Interleukin-11 Inhibits NF-κB and AP-1 Activation in Islets and Prevents Diabetes Induced with Streptozotocin in Mice

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This laboratory has reported that multiple low doses of streptozotocin (MLD-STZ) similarly upregulate the T helper (Th)1-type proinflammatory cytokines tumor necrosis factor (TNF)-α and interferon (IFN)-γ in islets of both the diabetes-susceptible male and the diabetes-resistant female C57BL/6 mice and that MLD-STZ downregulates the anti-inflammatory Th2-type cytokines interleukin (IL)-4 and IL-10, as well as the anti-inflammatory Th3-type cytokine-transforming growth factor (TGF)-β1 in islets of male, but not female, mice. Thus, diabetes is associated with a relative preponderance of local proinflammatory cytokines. Here, we investigated the effects of MLD-STZ on the anti-inflammatory cytokine IL-11 and the transcription factors nuclear factor (NF)-κB and activator protein (AP)-1, which are involved in gene activation of proinflammatory cytokines, and on the cytosolic kinase (IKK-α) of NF-κB inhibitor (IκB). Furthermore, the effect of recombinant human (rh)IL-11 on MLD-STZ diabetes, insulitis, cytokines, IKK-α, NF-κB, and AP-1 was analyzed in islets.

Interleukin-11 prevented diabetes without affecting insulitis; attenuated TNF-α and IFN-γ response; and stimulated IL-4 production and inhibited activation of IKK-α, NF-κB, and AP-1. The results demonstrated the potential of rhIL-11 in preventing MLD-STZ diabetes through enhancement of anti-inflammatory responses in islets. In this process, the transcription factors NF-κB and AP-1 might play a key role. Exp Biol Med 229:425–436, 2004

Key words: MLD-STZ diabetes; cytokines; NF-κB; AP-1; IKK-α

This study was supported by the German Research Foundation SFB 503 "Molecular and Cellular Mediators of Exogeneous Noxae," project B5, by the Foundation "*Das zuckerkranke Kind,*" and by the Ministry for Health, Bonn, Germany.

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Received October 18, 2003.
Accepted January 29, 2004.

1535-3702/04/2292-0001$15.00

In general, the balance between T helper (Th)1-type and Th2-type cytokine profiles is crucial in the pathogenesis of type 1 diabetes (1). Hence, a bias toward the proinflammatory Th1-type cytokines tumor necrosis factor (TNF)-α and interferon (IFN)-γ promotes inflammatory insulitis and diabetes (2, 3), whereas preponderance of the anti-inflammatory Th2-type cytokines interleukin (IL)-4 and IL-10 counteracts Th1-type cytokine effects and prevents ß-cell destruction (4–6). The regulatory, anti-inflammatory Th3-type cytokine transforming growth factor (TGF)-ß also suppresses Th1-type cytokine production (7), and transgenic overexpression of its gene targeted to ß-cells protects from diabetes (8). Reactive oxygen species (ROS) are likely to be the ultimate mediators of ß-cell destruction (9, 10). In male C57BL/6 mice whose diabetes has been induced with multiple low doses of streptozotocin (MLD-STZ), IL-4, IL-10, and TGF-β1 are downregulated in islets *ex vivo*, whereas IFN-γ and TNF-α are similarly upregulated in both diabetes-susceptible males and diabetes-resistant females.

Furthermore, in contrast to male mice, IL-4, IL-10, and TGF-β1 in islets of female mice are not affected by MLD-STZ (11). In chronic inflammatory disease, activation of both transcription factors—the ROS-sensitive nuclear factor (NF)-κB (12) and activator protein (AP)-1 (13)—are essential in cytokine gene activation and progression of pathogenesis. The pivotal role of NF-κB in MLD-STZ diabetes has been demonstrated in mice deficient in the p50 NF-κB subunit, because they are diabetes-resistant compared with their female counterparts (14). Thus, inhibition of NF-κB activation might be effective in preventing diabetogenesis. Treatment with the anti-inflammatory cytokine recombinant human (rh)IL-11 ameliorates disease signs in animal models of inflammatory disease (15), in human psoriatic lesions (16), and in the spontaneously diabetic NOD mouse (17) by shifting Th1-type cytokine responses toward anti-inflammatory Th2-type reactivities. Inasmuch as rhIL-11 has a strong anti-inflammatory potential, we studied

its effects on clinical, histologic, immunologic, and molecular parameters in MLD-STZ diabetes.

Materials and Methods

Animals. C57BL/6 mice of both sexes, 5–6 weeks old, were obtained from Harlan Winkelmann GmbH (Borchen, Germany). They were kept under specific pathogen-free conditions and were given a rodent diet (Ssniff, Soest, Germany) and drinking water *ad libitum*. They were 7–8 weeks old at the beginning of the experiments. The experiments were conducted in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 85-23, revised 1995) and the German Law on the Protection of Animals

Reagents. Recombinant human (rh) IL-11 was donated by W.L.T. (Wyeth Research, Cambridge, MA) with activities as described (17). All the reagents were obtained from companies residing in Germany. Streptozotocin, Taq polymerase, proteinase inhibitor, T4 polynucleotide kinase, and Moloney murine leukemia virus reverse transcriptase (MMLV-RT) were purchased from Roche Diagnostics GmbH (Mannheim); collagenase (0.42 U/mg) and trypsin (1.25 mg/ml) from Sigma (Deisenhofen); and PBS and TRIzol reagent from Life Technologies GmbH (Karlsruhe). Reagents for saponine buffer, hematoxylin, and sodium citrate buffer were obtained from Merck (Darmstadt), and fluorescein isothiocyanate (FITC)-coupled or phycoerythreine (PE)-coupled monoclonal antibodies against cytokines and isotype antibodies were obtained from PharMingen (Hamburg). Primer pairs were commercially synthesized by MWG Biotech .GmbH (Ebersberg) as follows: IL-11: 5′-TGCTGACAAGGCTTCGAGTAG-3′, 3′-CAGTCGAGTCTTTAACAACAGC-5′; IL-11R: 5′-CTGATGAAGGCACTT-ATGTCTG-3′, 3′- CATCTG-TTATCACTTCCTCCAAAG-5′; TGF-ß1: 5′-CTCC-CAC-TCCCGTGGCTTCTAG-3′, 3′-GTTCACACCTCGTTGT-ACACCTTG-5′; ß-actin: 5′-AAGTACCCCATTGAA-CATG-3′, 3′-AGGAGCAATGATCTTGATC-5′. NF-κB consensus oligonucleotides (5‘-AGTTGAGGGGACTTTC-CCAGGC-3‘), and AP-1 (5‘-CGCTTGATGAGTCAGCC-GGAA-3‘) were commercially synthesized by Promega (Mannheim).

Treatment of Mice. Streptozotocin was dissolved in sodium citrate buffer (18). To induce diabetes, male mice were injected ip with STZ, 40 mg/kg body wt each on 5 consecutive days (19). Interleukin-11 was dissolved in phosphate-buffered saline (PBS) and injected ip into male mice at a daily dose of 10 μg for 13 subsequent days: on the 4 days before the first STZ injection, 1 hr before each of the five STZ injections, and on the 4 days after the last STZ injection. Control groups received rhIL-11 only or remained untreated. The oral glucose tolerance test (OGTT) was performed (20) at weeks 4, 12, and 20 after the first STZ injection and in age-matched control groups. Streptozotocin–injected female mice served as controls to evaluate diabetes-associated effects in male mice.

Determination of Plasma Glucose. At weekly intervals, blood samples were collected (20). Diabetes was defined as a nonfasting blood glucose concentration greater than 11.1 mM for 3 or more consecutive weeks.

Islet Isolation. Islets were isolated by collagenase digestion (21) on day 1 after the third injection, on day 1 and/or 3 after the last STZ injection, and from age-matched controls.

