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PAPER

Immunization with a bacterial ATP-binding cassette transporter fragment suppresses autoimmunity and prolongs survival in MRL/*lpr* lupus-prone mice

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The present study was undertaken to better define the role of the U1 70 kDa antigen in a spontaneous murine model of systemic lupus erythematosus (SLE) by testing whether immunization with the U1 70 kDa polypeptide could alter the production of antibodies against U1 70 kDa or against other small nuclear ribonucleoproteins (snRNP), modify disease expression or alter survival. We found that, while immunization with a U1 70 kDa derived fusion protein (70 KFP) tended to delay the development of anti-snRNP antibodies in the sera of MRL/*lpr* mice, it had no effect on autoimmune-mediated renal disease or survival. Unexpectedly, it was found that MRL/*lpr* mice immunized with a 367 amino acid fragment of a bacterial ATP-binding cassette transporter, MFP, had prolonged survival compared to saline injection or U1 70 kDa immunization and that this was associated with a delay in the onset of SLE-like proliferative glomerulonephritis. This is the first study, to our knowledge, in which a bacterial ATP-binding cassette transporter was shown to be beneficial in treating a murine model of SLE. We report that MFP significantly prolonged longevity in the MRL/*lpr* murine model of SLE compared to saline injection or 70 KFP immunization and that improved survival was associated with a delay in the onset of SLE-like glomerulonephritis. *Lupus* (2000) **9,** 655–663.

Keywords: systemic lupus erythematosus; ATP binding cassette; small nuclear ribonucleoprotein; chaperone

Introduction

Systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD) are characterized by the presence of high levels of autoantibodies against complexes consisting of small nuclear ribonucleoprotein (snRNP) antigens associated with uridine-rich RNA (U), including U1 70 kDa, U1-6-Sm-B and U1-6-Sm-D.[1] The presence of anti-U1 70 kDa autoantibodies is associated with specific clinical manifestations of disease, including Raynaud's phenomenon, esophageal dysmotility, sclerodactyly and myositis.[2] Whether anti-U1 70 kDa antibodies have a direct role in mediating tissue injury and disease pathogenesis or are merely markers of disease remains to be determined. The following evidence has been published to support the concept that the U1 70 kDa antigen is itself central in driving the anti-U1 70 kDa immune response: (1) antibodies and T cells reactive with U1 70 kDa can be detected in human disease and in MRL/MpJ-*lpr* (MRL/*lpr*) lupus-prone mice;[1–5] (2) antibodies against U1 70 kDa are directed against functional domains of U1 70 kDa antigen;[6,7] (3) antibodies against U1 70 kDa exhibit isotype switching;[8] (4) T and B cell responses against U1 70 kDa are linked *in vivo*;[9] and (5) U1 70 kDa antigen is structurally modified during apoptotic cell death.[10,11]

As a model of human disease, MRL/*lpr* mice possess features of SLE and MCTD and have autoantibodies against snRNP polypeptides, including U1 70 kDa, Sm-B and Sm-D.[4,6,12,13] MRL/*lpr* mice are, therefore, a potentially useful model for studying the role of the anti-U1 70 kDa immune response in tissue injury and disease pathogenesis.

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In an attempt to better understand the role of snRNP polypeptides in disease pathogenesis in SLE, other investigators have tested the ability of the snRNP polypeptides, including Sm-B, Sm-D and U1-A, to modulate the immune response.[5,14–17] While peptide fragments of Sm-B and Sm-D can elicit an immune response, reports have differed on the clinical effects of such immunizations. Harley and colleagues reported that immunization of rabbits and mice with Sm-B-derived peptides could induce anti-Sm antibodies and cause a lupus-like syndrome in these animals, and intramolecular spreading of the immune response occured against both the U1 70 kDa particle of the snRNP complex and double-stranded DNA (dsDNA).[14,15] In contrast, Manson *et al* and Winska-Wiloch *et al* reported that immunization of New Zealand White rabbits, BALB/c, B10/brown and lupus-prone MRL/*lpr* mice with Sm-B or Sm-D-derived peptides resulted in an anti-peptide immune response, without enhancement of the production of pathogenetic anti-Sm antibodies, intramolecular spreading of the response to U1 70 kDa or dsDNA, or disease inducing or modifying effects.[16,17] Craft and colleagues demonstrated that immunization of normal B10.BR and C57BL/6J mice with a fusion protein of the human U1-A protein elicited anti-snRNP antibody production.[5] Immunization with recombinant human U1-A protein resulted in intramolecular spreading to other snRNP polypeptides, including U1 70 kDa.[5] Mozes and colleagues have shown that peptides derived from the complementarity-determining regions of an anti-DNA antibody bearing the 16/6 idiotype can induce experimental lupus in mice and have shown that a similar peptide is a dominant T cell epitope in NZB/NZW F_1 lupus-prone mice.[18,19] Finally, it has been shown that autoantigen-derived peptide immunization can result in amelioration of some autoimmune diseases. As examples, spontaneous onset diabetes mellitus in NOD mice, experimental myasthenia gravis in B6 mice, and experimental antiphospholipid syndrome in BALB/C mice were all suppressed by autoantigen-derived peptide immunization.[20–22]

