Clin Exp Immunol 1996; **105**:480–485

Enhanced natural but diminished antibody-mediated cytotoxicity in the lungs of MRL*lpr/lpr* mice

N. NILSSON & H. CARLSTEN *Departments of Clinical Immunology and Rheumatology, University of Göteborg, Göteborg, Sweden*

(Accepted for publication 14 May 1996)

SUMMARY

Human systemic lupus erythematosus (SLE) patients, as well as MRL*lpr/lpr* mice which develop a SLE-like disease, have decreased numbers and functional activity of systemic natural killer (NK) cells. In contrast, it has been found that among lymphocytes recovered from the bronchoalveolar lavage fluid of SLE patients, NK cells were increased in number, correlating with the severity of the lung engagement. The present study was undertaken to assay the capacity for natural killing in the lung compartment of MRL*lpr/lpr* mice compared with healthy congenic MRL +/+ and heterozygous MRL +/*lpr* mice. ^{51}Cr-labelled YAC-1 cells were injected intravenously to settle in the lungs where they were targeted for lysis by NK cells. YAC-1 cell killing inversely correlated with radioactivity remaining in the lungs after the assay, and was inhibited by antibody to the asialo-GM1 antigen expressed on NK cells. To analyse the capacity in the lung for cytolysis of non-NK cell-sensitive target cells, a similar *in vivo* ^{51}Cr-release assay was set up for antibody-mediated allospecific cytotoxicity. We demonstrate that MRL*lpr/lpr* mice throughout their lifespan display significantly increased natural cytotoxic activity in the lungs compared with MRL +/+ and MRL +/*lpr* mice, as demonstrated by more efficient killing of YAC-1 cells. In contrast, antibody-mediated allospecific cytotoxicity in the lungs was significantly less effective in the MRL*lpr/lpr* strain.

Keywords MRL*lpr/lpr* lungs cytotoxicity natural killer cells antibody

INTRODUCTION

Human systemic lupus erythematosus (SLE) patients have decreased number and functional activity of systemic natural killer (NK) cells [1,2]. MRL mice homozygous for the *lpr* gene develop a SLE-like disease characterized by polyclonal B cell activation and autoantibody production as well as impaired T cell functions [3,4]. These mice have also been shown to display impaired systemic NK cell activity [5,6]. In human SLE, the decrease of NK activity correlates with exacerbations of disease [7]. Also, in lupus-prone C57Bl/6*lpr/lpr* mice, age-dependent loss of NK cell function has been reported as crucial for the onset of autoimmune symptoms [6,8].

In contrast, it has been found that among lymphocytes recovered from the bronchoalveolar lavage fluid of SLE patients, $CD8^+$ T cells and NK cells were increased in number, correlating with the severity of lung engagement [9]. Our present study was undertaken to assay the *in vivo* capacity for natural killing in the lungs of MRL*lpr/lpr* mice compared with that of the healthy congeneic MRL +/+ and the heterozygous MRL +/*lpr* strains. To analyse the capacity in the lung for cytolysis of non-NK cell-sensitive target cells, an *in vivo* assay was set up for antibody-mediated allospecific cytotoxicity.

Correspondence: Nicklas Nilsson, Department of Clinical Immunology, Guldhedsg 10, S-413 46 Göteborg, Sweden.

MATERIALS AND METHODS

Mice

MRL*lpr/lpr* (H-2^k) and congeneic MRL +/+ (H-2^k), originally obtained from Harlan Olac Farm (Bicester, UK), were bred and maintained in the animal facility of the Department of Clinical Immunology, Göteborg. MRL*lpr/lpr* and MRL +/+ were mated to obtain the MRL +/*lpr* mice heterozygous for the *lpr* gene. Outbred SWISS (H-$2^{q/s}$) mice were used while setting up the *in vivo* allocytotoxicity assay. DBA/1 (H-2^q) and C57Bl/6 (H-2^b) mice were used as sources of allogeneic spleen cells. Mice were housed 5–10 in each cage under standard conditions of temperature and light, and fed laboratory chow and water *ad libitum*.

Depletion of NK cells

NK cells were depleted by *in vivo* treatment with antiserum to asialo-GM1 (Wako Chemicals, Germany). Asialo-GM1 is a

glycolipid expressed on mouse NK cells and a minor population of monocytes [10]. Antiserum (50 μl; 10 mg/ml) diluted in 0·5 ml PBS was given intravenously 24 h before assay. Mice treated with this dose of anti-asialo-GM1 have been shown to totally lack NK cell activity as measured *in vitro* [10].

