Recombinant chaperonin 10 suppresses cutaneous lupus erythematosus and lupus nephritis in MRL-(Fas)lpr mice

Onkar P. Kulkarni1, Mi Ryu1, Claudia Kantner1, Miklós Sárday2, Dean Naylor3, Daniel Lambert3, Richard Brown3 and Hans-Joachim Anders1

1Medizinische Poliklinik-Innenstadt, University of Munich, Munich, Germany, 2Department of Dermatology and Allergology, University of Munich, Munich, Germany and 3CBio Ltd, Eight Mile Plains, Queensland, Australia

Correspondence and offprint requests to: Hans-Joachim Anders; E-mail: hjanders@med.uni-muenchen.de

Abstract

Background. Systemic lupus erythematosus (SLE) is still treated with global immunosuppressants with serious toxicities. We hypothesized that endogenous immunosuppressive molecules might be able to control SLE manifestations more specifically. Heat shock protein 10, or chaperonin 10 (Cpn10), is a secretory molecule that can suppress innate and adaptive immunity.

Methods. Recombinant human Cpn10 (100 μg per mouse) was given intraperitoneally to healthy-appearing female MRL-(Fas)lpr mice from 12 to 22 weeks of age. At the age of 22 weeks, mice were analysed for treatment outcome by harvesting organs, plasma and urine.

Results. Cpn10 entirely prevented cutaneous lupus lesions as compared to vehicle-treated mice. Cpn10 also suppressed lupus nephritis as evident from serum creatinine levels, albuminuria and the scores of disease activity and chronicity. Autoimmune lung disease was unaffected by Cpn10 treatment while overall survival of mice was prolonged. Cpn10 did not have any major effects on either dendritic cell or B-cell counts except T cells in spleen, plasma interferon-gamma, tumour necrosis factor-alpha, interleukin-10, anti-nuclear autoantibody levels or markers of lymphoproliferation.

Conclusions. In summary, recombinant Cpn10 selectively prevents cutaneous lupus and suppresses nephritis in MRL-(Fas)lpr mice without affecting the underlying systemic autoimmune process. Hence, Cpn10 might be useful for the treatment of skin and kidney manifestations of SLE.

Keywords: chaperonin 10; cutaneous lupus erythematosus; immunomodulation; inflammation; lupus nephritis

Introduction

Systemic lupus erythematosus (SLE) is characterized by loss of tolerance and a polyclonal immune response against nuclear particles [1]. The ubiquitous distribution of nuclear autoantigens serves as an explanation for the diverse clinical manifestations of SLE, which include autoimmune inflammation of the skin, joints, kidney, lung, heart, brain, intestine, liver, etc. [1]. SLE treatment usually depends on unspecific immunosuppressive drugs like corticosteroids, anti-malarials, cyclophosphamide and mycophenolate mofetil. These drugs can cause serious toxicities and contribute to the mortality and morbidity of SLE [1]. To develop more specific immunomodulatory drugs with favorable toxicity profiles is an unmet need for SLE patients.
Several major challenges confront the development of SLE treatments, including variable disease activity, the diverse organ manifestation patterns and the 9-fold higher prevalence in females compared to males [1]. Once loss of tolerance occurs and autoreactive lymphocyte clones have expanded, autoimmune disease activity depends on the balance of pro- and anti-inflammatory signals, which may differ in different body compartments. The female preponderance in prevalence is thought to depend on the immunoregulatory role of oestrogens supported by the common onset of SLE in puberty and by pregnancy-associated disease activity [2]. In the pre-steroid era, however, gestational lupus remissions were observed at the same frequency as exacerbations [3], suggesting a balance of pro- and anti-inflammatory factors regulating lupus activity in pregnancy. Interestingly, repeated pregnancies protect MRL(Fas)-lpr mice, a lupus-prone strain developing systemic autoimmune disease between 12 and 22 weeks of age, from cutaneous lupus but accelerate lupus nephritis [4], hence, pregnancy-associated factors can promote organ-specific outcomes. In fact, fetal as well as maternal immunosuppressive factors assure alloimmune tolerance of the maternal immune system to the fetus. It is, therefore, tempting to speculate that immunosuppressive pregnancy-related factors could serve as a therapeutic tool to control autoimmunity and tissue inflammation.

