Amelioration of SLE-like manifestations in (NZBxNZW)F1 mice following treatment with a peptide based on the complementarity determining region 1 of an autoantibody is associated with a down-regulation of apoptosis and of the pro-apoptotic factor JNK kinase

Micha J. Rapoporta,b,1, Amir Sharabic,1, Dorit Aharonib, Olga Bloch b, Heidy Zinger c, Molly Dayanc, Edna Mozesc,*

a Department C of Internal Medicine, Assaf Harofeh Medical Center, Zerifin 70300, Israel

b Diabetes Research Laboratory, Assaf Harofeh Medical Center, Zerifin 70300, affiliated to Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv 69978, Israel

c Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

Received 19 January 2005; accepted with revision 13 September 2005

Available online 27 October 2005

Abstract

Treatment with peptides based on the complementarity determining regions (CDR) of murine and human monoclonal anti-DNA antibodies that bear the common idiotype, 16/6 Id, ameliorates disease manifestations of mice with either induced or spontaneous SLE. Aberrant expression and function of the p21Ras/MAP kinase pathway are associated with active SLE. Therefore, we examined the effect of treatment with a CDR1-based peptide of a human autoantibody (hCDR1) on the p21Ras pathway and SLE manifestations of SLE-prone (NZBxNZW)F1 mice. Untreated SLE-affected mice demonstrated increased expression of p21Ras and the phosphorylated active form of its down-stream element JNK kinase in conjunction with reduced hSOS and unchanged p120GAP, as compared to healthy controls. Amelioration of SLE manifestations following treatment with hCDR1 was associated with a diminished expression of phosphorylated JNK kinase, mainly in the T cell population that also exhibited reduced rates of apoptosis. Thus, hCDR1 therapy ameliorates SLE, at least in part, via down-regulation of the activity of the pro-apoptotic JNK kinase.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Lupus; CDR-based peptides; Apoptosis; p21Ras pathway; JNK kinase; Immunomodulation; (NZBxNZW)F1 mice

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune inflammatory disease with complex clinical manifestations. It is characterized by the increased production of pathogenic autoantibodies and the lack of T- and B- lymphocyte regulation. The latter are associated with various clinical manifestations including immune complex deposits in the kidneys and other organs [1].

Female (New Zealand Black (NZB) x New Zealand White (NZW))F1 mice develop spontaneously an SLE-like syndrome. Beginning at the age of 3–4 months, the mice produce autoantibodies with subsequent formation of immune complex deposits in the kidneys at the age of 7–8 months and death within 1 year [2].

SLE can be induced in naive (not SLE-prone) mice by an active immunization with the human monoclonal anti-DNA antibody that bears the 16/6 idiotype (Id) [3], or with the mouse anti-DNA 16/6Id (5G12) monoclonal antibody [4]. Immunized mice develop high levels of autoantibodies (e.g. anti-dsDNA, anti-nuclear antigens) accompanied by SLE-related clinical manifestations [3–5]. High homology was
sequences of the complementarity determining regions (CDR) induced SLE [6].

We previously demonstrated that two peptides based on the sequences of the complementarity determining regions (CDR) 1 and 3 of the murine anti-DNA 16/6Id monoclonal antibody [7] had beneficial effects in down-regulating SLE-associated immunological responses and in ameliorating the clinical manifestations of an induced experimental disease [8]. Further, we have shown that these CDR-based peptides prevented the spontaneous development of SLE in (NZBxNZW)F1 female mice [9] and could successfully treat an already established clinical SLE-like disease in these SLE-prone mice [10]. The CDR-based peptides were shown to immunomodulate the cytokine profile found in SLE by down-regulating the secretion of Th1-type, and Th2-type cytokines. In addition, these peptides caused a significant reduction of the pro-inflammatory cytokine tumor necrosis factor (TNF)-α, and an up-regulated secretion of the immunosuppressive cytokine transforming growth factor (TGF)-β [8,9].