The periplasmic maltose binding protein malE is one of a series of bacterial ATP-binding cassette (ABC) transporters that function together to form a pump that hydrolyses ATP to translocate substrate across cytoplasmic membranes.[23] A 367 amino acid fragment of MBP malE (MFP) has been widely utilized as a soluble fusion protein partner, which permits rapid purification of expressed fusion proteins.[24] MFP and its parent molecule, malE, have been shown to be capable of functioning as molecular chaperones, assisting in protein folding and intracellular transport of proteins.[25,26] Previously published studies, to our knowledge, have not examined the immunomodulatory effects of bacterial ATP-binding cassette transporters, such as malE. Other molecular chaperones, however, including heat shock protein (Hsp)-70 and Hsp-65, can significantly suppress autoimmune diseases.[27,28]

The present study was undertaken to better define the role of the U1 70 kDa antigen in autoimmune disease, by testing whether immunization with the U1 70 kDa polypeptide could modify disease expression in MRL/*lpr* mice. We did observe that immunization with a molecularly cloned human U1 70 kDa antigen (70 KFP) tended to suppress or delay subsequent development of anti-U1 70 kDa antibodies in MRL/*lpr* mice, although this trend did not achieve statistical significance. Treatment with 70 KFP had no effect upon survival, the production of anti-dsDNA antibodies, renal function or the pathologic development of proliferative glomerulonephritis. Unexpectedly, it was found that MRL/*lpr* mice immunized with a 367 amino acid fragment of a bacterial ATP-binding cassette transporter, MFP, had prolonged survival compared to saline injection or U1 70 kDa immunization and increased longevity was associated with delay in the onset of glomerulonephritis.

Materials and methods

Mice

Female MRL/*lpr* mice were purchased from Jackson Laboratories, Bar Harbor, ME, USA and maintained in American Association for Accreditation of Laboratory Animal Care-approved, pathogen-free conditions. Mice were assigned randomly to treatment and control groups and injected beginning at 6 weeks of age.

Longevity protocol

Mice were bled by venipuncture of the retro-orbital plexus under anesthesia at the start of the study and at intervals of 8 weeks. Mice were examined daily and any mouse that showed evidence of entering a phase of rapid terminal decline was sacrificed. The characteristics of terminal decline, which were consistent in severely ill animals, were significant weight loss (>15% body weight per week), edema and loss of vigor. Complete necropsies were performed. Organ samples were collected and placed in buffered formalin and processed for subsequent histologic

analysis, and hematoxylin and eosin-stained tissues were examined by light microscopy. Causes of death were determined based upon gross examination of the animals during necropsy and examination by light microscopy of tissues obtained at necropsy.

Antigens

The human U1 70 kDa protein was fused to a 367 amino acid fragment of the *E. coli* bacterial ATP-binding cassette transporter, malE, in the pMAL-c2 plasmid construct (New England Biolabs, Beverly, MA, USA). The final construct of U1 70 kDa and its expressed protein are subsequently referred to as 70 KFP. Fusion proteins from 70 KFP and MFP were expressed from their respective plasmids in *E. coli* and purified under otherwise identical conditions, as previously described.[9] The proteins were analyzed for purity by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting before injection into mice.[9] Antigens were analyzed for the presence of lipopolysaccharide contamination (Pierce, Rockford, IL, USA) and none was detected.

Immunization protocol

Mice ($n = 15$) in the 70 KFP group received one intraperitoneal injection of 100 µg of 70 KFP emulsified in 200 µl of complete Freund's adjuvant (CFA) (Sigma, St, Louis, MO, USA) as a primary immunization. Secondary immunizations were given as 100 µg of 70 KFP emulsified in 200 µl of incomplete Freund's adjuvant (IFA) (Sigma). Mice ($n = 15$) in the MFP group received one intraperitoneal injection of 100 µg of MFP emulsified in 200 µl of CFA as a primary immunization. Secondary immunizations were given as 100 µg of MFP emulsified in 200 µl of IFA. Mice ($n = 15$) in the saline control group received 200 µl 0.9 saline. Each injection in the secondary immunization series was given intraperitoneally once a week for 4 weeks, starting 1 week after the primary immunization.