Depletion of T cells

T cell depletion was performed according to the protocol described by Goldschmidt [11]. The MoAbs H129.19 (anti-CD4) and YTS169.4 (anti-CD8) were injected intraperitoneally, 0·4–0·8 mg and 0·4 mg, respectively. The efficacy of depletion was enhanced by injection of MAR18.5 (mouse anti-rabbit immunoglobulin) 0·6 mg 1 h later. Antibodies were kindly provided by Dr Rikard Holmdahl (Lund, Sweden). The efficacy of T cell depletion was controlled by FACS analysis of spleen lymphocytes.

Assessment of NK cell cytotoxicity in vivo

The mouse lymphoma cell line YAC-1 originally described by Kiessling *et al*. has been shown to be a target cell exclusively lysed by NK cells [12]. We have adopted an *in vivo* assay for NK cell cytotoxicity from Hanna & Fidler [13] as previously described [14]. YAC-1 lymphoma cells were labelled for 1 h with 200 mCi ^{51}sodium chromate (Amersham International, Aylesbury, UK) per 10^6 cells, washed in PBS $\times$3 and diluted to 3×10^5 cells/ml in PBS. Suspension (0·5 ml) containing $1{\cdot}5 \times 10^5$ radiolabelled cells was injected intravenously in a lateral tail vein. After 1 h, mice were killed by cervical dislocation and the remaining radioactivity in the lungs was detected in a Packard Cobra Autogamma counter. The total amount of radioactivity of the YAC-1 cells initially trapped in the lungs was calculated from animals killed within 1 min after injection. In mice pretreated with 50 μl of anti-asialo-GM1 thus lacking NK cell cytotoxic activity, typically 65% of the total initial radioactivity was still retained in the lungs after 1 h (depletion value). Thus, 35% of the radioactivity was released from the lungs by NK cell-independent mechanisms. Specific lysis of YAC-1 cells by NK cells was calculated by the formula: specific lysis = (1 − experimental value/depletion value) $\times$100% [1].

Induction of melanoma metastases

A murine melanoma cell line syngeneic with the C57Bl/6 mouse, B16, was kindly provided by Dr K. Hellstrand (Department of Clinical Virology, University of Göteborg). B16 (10^5) cells were injected intravenously, according to the protocol described by Hanna [15]. One month later, mice were killed and lungs harvested. Black spots on the lung surfaces, each representing a melanoma metastasis, were enumerated under a light microscope at $\times$10 magnification. Small metastases $<$ 1 mm in diameter, and large $>$ 1 mm, were separately enumerated.

Antibody-mediated allospecific cytotoxicity in vivo

Immunization of MRL +/+ and MRL*lpr/lpr* was performed by i.p. injection of a suspension in PBS of 5×10^7 freshly isolated spleen cells from naive DBA/1 mice. Controls received PBS intraperitoneally. Ten days later, an allospecific cytotoxic effector phase was elicited by i.v. injection of $1{\cdot}5 \times 10^5$ concanavalin A (Con A)-stimulated (in complete medium containing 1 μg/ml Con A, incubated at 37°C and 5% CO_2 for 48 h) and ^{51}Cr-labelled DBA/1 spleen cells. Retention of ^{51}Cr-labelled cells in lungs was measured 1 h after injection. The background level of ^{51}Cr retention was estimated in the control animals. Specific lysis of allogeneic target cells in immunized animals was calculated by the formula: specific lysis = (1 − immune value/non-immune value) $\times$100% [2].

Antibody-mediated allospecific cytotoxicity in vitro

MRL +/+ and *lpr/lpr* mice were immunized with 5×10^7 freshly isolated naive DBA/1 spleen cells. Ten days later sera were collected. In 96-well round-bottomed cell culture plates (Nunclon) serial dilutions of immune sera dissolved in medium were added to 5×10^3 Con A-stimulated (1 μg/ml, 48 h) and ^{51}Cr-labelled DBA/1 spleen cells in a final volume of 200 μl. Guinea pig serum, obtained from our animal facility, in a final concentration of 5% was added as a source of complement. As a positive control for maximal lysis of target cells a detergent, sodium dodecyl sulphate (Sigma), was added rather than serum. Samples were set in triplicates and incubated at 37°C for 4 h. The plates were centrifuged and cell-free supernatants containing released ^{51}Cr were harvested and counted in a Packard Cobra Autogamma Counter. Sera from non-immunized animals were used to assess spontaneous release of ^{51}Cr. Specific lysis of target cells by immune serum was calculated by the formula: specific lysis = (immune − naive/maximal − naive value) $\times$100% [3].