As a potential candidate for the treatment of SLE in patients, a peptide based on the sequence of CDR1 of the human monoclonal anti-DNA 16/6Id [11] has been designed and synthesized [12]. The peptide, designated hCDR1, was shown to inhibit efficiently and specifically the 16/6Id-induced in vitro proliferation and interleukin (IL)-2 production of peripheral blood lymphocytes of SLE patients. Furthermore, it was shown to down-regulate SLE manifestations in mice afflicted with either the spontaneous or induced experimental SLE [13].

Several defects of the signal transduction have been described in SLE [14]. These defects of biochemical signaling pathway in SLE T cells were divided to proximal, middle, and distal regions. The p21Ras proto-oncogene product is a key regulatory protein, which transduces a variety of extracellular signals, activating tyrosine kinase-associated and non-associated membrane receptors, leading to cell growth and differentiation [15]. It is involved in both T cell receptor activation and in IL-2 receptor activation, suggesting a pivotal role of this protein in mediating immunological responses [16]. The activity of p21Ras is determined by the ratio between its active GTP-bound and inactive GDP-bound forms. The GTPase activating protein, p120GAP, and other GAP-like proteins down-regulate the p21Ras activity by accelerating its relatively weak intrinsic GTPase activity, while the guanine nucleotide releasing factors (GNRF’s) such as ‘son of sevenless’ (SOS) enhance its activity by accelerating the dissociation of prebound-GDP [17,18]. Migration of SOS from the cytosol to the cell membrane and its association with p21Ras via an adapter protein, namely Grb2, is an early event in p21Ras pathway signaling [19,20]. Thus, the ratio of membrane and cytosolic fractions of SOS is an indirect indicator of p21Ras pathway signaling [21]. Generation of GTP-bound p21Ras results in activation of down-stream regulatory key enzymes including Raf-1 and the family of mitogen activated protein kinase (MAP-kinase), which ultimately regulates the transcription of cellular growth and differentiation genes [18]. We previously reported several signaling defects along the p21Ras pathway in SLE patients including reduced expression of hSOS, reverse migration of hSOS to the membrane following cell stimulation and constitutive up-regulated activity of the down-stream MAP kinase regulatory enzyme [22,23]. Most of these defects were more pronounced in patients with active disease suggesting that aberrant signaling of the p21Ras pathway may reflect disease activity in SLE patients.

Disregulated apoptosis is thought to play an important role in the pathogenesis of autoimmune diseases, including SLE [24–28]. It was shown to involve not only apoptosis-related genes such as lpr, gld, PD-1 and more [29–31], but also environmental factors such as ultraviolet light exposure and the use of certain drugs [28,32]. In addition, the existence of mitochondria hyperpolarization in conjunction with increased reactive oxygen species may partly explain the increased rate of spontaneous apoptosis in SLE T cells [33]. Recently, it has been shown that apoptotic lymphocytes are capable of accelerating the onset of lupus-like manifestations in (NZBxNZW)F1 female mice [34]. Further, SLE patients were reported to manifest increased rate of lymphocyte apoptosis [35], which correlates with disease activity [36]. These data underscore the role of dysregulated apoptosis in the pathogenesis of SLE. Recently, an important regulator of apoptosis was found to be the c-Jun NH2-terminal kinase (JNK), which is one of the down-stream elements of the p21Ras/MAP kinase pathway [37]. More recently, Niculescu et al. [38] reported an up-regulated activity of JNK in pathogenic T cells mediating SLE-like disease in the murine chronic graft-versus-host-disease model supporting the role of this apoptotic factor in the pathogenesis of SLE.

In the present study, we examined the expression and function of the p21Ras pathway and its correlation with disease activity in (NZBxNZW)F1 female mice with preexisting disease that were treated with the hCDR1 peptide. We further extended these observations to show that T cells were mainly affected by hCDR1 and that the rate of apoptosis and JNK activity along the p21Ras/MAP kinase signal transduction pathway is associated with the clinical status of SLE.