Detection of antibodies against small nuclear ribonucleoprotein and double-stranded DNA

The antigens used in the snRNP enzyme-linked immunosorbent assay (ELISA) were purified from rabbit thymus acetone powder (Pelfreez Biological, Roger, AR, USA) as described previously.[3] ELISA was done on sera obtained at 8 week intervals and from terminally ill mice.[2,29] Briefly, 96-well microtiter plates (Fisher, Pittsburgh, PA, USA) were coated with antigen, blocked with 1% non-fat milk and incubated with phosphate buffered saline (PBS) diluted mouse sera. After incubation, plates were washed with PBS with 0.025% Tween-20 four times, reincubated with peroxidase conjugated goat anti-mouse antibodies (Sigma), and visualized with addition of 100 µl of 20 µg/ml of *O*-diphenylenediamine dihydrochloride (Sigma) in 0.1 M citrate buffer with 0.01% hydrogen peroxide. Plates were read at 450 nm in a Microplate Autoreader (BIO-TEK Instrument, Winooski, VT, USA). Positive and negative controls were included in each experiment. Background absorbance of control wells was subtracted in calculating specific absorbance of antigen-coated wells.

Immunoblotting was done on serum samples to characterize the presence of autoantibodies reactive with individual U1 70 kDa U1-A, Sm-B and Sm-D snRNP polypeptides, as described.[9,30] Briefly, Jurkat cellular proteins were separated by SDS-PAGE and transferred onto nitrocellulose paper, which was then incubated with PBS diluted mouse sera (1:200), followed by washing and secondary incubation with peroxidase conjugated goat anti-mouse antibody. Specific binding was visualized using 4-chloro-1-naphtol as substrate. As positive controls, well characterized human sera were used in each experiment[9–31] normal sera served as negative controls.

Anti-dsDNA antibodies were measured in a modification of the Farr assay and reported as percent of ^{14}C-labeled DNA (DuPont, Boston, MA, USA) bound by 15 µl of mouse serum. Values greater than 20% binding indicated the presence of anti-dsDNA antibodies.[32,33]

Measurement of blood urea nitrogen and urinary protein

Urine was tested for albumin and graded on a scale of $0-4+$ according to a color chart provided by the manufacturer (Albustix, Bayer, Elkhart, IN, USA). A reading of $3+$ corresponds to a urine albumin concentration of at least 300 mg/dl, and a reading of $4+$ corresponds to >2000 mg/dl. Blood urea nitrogen (BUN) was measured in a colormetric assay (Sigma), and results were reported as mg/dl. The mean BUN level in stored frozen serum from normal female mice in this laboratory is 30 mg/dl.[34]

Renal histology and necropsy

Severity of glomerulonephritis was scored using a glomerular lesion count scoring system, in which it was determined that a numerical score ≥ 35 indicated the presence of proliferative glomerulonephritis. The number of specific abnormalities in 20 gloneruli in a 4 μm thick hematoxylin and eosin-stained cross section of each kidney were recorded. The glomerular abnormalities that were scored were: mesangial stalk thickening, mesangial stalk hypercellularity, focal glomerular hypercellularity, glomerular basement membrane thickening, generalized glomerular hypercellularity, fibrinoid degeneration, and crescent formation.[35]

Statistical analysis

Data were analyzed using Prism software package (GraphPad Software, San Diego, CA, USA) using Student's *t*-test for ordinal data and the binominal test for paired data. Survival was analyzed using Kaplan–Meier life table method. Results were expressed as mean ± standard error (s.e.).

Results

Survival was prolonged in the MFP treatment group

Contrary to the original hypothesis, 70 KFP immunization did not alter survival in MRL/*lpr* mice when compared to saline injection or MFP immunization (Figure 1). In contrast, MFP immunization did significantly prolong the survival of MRL/*lpr* mice compared to mice injected with either saline ($P = 0.005$) or 70 KFP ($P = 0.018$). The survival of the 70 KFP immunization group paralleled that of the saline injected control group. Injection of 70 KFP did not protect against or accelerate disease.

Saline injected control animals started to show signs of disease and die as early as 15 weeks of age, and only 50% of the saline-injected controls survived to 21 weeks of age. Similar to saline injected mice, 70 KFP immunized mice had 50% mortality at 22 weeks of age. In marked contrast, MFP immunized mice had improved longevity and 50% mortality was significantly prolonged to 34 weeks of age.

