The anti-inflammatory cytokine recombinant human interleukin-11 inhibits activation of the transcription factors NF-kB and AP-1 in pancreatic islets and prevents diabetes induced with multiple low doses of streptozotocin in male C57BL/6 mice

I n a u g u r a l – D i s s e r t a t i o n

zur
Erlangung des Doktorgrades der
Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Abdelhakim Lgssiar

aus Marrakesch (Marokko)

Veröffentlicht mit der Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Heinrich-Heine-Universität Düsseldorf

Dekan:	**Prof. Dr. rer. nat. Gerd Fischer**
Prodekan:	**Prof. Dr. rer. nat. Peter Westhoff**
1. Berichterstatter:	**Prof. Dr. med. Helga Gleichmann**
2. Berichterstatter:	**Prof. Dr. rer. nat. Frank Wunderlich**

Tag der mündlichen Prüfung: 24.05.2004

TABLE OF CONTENTS

1. INTRODUCTION 1

1.1 Type 1 diabetes -T1D -: general aspects..... 1

1.2 The Th1/Th2 concept..... 2

1.2.1 In immune reactions..... 2

1.2.2 In T1D..... 3

1.3 NF-κB and AP-1 in immune responses..... 5

1.3.1 NF-κB as regulator of inflammatory immune responses.. 5

1.3.2 AP-1 as regulator of inflammatory immune responses.... 7

1.4 The MLD-STZ diabetes model..... 7

1.4.1 Chemical structure of STZ and immuno-toxicology of MLD-STZ..... 7

1.4.2 Impact of cytokines and ROS..... 8

1.4.3 Key role of NF-κB in STZ-induced diabetes..... 10

1.4.4 Key role of AP-1 in MLD-STZ diabetes..... 11

1.5 IL-11..... 11

1.5.1 History and effects of rhIL-11 in animal models and in humans..... 11

1.5.2 Biochemistry and function of IL-11 receptor - IL-11R -..... 13

1.6 Zinc-ions -Zn^{2+}- and experimental diabetes..... 14

1.6.1 Biological functions of Zn^{2+}..... 14

1.6.2 Zn^{2+} supplementation and prevention of experimental T1D 14

2. SCOPE OF THIS THESIS..... 16

3. MATERIAL AND METHODS..... 17

3.1 Materials..... 17

3.1.1 Mice..... 17

3.1.2 Reagents..... 17

3.1.3 Buffers and solutions..... 20

3.1.3.1 Buffers and solutions for treatments of mice..... 20
3.1.3.2 Buffers and solutions for determination of plasma glucose..... 20
3.1.3.3 Buffers and solutions for islet isolation..... 21
3.1.3.4 Solution for separation of islets into single cells..... 21
3.1.3.5 Buffers and solutions for RNA electrophoresis..... 22
3.1.3.6 Buffers and solutions for FACS-analysis..... 22
3.1.3.7 Buffers and solutions for preparation of nuclear extracts..... 23
3.1.3.8 Buffers and solutions for electrophoretic mobility shift assay..... 24
3.1.3.9 Buffers and solutions for protein kinase assays..... 24
3.1.3.10 Buffers and solutions for histological examination..... 25
3.1.4 Technical equipment..... 26
3.1.5 One way material..... 27
3.2 Methods..... 28
3.2.1 Treatment of mice..... 28
3.2.1.1 Induction of MLD-STZ diabetes..... 28
3.2.1.2 Treatment with rhIL-11..... 29
3.2.1.3 Treatment of mice with Zn^{2+}-enriched drinking water..... 29
3.2.1.4 Oral glucose tolerance test (OGTT)..... 29
3.2.1.5 Determination of plasma glucose..... 30
3.2.2 Islets preparation..... 30
3.2.2.1 Isolation of islets..... 30
3.2.2.2 Separation of islets into single cells..... 31
3.2.2.3 Fixation of islet cells..... 31

3.2.2.4 Permeabilization of islet cells and staining with monoclonal antibodies..... 31
3.2.3 FACS-analysis and cytokine-producing cells..... 32
3.2.4 RNA preparation..... 32
3.2.4.1 RNA isolation from islets..... 32
3.2.4.2 Determination of RNA concentration..... 33
3.2.5 Reverse transcription polymerase chain reaction (RT-PCR)..... 33
3.2.5.1 Principles of the RT-PCR method..... 33
3.2.5.2 Synthesis of cDNA..... 34
3.2.5.3 PCR..... 34
3.2.5.4 Separation of PCR products..... 35
3.2.5.5 Quantification of PCR products..... 36
3.2.6 Electrophoretic mobility shift assay (EMSA)..... 36
3.2.6.1 Preparation of nuclear extracts..... 36
3.2.6.2 End-labelling of oligonucleotides..... 36
3.2.6.3 Removal of unincorporated label..... 37
3.2.6.4 DNA binding reaction using nuclear extracts..... 37
3.2.6.5 NF-κB and AP-1 activity by EMSA..... 37
3.2.7 Measurement of IKK-α activity..... 38
3.2.8 Histological examination..... 38
3.2.9 Data analysis..... 40