RNA Preparation and RT-PCR. Total RNA was extracted from pools of 800 to 1000 islets isolated from groups of 10 mice each as previously described (18). The RNA preparation was stored at -80°C until use. By using MMLV-RT, 1 μg of total RNA was reversely transcribed into cDNA, followed by amplification of the target genes by PCR (22). The RT reaction was amplified using Taq polymerase. The cycle numbers were chosen to be on the linear, or exponential, phase of the amplification of the three genes: 35 for IL-11, 32 for IL-11R, 30 for TGF-ß1, and 30 for ß-actin. The annealing temperature was 56°C for IL-11, 58°C for IL-11R, 55°C for TGF-ß1, and 55°C for ß-actin. For separation, the amplified PCR products, 8 μl of each (i.e., the target product and ß-actin), were loaded on 1% agarose gels containing ethidium bromide (0.1 μg/ml). The resulting bands were quantified with Lumi-Imager (Roche Diagnostics, Mannheim, Germany). The ratio of the intensity integral to the target PCR products to that of ß-actin was calculated. To exclude the possibility of genomic DNA contamination during RNA preparation, negative controls were set up for each PCR amplification using purified RNA as a template.

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay (EMSA). Between 500 and 700 islets isolated from groups of 7 mice each were separated into single cells by trypsin digestion for 5 mins at 37°C, using a syringe, and washed twice in PBS (11). Then the cells were separated into nuclei and cytosols using a lysis buffer for 10 mins on ice as described (23), with slight modification (24). The isolated nuclei were accumulated as pellet by centrifuging the lysed cells. The supernatant containing the cytosolic fraction was stored at -80°C until use for determination of IKK-α activity. The pellet was resuspended in a nuclear extraction buffer for 25 mins on ice and centrifuged. The supernatant containing the nuclear extract was stored at -80°C until use for determination of NF-κB and AP-1 activities. Double-stranded synthetic oligonucleotide probes for NF-κB and AP-1 were end-labeled using γ-^{32}P [dATP] (Hartmann Analytic, Braunschweig, Germany) and T4 polynucleotide kinase. The specificity of the NF-κB and AP-1 signals was approved by adding unlabeled (cold) consensus sequences in excess (10 pmol) for target competition. Binding reactions containing equal amounts of protein (4 μg) and labeled oligonucleotide probes were performed in binding buffer. The protein-DNA complexes were electrophoresed using a

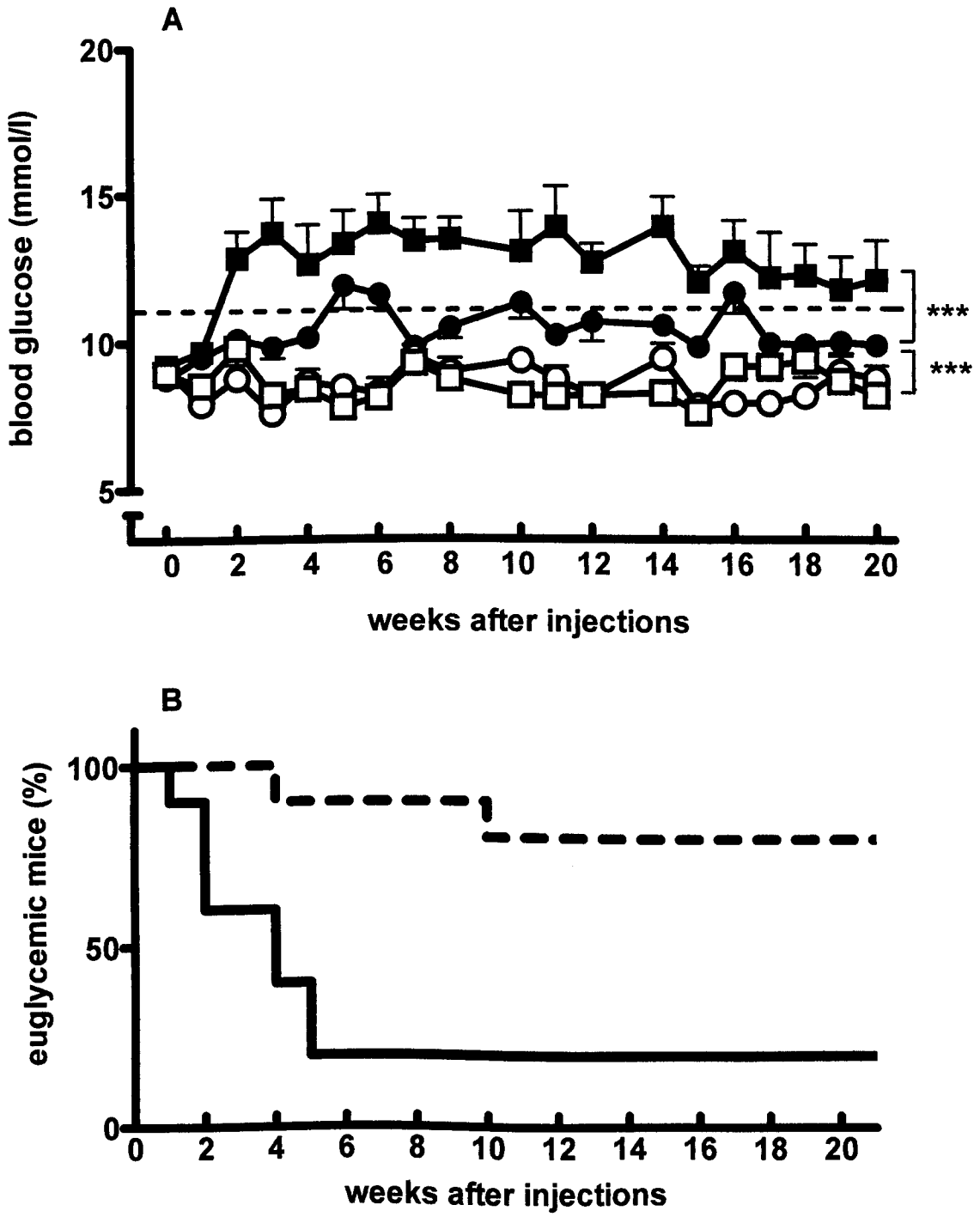
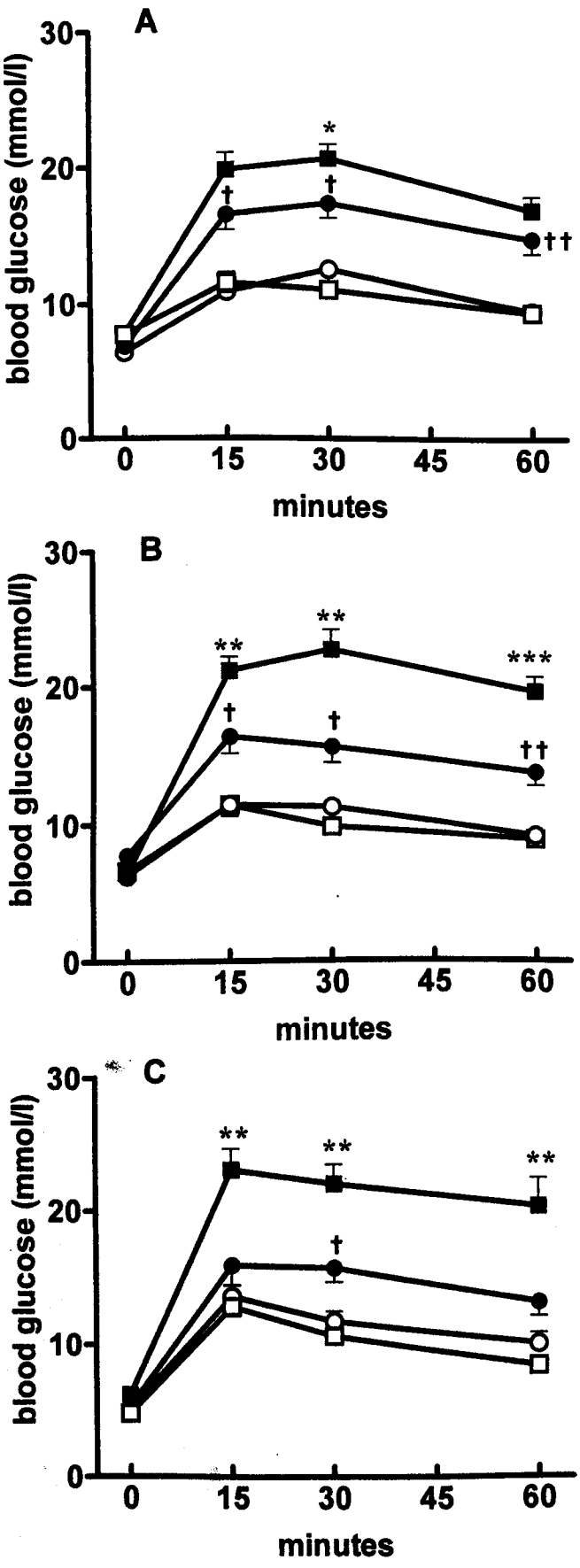


