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# Lymph node cell vaccination against the lupus syndrome of MRL/lpr/lpr mice

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Immunization with pathogenic lymphoid cells has been shown to induce resistance to disease in experimental animal models of cell mediated autoimmunity. In the present work we tested the effectiveness of this approach in a spontaneous murine autoimmune disease, the MRL/lpr/lpr (MRL/1) murine lupus model. We now report that the anti-DNA antibodies and glomerulonephritis of MRL/1 mice could be prevented by the adoptive transfer of spleen cells from MRL/+ mice that had been vaccinated with MRL/1 lymph node T lymphocytes, but not by direct vaccination of MRL/1 mice with their cells. These results indicate that the lupus of MRL/1 mice is susceptible to regulation by adoptive vaccination and that these autoimmune mice lack the ability to raise a suppressive response against their own pathogenic cells.

**Keywords:** Lupus; MRL-lpr/lpr; Vaccination; T cells

## Introduction

Immunization with autoimmune T cells has been shown to induce resistance to disease in experimental animal models of T cell-mediated autoimmune diseases. We have termed this procedure T cell vaccination. T cell vaccination was found to be successful in preventing experimental autoimmune encephalitis<sup>1</sup>, adjuvant arthritis<sup>2</sup> and experimental autoimmune thyroiditis<sup>3</sup>. Remission of established arthritis<sup>4</sup> and therapy of spontaneous mouse autoimmune diabetes<sup>5</sup> could also be achieved by T cell vaccination. The effectiveness of vaccination using cells in a systemic spontaneous autoimmune disease such as lupus has not been studied. To investigate this point, we tested the effect of cell vaccination in the model of lupus manifested by MRL/lpr/lpr (MRL/1) mice.

MRL/1 mice express the lpr gene which appears to be responsible for a massive proliferation of T cells in their lymph nodes. The mice appear healthy for the first 3 months of life after which the defective T cells begin proliferating<sup>6,7</sup>. The majority of these T cells are CD4-, CD8-, CD3+ and they appear to be in a state of spontaneous activation. They comprise most of the cells in the lymph nodes of 6 month old MRL/1 mice<sup>7,8</sup>. These T cells were shown to promote the production of autoantibodies, including anti DNA antibodies which

are deposited in the glomeruli and produce severe glomerulonephritis<sup>9-11</sup>.

In the present work, we tested two models of vaccination with the T cell enriched lymph node population of 6 month old MRL/1 mice: active and adoptive. In the active vaccination protocol, we vaccinated 6-week-old MRL/1 mice with sick MRL/1 lymph node cells. In the adoptive protocol, we first vaccinated MRL/+ congenic mice, which lack the lpr allele, against lymph node cells of 6-month-old MRL/1 mice. The anti-MRL/1 spleen cells of the MRL/+ mice were then transferred to 6-week-old MRL/1 mice. We now report that the lupus syndrome of MRL/1 mice can be suppressed by adoptive transfer of anti MRL/1 spleen cells. The malignant proliferation of MRL/1 T cells however was not affected. In contrast to adoptive vaccination, active vaccination of young MRL/1 mice did not have any effect on the course of their disease.

## Material and methods

### *Mice*

Female Mrl/lpr/lpr (MRL/1) and MRL/+ mice, 4 week old, were purchased from the Jackson Laboratories, Bar-Harbor, Maine, USA.

### *Materials*

H-labeled DNA was purchased from New England Nuclear, Boston, USA. Glutaraldehyde (25% solution)

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was purchased from Sigma Chemical Co. St. Louis, MO USA.

#### Culture medium

Dulbecco's modified Eagle's medium was purchased from Biological Industries, Kibbutz Beth Haemek, Israel. The culture medium contained 4.5 g/l of D-glucose, 1% sodium pyruvate, non-essential amino acids, buffer (pH 7.3), antibiotics, L-glutamine and 0.05% dimercaptoethanol.