Cellular mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity in vitro

Cell-mediated cytotoxicity was assayed thus. Freshly isolated spleen cells from MRL +/+ mice immunized with DBA/1 spleen cells as above were set in triplicates together with 10^4 Con A-stimulated and ^{51}Cr-labelled DBA/1 spleen cells in a final volume of 200 μl/well on 96-well round-bottomed cell culture plates. Effector : target cell ratios were 100–50–25–12 : 1, and the assay was run for 4 h at 37°C. To test antibody-dependent cell-mediated cytotoxicity (ADCC), target cells were preincubated for 30 min with immune serum diluted 1 : 2, washed in PBS and mixed with naive MRL +/+ spleen cells and peritoneal macrophages in effector:target cell ratios as above. Supernatants were collected and read as above to determine specific lysis of target cells.

Determination of serum immunoglobulin levels

Serum levels of IgG1, IgG2a, IgG3 and IgM were measured by the radial immunodiffusion technique [16]. Antiserum specific for the IgG subclasses and IgM as well as the standards for IgG were purchased from Sigma, whereas the IgM standard was a kind gift from Dr Leif Lindholm (Pharmacia, Göteborg, Sweden).

Statistical analysis

Comparisons between the means of specific lysis of target cells and serum levels of immunoglobulins were carried out by Student's *t*-test. Differences in the number of B16 melanoma metastases were estimated by the non-parametric Mann–Whitney test. Calculations were done on a Macintosh IIcx using the StatView program.

RESULTS

Enhanced lung NK cell cytotoxic activity in MRLlpr/lpr mice

Mice were injected intravenously with YAC-1 cells and NK cell-mediated cytotoxicity in the lungs was calculated as specific lysis. As shown in Fig. 1, MRL*lpr/lpr* mice displayed a higher *in vivo* clearance of YAC-1 cells than MRL +/+ mice. Increased lysis ($P < 0{\cdot}001$) of YAC-1 cells was seen in young ($<$ 15 weeks old), clinically healthy mice as well as in aged ($>$ 15 weeks), severely

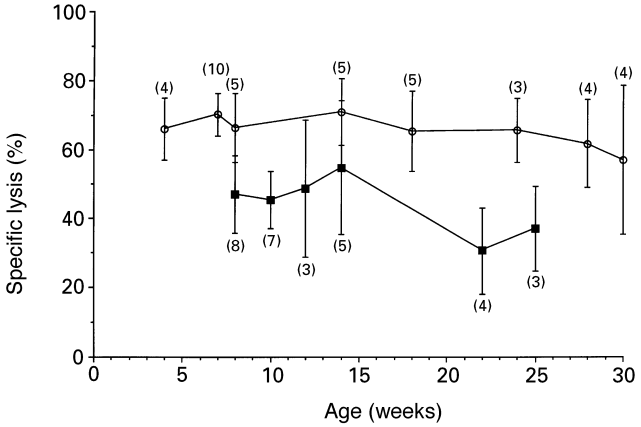

Fig. 1. Comparison of YAC-1 lysis cells in lungs of MRL +/+ (■) and MRL*lpr/lpr* mice (○). Female mice of different ages were injected intravenously with YAC-1 cells and *in vivo* natural killer (NK) cell-specific cytotoxic activity in the lungs was calculated. Number of mice in each group is indicated within parentheses in the figure. The symbols and bars represent means ± s.d.

diseased animals ($P < 0{\cdot}001$) (Fig. 1). Moreover, the difference in NK cell activity between MRL*lpr/lpr* and +/+ mice was demonstrated in both male and female mice. MRL +/*lpr* mice, heterozygous at the *lpr* gene locus, displayed similar YAC-1 lysis compared with that of age- and sex-matched +/+ mice (Fig. 2).

In the next experiment we calculated the number and size of lung metastases in MRL mice with different *lpr* gene status 1 month after i.v. injection of B16 melanoma cells. In Fig. 3 it is shown that the number of metastases was lower in MRL*lpr/lpr* mice compared with matched +/+ mice ($P = 0{\cdot}012$). However, a higher proportion of metastases in MRL*lpr/lpr* were larger than 1 mm. Heterozygous mice displayed a frequency of B16 melanoma metastases in between that of *lpr/lpr* and +/+ mice.