Materials and methods

Mice

Female (NZBxNZW)F1 mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). Mice were maintained under standard conditions in the animal facility of The Weizmann Institute of Science following the Institutional Animal Care and Use Committee procedures.

Peptide

A synthetic peptide with the following sequence GYYWSIRQPGKGEEWIG (hCDR1) [12] based on the
CDR1 of the human monoclonal anti-DNA antibody bearing the 16/6ld [11] was synthesized (solid phase synthesis by Fmoc chemistry) by Polypeptide laboratories (LA, USA) and used in this study. A peptide containing the same amino acids as the hCDR1, with a scrambled order (scrambled peptide), SKGIPYGGWPWEGWREYI, was used as a control. hCDR1 (TV-4710) is currently under clinical development for human SLE by Teva Pharmaceutical Industries Ltd.

Treatment of mice with the hCDR1

(NZBxNZW)F1 female mice at the age of 6 to 7 months were divided into groups of 10 mice each. Subcutaneous (s.c.) injections of the hCDR1 (50 μg/mouse), or the control-scrambled peptide (50 μg/mouse) were given once a week for 10 weeks. A control group of mice was treated with PBS. Mice at the age of 2–3 months were used in some of the experiments as healthy controls. It should be noted that mice of this age do not exhibit any of the clinical manifestations typical to SLE.

ELISA for the detection of dsDNA specific antibodies

For determination of anti-dsDNA antibodies, 96 well Maxisorb microtiter plates (Nunk, Denmark) were coated with poly-L-lysine (5 μg/ml) (Sigma, St. Louis, MO). The plates were then washed and coated with lambda phage dsDNA (5 μg/ml) (Boehringer, Mannheim). Thereafter, the plates were blocked with 10% FCS in PBS, and the diluted sera of the mice (1:50–1:1250) were incubated for 2 h. Plates were then washed and incubated for 90 min with goat anti-mouse IgG (gamma chain specific) conjugated to horseradish peroxidase (Jackson Immuno Research, West Grove, PA). Following washing, plates were incubated with the substrate, ABTS [2,2′-azino-bis-(3-ethylbenzene-thiazoline-6-sulfonic acid); Sigma] and read at 405 nm using an ELISA reader.

Proteinuria

Proteinuria was measured by a standard semi-quantitative test, using an Albustix kit (Bayer Diagnostic, Newbury, UK). Results were graded according to the manufacturer as: negative, + = 0.3 g/l, ++ = 1 g/l, +++ = 3 g/l or ++++ = ≥20 g/l.

Immunohistology

Mice were sacrificed 1–2 weeks after the end of treatment, and kidneys were removed and frozen immediately in liquid nitrogen. Frozen cryostat sections (6 μm) were air-dried and fixed in acetone. For the detection of Ig deposits, sections were incubated with FITC-conjugated goat anti-mouse IgG (gamma chain specific) (Jackson Immuno Research). Staining was visualized using a fluorescence microscope. The intensity of the immune complex deposits was graded on a scale of 0–3 as follows: 0—no immune complex deposits; 1—low intensity; 2—moderate intensity and 3—high intensity of immune complexes.

Antibodies and reagents

The following antibodies were used in the study: anti-CD3-FITC (clone 145-2C11) and anti-CD4-PE (clone GK1.5) and their matched isotype controls were obtained from Southern Biotechnology Associates. Anti-CD19-PE (clone 1D3) and its isotype control were obtained from Pharmingen. Rat purified ascites containing Y13–259 anti-p21v-H-ras mAb was kindly donated by Dr. G.B. Mills (Toronto General Hospital, Toronto Ontarion, Canada). Rabbit polyclonal antibodies (anti-GAP, anti-hSOS1), mouse monoclonal antibodies that react with mouse phosphorylated JNK forms and secondary goat anti-rabbit, anti-mouse and anti-rat IgG-HRP antibodies were purchased from Santa Cruz Biotechnology Inc. (CA, USA). Ficoll Hypaque and ECL Western blotting detection reagents were obtained from Pharmacia Biotech (Uppsala, Sweden). Kodak Biomax ML films were obtained from Eastman Kodak Company (USA). All other chemicals were reagent grade purchased from Sigma Chemical Co. (Sigma-Aldrich Israel Ltd.).