4. RESULTS..... 41
4.1 MLD-STZ reduce the mRNA expression of IL-11 and IL-11R in pancreatic islets of male C57BL/6 mice..... 41
4.2 rhIL-11 prevents MLD-STZ-induced hyperglycemia in male C57BL/6 mice..... 43
4.3 rhIL-11 ameliorates ß-cell function in MLD-STZ-injected male mice..... 44
4.4 rhIL-11 stimulates Th2-type cytokine responses in

islets of MLD-STZ-injected male mice..... 46
4.5 Effects of treatment with rhIL-11 on mRNA expression of endogenous IL-11, IL-11R, and TGFß-1..... 48
4.6 MLD-STZ stimulate NF-κB and AP-1 activity..... 50
4.7 rhIL-11 inhibits activation by MLD-STZ of NF-κB and AP-1..... 54
4.8 rhIL-11 inhibits stimulation by MLD-STZ of IKK-α activity in islets of male mice..... 55
4.9 rhIL-11 does not change insulitis..... 56
4.10 Zn^{2+}-enriched drinking water inhibits ex vivo stimulation by MLD-STZ of NF-κB and AP-1 activities in islets of male mice..... 58
4.11 Zn^{2+}-enriched drinking water prevents stimulation by MLD-STZ of the IKK-α activity in islets of male mice..... 60
4.12 Zn^{2+} enriched drinking water shifts MLD-STZ- induced cytokine responses from Th1-type to Th2-type in islets of male mice..... 61

5. DISCUSSION..... 64
6. SUMMARY..... 73
7. ZUSAMMENFASSUNG..... 74
8. REFERENCES..... 75
9. ACKNOWLEDGMENTS
10. ANNEX

ABBREVIATIONS

APCs:	antigen-presenting cells
BB:	BioBreeding
BSA:	bovine serum albumin
cDNA:	complementary DNA
CSF:	colony-stimulation factors
DEPC:	diethylpyrocarbonat
DTT:	dithiothreitol
EGTA:	ethylene glycol-bis-(ß-amino-ethyl ether)N,N,N´,N´-tetra-acetic acid
EMSA:	electrophoretic mobility shift assay
FBS:	fetal bovine serum
FCS:	fetal calf serum
FITC:	Fluorescein-isothiocyanate
GLUT:	glucose transporter
GVHD:	graft versus host diseases
H_2O_2:	hydrogen peroxide
HBSS:	Hank´s balanced salt solution
HCl:	hydrogen chloride
HEPES:	n-(2-hydroxyethyl)-piperazin-N´-2-ethansulfonic acid
IFN:	interferon
IL:	interleukin
ip:	intraperitoneally
Jak:	janus kinase
K-Al-sulphate:	potassium aluminium sulfate
$MgCl_2$:	magnesium chloride
MHC:	major histocompatibility complex
MLD-STZ:	multiple low doses of streptozotocin
mRNA:	messenger RNA
MT:	metallothionein
NaCl:	sodium chloride
NaF:	sodium fluoride

$NaHCO_3$:	sodium hydrogen carbonat
Na-jodate:	sodium jodate
Na-orthovanadate:	sodium orthovanadate
NF-κB:	nuclear factor κB
$NO^{\bullet}$:	nitrogen monoxide
NOD:	non-obese diabetic
OGTT:	oral glucose tolerance test
$^{\bullet}OH$:	hydroxyl radical
PBS:	phosphate buffered saline
PE:	phycoerythrin
Poly (di-dc):	polynucleotide (di-dc)
rh:	recombinant human
RNasin:	ribonuclease inhibitor
ROS:	reactive oxygen species
RT-PCR:	reverse transcriptase polymerase chain reaction
SDS:	sodium dodecyl sulfate
STAT:	signal transducer and activator of transcription
STZ:	streptozotocin
T1D:	type 1 diabetes
TGF:	transforming growth factor
Th:	T helper
TNF:	tumor necrosis factor
Zn^{2+}:	Zinc-ions

1. INTRODUCTION

1.1 Type 1 diabetes - T1D - : general aspects

Type 1 diabetes (T1D) develops usually in childhood or early adulthood and results from ß-cell destruction that leads to an absolute insulin deficiency and persistent hyperglycemia. Insulin has to be supplemented lifelong and despite the availability of a specific therapy, many patients will suffer from severe late complications damaging the retina of the eyes, kidneys, nerves, and blood vessels. Although remarkable progress has been achieved concerning therapeutic tools such as new insulins, insulin pumps, and self-monitoring of blood glucose levels, the evaluation of new prevention strategies for individuals at risk still remains an important public health issue (Alberti *et al*., 1998).

T1D is generally considered to be sequelae of a chronically progressing autoimmune disease (Atkinson and McLaren, 1994). Based on a genetic predisposition, environmental factors can initiate the disease process, followed by T cell-dependent inflammatory immune reactions. As environmental factors, toxins (Helgason and Jonasson, 1981) or viruses such as coxsackie virus (Pato *et al*., 1992; Schernthaner *et al*., 1985) and cytomegalovirus (Pato *et al*., 1992; Pak *et al*., 1988) have been reported to be implicated in the induction of T1D. Up to date, however, neither the ß-cell antigen(s) nor the autoreactive T cells in human are known. For ethical reasons, research with human pancreatic islets or ß-cells is not feasible. Therefore, to elucidate molecular mechanisms which could be involved in immune-mediated ß-cell destruction in human T1D, three animal models are of particular value: the non-obese diabetic (NOD) mice (Makino *et al*., 1980) and the BioBreeding (BB) rat (Crisa *et al*., 1992), which develop diabetes spontanously, and the model induced with multiple low doses of streptozotocin (MLD-STZ) as described by Like and Rossini (1976). All of them share pathologic features analogous to those in human such as infiltration of pancreatic islets with mononuclear cells (insulitis). Furthermore, T cell-dependent inflammatory immune reactions are a prerequisite in all three animal models.