Figure 1. Effect of interleukin-11 (rhIL-11) on diabetes induced with multiple low doses of streptozotocin (MLD-STZ) in male C57BL/6 mice. (A) blood glucose concentrations (means ± SE) over time in weeks. Mice were injected with MLD-STZ—either alone (black squares) or in addition to rhIL-11, that is, 13 injections of 10 μg each (black circles)—or with rhIL-11 (white circles) or remained untreated (white squares). Each group injected with MLD-STZ consisted of 10 mice and, for the control groups, 6 mice each were used. Interleukin-11 prevented (***, $P < 0.001$) hyperglycemia comparing the areas under the curves of the MLD-STZ-injected group versus the group injected with rhIL-11 in addition. Yet, the blood glucose concentrations of the latter group were still higher (***, $P < 0.001$) compared with the control groups. (B) Percentage of mice with euglycemia in the MLD-STZ-injected group (solid line) and the group injected with both MLD-STZ and rhIL-11 (dotted line). Interleukin-11 conferred protection against MLD-STZ diabetes.

nondenatured 6% polyacrylamide gel. The gels were dried and exposed to autoradiographic films. The films were scanned, and band intensity was quantified using TINA 2.09d quantification software (Raytest).

Measurement of IKK-α Activity. In principal, IKK-α activity was determined through phosphorylation of IκB as described (25), with slight modification. Briefly, stored fractions were mixed with equal amounts of the RL lysis



buffer (25 mM HEPES of pH 7.4, 150 mM NaCl, 20 mM ß-glycerphosphate, 2 mM EGTA, 50 mM NaF, 1 mM Na-orthovanadate, and 1% Triton X-100) containing a complete protease inhibitor cocktail set (Roche, Mannheim, Germany) and incubated with an anti-IKK-α antibody sc-7182 (Santa Cruz, CA) for 1 hr prior to addition of the protein A-sepharose beads and incubated further overnight at 4°C. Cytosolic kinase antigen-antibody immune complexes were recovered with protein A-sepharose beads (Sigma, Deisenhofen, Germany) and washed three times with lysis buffer and twice with kinase buffer (25 mM HEPES of pH 7.4, 20 mM MgCl, 20 mM ß-glycerphosphate, 0.5 mM EGTA, 0.5 mM NaF, and 0.5 mM Na orthovanadate). The beads with the bound immune complexes were incubated with a reaction solution of 15 µl containing kinase buffer, 1 µCi of [γ-^{32}P] ATP, and 2 µg of the IκB-α protein (1-317) (Santa Cruz, CA) as substrate for 30 mins at 37°C. The reactions were terminated by adding 15 µl of 2 × SDS-PAGE loading buffer, then this mixture was boiled for 5 mins. Samples were resolved with 12% SDS-PAGE and the phosphorylated substrates indicating kinase activity were visualized by autoradiography. The films were scanned, and band intensity was quantified using TINA 2.09d quantification software (Raytest).

Determination of Cytokine-Positive Cells by Flow Cytometry. Islet cell suspensions were prepared and stained with antibodies as previously described (11). Isotype controls were used to guide quadrant setting. Briefly, 10,000 cells were analyzed for one specified cytokine using a FACScalibur flow cytometer and the Cell Quest program (Becton Dickinson, Heidelberg, Germany).

Histologic Examination. For light microscopy, groups of 5 mice each, injected with rhIL-11 and/or MLD-STZ, were killed on day 12 after the first STZ injection. Control mice received rhIL-11 alone or remained untreated. For histology, sections were prepared as described (20, 26). Coded slides were independently examined for infiltrates with mononuclear cells at both islet-poles and intra-islet sites by two of the authors. Perivascular or periductular sites at islet poles were examined for presence of infiltrates. The degree of insulitis was scored as follows: 0 = no infiltrate; 1+ = mild infiltrate

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Figure 2. Effect of rhIL-11 on oral glucose tolerance tests (OGTT) in male C57BL/6 mice injected with MLD-STZ. Blood glucose values (means ± SE) before (0), at 15, 30, and 60 mins after a glucose load of 2.0 g/kg body wt at weeks 4 (A), 12 weeks (B), and 20 weeks (C) after the first STZ injection. Mice were treated with MLD-STZ, either alone (black squares) or in addition to 130 µg (13 injections of 10 µg each) rhIL-11 (black circles), or with rhIL-11 (white circles) or remained untreated (white squares). Each group injected with MLD-STZ consisted of 10 mice and, for the control groups, 6 mice each were used. Interleukin-11 ameliorated intolerance induced with MLD-STZ. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$ comparing MLD-STZ-treated versus MLD-STZ- plus rhIL-11-treated groups; $P <$ 0.05 and $=P < 0.01$ comparing MLD-STZ- plus rhIL-11-treated versus control groups.

Table 1. Effect of Treatment with Multiple Low Doses of Streptozotocin (MLD-STZ) and Interleukin-11 (rhIL-11) on Infiltrates with Mononuclear Cells in Pancreatic Islet of Male C57BL/6 Mice[a]

	Islets with mononuclear cell infiltrates, %					
	At islet poles		At intra-islet sites			
Treatment of mice	Absent	Present	0	1+	2+	3+
rhIL-11	85.7	14.3	96.1	2.7	1.2	0.0
MLD-STZ	47	53	87.1	7.1	3.6	2.2
rhIL-11 + MLD-STZ	34.3	65.7	85.2	8.3	4.4	2.1

[a] Data were obtained from at least 300 islets per group.

(≤30% of cells in islets); 2+ = moderate infiltrate (30%–75% of cells in islets); 3+ = severe infiltrate (>75% of cells in islets).

Data Analysis. Data presenting molecular analyses are means ± SE of three or four independent experiments. For statistical analysis, the unpaired ANOVA or Student's *t* test was used. $P < 0.05$ was considered statistically significant.

Results

Interleukin-11 Prevents MLD-STZ Diabetes Without Changing Insulitis. Although MLD-STZ induced hyperglycemia in male C57BL/6 mice, injections of rhIL-11 resulted in a reduction ($P < 0.001$) in blood glucose that persisted for the observation period of 20 weeks (Fig. 1A). Diabetes developed in 80% of the mice injected with MLD-STZ alone, but only in 20% of those receiving rhIL-11 in addition (Fig. 1B). Yet the blood glucose concentrations in the group treated with both rhIL-11 and MLD-STZ were still higher ($P < 0.001$) than those in the control groups (Fig. 1A). Treatment with rhIL-11 alone did not affect blood glucose concentrations. In concordance with the results shown in Figure 1 are those obtained with the OGTT (Fig. 2) as an *in vivo* measure of ß-cell function. Streptozotocin induced a severe glucose intolerance that was significantly ameliorated by rhIL-11. Yet the response to the glucose challenge was still significantly impaired compared with the responses obtained in the control groups at week 4 (Fig. 2A), week 12 (Fig. 2B), and week 20 (Fig. 2C) after the first injection of STZ. Interestingly, the deteriorated glucose tolerance in the group treated with MLD-STZ alone persisted on a similar level over 20 weeks, whereas incremental improvement to near normal developed with time in the group receiving rhIL-11 in addition (Fig. 2C). Apparently, rhIL-11 had a long-lasting effect in protecting ß-cell function from MLD-STZ damage.