Isolation of T and B cell populations

Petri dishes were precoated (overnight, 4°C) with goat anti-mouse Ig (15 μg/ml in 5 ml PBS), then washed three times. Spleen cells were depleted of RBC using ACK and then incubated (70 min, 4°C in RPMI 1640 containing 10% FCS and antibiotics) on the coated plates. The non-adherent cells, which were mainly T cells (>85%, as assessed by FACS analysis), were then collected and washed in RPMI 1640. The adherent cells, which were mainly B cells (>75%, as assessed by FACS analysis), were washed in RPMI 1640, and after 10 min incubation on ice the cells were collected by a vigorous pipetation.

Preparation of splenocytes

Spleen cells were dispersed into 3 ml RPMI 1640 with 10 mM HEPES, supplemented with 10% FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin and 10 mM L-glutamine. The cell suspension was passed through a wire mesh filter and splenic lymphocytes were then separated from erythrocytes by means of Ficoll–Hypaque gradient centrifugation.

Preparation of cell lysates

Spleen cells and isolated T cells and B cells were washed and incubated for 15 min on ice in the presence of cold lysis buffer containing: HEPES (50 mM, pH 7.2), NaCl (150 mM), MgCl2 (1.5 mM), EGTA (1 mM), Triton X-100 (1%), glycerol (10%), Na-orthovanadate (1 mM), Na-pyrophosphate (30 mM), PMSF (1 mM), leupeptin (10 μg/ml) and aprotinin (10 μg/ml) and centrifuged at 14,000 rpm for 10 min at 4°C.
Protein content in supernatants was detected by Bradford method.

Western blot analysis

Lysates were boiled for 5 min in the presence of sample buffer. Equal amounts of proteins (30 μg/lane) were separated on SDS-PAGE and transferred to nitrocellulose membranes. Non-specific binding sites were blocked by 1 h incubation with 1% BSA and the nitrocellulose membranes were reacted for 1 h at 24°C with primary Y13-259 anti p21v-H-ras rat monoclonal antibodies (1: 10 dilution), rabbit anti-GAP polyclonal antibody (1:100), rabbit anti-hSOS1 polyclonal antibody (1:200) and mouse monoclonal antibody which recognizes the phosphorylated forms of the activated JNK-kinase enzyme (1:100), followed by six washings. Membranes were incubated for 1 h at 24°C with secondary goat anti-rat IgG (1:10,000), anti-rabbit IgG (1:10,000) and anti-mouse IgG (1:1000) conjugated to HRP followed by six washings. Nitrocellulose membranes were then incubated with ECL Western blotting detection reagents for 1 min and then exposed to Kodak Biomax film for 15 s. Protein expression was determined by photodensitometry (Computing densitometer, Molecular Dynamics, Model 300A; Eugene OR).

TUNEL assay

Apoptosis, as evidenced by fragmented DNA, was determined using the In Situ Death Detection Kit (Roche, Indianapolis, IN) based on TUNEL technology, according to the protocol supplied by the manufacturer. Each sample was accompanied by a negative control, consisted of uridine triphosphate (UTP) labeled with fluorescein, and a positive control, consisted of DNase I, grade I (Roche, Indianapolis, IN) based on TUNEL technology, according to the protocol supplied by the manufacturer. Each sample was incubated with ECL Western blotting detection reagents for 1 h at 24°C with secondary goat anti-rat IgG (1:10,000), anti-rabbit IgG (1:10,000) and anti-mouse IgG (1:1000) conjugated to HRP followed by six washings. Nitrocellulose membranes were then incubated with ECL Western blotting detection reagents for 1 min and then exposed to Kodak Biomax film for 15 s. Protein expression was determined by photodensitometry (Computing densitometer, Molecular Dynamics, Model 300A; Eugene OR).