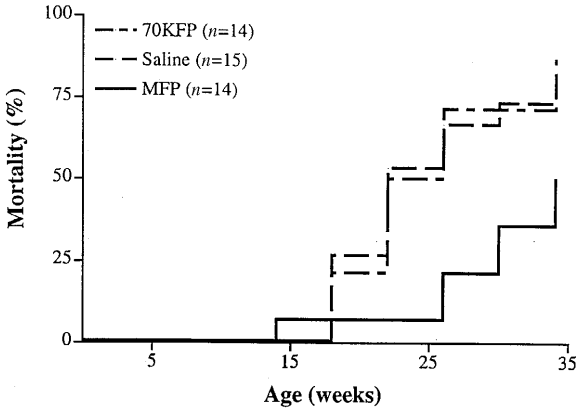

Figure 1 Mortality versus time (weeks). Two early deaths, one in the 70 KFP group and one in the MFP group, resulted from injection accidents and mortality was calculated on the basis of $n = 14$ mice at risk in each of these groups. The plot is carried out to 33 weeks, at which point 50% of the mice in the MFP group had died spontaneously of autoimmune renal disease and vasculitis. Survival was significantly prolonged in the MFP treatment group when analyzed by the method of Kaplan–Meier versus 70 KFP immunization ($P = 0.018$) and versus saline injection ($P = 0.005$). 34 of the 45 mice in the study died with glomerulonephritis and arteritis. The BUN concentration in terminal serum was greater than 85 mg/dl in 26 of these animals, and this finding supported the impression that autoimmune renal disease and renal failure resulted in death. Two mice in the 70 KFP group, 2 saline control mice and 5 mice in the MFP group were killed at the age of 38 weeks to end the study and are not included in this figure.

Immunization with MFP or 70 KFP delays development of anti-small nuclear ribonucleoprotein polypeptide antibodies

To determine the effect of immunization with a U1 70 kDa derived fusion protein, 70 KFP, on the level of total anti-U1-snRNP antibodies, these were quantitated in mice from all groups by ELISA on sera obtained at baseline and at 8 week intervals from surviving mice to the age of 38 weeks (Figure 2). At 14 and 22 weeks of age, the 70 KFP and MFP immunized groups exhibited a trend toward lower levels of anti-snRNP antibodies compared to the saline injected group. This apparent suppression reached statistical significance only in the MFP immunized group versus the saline injected group ($P = 0.038$) at 14 weeks of age.

To characterize individual anti-snRNP polypeptide reactivities present in individual serum samples, immunoblotting was done on all surviving mice in each group at baseline and at 14, 22, 30 and 38 weeks of age. Mice were uniformly negative at the age of 6 weeks. Table 1 displays results of serial testing in each mouse that had antibody reactivities that were detected as clearly delineated bands. Results confirmed the ELISA findings. 70 KFP and MFP

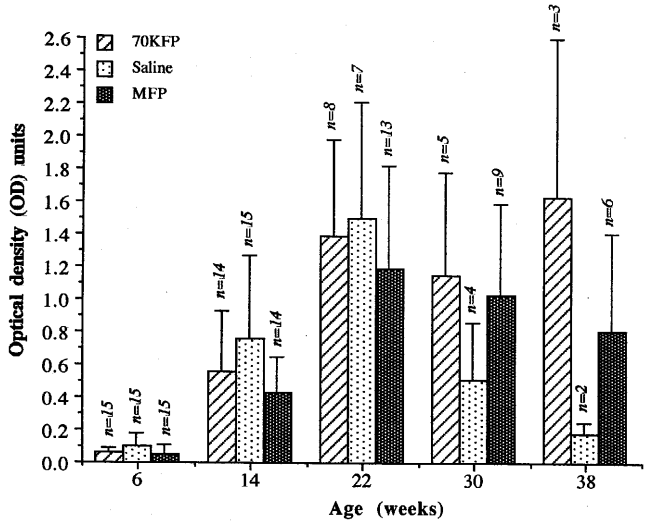

Figure 2 Anti-snRNP antibodies. Sera were obtained starting at 6 weeks of age and at 8 week intervals. Anti-U1-snRNP antibodies were quantitated by ELISA using immunoaffinity purified snRNP from rabbit thymus acetone powder as an antigen. At 14 and 22 weeks of age the 70 KFP and MFP immunized groups had tendencies to have lower levels of anti-snRNP antibodies compared to the saline-injected group. The apparent reduction in anti-snRNP antibodies was statistically significant only in MFP versus saline ($P = 0.038$). At later time points there were no significant differences between the groups. The number of mice surviving in each group is shown above the error bars.