#### Binding of antibodies to DNA

Antibody binding to DNA was measured using the Millipore filter assay<sup>12</sup>. Sera were prepared in serial dilutions in 0.2 M borate saline buffer (pH 8.0). Ten  $\mu$ l of <sup>3</sup>H-labeled DNA (approximately 6000 cpm) were added to 100  $\mu$ l of the antibody in various dilution's. The mixture was incubated at 37°C for 30 min and for an additional 60 min at 4°C, and then was aspirated through 0.45  $\mu$ m nitocellulose filters (Milford, Bedford, MA) by vacuum. The filters were washed with two aliquots of 3 ml borate buffer and placed separately in plastic vials. The filters were dried by incubation at room temperature for at least 16 h and read with toluene based scintillation fluid in a beta counter.

#### Active vaccination

Cells were obtained from lymph nodes and spleens of 6 month old MRL/1 mice or from MRL/+ mice. The cells were treated with 0.3% glutaraldehyde solution for 15 min<sup>13</sup> and then washed four times with PBS. Active vaccination was done with  $20 \times 10^6$  cells administered i.p. into 6-week-old MRL/1 or MRL/+ mice. The vaccination was repeated 10 days later. The mice were then observed for development of lupus.

#### Adoptive vaccination

Six-week-old MRL/+ were actively vaccinated as above. One week after the second vaccination the MRL/+ mice were killed and  $20 \times 10^6$  spleen cells were adoptively transferred i.v. into 6-week-old MRL/1 mice. The adoptive transfers were repeated 10 and 20 days later. The same protocol was used for the adoptive vaccination in groups 3 and 4.

#### Treatment protocols

Table 1 summarizes the treatments of four groups of 20–30 six-week-old MRL/1 mice: Group 1 was treated

**Table 1** Treatment protocols of MRL/L mice

Group	Treatment
1	PBS
2	Active vaccination with MRL/1 lymphocytes
3	Adoptive transfer of spleen cell from MRL/+ mice vaccinated with MRL/1 lymph node cells
4	Adoptive transfer of spleen cells from MRL/+ mice vaccinated with MRL/+ lymph node cells

with PBS. Group 2 was treated by active cell vaccination. These mice were actively vaccinated twice with spleen cells derived from 6-month-old MRL/1 mice suffering from overt lupus. Groups 3 and 4 were tested for the effect of adoptive transfer of immunized MRL/+ spleen cells. Mice in group 3 received three i.v. injections of spleen cells from MRL/+ mice that had been actively vaccinated twice with MRL/1 cells. The mice in group 4 received three i.v. injections of spleen cells from MRL/+ mice that had been vaccinated twice with MRL/+ lymphocytes. At the age of 6 months, serum and urine samples were taken from five mice in each group, and tested individually for urinary protein excretion and for serum anti-DNA antibody levels. The mice were then killed and their kidneys examined by light microscopy and immunofluorescence for glomerulonephritis. The spleens and lymph nodes were examined for lymphoid hyperplasia. The whole experiment was repeated twice with similar results.

#### Light microscopy

Tissue samples obtained from kidneys, lymph nodes and spleens were fixed in 10% formalin and embedded in paraffin. Six  $\mu$ m sections were stained with hematoxylin–eosin and PAS stains.

Morphological parameters of autoimmune disease were evaluated by an observer blind to the groups. The code was broken only at the end of the entire study. Histology was graded as follows: normal kidneys, or mild glomerulonephritis (glomeruli slightly hypercellular), +1; moderate glomerulonephritis (glomeruli hypercellular with focal changes and foci necrosis in a few glomeruli), +2; severe glomerulonephritis (diffuse proliferative glomerulonephritis with crescents and necrosis, some glomeruli are completely destroyed or fibrotic and there is tubular atrophy), +3.