Statistical analysis

Mann–Whitney and unpaired Student’s t test were used for the evaluation of significant differences between treated and untreated groups. Values of $P \leq 0.05$ were considered significant.

Results

Treatment with hCDR1 ameliorates disease manifestations of (NZBxNZW)F1 female mice with established SLE

(NZBxNZW)F1 female mice at the age of 6–7 months, when lupus-like manifestations were already observed, were treated with 10 weekly s.c. injections of hCDR1 (50 μg/mouse/week), or a scrambled peptide (50 μg/mouse/week), or the vehicle (PBS). Mice were followed for disease manifestations. Two weeks after the end of the treatment, mice were sacrificed and their kidneys were evaluated for immune complex deposits. Table 1 summarizes the clinical evaluations of a representative experiment. (NZBxNZW)F1 female mice treated with PBS alone had high levels of anti-dsDNA autoantibodies. Treatment with the hCDR1 significantly reduced the levels of the anti-dsDNA autoantibodies ($P = 0.0001$). In addition, a marked decrease in proteinuria levels was recorded following treatment with hCDR1, in comparison to the untreated mice. Kidney sections obtained from the vehicle-treated (NZBxNZW)F1 female mice at the age of 9 months revealed diffuse pattern of immune complex deposits (total IgG) in their glomeruli. The number and intensity of the immune complexes were significantly reduced ($P = 0.01$) in the hCDR1-treated mice. None of the mentioned parameters were improved in response to treatment with a scrambled peptide. These data demonstrate that treatment with the hCDR1 is specific and results in a significant amelioration of disease manifestations.

The p21Ras signal transduction pathway

Expression of p21Ras

The p21Ras pathway is involved in cell growth and differentiation, but also in mediating immunological responses. Therefore, we studied whether treatment with hCDR1 affects its expression in SLE-afflicted mice. As can be seen in Fig. 1, the expression of p21Ras in whole cell lysate was approximately 2-fold increased in spleen cells of (NZBxNZW)F1 female mice with established disease as compared to healthy controls. Treatment with the hCDR1 for 10 weeks did not modify the expression of p21Ras. No correlation was found between p21Ras expression and any of the clinical or laboratory parameters of treated and untreated mice (data not shown). These data suggest that the p21Ras expression is increased in spleen cells of SLE-afflicted (NZBxNZW)F1 mice, as compared to healthy controls, regardless of disease activity or treatment with hCDR1.

Expression of p21Ras regulatory elements hSOS1 and p120GAP

To further examine the regulation on p21Ras pathway in (NZBxNZW)F1 female mice, we studied the expression of the p21Ras regulatory elements hSOS1 and p120GAP in whole cell lysate. Fig. 1 demonstrates that levels of hSOS1 were 3-fold reduced in both vehicle- and hCDR1-treated (NZBxNZW)F1 female mice as compared to healthy controls. In contrast, no difference was observed in the mean levels of p120GAP in any of the groups. These data demonstrate that the expression of the p21Ras stimulatory element hSOS1 is down-regulated in spleen cells of (NZBxNZW)F1 female mice with established disease regardless of disease activity or hCDR1 treatment while the expression of its inhibitory element p120GAP remains normal.

JNK kinase activity

To further investigate the p21Ras pathway, we examined the activity of the p21Ras down-stream key regulatory enzyme
JNK kinase in established lupus in (NZBxNZW)F1 female mice and the effect of hCDR1 treatment. The expression of the phosphorylated active form of JNK kinase (pJNK) in whole cell lysates was 8-fold increased in the vehicle-treated mice as compared to healthy controls (Fig. 1). A 10-week treatment with the hCDR1 resulted in a significant decrease of 33% (P < 0.05) in the level of pJNK (Fig. 1). To find out the cell type that was affected by the hCDR1, we tested pJNK expression in isolated T and B cell populations. The results presented in Fig. 2 show that the up-regulated expression of pJNK in splenocytes from SLE-afflicted mice occurred prominently in the T cell population. Accordingly, the expression of pJNK was significantly increased (P = 0.01) in the isolated T cells of mice with established disease as compared to T cells of young healthy mice (Fig. 2). Treatment with hCDR1 significantly reduced (P = 0.03) the over-expression of pJNK. The effect on pJNK expression in T cells was specific to hCDR1 since treatment with the control scrambled peptide did not affect significantly pJNK expression in the cells (Fig. 2). The expression of pJNK in the B cell population was comparable in both the healthy and the diseased mice and neither hCDR1 nor the scrambled peptide modified it (data not shown).