As depicted in Table 1, the degree of insulitis was similar in the mice injected with MLD-STZ alone and those receiving rhIL-11 in addition to MLD-STZ. The minor infiltrates in rhIL-11-injected controls could have resulted from a nonspecific reaction to the injections. Consistent with previous findings (20, 26), no infiltrates were seen in islets of pancreas sections from untreated controls (data not shown).

Streptozotocin Reduces mRNA Levels of IL-11 and IL-11R in Male but Not Female Mice. To analyze effects of MLD-STZ on the mRNA levels of IL-11 and its receptor IL-11R, islets of MLD-STZ- and solvent-injected mice were used. To evaluate an association between changes and diabetes susceptibility, the islets of MLD-STZ- and solvent-injected female mice were analyzed as well. In islets of male mice, reduction of IL-11 mRNA levels was already severe ($P < 0.001$) on day 1 after the last STZ injection and was even more pronounced on day 3 thereafter (Fig. 3A). The IL-11R mRNA levels were not reduced ($P < 0.001$) until day 3 after the last STZ injection (Fig. 3B). In islets of female mice, in contrast, MLD-STZ did not significantly change the mRNA levels of either IL-11 or IL-11R (Figs. 3A and B, respectively).

Interleukin-11 Shifts MLD-STZ Responses Toward Anti-Inflammatory Reactions. Treatment with rhIL-11 deviated MLD-STZ-induced, inflammatory *ex vivo* responses in islets toward anti-inflammatory reactions (Fig. 4). The percentage of cells producing the Th1-type proinflammatory cytokines TNF-α and IFN-γ remained close to the constitutive levels found in untreated control mice. In contrast, the percentage of cells producing the Th2-type anti-inflammatory cytokine IL-4 was increased and by far exceeded the value of the control group. Interleukin-11 by itself did not alter the constitutive cytokine profiles found in untreated controls (Fig. 4). It also prevented severe reduction of the mRNA levels of IL-11 and IL-11R induced by MLD-STZ, a significant increment, yet clearly below constitutive levels as calculated for IL-11R mRNA. The MLD-STZ-induced reduction of TGF-ß1 was not changed by rhIL-11 (Fig. 5).

Interleukin-11 Inhibits Activation of NF-κB, AP-1, and IKK-α by MLD-STZ. The specificity of NF-κB and AP-1 activity determinations was verified by cold target competition analyses (Fig. 6). The unlabeled consensus sequences completely prevented registration of signals.

Streptozotocin increased the *ex vivo* activity of NF-κB ($P < 0.001$) in islets isolated from C57BL/6 male mice on day 3 after the last STZ injection. In islets of female donors, however, a transient reduction ($P < 0.01$) of the NF-κB activity was induced only on day 1 after the last STZ injection; on day 1 after the third and day 3 after the fifth

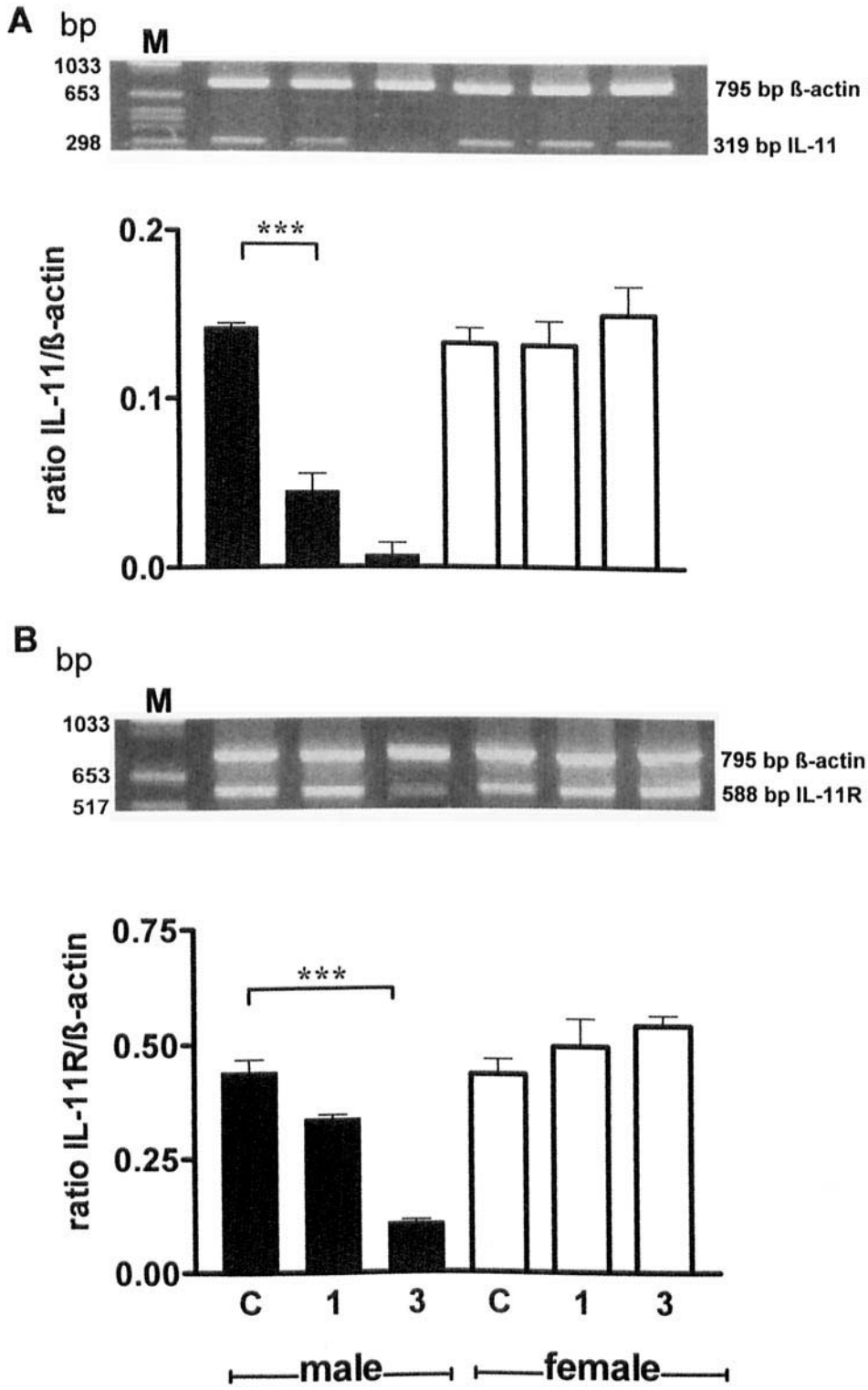

Figure 3. RT-PCR determination of rhIL-11 (A), IL-11R (B), and ß-actin mRNA in pancreatic islets isolated from of C57BL/6 mice of both sexes that had been injected with multiple low doses of streptozotocin (MLD-STZ) or with the solvent of STZ as controls (C). Islets were isolated on either day 1 or day 3 after the fifth STZ injection. MLD-STZ treatment resulted in profound reduction of the mRNA levels of IL-11 and IL-11R in islets of male mice, whereas the levels in islets of female mice remained unaffected. The level of ß-actin mRNA remained unchanged in islets of both sexes. Means ± SE of the ratio of IL-11 to ß-actin mRNA indicate a reduction (***, $P < 0.001$) on day 1 after the last STZ injection, which was even more pronounced on day 3 thereafter compared with the ratio of untreated controls. Means ± SE of the ratio of IL-11R to ß-actin mRNA indicated a reduction (***, $P < 0.001$) on day 3 after the last STZ injection compared with the ratio of untreated controls. M, marker. bp, base pairs.