#### Immunofluorescent staining

Cryostat sections from snap frozen kidneys of the above mice were rinsed in phosphate buffered saline (PBS, pH = 7.4), and incubated for 60 min at room temperature with rabbit antibodies to mouse immuno-

globulins conjugated with fluorescein 1/20 (Dakopaths, Denmark).

### Statistical methods

The chi square analysis was used to test differences in mortality during the last months of life between group 3 and the other groups. The rest of the statistical evaluation was carried out using the Mann-Whitney-Wilcoxon test to compare differences between the groups.

## Results

### Effect of active vaccination of MRL/1 mice

We actively vaccinated 6-week-old MRL/1 mice with lymph node cells derived from 6-month-old MRL/1 mice and observed the mice. As can be seen in Tables 2 and 3, active vaccination of MRL/1 mice decreased the anti-DNA antibody level. This change however was not statistically significant and no effects on the amount of urinary protein and on mice survival were observed. Histopathological findings in the kidneys of these mice (group 2), were similar to the findings in the control

**Table 2** The effect of active and adoptive vaccination on urinary protein and serum anti-DNA antibody level<sup>a</sup>

Group <sup>b</sup>	Serum anti-DNA <sup>c</sup> (mean cpm $\pm$ SE)	Urinary protein <sup>e</sup>
1	2088 $\pm$ 244	+3 < 500 mg/dl
2	1695 $\pm$ 336	+3 < 500 mg/dl
3	1279 $\pm$ 124 <sup>d</sup>	traces < 30 mg/dl
4	2221 $\pm$ 365	+3 < 500 mg/dl

<sup>a</sup> Representative results of one of two experiments.

<sup>b</sup> See Table 1.

<sup>c</sup> Serum was tested by a filter assay (12).

<sup>d</sup>  $P < 0.05$  as compared to groups 1 and 4.

<sup>e</sup> Tested by Albustick (Meditest, Macherey-Nagel). Results did not differ between individual mice in each group.

**Table 3** The effect of active and adoptive vaccination on survival

Group	No. of mice	Median age of death (weeks)	% survival at 6 months
1	20	18	35
2	20	16	35
3	30	24	50 <sup>a</sup>
4	20	18	25

<sup>a</sup>  $P = 0.06$  compared to group 4.

mice (group 1). All the mice in both groups developed moderate to severe glomerulonephritis. Most of the mice had diffuse proliferative glomerulonephritis with capillary loop necrosis of affected glomeruli and hyaline deposits similar to the hyaline thrombi described in human SLE.

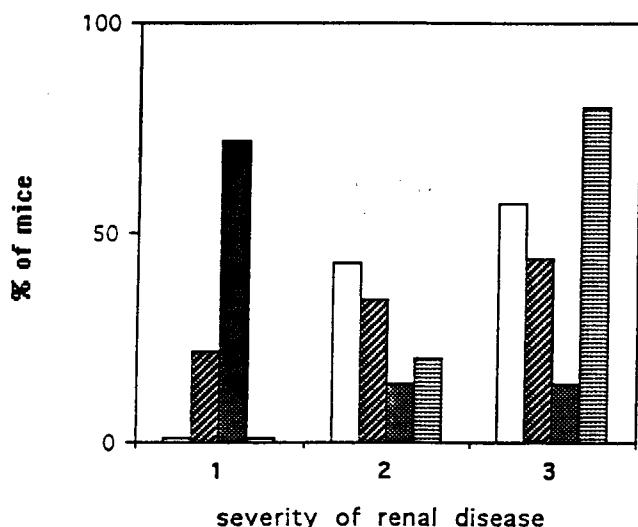
Wire loop lesions were a common finding and many of the glomeruli showed crescent formation. Secondary tubular atrophy was also noted.

Immunofluorescence study of these kidneys showed extensive global granular peripheral and mesangial deposits of immunoglobulin.