Taken together, these data indicate that the late signaling of the p21Ras pathway is up-regulated in the T cells of (NZBxNZW)F1 female mice with established disease and that this activity is reduced by treatment with the hCDR1 peptide.

**JNK kinase activity is associated with the clinical status of (NZBxNZW)F1 female mice with established disease**

We determined whether the reduced activity of JNK kinase following treatment with the hCDR1 is associated with the amelioration of disease manifestations. The results showed that the intensity of immune complex deposits in kidneys of individual mice was associated with JNK kinase activity. As exemplified in Fig. 3, kidney sections of the vehicle-treated mouse designated "1/3", which was also found to have high activity of JNK kinase, had diffuse glomerular IgG immune complex deposits in comparison to young health mice represented by mouse "4/1". In contrast, no such deposits were found in the kidney of an age-matched hCDR1-treated mouse designated "3/2", with the low JNK kinase activity (Figs. 3E, F). Fig. 3 shows also that amelioration in the kidney disease was specific to hCDR1 because treatment with a control scrambled peptide, as

---

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Anti-dsDNA autoantibodies Mean ± SEM (O.D.)</th>
<th>Proteinuria Mean ± SEM (g/L)</th>
<th>Intensity of ICD Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>2.3 ± 0.2</td>
<td>7.20 ± 9.08</td>
<td>2.22 ± 1.03</td>
</tr>
<tr>
<td>Scrambled peptide</td>
<td>2.2 ± 0.3</td>
<td>5.13 ± 8.36</td>
<td>2.50 ± 0.22</td>
</tr>
<tr>
<td>hCDR1 peptide</td>
<td>1.5 ± 0.3</td>
<td>0.81 ± 1.09</td>
<td>0.90 ± 1.04</td>
</tr>
</tbody>
</table>

* Mice (n = 10/group) were injected subcutaneous. Treatment was given once a week for total 10 times.

**Fig. 1. Decreased expression of phosphorylated JNK kinase following treatment with hCDR1.** (A) Representative Western blot demonstrating the expression of p21Ras, p120GAP, p175 SOS and the phosphorylated form of JNK kinase in (NZBxNZW)F1 female mice representing the various groups. Densitometry units were determined to be 1374, 2052 and 262 for pJNK in hCDR1-, vehicle-treated and healthy controls, respectively. (B) Expression of p21Ras, p120GAP, p175 SOS and phosphorylated JNK kinase in hCDR1 and vehicle (PBS)-treated mice (n = 5) was determined by photodensitometry. Data are mean ± SD and expressed as percent of healthy control (indicated by the dashed line).
represented by mouse “2/1”, did not reduce the intensity of glomerular deposits (Figs. 3G, H).

Decreased JNK kinase activity is associated with reduced apoptosis of T cells of (NZBxNZW)F1 female mice with established SLE.

To determine whether the reduced JNK kinase activity mediated by hCDR1 treatment is associated with decreased apoptosis, we examined apoptosis in spleen cells and in spleen-derived T cells and B cells of the SLE-afflicted (NZBxNZW)F1 female mice. Fig. 4A demonstrates TUNEL staining mean values (±SD) of three experiments. As can be seen in the figure, the diseased mice that were treated with the vehicle alone were characterized by increased number of TUNEL [+] cells in comparison to healthy control mice ($P = 0.002$). In contrast, treatment with the hCDR1 during 10 weeks resulted in a significant reduced rate of apoptosis, as compared to the mice treated with the vehicle only ($P = 0.006$). Treatment with a scrambled peptide resulted in an insignificant decrease of apoptotic cells (Fig. 4A).