1.2 The Th1/Th2 concept

1.2.1 In immune reactions

Very briefly, the Th1/Th2 concept of T helper (Th)1 versus Th2 cells was first described in mice (Mosmann *et al*., 1989; Fitch *et al*., 1993; Powrie *et al*., 1993) and later in humans (Romagnani *et al*., 1992). Based on their surface marker proteins, mature T cells are classified into CD4+ Th cells and CD8+ cytotoxic cells, which recognize antigens associated with major histocompatibility complex (MHC) class II and class I molecules, respectively. Classification of CD4+ T cells into the subsets Th1 and Th2 cells is based on the profile of cytokines they produce. Th1 cells secrete cytokines associated with inflammation such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-2, Th2 cells produce cytokines associated with anti-inflammatory reactions such as IL-4, IL-5, IL-6, IL-10, and IL-13.

In general, Th1 cells and their mediators activate cellular immune responses, whereas Th2 cells are required in the stimulation of humoral immunity (Mosmann *et al*., 1996; Fearon *et al*., 1996; Elenkov *et al*., 1999; Trinchieri *et al*., 1995). Another Th cell type with a unique cytokine production pattern are Th3 cells, which appear to be a $CD4^+$ immune regulatory cell that secretes the transforming growth factor (TGF)-ß (Chen Y *et al*., 1994; MacDonald *et al*., 1998; 1999). The inhibition of Th1 and Th2 responses are mutually. Thus, e.g., IFN-γ inhibits Th2 cell activities, whereas IL-4 inhibits Th1 responses (Gajewski *et al*., 1989; Fiorentono *et al*., 1989). Consequently, a disturbance of the balance between Th1 and Th2 responses and dominance of one subset leads to a polarized immune response and may play a key role in protection or promotion of different immunopathological reactions.

A scheme depicting the principal differenciation of Th1, Th2, and Th3 phenotypes from undifferentiated Th0 cells is presented in Fig. 1.