immunization tended to delay the onset of anti-snRNP polypeptide antibody production. At 14 weeks of age, sera from 6/15 (40%) of saline injected mice had detectible antibodies to Sm-B and/or Sm-D snRNP polypeptide antigens, but only 3/14 (21%) sera from 70 KFP immunized, and 3/14 (21%) sera from MFP immunized mice had detectible autoantibodies to U1 70 kDa and/or Sm-B/Sm-D antigens. At 22 weeks of age, 4 mice from the 70 KFP injected group developed new reactivity to U1 70 kDa or Sm polypeptide and 3 mice from the MFP immunized group developed new reactivity to U1 70 kDa or Sm polypeptides. In contrast, only one of the survivors in the saline injected group showed additional new snRNP polypeptide autoantibody reactivity. At 30 weeks of age, a mouse from the MFP immunized group developed reactivity with U1 70 kDa, Sm-B and Sm-D for the first time. Of the animals which survived, anti-snRNP polypeptide reactivities were sustained in all except for 2 mice in the MFP injection group, which lost reactivity to Sm after 22 weeks of age.

Anti-dsDNA antibodies were not lowered in treatment groups

Anti-dsDNA activity is shown in Figure 3. There was a trend for the anti-dsDNA binding to be higher in the 70 KFP and MFP immunized groups compared to the saline injected group at 22, 30 and 38 weeks of age, but these differences were only statistically significant at 30 weeks of age; 70 KFP versus saline ($P = 0.024$) and MFP versus saline ($P = 0.029$). Immunization with 70 KFP did not enhance anti-dsDNA antibodies at 14 or 22 weeks of age.

Renal function was maintained and renal histologic severity scores were lower in the MFP treatment group

As shown in Figure 4A, animals with urine albumin excretion of 300 mg/dl or greater were observed in all groups as early as 14 weeks of age. At 30 weeks of age, the MFP immunized group had significantly fewer mice with 3+ to 4+ albumin excretion compared to saline injected controls ($P = 0.012$). Figure 4B shows that there was a trend of increasing BUN concentration in all treatment groups over time. This increase in BUN was least prominent in MFP immunized animals when compared to the 70 KFP immunized or saline injected

Table 1 Anti-snRNP polypeptide reactivity among 70 KFP immunized, MFP immunized and saline injected mice as detected by immunoblotting

70 KFP (age in weeks)				*MFP (age in weeks)*				*Saline (age in weeks)*			
14	*22*	*30*	*38*	*14*	*22*	*30*	*38*	*14*	*22*	*30*	*38*
70 kDa	B, D	B, D	B, D	70 kDa, B, D	70 kDa, B, D	B, D	B, D	B, D	B, D	B, D	B, D
B, D	Dead	Dead	Dead	70 kDa, B, D	B, D	Dead	Dead	B, D	B, D	B, D	B, D
B, D	Dead	Dead	Dead	B, D	B, D	Dead	Dead	B, D	B, D	B, D	Dead
neg.	70 kDa B, D	70 kDa, B, D	Dead	neg.	B, D	neg.	neg.	B, D	B, D	Dead	Dead
neg.	70 kDa B, D	B, D	B, D	neg.	B, D	neg.	neg.	B, D	B, D	Dead	Dead
neg.	70 kDa	Dead	Dead	neg.	neg.	70 kDa, B,	Dead	B	70 kDa, B, D	Dead	Dead
neg.	70 kDa	Dead	Dead	neg.	70 kDa, B, D	B, D	B, D				

Only those animals that developed antibody reactivities detectible by immunoblotting are shown here. There were 7/14 (50%) positive in the 70 KFP group, 7/14 (50%) in the MFP groups and 6/15 (40%) in the saline group which developed anti-snRNP antibodies detectible by immunoblotting. neg. = none detected.