Because the enhanced expression of pJNK was shown to originate from T cells rather than B cells of the SLE-afflicted mice, we determined the rate of apoptosis in the two cell populations. As can be seen in Fig. 4B, the frequency of apoptosis in CD4$^+$ cells in mice with established lupus was significantly higher ($P = 0.001$) than that in young healthy mice. Treatment with hCDR1 resulted in a significant reduction ($P = 0.01$) in apoptosis of CD4$^+$ cells in comparison to diseased mice that were treated with the vehicle. Treatment with the scrambled peptide resulted in an insignificant reduction in the apoptosis of CD4$^+$ cells. The frequency of apoptosis in B (CD19$^+$) cells was comparable between healthy and diseased mice and it was not changed by the treatments (Fig. 4B). Thus, the reduced activity of JNK kinase following treatment with hCDR1 is associated with low rates of apoptosis, and the effect

---

Fig. 2. Treatment with hCDR1 reduces specifically the expression of phosphorylated JNK in CD4$^+$ cells of (NZBxNZW) F1 mice. (A) Representative Western blot demonstrating the expression of pJNK in (NZBxNZW)F1 female mouse “1/3” representing the vehicle-treated group, mouse “2/1” representing the scrambled peptide-treated group and mouse “3/2” representing the hCDR1-treated group. The pJNK expression in T cells of healthy controls was obtained from pooled T cell population of individual young mice. (B) Expression of phosphorylated JNK kinase in isolated T cells of mice treated with hCDR1, control scrambled peptide or vehicle was determined by photodensitometry. Data are mean ± SD and expressed as densitometry units.

Fig. 3. Immune complex deposits in kidneys of (NZBxNZW)F1 female mice matched with JNK kinase activity. Three groups of mice ($n = 10$) at the age of 6 months were treated with subcutaneous injections of either the hCDR1, the scrambled peptide or the vehicle once a week during 10 weeks. Healthy control mice also were assessed. All mice in the experiments were tagged and further analyzed for JNK kinase activity at the end of treatment course. Kidney sections representing mouse “1/3” of the vehicle-treated group [A (×100), B (×400)], mouse “4/1” of the healthy controls [C (×100), D (×400)], mouse “3/2” of the hCDR1-treated group [E (×100), F (×400)] and mouse “2/1” of the scrambled peptide-treated group [G (×100), H (×400)].
Discussion

This report demonstrates for the first time that the beneficial clinical and immunological effects of treatment with hCDR1 in an established spontaneous SLE-like disease of (NZBxNZW)F1 female mice are associated with reduced activity of the pro-apoptotic factor JNK kinase and with decreased apoptosis in the T cell population.

The elucidation of the mechanisms underlying the beneficial effects of the hCDR1 is of great importance. We have previously demonstrated in the (NZBxNZW)F1 female mouse model and in the 16/6Id-induced experimental SLE model that the amelioration of disease manifestations following treatment with peptides based on the CDR of an anti-DNA antibody is associated with the down-regulation of the pathogenic cytokines, namely, IFN-γ, IL-10 and TNF-α. In contrast, the immunosuppressive cytokine TGF-β was shown to be up-regulated in the mice treated with the CDR-based peptides [8,10,13]. However, the intracellular signaling mechanisms associated with and possibly underlying these treatment-mediated changes in the cytokine patterns remained unclear. Our previous findings in lymphocytes of SLE patients suggested that the prominent up-regulated constitutive MAP kinase activity feeds back up-stream to suppress the early signaling events of this pathway [23].