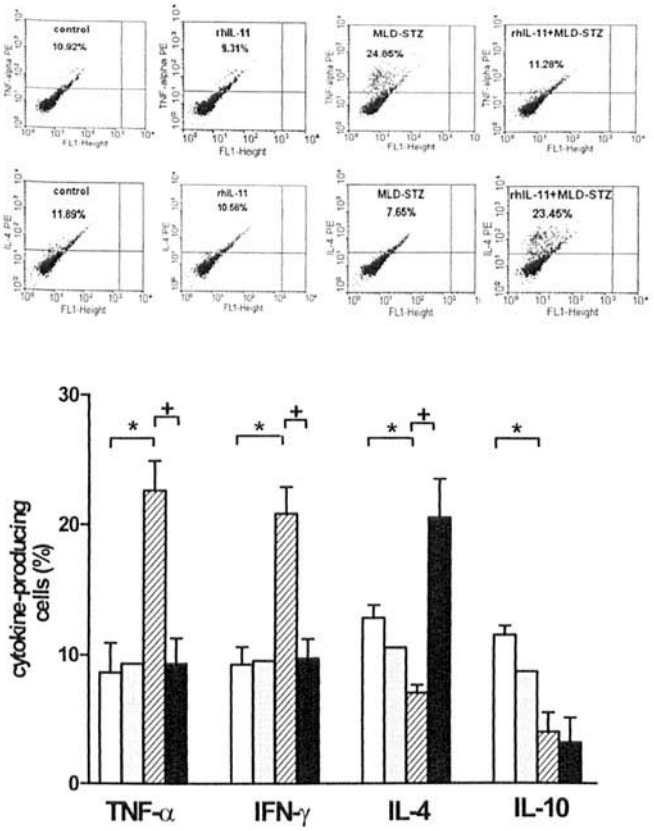

Figure 4. Effect of rhIL-11 on the percentage of cytokine producing-cells in islets isolated from male C57BL/6 mice that had been injected with MLD-STZ—either alone (striped bars) or in addition to rhIL-11 (black bars)—or with rhIL-11 alone (pointed bars) or remained untreated (white bars) as controls. Islets were isolated on day 3 after the fifth STZ injection and from age-matched controls. Dotplots for TNF-α and IL-4 are shown. Interleukin-11 attenuated TNF-α- and IFN-γ-producing cells and stimulated production of IL-4. Interleukin-11 did not affect the constitutive cytokine profile. Because rhIL-11 alone had no effects, this control experiment was conducted only once. Means ± SE of three independent experiments are given. *, $P < 0.05$ comparing MLD-STZ-treated versus untreated control mice. +, $P < 0.05$ comparing MLD-STZ-treated versus MLD-STZ- plus rhIL-11-treated groups.

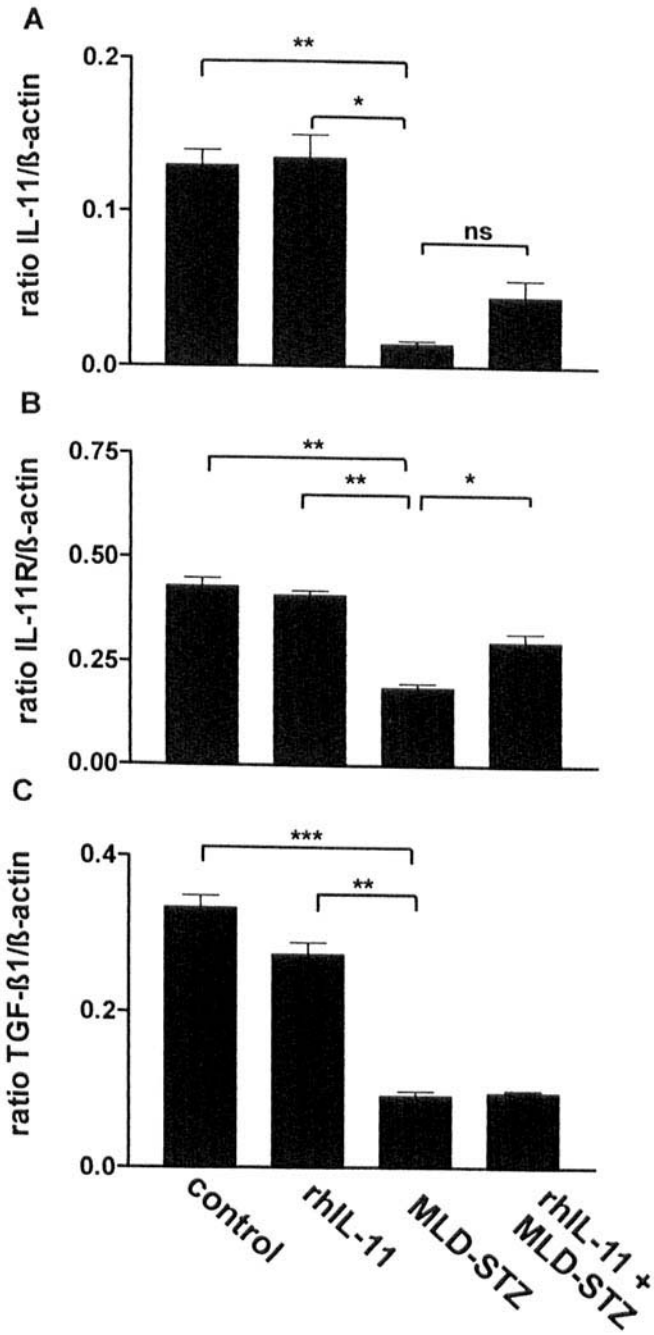

Figure 5. Effect of rhIL-11 on levels of mRNA expression of of IL-11, IL-11R, and TGF-ß1 in islets isolated from male C57BL/6 mice that had been injected with MLD-STZ—either alone or in addition to rhIL-11—or remained untreated or had received rhIL-11 alone as controls. Islets were isolated on day 3 after the last STZ injection. Streptozotocin profoundly reduced the expression of all three target genes; additional treatment with rhIL-11 exerted a significant change only on the expression of IL-11R. Injections with rhIL-11 alone did not change the expression. Means ± SE of the ratio of the target gene over ß-actin are given. ns, not significant. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

STZ injection the mean activity remained below that of the control values (Fig. 7A). The AP-1 activity (Fig. 7B) was significantly increased on day 3 after the fifth STZ injection in islets of both sexes. Treatment of male mice with rhIL-11 in addition to MLD-STZ, in contrast, inhibited ($P < 0.001$) activation of both NF-κB and AP-1 (Fig. 8).

In the signal cascade of NF-κB activation, its inhibitor protein IκB is phosphorylated by IKK-α and subsequently ubiquitinated or proteolytically degraded. Therefore, the observation that rhIL-11 inhibits the MLD-STZ-induced activation of NF-κB prompted investigations of IKK-α activity. Multiple low doses of streptozotocin alone stimulated IKK-α activity ($P < 0.001$) on day 3 after the last STZ injection. This stimulation, however, was attenuated ($P < 0.01$) by additional treatment with rhIL-11 (Fig. 9), which prevented nuclear translocation of NF-κB.