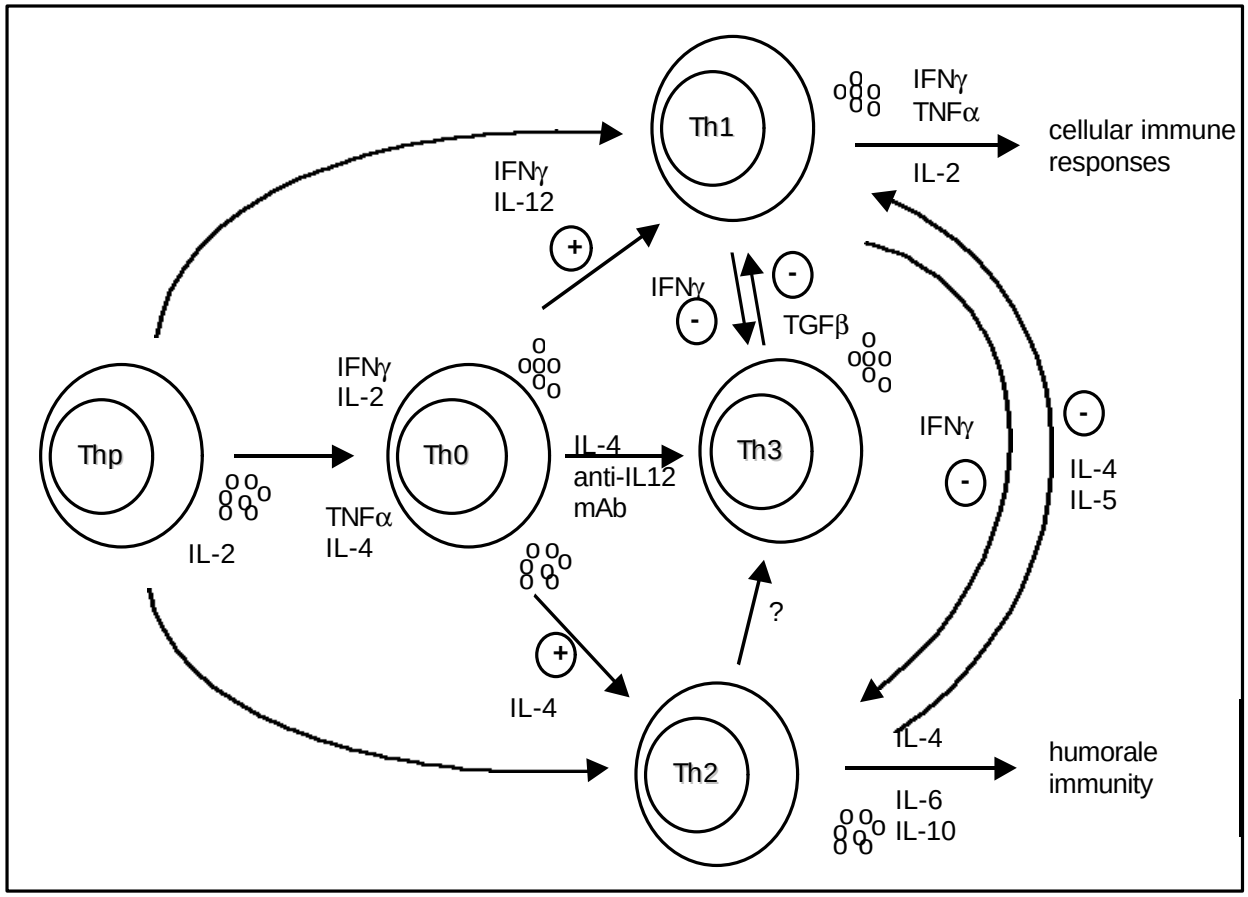

Fig. 1. Unstimulated T cells defined Thp (precursor of T helper cells) can undergo differentiation to a bipotential Th0 cell upon secretion of IL-2 by the innate immune system. The cytokines produced by Th0 cells are decisive in the differentiation to Th1 or Th2 cells. The main inducers of the Th1 cells are IL-2 and IFN-γ, of Th2 cells IL-4, and of Th3 cells IL-4 and monoclonal antibodies against IL-12. These three subpopulations are equipped with a mechanism to negatively regulate the expansion of the other subset. Th1 cells promote mainly cellular immunity, Th2 cells are more implicated in the humoral immunity by stimulating B cells to secrete antibodies, and Th3 cells produce TGF-ß that inhibits the differentiation to Th1 cells.

1.2.2 In T1D

The relevance of the Th1/Th2 concept for T1D was proposed and extended by Rabinovitch (1994) suggesting that an immuno-regulatory defect is associated with an autoimmunue response to the ß-cell in diabetes-susceptible individuals. The deviation of the cytokine profile toward a Th1-type in the microenvironment of islet ß-cells results in pro-inflammatory cytokine production, inhibition of Th2-type cytokines, and production of free radicals that may be directly toxic to ß-cells (Fig. 2).