Our current findings in (NZBxNZW)F1 female mice add further support to this concept indicating that p21Ras/MAP kinase aberrant signaling is common to murine and human SLE. These data are in agreement with other reports demonstrating multiple/multi-level early, middle and late intracellular signaling aberrations in lymphocytes of these patients including the generalized increased overall protein phosphorylation following cell stimulation [14]. Our data in (NZBxNZW)F1 female mice are also supported by the recent report by Niculescu et al. demonstrating an increased constitutive activity of JNK kinase in SLE-prone mice in another model of lupus-like chronic graft-versus-host disease (GVHD) [38].

Some but not all of the p21Ras/MAP kinase signaling defects in human SLE patients such as aberrant hSOS membrane cytoplasmic ratio and the reduced responsiveness of this ratio to cell stimulation were reported in active but not in non-active patients. This suggested a correlation between SLE disease activity and the extent of functional inadequacy of this pathway. It is of interest that immunomodulating therapy with linomide resulting in prevention of a different autoimmune disease, namely autoimmune diabetes of NOD mice, was also associated with disappearance of p21Ras functional defect [39]. These reports are in agreement with our current findings demonstrating over-expression of pJNK in splenocytes from (NZBxNZW)F1 female mice with established lupus in comparison to disease-free mice. Furthermore, the increased expression of pJNK was revealed in the T cells and not in the B cells as detected in experiments utilizing isolated cell populations. Here, we show that treatment with hCDR1 improves the clinical and immunological parameters of the disease together with a significant decrease in JNK kinase activity of the T cells.

The cause of hCDR1-mediated decrease in JNK phosphorylation is not clear as the expression of p21Ras and its regulatory factors remained unchanged in the treated animals. This may indicate that treatment with hCDR ameliorated a preexisting functional defect of this pathway in SLE [24,40], or circumvented this defect using several other downstream signaling pathways such as p38 that interact with p21Ras pathway to regulate lymphocyte activation and function [41]. This concept is supported by our previous reports of reversible functional defects along the p21Ras pathway to regulate lymphocyte activation and function [41].
The implications of increased JNK kinase activity in lupus are not fully understood yet, and the roles of MAPK family members in apoptosis remain controversial. Thus, the JNK pathway is reported to facilitate apoptotic [37] but also anti-apoptotic processes [44,45]. In this study, we found that splenocytes from the diseased mice with the enhanced pJNK expression exhibited a high rate of apoptosis comparing to young free of disease (NZBxNZW)F1 female mice. Furthermore, whereas apoptosis rate of B cells was comparable in both healthy and diseased mice, the apoptosis rate of T cells was significantly higher (P = 0.001) in the SLE-afflicted mice. As demonstrated, the T cells from the diseased mice simultaneously expressed high JNK activity and increased rate of apoptosis, and the observed amelioration in lupus clinical manifestations following treatment with hCDR1 occurred together with a significant reduction in both JNK activity and apoptosis rate of the splenocytes, and more prominently of the T cells (Figs. 2 and 4). In contrast, there were no differences in JNK activity and apoptosis of B cells in neither healthy nor diseased mice, and the effect of hCDR1 on JNK activity and apoptosis of B cells was minimal and insignificant.

Another functional aspect of JNK kinase is concerning the Th1 cytokine generation [46–49]. High activity of JNK kinase is expected to promote Th1 effector cell maturation with the subsequent increased secretion of IFN-γ, known to be pathogenic in several models of murine lupus [50–53]. This is in line with our previous findings showing the down-regulation of the “pathogenic” Th1-type cytokine, IFN-γ, after treatment with the CDR-based peptides [8,10].

In conclusion, treatment with the hCDR1 peptide ameliorates beneficially the clinical manifestations of SLE in (NZBxNZW)F1 female mice. This improvement is associated with decreased constitutive activity of JNK kinase and of apoptosis in the T cells of the diseased mice. These findings underscore the role of the pro-apoptotic JNK kinase and the p21Ras/MAP kinase pathway in the pathogenesis of SLE-like disease in (NZBxNZW)F1 female mice.

Acknowledgment

This work was supported (E.M.) by Teva Pharmaceutical Industries Ltd., Israel.

References

M.J. Rapoport et al. / Clinical Immunology 117 (2005) 262 – 270