### Effect of adoptive transfer of vaccination

To test whether adoptive cell vaccination could have an effect on the development of lupus, we immunized MRL/+ mice with MRL/1 or MRL/+ cells, and transferred their spleen cells to young, 6-week-old MRL/1 mice (groups 3 and 4). In contrast to active vaccination, adoptive transfer of anti- MRL/1 cells (group 3) was effective. There was a significant decrease in the levels of anti-DNA antibodies in the serum and in the excretion of protein in the recipient MRL/1 mice (Table 2).

The histopathological findings are shown in Figure 1. All the mice in groups 1 and 4 developed moderate to severe glomerulonephritis. In contrast most of the mice in group 3 manifested kidneys with either normal glomeruli or with mild glomerulonephritis with some



**Figure 1** Severity of glomerular lesions in MRL/1 mice treated with PBS (group 1, □) or vaccinated with lymph node cells from an adult MRL/1 mouse [group 2, ▨, or treated with adoptive transfer of cells from MRL/+ mice that were vaccinated with MRL/1 cells (group 3, ▨) or with MRL/+ cells (group 4, ▨). and 3 are grades of severity of the nephropathy, as described in the methods.

mesangial hypercellularity and increase of mesangial matrix.

Immunofluorescence examination of the mildly affected kidneys showed fine granular peripheral segmental deposits of immunoglobulin. Some of the kidneys had predominant involvement of the mesangium with diffuse segmental deposits. Ranking of the renal light microscopy was significantly lower in group 3 compared to the other groups ( $P < 0.01$ ). However, there was no noticeable decrease in the lymph node and spleen lymphoid hyperplasia. Table 3 shows that the proportion of deaths during the last 3 months of life was marginally lower in group 3 than in group 4 ( $P = 0.06$ ).

## Discussion

In the present work we studied whether vaccination with MRL/1 lymphoid cells can lead to suppression of spontaneous lupus. Adoptive transfer of spleen cells from immunized MRL/1 mice was shown to suppress the development of lupus in the MRL/1 mice. Both anti DNA antibodies and kidney disease were suppressed. This effect was specific for spleen cells obtained from MRL/+ mice that had been vaccinated with MRL/1 cells (group 3). The lack of effect after direct vaccination with MRL/1 cells (group 2), indicates that the effects is due to the MRL/+ cells and not to contamination of the MRL/+ spleen cells by the vaccinating MRL/1 cells. No effect was observed when the cells were transferred from MRL/+ mice that were vaccinated with MRL/+ cells, indicating that suppression of lupus cannot be obtained merely by transfer of MRL/+ cells to the MRL/1 mice, but that it most probably resulted from anti-MRL/1 immunity which had been induced by vaccination of the donor MRL/+ mice. This suggests that MRL/+ mice can respond to at least one antigen on MRL/1 cells. Moreover, the responding MRL/+ lymphocytes must have been able to operate in the MRL/1 mice and suppress the mechanism responsible for lupus. Since MRL/1 and MRL/+ mice differ only at the lpr locus<sup>7</sup>, it is possible that the target MRL/1 antigen is a direct product of the lpr gene or dependent indirectly on the activity of the lpr gene. Further analysis of the putative target antigen could be facilitated by development of an *in vitro* assay of reactivity of vaccinated MRL/+ mice to MRL/1 cells. However, we have not yet succeeded in developing such an assay.

The lpr gene appears to be expressed in T cells and results in the aberrant expression of the fas gene product, a cell surface protein capable of inducing an apoptotic signal<sup>14</sup>. As a result of the mutations in this gene the activation-induced cell death is rendered inoperative, leading to a defect in the elimination of lymphocyte that