Early pregnancy factor (EPF) has been proposed as such a maternal immunosuppressive factor preventing embryonic loss during early pregnancy [5, 6]. Initial studies identified EPF as an extracellular form of heat shock protein 10 (Hsp10) [7], also known as chaperonin 10 (Cpn10); however, other studies suggest that EPF is comprised of several entities. Nevertheless, Cpn10 has been found in sera during early pregnancy and has clear immunomodulatory properties. Cpn10 is a highly conserved heptamer of 10 kDa subunits that intracellularly fulfils an essential role in mitochondrial protein folding and its synthesis is highly induced by cellular stress [8, 9]. Some Hsp50 have shown to act as damage-associated molecules in the extracellular space where they can alert the innate immune system by activating Toll-like receptors (TLR) [10, 11]. Cpn10 may prevent such interactions and thereby act as an inducible and secreteable immunosuppressive factor during cell stress [9, 12]. Do these characteristics predict potential immunosuppressive effects of Cpn10 on inflammation and autoimmunity in vivo? Recombinant Cpn10 suppressed systemic tumour necrosis factor-alpha (TNF-α) and chemokine (C-C motif) ligand 5 (CCL5) induction and induced interleukin (IL)-10 after lipopolysaccharide (LPS) injection in female Balb/c mice [12]. Furthermore, recombinant Cpn10 injections prolonged skin allograft survival in rats [13], suppressed adjuvant arthritis in rats [14, 15] and experimental encephalomyelitis in rats and mice [16]. Based on these findings, recombinant Cpn10 is currently being evaluated in phase II trials and has already shown to be effective in rheumatoid arthritis [17] and in psoriasis vulgaris [18], while a non-significant trend towards less new brain lesions on magnetic resonance imaging was noted in multiple sclerosis patients within 16 weeks of Cpn10 treatment [19].

We therefore hypothesized that recombinant Cpn10 would suppress experimental lupus of MRL(Fas)-lpr mice and that a potential therapeutic effect would be most prominent on cutaneous lupus.