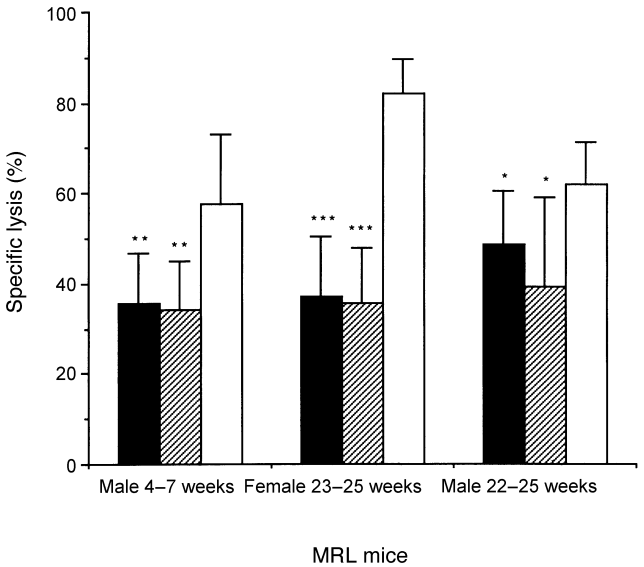

Fig. 2. MRL +/+ (■), MRL*lpr/lpr* (□) and the heterozygous MRL +/*lpr* mice (▨) were assayed for *in vivo* YAC-1 cell lysis in lungs; $n = 7$–11 in each group. The symbols and bars represent means ± s.d. Statistically significant differences *versus* MRL*lpr/lpr* mice: *$P < 0{\cdot}05$; **$< 0{\cdot}01$; ***$< 0{\cdot}001$.

Antibody-mediated allocytotoxicity

In order to study antibody-mediated allocytotoxicity in lungs of MRL*lpr/lpr* and MRL +/+ mice a novel *in vivo* assay was established. In methodological experiments SWISS mice were immunized and challenged with allogeneic DBA/1 spleen cells (see Materials and Methods). Cytotoxicity was measured by calculating the decrease in retention of ^{51}Cr in lungs. After 1 h only 5% of injected ^{51}Cr-labelled DBA/1 cells remained in the lungs of immunized mice compared with 30% in non-immunized controls (data not shown). To exclude NK and T cell-mediated lysis of allogeneic cells several depletion experiments were performed. In summary, neither depletion of NK cells with anti-asialo-GM1, nor depletion of $CD8^+$ and $CD4^+$ T cells with MoAbs (H129.19, YTS169.4) did alter the effector phase of allogeneic cytolysis in immunized mice (data not shown). In contrast, i.v. transfer of 0·5 ml of serum from immunized mice to naive mice 1 h before challenge resulted in significantly decreased retention of ^{51}Cr-labelled cells compared with mice receiving non-immune serum ($P < 0{\cdot}05$). We also considered cell-mediated cytolysis as an alternative target cell killing mechanism, but immune spleen cells could not *in vitro* induce target cell lysis.

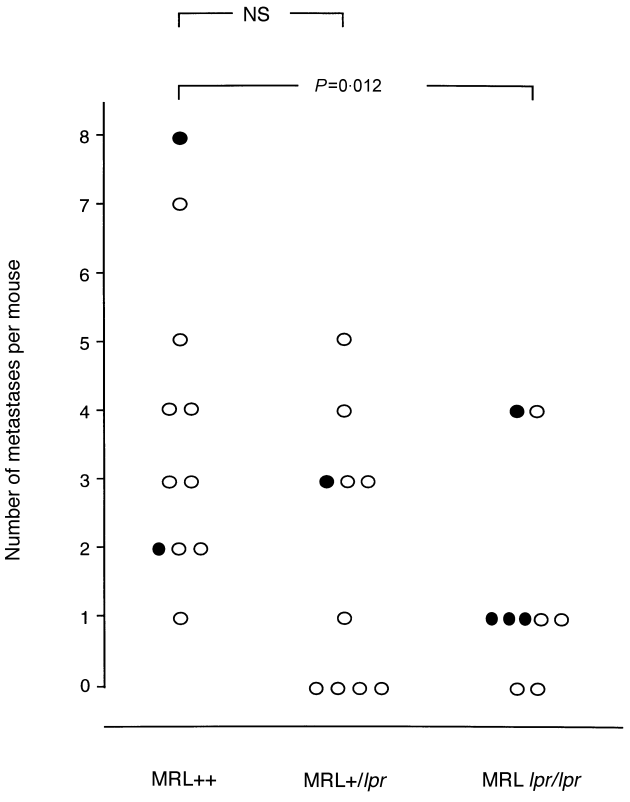

Fig. 3. Comparison of development of B16 melanoma lung metastases in MRL mice with different *lpr* gene status. Male MRL +/+, +/*lpr* and *lpr/lpr* mice 24–26 weeks old, $n = 9$–11 in each group, were administered intravenously with B16 melanoma cells. The number of lung metastases was determined 1 month later. ○, Metastases sized < 1 mm; ●, at least one metastasis was > 1 mm. Significance compared with MRL++ is indicated. NS, Not significant.