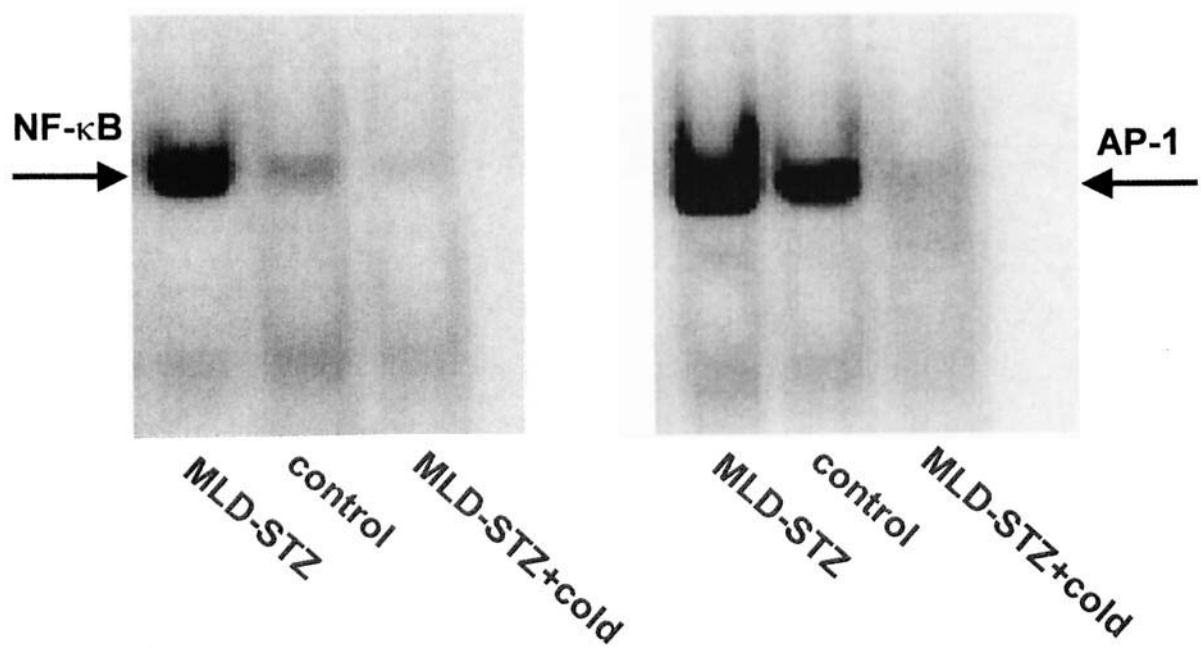

Figure 6. Nuclear factor (NF-κB) and activator protein (AP-1) activity in islets of C57BL/6 male mice that had received five injections of STZ or the solvent of STZ as control. The islets were isolated on day 3 after the last injection. Addition of the unlabeled consensus sequences (cold) abolished the MLD-STZ-induced activity signals of both NF-κB and AP-1 and proved specificity of the labeled reagents.

Discussion

The present results are the first to demonstrate that treatment with the multifunctional anti-inflammatory cytokine rhIL-11 prevented MLD-STZ diabetes in mice. A total of 130 μg rhIL-11, given at equal doses for 13 subsequent days, long-lastingly abrogated MLD-STZ-induced hyperglycemia. Yet, the blood glucose levels remained significantly higher compared with the levels of the control groups that remained untreated or were injected with rhIL-11 alone. Consistent with the beneficial rhIL-11 effect on diabetes are the OGTT data. The markedly increased blood glucose levels on glucose challenge in MLD-STZ-injected mice were significantly reduced by rhIL-11 and gradually decreased to near normal during the weeks after treatment.

The mechanism through which rhIL-11 affords protection from MLD-STZ diabetes most likely relies on its potent immuno-modulating activities to inhibit inflammatory reactions and stimulate anti-inflammatory responses. Thus, *ex vivo*, rhIL-11 reduced the local MLD-STZ-induced Th1-type cytokines TNF-α and INF-γ in isolated islets and stimulated the production of Th2-type cytokine IL-4. The percentage of IL-4-producing cells markedly surmounted that in islets of control mice. Moreover, rhIL-11 inhibited activation by MLD-STZ of the transcription factors NF-κB and AP-1, as well as the kinase IKK-α of the NF-κB inhibitor IκB. Because NF-κB and AP-1 participate in the transcriptional regulation of cytokine genes (27, 28) and because their activation results in pro-inflammatory cytokine production, these transcription factors may be key regulators in the pathway of local cytokine responses involved in immune-mediated ß-cell destruction by MLD-STZ.

The shift of MLD-STZ-induced cytokine responses by rhIL-11 toward anti-inflammatory reactions is assumed to be secondary to an enhancement of the activity of NF-κB inhibitors that bind NF-κB in the cytosolic fraction and prevent its nuclear translocation (29). Our data on the central role of NF-κB in MLD-STZ diabetogenesis substantiate recent findings indicating that mice deficient in the p50 subunit of NF-κB targeted to ß-cells are resistant to MLD-STZ diabetes, whereas no effect was exerted on diabetes induced with a single high toxic dose of STZ (14). Because T cell-dependent inflammatory reactions are stimulated only in the MLD-STZ model—but not after injection of a single toxic dose of STZ—NF-κB, obviously, regulates MLD-STZ-induced immune responses. The failure