have been propagated in response to antigenic stimulation as observed in the MRL/1 mice<sup>15</sup>. Although the events leading from this accumulation of lymphocytes to the production of autoantibodies and thereafter to glomerulonephritis in lupus are not fully understood, there is evidence indicating that T lymphocytes play a major role in this process. MRL/1 mice, but not MRL/+ mice, spontaneously develop massive T cell proliferation and show abnormalities in T cell function<sup>7</sup>. The importance of T cells has been confirmed in experiments in which thymectomy prevented disease in MRL/1 mice; subsequent transplantation of the thymus restored the disease<sup>16,17</sup>. It was also shown that T cells derived from MRL/1 mice produce factors that induce secretion of immunoglobulins by unstimulated B cells, including the production of anti DNA antibodies<sup>10,11</sup>. Although proliferating T cells in MRL/1 mice are polyclonal, a preferential subset of lymphocytes expressing V $\beta$  8 genes was described<sup>18</sup>. Similarly, a higher frequency of autoreactive T cells recognizing self-MHC epitopes was found<sup>10</sup>. The existence of an unusual population of CD4-/CD8-TCR+ T cells in the blood of lupus patients has also been described<sup>19</sup>. These T cells were shown to induce the production of pathogenic cationic anti-DNA antibodies *in vitro*. Treatment of MRL/1 mice with monoclonal antibodies directed to all T cells, or to CD4+ T-cells was found to suppress disease<sup>20</sup>. Thus, T cells may be critical in both human and MRL/1 lupus.

Note that the MRL/+ spleen cells suppressed the expression of lupus nephritis in the recipient MRL/1 and postponed the median day of death, but they did not abolish the mortality due to the massive lymphoproliferation of T cells. This indicates that the MRL/1 lupus syndrome can be separated from the T cell proliferative disease and that the lupus syndrome is more susceptible to manipulation than is the T cell proliferation. In contrast to the effect of adoptive vaccination, active vaccination of young 6-week-old MRL/1 mice with lymphoid cells of 6-month-old MRL/1 mice did not lead to suppression of disease. No significant suppression of anti-DNA antibodies or kidney disease was noticed in the vaccinated mice.

A previous report<sup>21</sup> showed, in contrast to our results, that active T cell vaccination significantly reduced the development of the interstitial nephritis, ulcers, arthritis, lymphadenopathy and anti DNA in MRL/1 mice. One explanation for the differences is the amount of cells used to vaccinate. De Alborian *et al.*<sup>21</sup> used as few as  $2.5 \times 10^5$  cells per mouse, while we injected  $20 \times 10^6$  cells/mouse. Indeed, when De Alborian used  $5 \times 10^6$  cells to vaccinate, no beneficial result was obtained, similar to our findings.

It is possible that MRL/1 mice are not able to respond to the MRL/1 marker in the way that MRL/+

mice are able to respond. MRL/1 mice may be tolerant to the large amount of the MRL/1 marker to which MRL+ mice can respond. However, small amounts of lpr cells may cause an escape from such tolerance, and induce the accumulation of protective T lymphocytes, as may occur in MRL/+ mice treated with large amounts of lpr cells. The failure of MRL/1 mice to mount an immune response against their own bulk of pathogenic cells may actually form the basis for the development of their chronic autoimmune disease.

The mechanisms of vaccination of the MRL/+ mice against MRL/1 cells are now under study. T cell vaccination against autoimmune diseases is usually done with syngeneic autoimmune T cells. In the present experiments, we do not know if the immunogenic elements in the vaccine of MRL/1 lymphoid cells were actually the abnormal MRL/1 T cells. The vaccine certainly contained these T cells because they constitute the vast majority of cells in the lymphoid organs. Moreover in T cell vaccination against autoimmune disease, the target antigen on the autoimmune T cells appears to be the antigen receptor<sup>22</sup> or a peptide of the antigen receptor<sup>23</sup>. In the present experiment we have no information about the lpr marker – which is most probably not an antigen receptor. Nevertheless, the experiments reported here indicate that the lupus-like autoimmune syndrome of MRL/1 mice, like the other autoimmune disease models, is susceptible to manipulation by a form of cellular vaccination. Note however, that the mice in need of treatment could not themselves respond actively to the vaccination. An immune system that is intrinsically defective may not be able to repair itself.

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