Materials and methods

Production and purification of Cpn10

Mammalian Cpn10 (SwissProt P61604) was acetylated on alanine residue 2 following removal of the initiation methionine [20, 21]. As Escherichia coli cannot acetylate proteins at the N-terminus, recombinant human Cpn10 was engineered with an additional glycine residue at Position 2, to mimic the native acetyl group [22]. Recombinant Cpn10 was produced from the pET30 (Novagen/Merck, Darmstadt, Germany) in BL21(DE3) cells (Invitrogen, Mulgrave, Australia). Briefly, cells were grown at 37°C in Luria broth and 50 μg/mL kanamycin until O.D.600nm ~ 0.5 and expression was induced for 4 h with 0.5 mM isopropyl-β-D-thiogalactopyranosid. The soluble cellular lysate, buffered in 25 mM Tris–HCl pH 7.5, was mixed with 0.1% (w/v) polyethyleneimine (Sigma–Aldrich, Sydney, Australia) and incubated on ice for 5 min prior to centrifugation (15 300 g for 30 min). All subsequent chromatography steps were carried out at room temperature. The clarified supernatant was applied to an SP Sepharose Big Bead column (GE Biosciences, Sydney, Australia), equilibrated in 25 mM Tris–HCl pH 7.5 and bound Cpn10 was eluted with a linear gradient from 0 to 400 mM NaCl. Fractions containing Cpn10 were adjusted to 25 mM Tris–HCl pH 7.5 + 2 M (NH4)2SO4 and applied to a Butyl-S-Sepharose 6 Fast Flow column (GE Biosciences) equilibrated in the same buffer. Bound Cpn10 was eluted with a linear gradient from 2–0.8 M (NH4)2SO4. Fractions containing Cpn10 were dialysed against 25 mM Tris–HCl pH 8 + 1 mM EDTA (ethylene diamine tetraacetic Acid) and applied to Capto-Q column (GE Biosciences) equilibrated in the same buffer. The unbound fraction, containing essentially pure Cpn10, was dialysed into 50 mM Tris–HCl pH 7.6 + 150 mM NaCl, formulated to 5 mg/mL, filtered through a 0.2-μm membrane and stored at ~80°C. Aliquots were thawed once prior to use. Cpn10 formulation buffer was prepared from ultra-pure reagents and filtered through an Acrodisc 0.2-μm unit with a Mustang E membrane to remove trace amounts of pro-inflammatory contaminants ( Pall Corp., Ann Arbor, MI, cat. no. M52725E). All batches of Cpn10 had similar molar activity as GroES in GroEL-mediated rhodanese refolding assays [23] (data not shown). LPS contamination of Cpn10 was determined to be <0.01 endotoxin EU/mL of formulation buffer utilizing the Endosafe PTS unit (Charles River Laboratories, Wilmington, MA). The presence of other TLR ligands was determined by incubating Cpn10 and the storage buffer with the RAW264.7 cell line stably transfected with the pNiFty2-Luc plasmid, a luciferase reporter construct highly and rapidly responsive to Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) stimulation (Inviogene). Silver staining sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis was achieved using the ammoniacal method. The concentration of Cpn10 was determined spectrophotometrically assuming an extinction coefficient of 0.412 at 280 nm for a 1-mg/mL solution of pure Cpn10.

Animals and experimental protocol

Eight-week-old female MRL(Fas)-lpr mice were obtained from Harlan Winkelmann (Borchern, Germany) and kept under normal housing conditions in a 12-hour light and dark cycle. Water and standard chow (Sniff, Soest, Germany) were available ad libitum. At the age of 12 weeks, groups of 11 mice each started to receive either 100 μg Cpn10 in 100 μL of vehicle (50 mM NaCl, 150 mM NaCl, pH 7.6) or vehicle only by intraperitoneal (i.p.) injection on alternative days. All mice were sacrificed by cervical dislocation at the end of Week 22. All experiments were performed according to German animal protection laws and had been approved by the local government authorities.

Evaluation of systemic lupus

Urinary albumin concentrations ratios, serum creatinine levels and plasma cytokine concentrations were determined by enzyme-linked immunosorbent assay (ELISA) as previously described [24]. Two micrometre paraffin sections for haematoxylin–eosin and periodic acid–Schiff stains were prepared from skin, lung and kidney tissue samples following routine protocols [25]. Skin lesions were scored semi-quantitatively as described [26, 27]. In brief, haematoxylin–eosin skin sections were graded for the degree of acanthosis, mild (1) to very markedly thickened dermis (3); hyperkeratosis, mild (1) or markedly increased amount of keratin (2); interface lichenification, focal (1) or
extensive damage to basal cell layer (2); inflammation, sparse (1) or heavy
dermal lymphocytic infiltrates (2); fibrosis, increased dermal collagen with
slight (1) or markedly thickened dermis (2); vessels, focal (1) or diffuse (2)
dilated vessels with haemorrhage and ulcer, absence (0) or presence (1) of
ulcer or erosion. The severity of the renal lesions was graded using the
indices for activity and chronicity as described for human lupus nephritis
[28]. Immunostaining was performed as described using the following
primary antibodies: anti-mouse Mac-2 (Cedarlane, Ontario, Canada), rat
anti-F4/80 (1:50; Serotec, Oxford, UK), anti-mouse CD3 (1:100; Serotec),
ant-mouse IgG (Caltag Laboratories, Burlingame, CA). The severity of the
glomerular IgG deposits as well as that of peribronchial inflammation was
graded semiquantitatively from 0 to 4 as described [29].