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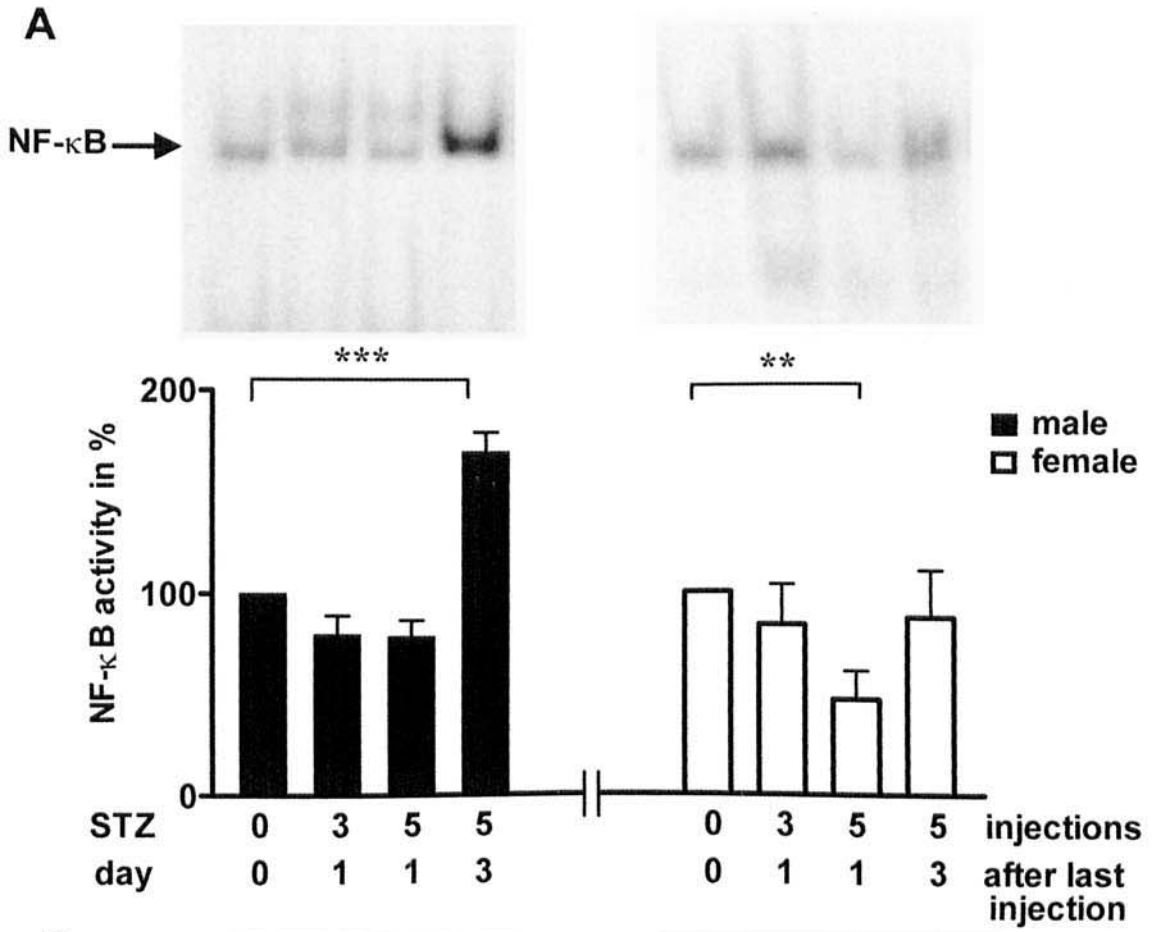
Figure 7. NF-κB (A) and AP-1 (B) activity in islets of C57BL/6 mice of both sexes that had received three or five injections of STZ or five injections of the solvent of STZ (0) as control. The NF-κB activity was increased (***, $P < 0.001$) on day 3 after the fifth STZ injection in islets of male mice compared with the activity in control islets. In islets of female donors, in contrast, a transient reduction (**, $P < 0.01$) of the activity was induced on day 1 only after the fifth STZ injection. The AP-1 activity was up-regulated (*, $P < 0.05$; **, $P < 0.01$) in islets isolated from both sexes on day 3 after the fifth STZ injection.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NF-κB
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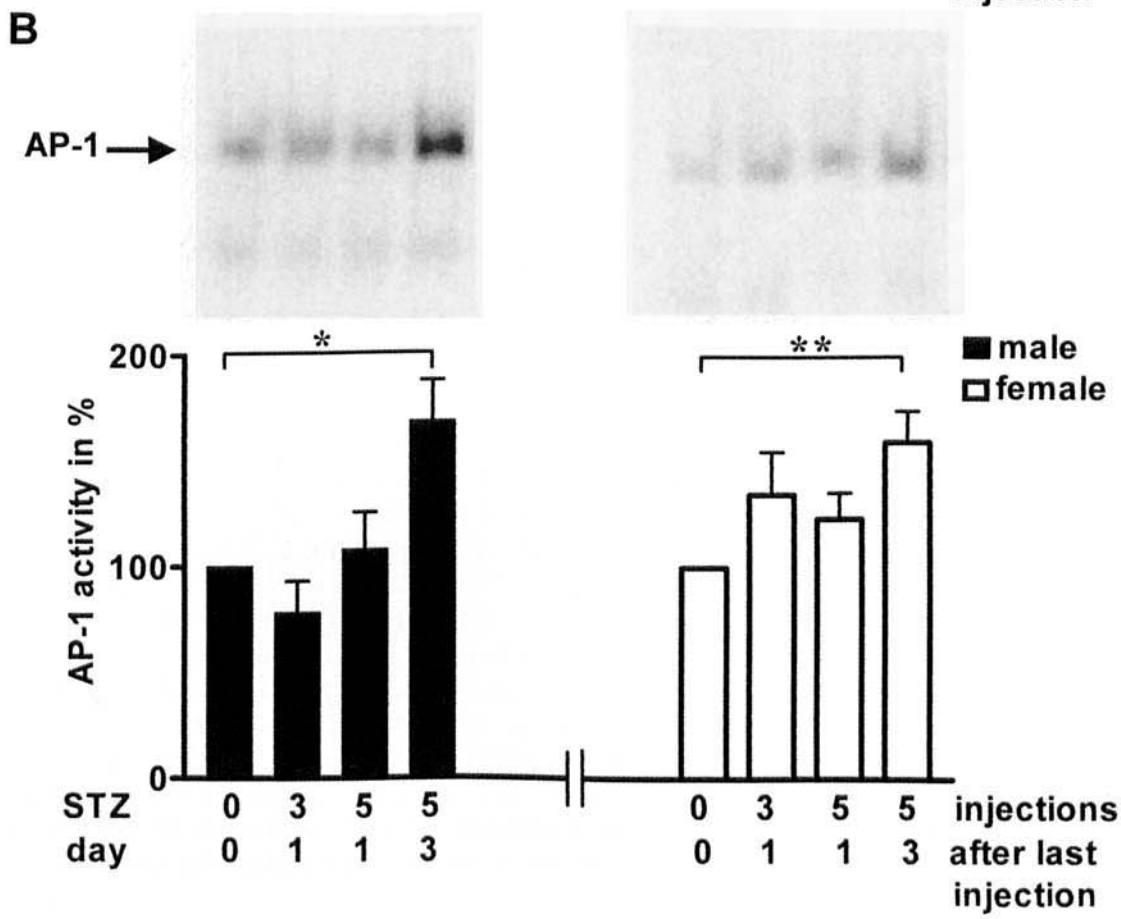
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AP-1
*
**
200
100
0
AP-1 activity in %
male
female
STZ 0 3 5 5 0 3 5 5 injections
day 0 1 1 3 0 1 1 3 after last injection

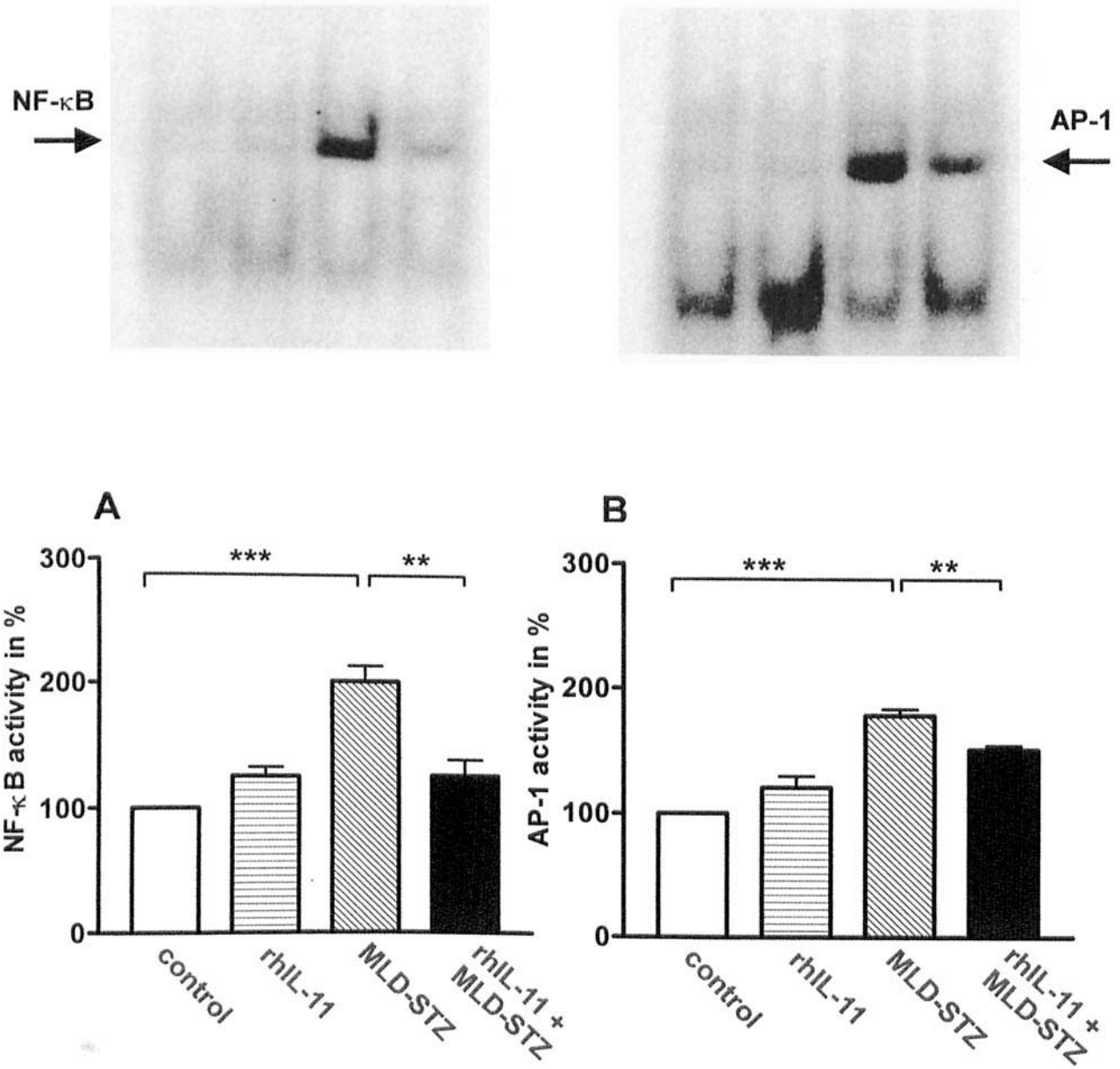

Figure 8. Effect of rhIL-11 on NF-κB (A) and AP-1 (B) activity in islets isolated from male C57BL/6 mice that had been injected with MLD-STZ—either alone or in addition to rhIL-11—or with the solvent of STZ or rhIL-11 alone as controls. Islets were isolated on day 3 after the last of five STZ injections. Interleukin-11 reduced (**, $P < 0.01$) the MLD-STZ-induced (***, $P < 0.001$) activity of NF-κB and AP-1. Interleukin-11 alone did not affect NF-κB or AP-1 activity.