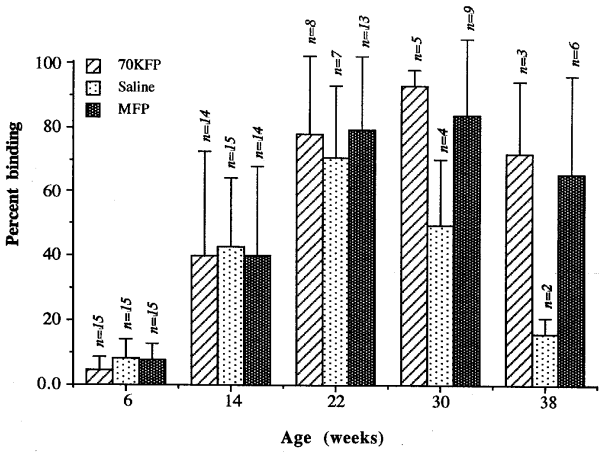

Figure 3 Anti-double-stranded DNA (dsDNA) antibodies sera were obtained at 6 weeks of age and at 8 week intervals. Anti-dsDNA antibodies were quantitated by a radioimmunoprecipitation assay and results are shown as percent binding. Anti-dsDNA activity had a tendency to be suppressed in the 70 KFP or MFP immunized groups at 22, 30 and 38 weeks of age. This trend was statistically significant only at 30 weeks of age for saline versus 70 KFP ($P = 0.024$) and versus MFP ($P = 0.029$). Immunization with 70 KFP did not induce anti-dsDNA antibodies.

groups. Due to high mortality among 70 KFP and saline treatment group after 20 weeks of age, these differences did not achieve statistical significance, however.

Glomerular lesion counts in all mice that either died with renal disease and vasculitis, or were killed at an advanced age to end the study, are shown in Figure 5. The MFP treated animals had, as a group, less histological evidence of advanced glomerulonephritis that did mice injected with saline or immunized with 70 KFP. Saline controls had a mean renal severity score of 60. 70 KFP treated mice had a mean renal severity score of 66. Mice that received MFP immunization had a mean renal severity score of 46, which was significantly lower than the saline ($P = 0.037$) or 70 KFP ($P = 0.013$) groups. There was no correlation between the presence of anti-U1 70 kDa antibodies and renal function or renal severity scores.

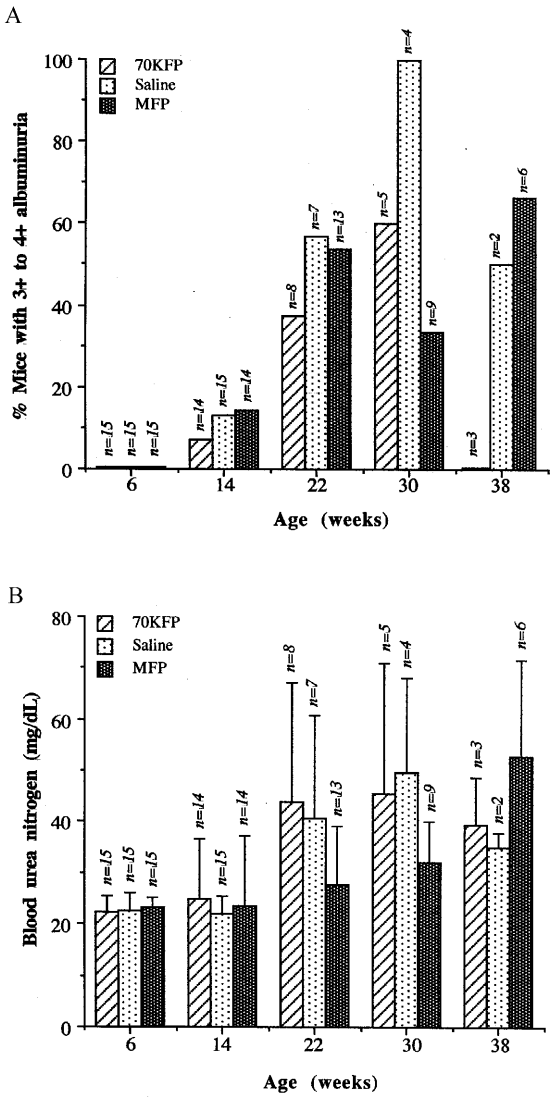

Figure 4 The effect of 70 KFP immunization, and MFP immunization and saline injection on renal function as measured by urinary albumin excretion and blood urea nitrogen (BUN). (A) MFP immunized animals tended to have less urinary albumin excretion at 22 and 30 weeks of age compared to 70 KFP immunization or saline injection. At 30 weeks of age, the MFP immunized group had significantly higher urinary albumin excretion than the saline injected group ($P = 0.012$). (B) BUN increased in all groups over time, but had a tendency to be lower in the MFP treated animals versus the MFP and saline injected groups. These differences were not statistically significant.