Lupus autoantibodies

Serum antibody levels were determined by ELISA as described [27]. Anti-
dsDNA antibodies: NUNC maxisorp ELISA plates were coated with poly-
t-lysine (Trevigen, Gaithersburg, MD) and mouse embryonic stem cell
dsDNA. After incubation with mouse serum, dsDNA-specific IgG were
detected by ELISA (Bethyl Labs, Montgomery, TX). Anti-nucleosome:
NUNC maxisorp ELISA plates were coated with histones (5 μg/mL) and
mouse embryonic stem cell dsDNA (1 μg/mL) overnight. Prior to the coat-
ing of the sample wells with histones and dsDNA, plates were layered with
dulti-lysine (Trevigen) for 1 h at room temperature followed by washing
with wash buffer. After overnight coating with histones and dsDNA, serum
samples were analysed for anti-nucleosome IgG by using mouse IgG de-
tection kit (Bethyl Labs). Reference serum with specific IgG was used as a
positive control and to calculate autoantibody concentrations.

Flow cytometry

Flow cytometry was performed using a FACScalibur flow cytometer as
described [27]. The following primary antibodies were procured from BD
Pharmingen: anti-mouse CD4-APC, CD8-PerCP, CD3-FITC, CD25-PerCP,
CD45R-APC, CD11c PE and MHC II-FITC.

RNA preparation and real-time quantitative (TaqMan) reverse
transcription–polymerase chain reaction

Reverse transcription and real-time reverse transcription–polymerase
chain reaction from total renal RNA or renal cell types isolated by magnetic
bead isolation was prepared as described [29]. SYBR Green Dye detection
system was used for quantitative real-time polymerase chain reaction on
Light Cycler 480 (Roche, Mannheim, Germany). Gene-specific primers
(300 nM; Metabion, Martinsried, Germany) were used as listed in Table 1.
Controls consisting of ddH2O (double-distilled water) were negative for
controls. Gene-specific primers (300 nM; Metabion, Martinsried, Germany) were used as listed in Table 1. Controls consisting of ddH2O (double-distilled water) were negative for
target and housekeeper genes.

Statistical analysis

Data were expressed as mean ± SEM. Comparison between groups were
performed using Student’s t-test. A value of P < 0.05 indicated statistical
significance.

Table 1. Primers used for real-time reverse transcription–polymerase chain reaction

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession number</th>
<th>Sequence</th>
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<tr>
<td>CCL2</td>
<td>NM 011333</td>
<td>Forward primer: 5'- CCTGCTGTTCACAGTTGCC-3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Revers primer: 3' - ATTGGGATCATCTTGCTGGT-5'</td>
</tr>
<tr>
<td>CCL5</td>
<td>NM 013653</td>
<td>Forward primer: 5' - CCACTTCTCTCTGTTGTTG-3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Revers primer: 3' - GTGCCAACAGTCAGGATAGAT-5'</td>
</tr>
<tr>
<td>IL-6</td>
<td>NM 031168</td>
<td>Forward primer: 5' - TGAATGCACTTTGCAAGAAAAC-3'</td>
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<tr>
<td></td>
<td></td>
<td>Revers primer: 3' - ACCAGAGAAAATTTTCAATAGGC-5'</td>
</tr>
<tr>
<td>TNF-α</td>
<td>NM 013693</td>
<td>Forward primer: 5' - ACCAGAGAAAATTTTCAATAGGC-3'</td>
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<tr>
<td></td>
<td></td>
<td>Revers primer: 3' - GAGGGTCTGGGCCATAGAACT-5'</td>
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<tr>
<td>IL-12p40</td>
<td>NM 008352</td>
<td>Forward primer: 5' - AGCATGAGCTTCCCCCTGA-3'</td>
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<td></td>
<td></td>
<td>Revers primer: 3' - AGTCCTTTGGTTCAGTCGTTG-5'</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>NM 008337</td>
<td>Forward primer: 5' - ACAACCAAGGGCAAAGGATG-3'</td>
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<td>Revers primer: 3' - TGAATGCACTTTGCAAGAAAAC-5'</td>
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<tr>
<td>IL-10</td>
<td>NM 010548</td>
<td>Forward primer: 5' - ATGGATTTAATCCCTGTTGA-3'</td>
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<tr>
<td>18S RNA</td>
<td>NR 003278</td>
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<tr>
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<td></td>
<td>Revers primer: 3' - AGGGCTCTACAAACCATCC-5'</td>
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same neck area of all mice (Figure 2B). As such, detailed scoring of seven different lupus skin disease parameters as well as the composite score displayed significant suppression of skin disease by Cpn10 (Figure 2C). Thus, Cpn10 treatment prevents cutaneous lupus in MRL(Fas)-lpr mice.