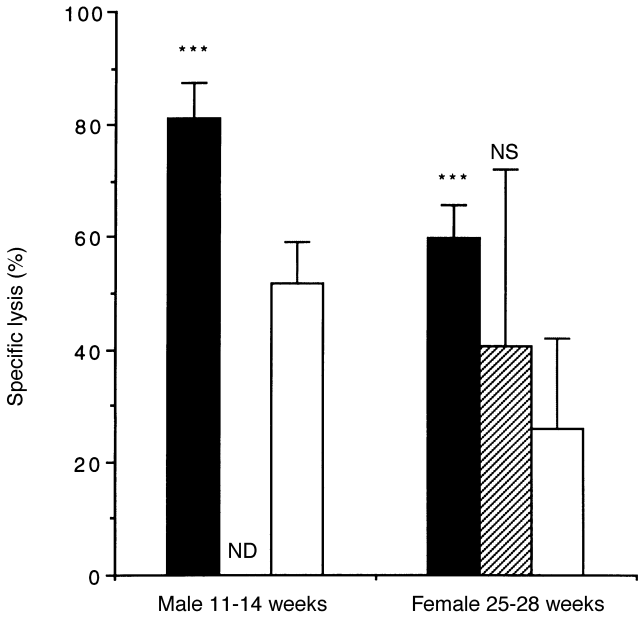

Fig. 4. Comparison of *in vivo* allocytotoxicity in lungs of MRL mice with different *lpr* gene status. MRL +/+ (■), +/*lpr* (▨) and *lpr/lpr* (□) mice were immunized intraperitoneally with freshly isolated DBA/1 spleen cells and 10 days later challenged with concanavalin A-expanded and ^{51}Cr-labelled DBA/1 cells; $n = 5$–10 in each group. The symbols and bars represent means ± s.d. ***$P < 0{\cdot}001$ compared with MRL*lpr/lpr*. NS, Not significant; ND, not done.

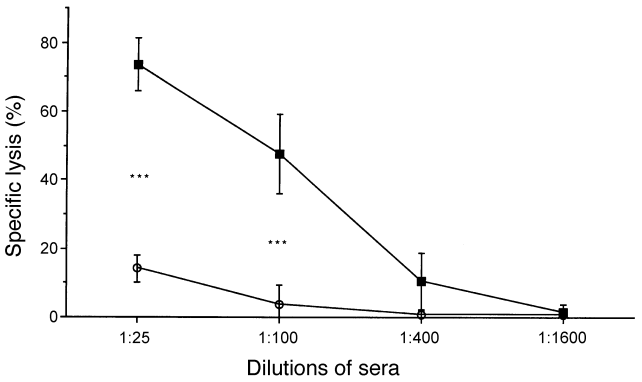

Fig. 5. Comparison of *in vitro* allocytotoxicity in MRL*lpr/lpr* (○) and MRL+/+ mice (■). Sera from 11 to 12-week-old male mice were taken on day 10 after immunization with freshly isolated DBA/1 spleen cells. An *in vitro* cytotoxicity assay was run with concanavalin A-stimulated, ^{51}Cr-labelled DBA/1 spleen cells as target cells. Sera were serially diluted and set in triplicates; $n = 6$ in each group. The symbols and bars represent means ± s.d. ***$P < 0{\cdot}001$.

Neither could naive spleen cells lyse target cells preincubated with immune serum, which made ADCC an unlikely lytic mechanism. Immune serum together with guinea pig serum (as complement source) lysed target cells (see Results). In the absence of immune serum, guinea pig serum by itself or together with non-immune serum failed to lyse target cells.

*Impaired antibody-mediated allocytotoxicity in MRL*lpr/lpr *mice*

MRL mice with different *lpr* gene status were immunized and challenged with allogeneic DBA/1 spleen cells. Specific lysis of target cells in lungs was significantly lower in MRL*lpr/lpr* mice compared with +/+ mice (Fig. 4). A similar degree of suppression of antibody-mediated allocytotoxicity was obtained in young and old female mice. In addition, female heterozygous MRL +/*lpr* mice displayed lytic capacity in between that of matched homozygous +/+ and *lpr/lpr* mice (Fig. 4).

To confirm this *in vivo* finding we set up an *in vitro* assay where serial dilutions of sera from immunized MRL*lpr/lpr* and +/+ mice were mixed with Con A-expanded and ^{51}Cr-labelled DBA/1 cells in medium supplemented with guinea pig serum as a complement source. In Fig. 5 it is clearly demonstrated that sera from immunized MRL +/+ mice induce cytolysis of DBA/1 target cells significantly better than sera from sex- and age-matched MRL*lpr/lpr* mice.