Discussion

The goal of this study was to test the hypotheses that immunization of MRL/*lpr* mice with 70 KFP would enhance the anti-U1 70 kDa snRNP antibody response and that this would alter disease expression in this murine model of SLE. As shown in Figure 2, anti-snRNP antibodies measured in ELISA were not enhanced in 70 KFP immunized MRL/*lpr* mice. Overall, anti-snRNP antibody levels were lower in both 70 KFP and MFP immunized groups compared to saline injected mice, although this trend was only statistically significant at 14 weeks of age ($P = 0.038$) (Figure 2). Antibodies to individual U1-specific snRNP polypeptides (70 kDa, A and C) and to Sm-specific polypeptide (Sm-B and Sm-D) were tested by immunoblotting (Table 1). Overall, the immunoblotting results confirmed the finding of the anti-snRNP ELISA, showing that at 14 weeks of age anti-snRNP polypeptide antibodies were detected most frequently

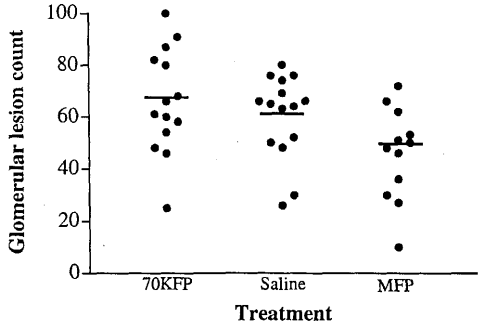

Figure 5 The effects of 70 KFP immunization, MFP immunization and saline injection on glomerular lesion counts. MFP treated animals had significantly lower renal severity scores than did mice injected with saline or 70 KFP. 70 KFP treated mice had a mean renal severity score of 66. Mice that had received MFP immunization had a mean renal severity score of 46, which was significantly lower than saline ($P = 0.037$) or 70 KFP ($P = 0.013$) groups.

in the saline injected group (Table 1). Only 46% of MRL/*lpr* mice developed anti-snRNP antibodies during their lifespan, which is similar to findings of Eisenberg, Cohen and colleagues.[12,13] Although the groups were small, the presence of anti-U1 70 kDa antibody did not appear to protect renal function in the present study.

No previously published study has, to our knowledge, examined the effect of immunization of MRL/*lpr* mice with recombinant U1-70 kD antigen. Studies have examined the effect of injection of U1-snRNP, U1-A, Sm-B or Sm-D antigens in a variety of normal rabbits, mice, and in lupus-prone MRL/*lpr* mice.[5,14–17] Harley and colleagues have shown that immunization of normal rabbits and mice with a peptide fragment of Sm-B can induce an anti-Sm-B antibody response, which then spreads to induce antibodies against other snRNP polypeptides (including U1 70 kDa) and against dsDNA. Furthermore, they found that Sm-B peptide (PPPGMRPP) immunization resulted in a lupus-like syndrome in rabbits and mice.[14] Isenberg and colleagues, in contrast, reported that immunization of New Zealand White rabbits, BALB/c, B10/brown and MRL/*lpr* mice with Sm-B or Sm-D antigen-derived peptides resulted in anti-peptide antibodies, but did not result in the emergence of autoantibodies against the intact Sm antigen in normal animals, nor did peptide immunization enhance the spontaneous production of anti-Sm antibodies in MRL/*lpr* mice.[16,17] Craft and colleagues have demonstrated that immunization of normal C57BL/6J and B10.BR mice with a fusion protein of the human U1-A protein can elicit anti-snRNP antibody production.[5] Immunization with recombinant human U1-A protein also results in intramolecular spreading to alter the production of antibodies reactive with other snRNP polypeptides, including U1 70 kDa.[5] Finally, immunization of normal mice with purified human anti-U1-RNP IgG resulted in induction of murine antibodies directly predominantly against the U1 70 kDa peptide of the RNP complex.[36]

In the present study, 70 KFP did not enhance anti-U1 70 kDa antibody product, did not induce the production of anti-Sm-B, nor anti-Sm-D or dsDNA antibodies, and did not accelerate disease in MRL/*lpr* mice. Potential technical factors, which explain the failure to detect an antibody response to 70 KFP, could include inadequate dose of antigen used, incorrect method of delivery of the antigen, or the use of intact antigen rather that peptide fragments of the antigen. The concentration of antigen and immunization scheduled in the present study was similar to those used successfully in other published studies, which argues that these factors did not influence the outcome reported here.[21,22] In the present study, the development of anti-MFP antibodies could readily be detected as appearing in immunized mice after 14 weeks of age (but not in saline injected mice), as evidence that the dose of antigen and the injection schedule used were adequate to elicit an immune response (Figure 6). Finally, in preliminary experiments immunodominant synthetic peptide fragments of U1 70 kDa have been tested, and also failed to enhance anti-snRNP antibody production (Hoffman *et al*, unpublished), similar to results observed here using the intact U1 70 kDa antigen.