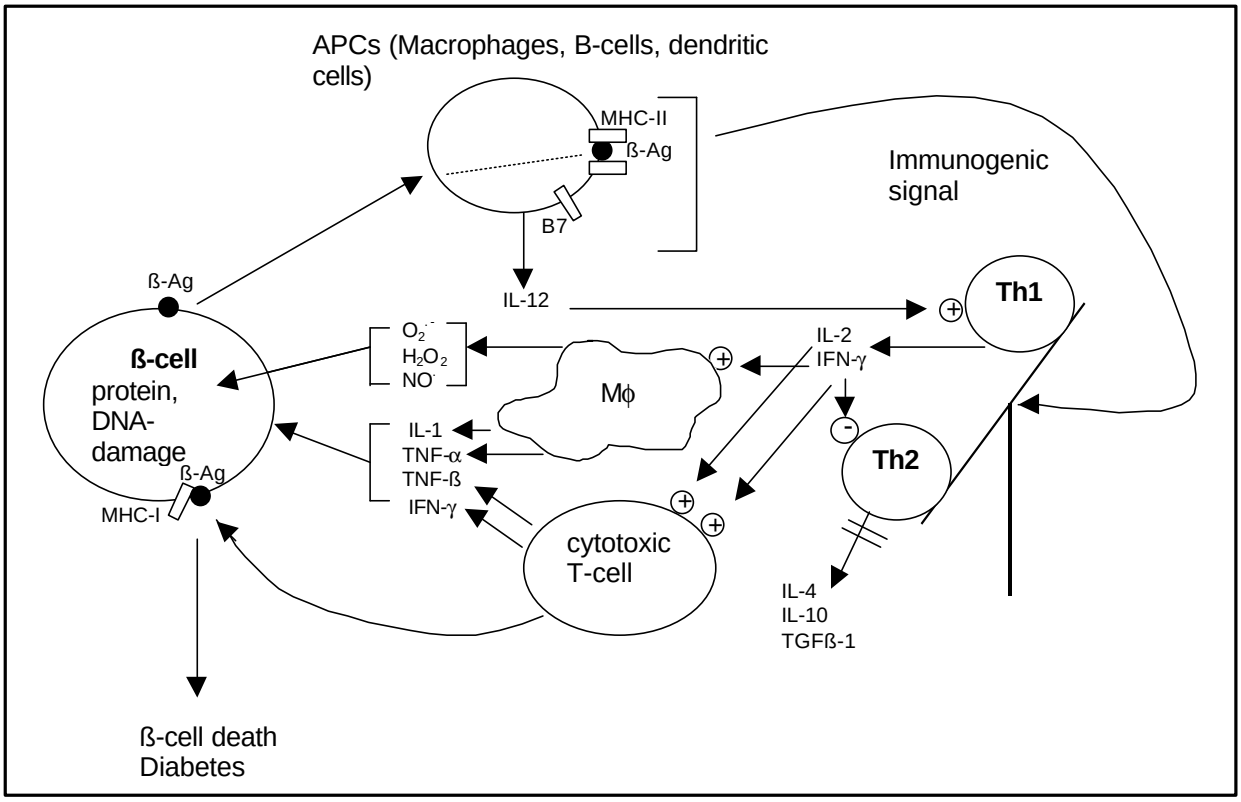

Fig. 2. Hypothesis of initiation and progression of ß-cell destruction. ß-cell-specific autoantigens are processed and presented by antigen-presenting cells (APCs) in association with MHC class II molecules and accessory molecules such as B7. The APCs secrete IL-12 and promote the differentiation of Th0 (not shown) to Th1 cells. The Th1-cells secrete IL-2 and IFNγ which inhibit the production of IL-4 and IL-10. IFNγ also stimulates macrophages to release the pro-inflammatory cytokines TNF-α and IL-1ß as well as free radicals, which can be toxic to ß-cells. Above that, IL-2 and IFNγ can stimulate cytotoxic T cells, which cause, recognizing specific autoantigens on ß-cells, death or apoptosis and eventually diabetes (reproduced from Rabinovitch 1994, with slight modifications).

The data of expression of Th1-type cytokines at mRNA and/or protein levels in the insulitis lesion of animal models as well as humans suggest their central role in the pathogenesis of T1D. Thus, in few studies in patients, only IFN-α (Foulis *et al.*, 1987; Huang *et al.,* 1995; Somoza *et al*., 1994) and IFN-γ (Foulis *et al*., 1991; Yamagata *et al*., 1996) were found to be associated with ß-cell destructive insulitis. Investigations in NOD mice (Liblau *et al*., 1995), BB rats (Katz *et al*., 1995) as well as mice rendered diabetic with MLD-STZ (Müller *et al*., 2002) showed a marked increase of the two pro-inflammatory Th1-type cytokines TNF-α and IFN-γ. In contrast, treatment with the anti-inflammatory Th2-type cytokines IL-4 (Cameron *et al*., 2000) and IL-10 (Pennline *et al*., 1994; Rabinovitch *et al*., 1995) as well as the tansgenic overexpression of the regulatory

anti-inflammatory Th3-type cytokine TGF-ß1 targeted to ß-cells prevented diabetes in NOD mice (King *et al*., 1998). The protective effect was associated with suppression of Th1-type cytokines that coincided with a benigne, non-destructive insulitis without any significant loss of ß-cells (Rabinovitch, 1998; Rabinovitch and Suarez-Pinzon, 1998; Hirai *et al*., 2000).

Obviously, Th1-type cytokines in the micromilieu of the pancreatic islets are mediators of ß-cell destruction and diabetes, whereas intervention with Th2-type cytokines protects ß-cells and prevents diabetes development. Thus, it is justified to search for interventional strategies shifting deleterious Th1-type immune responses toward protective Th2-type reactivities. Therefore, the goal of the present work was to evaluate the effect of another anti-inflammatory cytokine, the recombinant human (rh)IL-11 on MLD-STZ diabetes.

1.3 NF-κB and AP-1 in immune responses

1.3.1 NF-κB as regulator of inflammatory immune responses

Several studies have discovered that there are several reactive oxygen species (ROS)-sensitive biological molecules that are sensitive to low concentrations of ROS and decisive in cell signalling. One potential target of ROS activation is the nuclear transcription factor (NF)-κB.

NF-κB was first identified as a B cell nuclear factor that binds to an intronic enhancer of the immunoglobulin κ-light chain gene (Sen and Baltimore, 1986). NF-κB has been detected in essentially all cell types and appears to play a central role in regulating gene activation of cytokines (Baeuerle *et al*., 1994; Chen F *et al*., 1999). Therefore, development of modulatory strategies targeting this transcription factor may provide a novel therapeutic strategy for the treatment or prevention of various diseases, because dysregulation of NF-κB by different agents is associated with a wide range of human disorder including inflammatory bowel disease (Neurath *et al*., 1998), rheumatoid

arthritis (Foxwell *et al*., 1998), asthma (Barnes *et al*., 1998), and other inflammatory diseases.

As illustrated in Fig. 3, NF-κB dimers are normally arrested in the cytosol by inhibitor proteins called IkBs. Upon extracellular signals such as ROS (Baeuerle *et al*., 1988; Baldwin *et al*., 1996) or the inflammatory cytokines IL-1 and TNF-α (Mellits *et al*., 1993; Sun *et al*., 1995; Tan *et al*., 1994), phosphorylation and rapid dissociation of IκBs from NF-κB is induced. The phosphorylated IκBs are then ubiquitinated and degraded by the cytoplasmic 26S proteasome. This phosphorylation releases NF-κB dimers from the cytoplasmic NF-κB-IκB complex proteins, so that it can translocate to the nucleus and bind to κB promoter sequence of target genes, including transcription of pro-inflammatory genes.