*Increased immunoglobulin levels in young MRL*lpr/lpr *mice*

As shown in Table 1, young premorbid MRL*lpr/lpr* mice had significantly higher levels of immunoglobulins than both MRL +/+ and MRL +/*lpr*. However, there was a tendency in the heterozygous F_1 strain for increased serum immunoglobulin levels, as demonstrated by significantly higher concentrations of IgG2a and IgM compared with the +/+ parental strain (Table 1).

DISCUSSION

Our present study demonstrates that MRL mice homozygous at the *lpr* gene locus display increased *in vivo* natural cytotoxic activity in the lung, compared with homozygous congeneic MRL +/+ and heterozygous MRL +/*lpr* mice. This finding indicates recessively inherited augmentation of natural cytotoxicity in *lpr*-bearing mice. The functional relevance of our finding is further supported using the B16 cell line for melanoma tumour growth in the lungs with 1 month follow-up time, where old male MRL*lpr/lpr* mice cleared primary melanoma metastases more efficiently than MRL +/+ mice. However, MRL*lpr/lpr* mice showed a tendency to have more large metastases, indicative of a defect in the adaptive, T cell-dependent response to established allogeneic tumours.

Our results contradict earlier data, which showed that spleen cells from mice homozygous for the *lpr* gene on different backgrounds (MRL, C3H, C57Bl/6) have depressed *in vitro* NK activity [5,6]. However, hepatic non-parenchymal cells from 2 and 4-month-old MRL*lpr/lpr* mice have been shown to display increased YAC-1 cell killing compared with cells isolated from congeneic matched MRL +/+ mice [17]. These data suggest that NK cell activity in MRL*lpr/lpr* mice is highly variable between different anatomical compartments. Also, the functional capacity of T cells is dependent on the anatomical site studied. Thus, *lpr*-bearing mice display a less aberrant T cell phenotypic distribution in the mucosa-associated lymphoid tissues of lungs and female genital tract compared with peripheral blood and spleen [18].

The role of NK cells in lupus is still obscure. In human SLE as well as in the mouse counterpart systemic NK activity is decreased during the progression of disease [1,2,5–8]. However, in lungs of SLE patients an increased number of $CD56^+/CD16^+/CD3^-$ NK cells has been associated with up-regulated local production of oxygen radicals and with impaired pulmonary diffusing capacity [9]. Lungs of diseased MRL*lpr/lpr* mice show extensive perivascular and peribronchial lymphocyte infiltrates [4]. Our finding that lung NK cells are activated throughout the life span of these mice

Table 1. Serum immunoglobulin levels (mg/ml) ± s.d. in male MRL mice aged 4–7 weeks; $n = 8$–11 in each group

	Immunoglobulins (mg/ml)			
Mice	IgG1	IgG2a	IgG3	IgM
MRL +/+	0·35 ± 0·20** NS	< 0·25*** $P = 0·002$	0·16 ± 0·08* NS	0·05 ± 0·02*** $P = 0·002$
MRL +/*lpr*	0·47 ± 0·55**	0·77 ± 0·58***	0·15 ± 0·08*	0·09 ± 0·02***
MRL*lpr/lpr*	2·90 ± 2·28	3·13 ± 1·36	1·37 ± 1·43	0·17 ± 0·04

Significance of the differences between the means of MRL +/*lpr* and MRL +/+ are also indicated. NS, Not significant. *$P < 0·05$; **$P < 0·01$; ***$P < 0·001$ compared with the level of MRL*lpr/lpr* mice.

could indicate a role for NK cells in the etiology of lung disease. Indeed, Gray [19] has shown that $CD8^+$ T cells can induce production of transforming growth factor-beta (TGF-β) in human NK cells. Increased TGF-β gene expression has been found in bronchoalveolar mononuclear cells from humans with systemic autoimmune disease with lung involvement [20]. In addition, elevated production of TGF-β has been implicated in the defective neutrophil function of MRL*lpr/lpr* mice [21]. Possibly this contributes to the steadily progressive lung fibrosis observed in SLE patients, as TGF-β is known to induce collagen production from fibroblasts *in vivo* [22]. To address this hypothesis studies of cytokine production of lung NK cells as well as lung pathology in B6*lpr/lpr* mice susceptible to long-term depletion of NK cells with monoclonal NK1.1 antibodies are under way.