of rhIL-11 to prevent insulitis may lie in its potential to shift, but not to abrogate, immune responses. Conceivably, the majority of the infiltrating mononuclear cells were non-specifically recruited, and they remained functionally inert. Therefore, similar to the present observations, no effects on MLD-STZ-induced islet infiltrates have been observed with other approaches to prevent MLD-STZ diabetes (20, 26). Obviously, attenuation of TNF-α and INF-γ production, stimulation of IL-4 production, and prevention of activation of IKK-α, NF-κB, and AP-1 by rhIL-11 sufficed to protect against MLD-STZ diabetes. The finding that rhIL-11 did not abrogate reduction of the anti-inflammatory Th3-type cytokine TGF-ß1 is comparable to *in vitro* observations using murine macrophages (30).

In trying to dissect the cascade of molecular interactions in MLD-STZ diabetogenicity, we propose that the pathway is activated by two different STZ effects that are possibly initiated by ROS: (i) direct toxicity on the essential ß-cell molecule GLUT2 (18) and (ii) T cell-dependent stimulation of TNF-α and INF-γ cytokines and activation of IKK-α and NF-κB. Evidence for these two initial effects is the following: (i) ROS can be generated in islets both *in vitro* with STZ (31) and *ex vivo* from MLD-STZ-treated male but not female C57BL/6 mice (32); and (ii) STZ is an

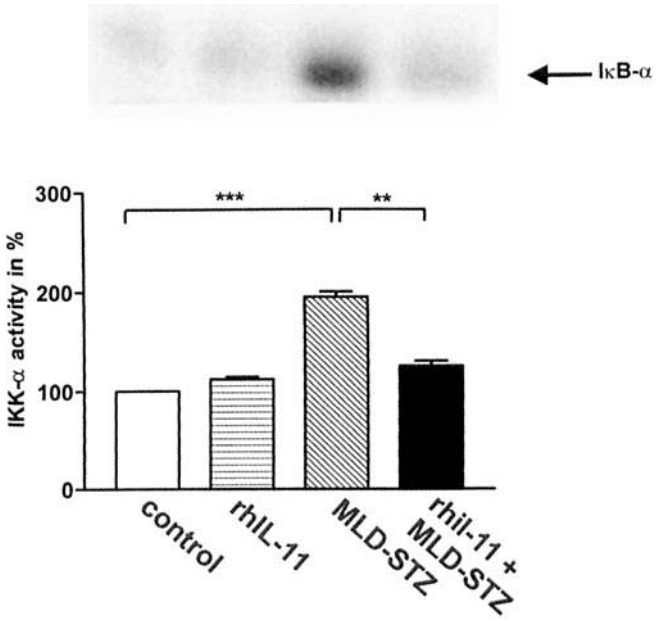

Figure 9. Effect of rhIL-11 on cytosolic kinase-α (IKK-α) activity in islets from male C57BL/6 mice that had been injected with MLD-STZ—either alone or in addition to rhIL-11—or with the solvent of STZ or rhIL-11 alone as controls. Islets were isolated on day 3 after the last of five STZ injections. Cytosolic kinase-α activities were measured by an *in vitro* kinase assay using IκB-α (1-317) as a substrate. Interleukin-11 reduced (**, $P < 0.01$) the MLD-STZ-induced (***, $P < 0.001$) activity of IKK-α. Interleukin-11 alone did not affect IKK-α activity. Means ± SE of two independent experiments.

antigen for T cells *in vivo* (33), and the T cell activation might be triggered by ROS, as elaborated for T cell lines (34). In response to STZ, Th1-type cytokines and other immune cells may transiently produce low levels of ROS that activate NF-κB (35). Subsequently, this might result in gene activation of proinflammatory cytokines which, in turn, activate and are activated by NF-κB. This regulatory circuit amplifies and sustains local inflammatory responses, reduces the anti-inflammatory activities, and finally results in ß-cell damage. Noteworthy, both effects of STZ are required for diabetes induction, because the disease process is abrogated by either inhibiting uptake of STZ through the GLUT2 (18, 36) or suppressing inflammatory immune responses (37). With regard to the effect of inflammatory immune reactions, NF-κB and AP-1 are, apparently, pivotal regulators of local cytokine gene activation in MLD-STZ diabetes. TNF-α and INF-γ are similarly up-regulated by MLD-STZ in islets of C57BL/6 mice of both sexes, whereas reduction of the anti-inflammatory cytokines IL-4, IL-10 (11), and IL-11—as well as activation of NF-κB and AP-1—are associated with diabetes only in male C57BL/6 mice (present data). Once NF-κB and AP-1 are activated, the continuously vicious rebound effect between these transcription factors and proinflammatory gene activation sustains the local, chronic inflammatory process and reduces the anti-inflammatory potential. As a consequence of this cycle, it is also possible that, beyond day 3 after the last STZ injection, the up-regulation of TNF-α and INF-γ by MLD-STZ in islets of male mice exceeds that of female donors. In that case, the absolute instead of the relative imbalance between Th1- and Th2-type cytokines might be decisive for diabetogenesis. Analyses at later time points, however, are hindered by loss of ß-cells because of the method applied.

Having demonstrated the effect of rhIL-11 in rescuing ß-cells from MLD-STZ–induced inflammatory damage, we believe that further investigation is required to resolve the cascade of cellular and molecular effects by which rhIL-11 attenuates noxious immune reactions in pancreatic islets. It is necessary to define the cell(s) targeted by rhIL-11 and to analyze additional transcription factors such as signal transducer and activator of transcription (STAT) involved in immune cell activation. Such information might help establish schedules for monitoring treatment with rhIL-11 in clinical settings.

Interleukin-11 has been shown to inhibit NF-κB activation (29), to ameliorate disease signs in psoriatic lesions by selective down-regulation of proinflammatory pathways and up-regulation of anti-inflammatory cytokines in lesional skin (16), and to retard diabetes manifestation in NOD mice, which is associated with a decrement of TNF-α and INF-γ levels in the serum (17). Above that, as shown in the present data, rhIL-11 prevents MLD-STZ diabetes by attenuating NF-κB and AP-1 activation and deviating the local cytokine profile in pancreatic islets from inflammatory reactions toward anti-inflammatory responses. Because treatment with rhIL-11 is not accompanied by serious side effects (16, 38), further clinical evaluation of this cytokine is warranted for intervention in individuals at high risk for type 1 diabetes.

In conclusion, MLD-STZ diabetes was persistently prevented with injections of the pleiotropic cytokine rhIL-11, which prevented activation of NF-κB and AP-1 and shifted the local inflammatory Th1-type cytokine responses in pancreatic islets to an anti-inflammatory profile. Because rhIL-11 has been successfully used in preventing chronic inflammatory diseases in animal models and in humans, its use for intervention in individuals at risk for type 1 diabetes should be considered.

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