994; **6**:55–69.

27 Eisenberg RA, Sobel ES, Reap EA, Halpern MD, Cohen PL. The role of B cell abnormalities in systemic autoimmune syndromes of *lpr* and *gld* mice. Semin Immunol 1994; **6**:49–54.

28 Reap EA, Leslie D, Abrahams M, Eisenberg RA, Cohen PL. Apoptosis abnormalities of splenic lymphocytes in autoimmune lpr and gld mice. J Immunol 1995; **154**:936–43.

29 Isenberg DA. Systemic lupus erythematosus. Hospital Update 1993; **19**:598–606.

30 McCarty DJ, Manzi S, Medsger TA, JR, Ramsey-Goldman R, LaPorte

RE, Kwoh KC. Incidence of systemic lupus erythematosus: race and gender differences. Arthritis Rheum 1995; **38**:1260–70.

31 Andrews BS, Eisenberg RA, Theofilopoulos AN *et al.* Spontaneous murine lupus-like syndromes. Clinical and immunopathological manifestations in several strains. J Exp Med 1978; **148**:1198–215.

32 Buttyan R, Olsson CA, Pintar J, Chang C, Bandyk M, Ng PY, Sawczuk IS. Induction of the TRPM-2 gene in cells undergoing programmed cell death. Mol Cell Biol 1989; **9**:3473–81.

33 Rozzo SJ, Eisenberg RA, Cohen PL, Kotzin BL. Development of the T cell receptor repertoire in *lpr* mice. Semin Immunol 1994; **6**:19–26.

34 Singer GG, Abbas AK. The fas antigen is involved in peripheral but not thymic deletion of T lymphocytes. Immunity 1994; **1**:365–71.

35 Nishimura Y, Ishii A, Kobayashi Y, Yamasaki Y, Yonehara S. Expression and function of mouse Fas antigen on mature and immature T cells. J Immunol 1995; **154**:4395–403.

36 Murakami M, Tsubata T, Okamoto M, Shimizu A, Kumagai S, Imura H, Honjo T. Antigen-induced apoptotic death of Ly-1 B cells responsible for autoimmune disease in transgenic mice. Nature 1992; **357**:77–80.