The mechanism whereby NK cells are activated in lungs of MRL*lpr/lpr* mice is not known. One possibility is that cells in the perivascular infiltrates in the lungs of lupus mice produce interferon-gamma (IFN-γ) and amine inflammatory mediators (histamine, serotonin) known to be potent activators of NK cells [23,24].

The next finding in this study showing decreased antibody-dependent allocytotoxic capacity in lungs and in sera from homozygous *lpr*-bearing MRL mice was more expected. In agreement with many authors we have demonstrated early onset polyclonal B cell activation but weak antigen-specific antibody responses in these mice [25]. Furthermore, lack of production of and responsiveness to IL-2 [26–28] as well as defective proliferative, cytolytic [29] and inflammatory T cell-dependent responses [30] to various antigens are typical immunological aberrancies in MRL*lpr/lpr* mice. Since antibody responses to protein antigens are dependent on T cell help it has been proposed that T cell anergy found in MRL*lpr/lpr* mice is the major factor resulting in failure to produce adequate levels of antigen-specific circulating antibodies after immunization of these mice. However, it can not be ruled out that the high levels of circulating immunoglobulins in itself will *in vivo* impede specific antigen presentation via binding of immunoglobulin to Fc receptors on antigen-presenting cells. Earlier studies [31] have shown that heterozygous *lpr* mice display early life defects of T cell responses as well as polyclonal B cell activation. MRL +/*lpr* mice are also characterized by T cell-mediated disease manifestations such as full-blown sialadenitis and low-grade glomerulonephritis [32]. Again, we show heterozygous MRL +/*lpr* to display a low degree of polyclonal B cell activation and an allocytotoxic response which tends to be intermediate between that of the parental strains.

In conclusion, we show an increased capacity for natural cytotoxicity in the lungs of lupus-prone mice, which precedes lupus disease and persists in spite of it. This points to a different immunological setting in the lung compartment compared with the systemic compartment in lupus, as seems to be the case for human SLE sufferers [9]. Thus, the MRL*lpr/lpr* mouse model could be used to further dissect immunological mechanisms of the lung engagement in systemic autoimmune disease.

ACKNOWLEDGMENTS

We thank Mrs Lena Svensson and Mrs Margareta Verdrengh for their expert technical assistance and Dr Andrzej Tarkowski for valuable comment on the manuscript. This study was supported by grants from the Göteborg Medical Society, the Swedish Society of Medicine, the Swedish Association against Rheumatism, the King Gustav V′s 80 years foundation, the Börje Dahlin foundation, the Medical Faculty of the University of Göteborg (LUA) and the Swedish Medical Research Council.

REFERENCES

1 Altman A, Theofilopoulos A, Weiner R, Katz D, Dixon F. Analysis of T cell function in autoimmune murine strains. J Exp Med 1981; **154**:791–808.
2 Andrews B, Eisenberg R, Theofilopoulos A *et al.* Spontaneous murine lupus-like syndromes. J Exp Med 1978; **148**:1198–215.
3 Carlsten H, Nilsson L-Å, Tarkowski A. Impaired cutaneous delayed-type hypersensitivity in autoimmune MRL lpr/lpr mice. Int Archs Allergy Appl Immunol 1986; **81**:322–5.
4 Carlsten H, Tarkowski A. Expression of heterozygous lpr gene in MRL mice. I. Defective T-cell reactivity and polyclonal B-cell activation. Scand J Immunol 1989; **30**:457–62.
5 Carlsten H, Tarkowski A, Jonsson R, Nilsson L-Å. Expression of heterozygous lpr gene in MRL mice. II Acceleration of glomerulonephritis, sialadenitis and autoantibody production. Scand J Immunol 1990; **32**:21–28.
6 Dauphinée M, Kipper S, Wofsy D, Talal N. Interleukin 2 deficiency is a common feature of autoimmune mice. J Immunol 1981; **127**: 2483–7.
7 Deguchi Y, Kishimoto S. Spontaneous activation of transforming growth factor-beta gene transcription in broncho-alveolar mononuclear cells of individuals with systemic autoimmune diseases with lung involvement. Lupus 1991; **1**:27–30.
8 Erkeller-Yusel F, Hulstaart F, Hannet I, Isenberg D, Lydyard P. Lymphocyte subsets in a large cohort of patients with systemic lupus erythematosus. Lupus 1993; **2**:227–31.
9 Goldschmidt TJ, Holmdahl R, Klareskog L. Depletion of murine T cells by *in vivo* monoclonal antibody treatment is enhanced by adding an

autologous anti-rat κ chain antibody. J Immunol Methods 1988; **111**:219–26.