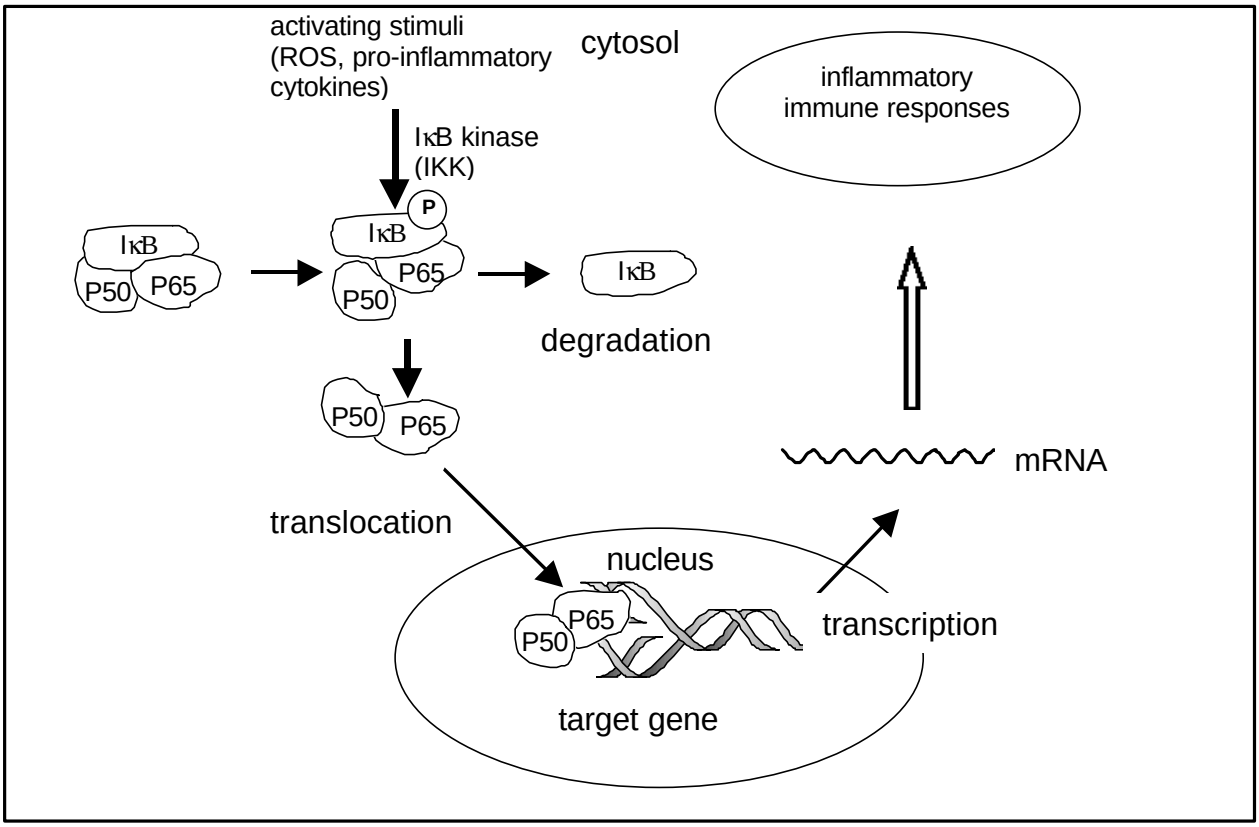

Fig. 3. Simplified signal cascade of NF-κB activation. In response to extracellular inducers such as ROS or pro-inflammatory cytokines, IκB kinases (IKK) activate and phosphorylate IκBs. Phosphorylated IκBs are recognized and degraded by proteasome-mediated degradation of ĸBs. The activated P65/P50 dimerize and translocate to the nucleus for gene activation of inflammatory immune responses.

1.3.2 AP-1 as regulator of inflammatory immune responses

The activator protein (AP)-1 is a key transcription factor that plays a central role in the control of T cell proliferation induced by growth factors (Angel *et al*., 1991) and cell death (Shaulian *et al*., 2002)). It is a homo- or heterodimer composed of the proto-oncogene products c-Jun and c-Fos that binds to a large number of cellular promoters at the DNA sequence TGAGTCA (Chiu *et al*., 1988; Curran *et al*., 1988). Like NF-κB, AP-1 also plays a critical role in the regulation of several pro-inflammatory cytokines (Gottschalk *et al*., 1993; Rao *et al*., 1994). Therefore, the inhibition of AP-1-mediated transcriptional activation seems to be a novel powerful therapeutic strategy for treating inflammatory diseases.

1.4 The MLD-STZ diabetes model

1.4.1 Chemical structure of STZ and immuno-toxicology of MLD-STZ

STZ is derived from the soil micoorganism *streptomyces achromogenes* and has a molecular weight of 265 kDa. It is a glucosamine-nitrosourea compound that was originally found to be an antibiotic for a wide spectrum of organisms (Lewis *et al*., 1960). The structure is composed of a nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other (Fig. 4).