Cpn10 ameliorates diffuse proliferative lupus nephritis in MRL(Fas)-lpr mice

Lupus nephritis is a common and potentially life-threatening organ manifestation of human SLE [1] and a characteristic of lupus-like autoimmunity in MRL(Fas)-lpr mice. In the present study, vehicle treated 22-week-old female MRL(Fas)-lpr mice showed diffuse proliferative lupus nephritis with major glomerular and tubulointerstitial abnormalities (Figure 3). Cpn10 treatment significantly reduced renal immunopathology as evidenced by semi-quantitative morphometrical evaluation, e.g. by assessing the activity and chronicity scores of lupus nephritis. The activity score, a composite score of mostly glomerular abnormalities, was significantly reduced by Cpn10 (Figure 4D). The chronicity score, a composite score of glomerular and interstitial scarring, was also significantly reduced (Figure 4E). Silver stains of renal sections illustrate the improvement on extracellular matrix production within glomeruli (less fibrocellular crescents) as well as in the interstitial compartment (Figure 3). The improvement of structural glomerular damage was associated with less glomerular Mac-2 macrophages as well as periglomerular and interstitial F4/80$^+$ monocytic cells and CD3$^+$ T cells (Figures 3 and 4A–C). Nextly, we determined serum creatinine levels as a functional marker of renal excretory function and urinary albumin/creatinine ratio as a marker of proteinuria, i.e. glomerular filtration barrier dysfunction. Cpn10 treatment was associated with a significant decline in serum creatinine and a non-significant trend towards lower albuminuria (Figures 4F and G). Therefore, we conclude that beyond preventing cutaneous lupus Cpn10 treatment suppresses lupus nephritis and renal dysfunction in MRL(Fas)-lpr mice.

Cpn10 reduces intra-renal expression of pro-inflammatory cytokines in MRL(Fas)-lpr mice

We speculated that Cpn10 would suppress intra-renal inflammation and therefore nephritis by inhibiting pro-inflammatory signals. Hence, we studied the messenger RNA (mRNA) expression levels of various pro-inflammatory...
cytokines that have previously been shown to contribute to renal immunopathology in MRL(Fas)-lpr mice [24, 30, 31]. Cpn10 treatment was associated with a significant reduction of intra-renal monocyte chemotactic protein-1 (MCP-1)/chemokine (C-C motif) ligand 2 (CCL2), IL-6 and TNF-α mRNA expression (Figure 5). There was a trend towards lower levels of IFN-γ and RANTES/CCL5 mRNA levels while the expression levels of IL-12p40 and the anti-inflammatory cytokine IL-10 were unaffected by Cpn10 (Figure 5). We conclude that Cpn10-mediated protection from diffuse proliferative lupus nephritis in MRL(Fas)-lpr mice is associated with lower mRNA expression of MCP-1/CCL2, IL-6 and TNF-α.