10 Gray JD, Hirokawa M, Horwitz DA. The role of transforming growth factor β in the generation of suppression: an inter-action between $CD8^+$ T and NK Cells. J Exp Med 1994; **180**: 1937–42.

11 Groen H, Aslander M, Bootsma H, van der Mark ThW, Kallenberg CGM, Postma DS. Bronchoalveolar lavage cell analysis and lung function impairment in patients with systemic lupus erythematosus (SLE). Clin Exp Immunol 1993; **94**:127–33.

12 Habu S, Fukui H, Shimamura K *et al. In vivo* effects of anti-asialo GM1. 1. Reduction of NK activity and enhancement of transplanted tumor growth in nude mice. J Immunol 1981; **127**:34–38.

13 Hanna N, Fidler IJ. Role of natural killer cells in the destruction of circulating tumor emboli. JNCI 1980; **65**:801–9.

14 Hanna N, Schneider M. Enhancement of tumor metastasis and suppression of natural killer cell activity by beta-estradiol treatment. J Immunol 1983; **130**:974–80.

15 Hellstrand K, Asea A, Hermodsson S. Role of histamine in natural killer cell-mediated resistance against tumor cells. J Immunol 1990; **145**:4365–70.

16 Hellstrand K, Hermodsson S. Serotonergic 5-$HT_{1\,A}$ receptors regulate a cell contact-mediated interaction between natural killer cells and monocytes. Scand J Immunol 1993; **37**:7–18.

17 Ibraghimov AR, Lynch RG. T cell specialization at environmental interfaces: T cells from the lung and female genital tract of *lpr* and *gld* mice differ from their splenic and lymph node counterparts. Eur J Immunol 1994; **24**:1848–52.

18 Karashima A, Taniguchi K, Himeno K, Kawano Y-I, Toshitani A, Nomoto K. Does depression of NK activity cause lymphadenopathy in lpr mice? Cell Immunol 1988; **115**:484–90.

19 Kiessling R, Klein E, Wigzell H. 'Natural' killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol 1975; **5**:112–7.

20 Lowrance JH, O'Sullivan FX, Caver TE, Waegell W, Gresham HD. Spontaneous elaboration of transforming growth factor beta suppresses host defense against bacterial infection in autoimmune MRL/lpr mice. J Exp Med 1994; **180**:1693–703.

21 Magilavy DB, Steinberg AD, Latta SA. High hepatic natural killer cell activity in murine lupus. J Exp Med 1987; **166**:271–6.

22 Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965; **2**:235–54.

23 Nilsson N, Carlsten H. Estrogen induces suppression of natural killer cell cytotoxicity and augmentation of polyclonal B cell activation. Cell Immunol 1994; **158**:131–9.

24 Pan L-Z, Dauphinée MJ, Ahmed A, Talal N. Altered natural killer and natural cytotoxic cellular activities in lpr mice. Scand J Immunol 1986; **23**:415–24.

25 Roberts AB, Sporn MB, Assoian RK. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. Proc Natl Acad Sci USA 1986; **83**:4167–71.

26 Scott CF, Tsurufuji M, Lu CY, Finberg R, Sy MS. Comparison of antigen-specific T cell responses in autoimmune MRL/Mp-lpr/lpr and MRL/Mp- +/+ mice. J Immunol 1984; **132**:633–9.

27 Sibbitt WL, Gibbs DL, Kenny C, Bankhurst AD, Searles RP, Ley KD. Relationship between circulating interferon and anti-interferon antibodies and natural killer cell activity in systemic lupus erythematosus. Arthritis Rheum 1985; **28**:624–9.

28 Strannegård Ö, Hermodsson S, Westberg G. Interferon and natural killer cells in systemic lupus erythematosus. Clin Exp Immunol 1982; **50**:246–51.

29 Takeda K, Dennert G. The development of autoimmunity in C57Bl/6 *lpr* mice correlates with disappearance of natural killer type 1-positive cells: evidence for their suppressive action on bone marrow stem cell proliferation, B cell immunoglobulin secretion, and autoimmune symptoms. J Exp Med 1993; **177**:155–64.

30 Theofilopoulos A, Dixon F. Etiopathogenesis of murine SLE. Immunol Rev 1981; **55**:179–216.

31 Theofilopoulos AN, Dixon FJ. Murine models for systemic lupus erythematosus. Adv Immunol 1985; **37**:269–393.

32 Wofsy D, Murphy ED, Roths JB, Dauphinée MJ, Kipper SB, Talal N. Deficient interleukin 2 activity in MRL/Mp and C57BL/6 J mice bearing the lpr gene. J Exp Med 1981; **154**:1671–80.