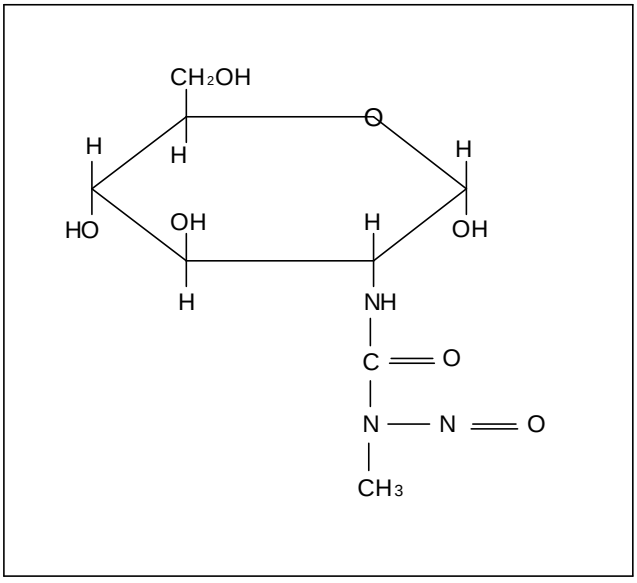

Fig. 4. Structure of streptozotocin

STZ is a relatively selective toxin for pancreatic ß-cells. It is rapidly transported into the ß-cell via its glucose transporter 2 (GLUT2) (Schnedl *et al*., 1994; Wang and Gleichmann, 1998) and used as an agent to induce diabetes in laboratory animals. Like and Rossini (1976) have been the first to report that MLD-STZ, i.e., 40 mg STZ/kg body weight each injected intraperitoneally (ip) on five consecutive days, induce diabetes in CD-1 male mice. Since then, abundant experiments have been performed to analyze the pathogenic pathway in MLD-STZ diabetes. Noteworthy, MLD-STZ diabetes develops only in male mice of susceptible strains, whereas female mice are resistant (reviewed in: Wilson and Leiter, 1990). Thus, when searching for diabetes-associated symptoms, MLD-STZ-injected female mice serve as an ideal control group. MLD-STZ exerts two different effects, namely lesions on ß-cells (Like and Rossini, 1976; Wang and Gleichmann, 1998) followed by T cell-dependent inflammatory immune reactions (Like and Rossini, 1976; Nakamura *et al*., 1984) which are STZ-specific (Klinkhammer *et al*., 1988). As early ß-cell lesion, the GLUT2 has been reported to be a preferential, if not selective, target for STZ-mediated toxicity (Wang and Gleichmann, 1998). Diabetes will gradually develop only, when both effects are mediated, because prevention of either the toxicity by pretreatment with 5 thio-D-glucose or D-glucose (Wang *et al*., 1993) or suppression of T cell-mediated immune reactions (Rossini *et al.,* 1978) is preventive.

1.4.2 Impact of cytokines and ROS

Cytokines are local messengers with low molecular weight that are involved in a variety of important signals such as development of the immune system, cell growth and differentiation, haematopoesis, the inflammatory and anti-inflammatory cascade. Cytokines are not only produced by cells of the immune system, but also by other cells such as keratinocytes, when damaged by ultraviolet irradiation (Shreedhar *et al*., 1998). More than 30 immunologically active cytokines have been identified, which are generally grouped as ILs, IFNs, TNFs, and colony-stimulation factors (CSFs) (Thorpe *et al*., 1992). A major effect of the cytokines is to induce immune responses by stimulating interactions between blood and tissues, so that large numbers of circulating cells of the immune system can be recruited to the site of damages and/or invading particles or antigens with the potential to damage cell organelles. Cytokines are frequently regulated

in cascades and the specificity of the reponse to cytokines is provided by unique receptors. These interactions of cytokines and their receptors are a necessary component of cytokine responses.

Prior to ß-cell destruction, islets are infiltrated with numerous immune cells including dendritic cells, macrophages, B and T cells. Each of these cell types has the capacity to secrete cytokines that either promote diabetes progression or prevent disease induction. Recently, colleagues from our laboratory have reported on the effect of MLD-STZ on Th1-, Th2-, and Th3-type cytokine profiles in the micromilieu of pancreatic islets of mice of both sexes (Müller *et al*., 2002). Since only male mice of susceptible strains develop MLD-STZ diabetes, whereas the female mice are resistant, it was possible to evaluate an association between cytokine profiles in islets and diabetes. They have observed for the first time that MLD-STZ similarly stimulated the production of the pro-inflammatory Th1-type cytokines TNF-α and IFN-γ in islets of C57BL/6 mice of both sexes. However, the levels of the anti-inflammatory Th2-type cytokines IL-4 and IL-10 were significantly reduced in male mice only. These functional results were in line with MLD-STZ effects on the levels of mRNA expression of the cytokines. Furthermore, MLD-STZ down-regulated the mRNA expression of the anti-inflammatory Th-3 type cytokine TGFß-1 only in islets of male, but not female, mice. Thus, MLD-STZ diabetes is associated with local reduction of anti-inflammatory cytokines. This reduction, most likely, results in an imbalance with a relative preponderance of pro-inflammatory cytokines that may augment their ß-cell-destructive potential.

IL-11 is a pleiotropic cytokine with potent anti-inflammatory activity that prevented spontaneous diabetes in NOD mice (Nicoletti *et al*., 1999). Therefore, the goal of the present work was to evaluate the impact of rhIL-11 on MLD-STZ diabetes, insulitis, and local cytokine profiles in isolated islets *ex vivo.*

There is solid evidence that ROS can mediate ß-cell destruction (Oberley *et al*., 1988). In this context, recent data of colleagues from our laboratory are of particular interest. They have reported for the first time that the generation of hydroxyl radical ($^{\bullet}OH$), the most toxic species of the group of ROS, and of hydrogen peroxide (H_2O_2) is stimulated

in isolated islets by STZ *in vitro* (Gille *et al*., 2002). Above that, together with my colleagues we have reported that also MLD-STZ stimulate H_2O_2 generation in pancreatic islets *ex vivo* of diabetes-susceptible male, but not female, C57BL/6 mice (Friesen *et al*., in press). Evidently, ROS are involved in the pathogenic pathway of MLD-STZ diabetes. Furthermore, MLD-STZ-induced ROS generation may be part of the T cell-dependent inflammatory immune reactions, since ROS can stimulate T cell activation (Los *et al*., 1995). Although another free radical, nitrogen monoxide ($NO^{\bullet}$) also contributes to the cytotoxic effect of STZ on pancreatic islet cells (Turk *et al*., 1993; Kröncke *et al*., 1995), its effect as a direct mediator of ß-cell toxicity has not been unequivocally proven (Sternesjö *et al*., 1997; Papaccio *et al*., 2000) and still remains to be resolved.