Cpn10 does not affect lung disease in MRL(Fas)-lpr mice

Lungs are also commonly affected by autoimmune tissue injury in MRL(Fas)-lpr mice, namely by moderate to severe peribronchiolar and perivascular T-cell and macrophage infiltrates [32]. We also observed such infiltrates in

Fig. 2. Cpn10 and skin disease in MRL(Fas)-lpr mice. (A) Mice of both groups were regularly checked for skin manifestations, which typically occur in the facial or neck/back area. The image shows representative mice from both groups at 22 weeks of age. (B) Representative images of haematoxylin–eosin-stained skin biopsies from selected groups are shown as indicated (original magnification ×100). (C) The semiquantitative assessment of skin pathology allowed to calculate a composite skin injury score, which was derived from adding the scores of seven different criteria (C) as indicated. Data represent means ± SEM. **P < 0.01, ***P < 0.0001 versus vehicle control group.
Fig. 3. Cpn10 and lupus nephritis in MRL(Fas)-lpr mice. Renal sections of 22-week-old MRL$^{lpr/lpr}$ mice were stained with periodic acid-Schiff, silver, F4/80, Mac-2 and CD3 as indicated. Note that glomerular hypercellularity, focal segmental sclerosis, global sclerosis and leukocyte infiltrates observed in vehicle-treated mice are absent or reduced in mice that had received recombinant Cpn10. Images are representative for 7–12 mice in each group, original magnification $\times 200$ to $\times 400$. 
22-week-old MRL(Fas)-lpr mice that had received Cpn10 treatment (Figure 6). This was evaluated by a semiquantitative lung injury score ranging from 0 to 4 (vehicle: 2.0 ± 0.2 versus Cpn10: 1.9 ± 0.3, P = 0.83). Obviously, Cpn10 treatment improves skin and kidney disease but not lung disease in autoimmune MRL(Fas)-lpr mice.

Cpn10 and markers of systemic autoimmunity in MRL(Fas)-lpr mice

Cpn10 has obviously profound effects on dermal and renal autoimmune tissue inflammation in MRL(Fas)-lpr mice. We next questioned whether these findings are associated with effects of Cpn10 on systemic autoimmunity of MRL(Fas)-lpr mice. Systemic autoimmunity in SLE manifests in many ways, i.e. lymphoproliferation, polyclonal expansion of autoreactive lymphocytes, autoantibody production and eventually increase of circulating cytokine levels. Total spleen weight, a marker of lymphoproliferation, was not affected by Cpn10 treatment (Figure 7A) but we also quantified splenocyte numbers more specifically by flow cytometry. Cpn10 had a small but significant effect on splenic CD3+ T cells, which was due to lower numbers of CD4+ T cells and CD4/CD8 double negative ‘autoreactive’ T cells (Figure 7B). CD8+ ‘cytotoxic’ T cells and CD4+/CD25+ ‘regulatory’ T cells were unaffected by Cpn10 treatment. B220+ cells, mainly including various B-cell subsets, were reduced in Cpn10-treated MRL(Fas)-lpr mice, whereas CD11c+ dendritic cells and including those expressing the activation marker MHC II were increased in number (Figure 7C). Thus, Cpn10 has no major effect on overall lymphoproliferation but reduces CD4+ T cells and autoreactive T cells and rather
increases the number of activated dendritic cells. These findings suggest distinct immunoregulatory functions of Cpn10 in lupus and exclude major unspecific immunosuppressive effects. How do these findings relate to the production of lupus autoantibodies, immune complex disease and plasma cytokine levels? We did not detect any significant effect of Cpn10 treatment on the plasma levels of either total IgG or dsDNA antibodies or anti-nucleosome IgG in MRL(Fas)-lpr mice (Figure 8A). This was consistent with identical glomerular IgG deposition, suggesting that the protective effect of Cpn10 on renal pathology was independent of glomerular immune complex deposition (Figure 8B). Cpn10 significantly reduced the plasma levels of TNF-α and gave a non-significant trend towards lower levels for IFN-γ (Figure 8C). Together, we conclude that Cpn10 does not consistently suppress systemic autoimmunity in terms of preventing lymphoproliferation or the production of DNA autoantibodies but Cpn10 specifically suppresses the expansion of CD4/CD8 double negative autoreactive T cells and plasma TNF-α levels.