When the effects of immunization on renal function were examined, it was observed that MFP slowed the progression of renal disease, as measured

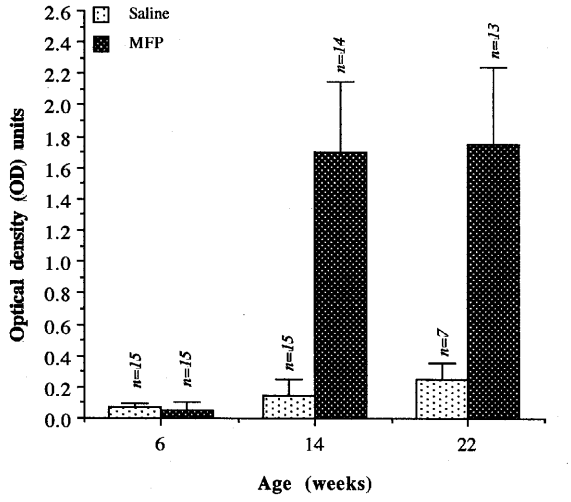

Figure 6 The effects of MFP injection on generating an anti-MFP antibody response. Animals immunized by MFP, but not saline, generated anti-MFP antibodies at 14 and 22 weeks of age. The MFP immunized group had significantly high anti-MFP antibodies than the saline injected groups ($P < 0.01$).

by urinary albumin excretion and BUN when compared to saline or 70 KFP injection (Figures 4A and 4B). Histologic examination of the kidneys confirmed that MFP slowed the progression of renal disease as measured by counting lesions in a specified number of glomeruli (Figure 5). Histologic examination of tissues also confirmed that glomerulonephritis and vasculitis were the primary causes of spontaneous death in all 3 groups of mice. A second analysis of the glomerular lesion counts included only mice in each group with recoverable tissue that died spontaneously of renal disease and vasculitis. This analysis showed that the lesions counted in the MFP group (mean = 48) were significantly suppressed compared to the saline injected controls (mean = 65; $P = 0.028$).

The MFP used in this study was derived from periplasmic maltose binding protein malE, one of a series of bacterial ATP-binding cassette transporters. In bacteria, these function to hydrolyze ATP and translocate substrate across the cytoplasmic membrane. MFP is a 367 amino acid fragment of maltose binding protein malE, which is widely utilized as a soluble fusion protein in the pMAL-c2 plasmid cloning vector (New England Biolabs). The maltose binding protein malE and MFP have been demonstrated to have chaperone-like properties[25,26] and functionally they are related to the Hsp protein superfamily. It is well known that members of the Hsp superfamily can have immunomodulatory effects in several animal models of autoimmunity, including diabetes mellitus in NOD mice and collagen induced arthritis in the rat.[27,28,37] In other relevant studies, an extract of *E. coli*, OM-89 or Subreum™ has been reported to slow the progression of renal disease in MRL/*lpr* mice and Hsp has been implicated as an active ingredient in OM-89.[38,39]

We hypothesize, based upon structural and functional similarities, that MFP has immunomodulatory properties similar to Hsp. These effects may be selective in inhibiting glomerular injury in that the predominant beneficial effect observed was on amelorating renal disease rather than diminishing autoantibody production. Taken together, these data suggest that the Hsp-like or molecular chaperone properties of MFP may play a role in its mechanism of action. Additional studies will be required to prove the mechanism by which MFP modulates renal disease in the MRL/*lpr* murine model of SLE.

In summary, the present study was undertaken to examine the role of the U1 70 kDa antigen in the MRL/*lpr* murine model of SLE. We found that, while immunization with 70 KFP tended to delay the development of expression of anti-snRNP antibodies in the sera of MRL/*lpr* mice, it did not affect the development or progression of proliferative glomerulonephritis, or alter survival. Unexpectedly, it was found that MRL/*lpr* mice immunized with a 367 amino acid fragment of a bacterial ATP-binding cassette transporter, MFP, had prolonged survival compared to saline injection or U1 70 kDa immunization and this was associated with a delay in the onset of SLE-like proliferative glomerulonephritis. This is the first report, to our knowledge, in which a bacterial ATP-binding cassette transporter has acted as a therapeutic agent in autoimmune disease. We report that MFP can modulate renal disease in an murine model of SLE. Future studies to examine the molecular mechanisms of the protective effect of MFP in autoimmune-mediated renal disease are warranted.

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