Discussion

Our data now extend such previous studies on the cutaneous and renal manifestations of lupus-like systemic autoimmunity of MRL(Fas)-lpr mice. Our data show that systemic administration of Cpn10 has local anti-inflammatory effects as it reduced the levels of tissue cytokine expression and tissue pathology in the absence of a major effect on circulating lupus autoantibody levels or immune complex disease. This became evident especially in the kidney where Cpn10 treatment prevented glomerular inflammation and pathology despite identical glomerular IgG deposition. This finding argues that Cpn10 elicits its protective effects mainly by local anti-inflammatory actions. Despite profound effects on renal pathology, there was only a trend towards less proteinuria in Cpn10-treated mice. This could refer to the high inter-individual variability of proteinuria and small group size or due to a limited anti-inflammatory effect of Cpn10 effect on podocytes.

Recombinant Cpn10 was particularly effective in preventing cutaneous lupus, a finding which may associate with the observation that multiple pregnancies have a profound effect on cutaneous lupus of MRL(Fas)-lpr mice [4]. Our observation that Cpn10 did not affect autoimmune lung disease confirms previous reports that had found dissociated outcomes for lung disease and other disease manifestations.
in MRL(Fas)-lpr mice. For example, intercellular adhesion molecule-1 and TNF-α have a non-redundant role in T-cell mediated in lung disease of MRL(Fas)-lpr mice while cutaneous and renal manifestations rather develop from immune complexes [33, 34].

Recombinant Cpn10 had no consistent or profound effect on markers of systemic autoimmunity. Although our flow cytometric analysis indicated an increased activation of splenic dendritic cells, the numbers of CD4 T cells and CD4/CD8 double negative T cells were rather reduced. These effects did not significantly modulate the production of immunoglobulins or dsDNA autoantibodies. Therefore, we conclude that Cpn10 suppressed skin and renal disease in MRL(Fas)-lpr mice rather via a local anti-inflammatory effect, as previously demonstrated [9, 12]. The treatment of many autoimmune diseases has been improved by anti-inflammatory drugs with only minor effects on adaptive immunity. Unfortunately, none of these drugs have thus far been proven to be beneficial in SLE in clinical trials. We have previously shown that targeting the pro-inflammatory chemokine MCP-1/CCL2 prevents skin, lung and kidney disease in MRL(Fas)-lpr mice [24]. Furthermore, combining MCP-1/CCL2 blockade with low-dose cyclophosphamide was found to be as effective as high-dose cyclophosphamide in suppressing SLE but avoids the T-cell ablation and myelosuppression associated with high-dose cyclophosphamide treatment [29]. The data of the present study would render Cpn10 an eligible candidate for a similar combination therapy approach for severe lupus nephritis or cutaneous lupus.

In summary, recombinant Cpn10 treatment prevents cutaneous lupus and reduces lupus nephritis in MRL(Fas)-lpr mice with SLE. Lung disease remained unaffected by Cpn10 suggesting that autoimmune lung pathology involves

Fig. 8. Cpn10 and humoral markers of systemic autoimmunity in MRL(Fas)-lpr mice. Plasma samples were obtained at 22 weeks of age from mice of all groups and total serum IgG levels (A), anti-dsDNA IgG and anti-nucleosome IgG levels or the plasma concentrations of IFN-γ and TNF-α (C) were determined by Elisa. Renal sections were stained for mouse IgG to assess glomerular immune complex deposits (B). The figure shows representative images from 22-week-old mice of both groups (original magnification ×400) as well as the semiquantitative assessment (B). Data are means ± SEM. * P < 0.05 versus vehicle group.
disease pathomechanisms, which are different from cutaneous lupus and lupus nephritis. These data identify recombinant Cpn10 treatment as a potential approach to modulate cutaneous lupus and lupus nephritis.

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