1.4.3 Key role of NF-κB in STZ-induced diabetes

As already mentioned above (1.4.2), in animal models of T1D, islet infiltration with inflammatory immune cells occurs prior to ß-cell destruction. The destruction of the insulin-producing ß-cells may result from direct attacks by ROS produced by inflammatory immune cells (Nathan *et al*., 1986). ROS can be produced by pro-inflammatory cytokines such as IL-1ß, TNF-α, and IFN-γ (Rabinovitch *et al*., 1996; Rabinovitch and Suarez-Pinzon, 1998). Recently, few studies have shown that activation of the ROS-sensitive NF-κB may be a key cellular signal in initiating the sequence of events leading to ß-cell destruction. Thus, NF-κB activation has been observed in pancreatic tissue of CD1 mice rendered diabetic with MLD-STZ (Ho *et al.*, 2001). The authors concluded the activation to be due to ROS generation. Support for a pivotal role of NF-κB in MLD-STZ diabetes has also been demonstrated in mice deficient for the p50 NF-κB subunit, since they are diabetes-resistant compared with their wild-type counterparts (Mabley *et al*., 2002). Thus, inhibition of NF-κB activation may be effective in preventing diabetogenesis.

Based on the above cited reports and own data on the protective effect of rhIL-11 toward MLD-STZ diabetes, the *ex vivo* activation of NF-κB in pancreatic islets as target for

MLD-STZ and rhIL-11 was studied. The manuscript on the results obtained has been accepted for publication (Lgssiar *et al*., in press) and the data will be presented in detail in my dissertation.

1.4.4 Key role of AP-1 in MLD-STZ diabetes

So far, no data are available on the role of AP-1 in the pathogenesis of T1D. Therefore, analyses on AP-1 activity in pancreatic islet of MLD-STZ-injected mice have been performed. The results have been accepted for publication (Lgssiar *et al*., in press) and the data will be presented in detail in this dissertation.

1.5 IL-11

1.5.1 History and effects of rhIL-11 in animal models and in humans

IL-11 is a pleiotropic cytokine that was originally identified as a soluble polypeptide produced by PU-34 primate bone marrow stromal cell lines (Paul *et al*., 1990) and first cloned from a human fetal lung fibroblast cell line (Paul *et al*., 1990; Taga *et al*., 1997). IL-11 is a 178-amino acid with a molecular mass of 19 kDa, non-glycosylated multifunctional cytokine that posesses pleiotropic effects both inside and outside of the immune system (Du *et al*., 1994; Trepicchio *et al* ., 1998). IL-11 is a member of the IL-6 family including IL-6, leukemia inhibitory factor, ciliary neurotropic factor and cardiotrophin that activates cells via the ubiquitous gp-130 signal transduction pathway (Trepicchio *et al*., 1996; Chérel *et al*., 1995). IL-11 is produced by several cells within the central nervous system, the thymus, the lung, skin, and connective tissue, and murine IL-11 is expressed at high levels in the *hippocampus* and expression is developmentally regulated in the testes (Du *et al*., 1996).

Alone or in association with other cytokines, rhIL-11 has been shown to exhibit a range of effects on both the proliferation and differentiation of a variety of hematopoietic and non-hematopoietic cell types (Du *et al*., 1994). It has been approved for clinical use in the treatment of thrombocytopenia following cancer chemotherapy because of its ability

to stimulate megakaryopoiesis and thrombopoiesis (Cantor *et al*., 2003). Recent studies have demonstrated that rhIL-11 has anti-inflammatory activity *in vitro* and *in vivo* and reduces production of pro-inflammatory mediators such as TNF-α, IL-12p40, IL-1ß, and $NO^{\bullet}$ from murine activated macrophages (Trepicchio *et al*.,1996; Leng *et al*., 1997). Several studies have demonstrated that rhIL-11 modulates T cell function including the suppression of Th1 differentiation as monitored by inhibition of IL-12-induced IFN-γ production and enhanced Th2 responses (Schwertschlag *et al*., 1999).

IL-11 has been widely reported to have a potent anti-inflammatory effects in a variety of different animal models and in patients with acute and chronic inflammation including inflammatory bowel disease, rheumatoid arthritis, hepatitis, and various infection-endotoxemia syndromes (Peterson *et al*., 1998; Walmsley *et al*., 1998; Trepicchio *et al*., 2001). In the HLA-B27 rat model of chronic inflammatory bowel disease, treatment with rhIL-11 reduces gross and histologic colonic lesions (Keith *et al*., 1994). The amelioration of the disease signs is associated with the reduction of pro-inflammatory cytokines including IFN-γ, IL-1ß, TNF-α, and IL-12 p40 at the RNA levels in the colon (Peterson *et al.,* 1998). Studies in animal models of systemic inflammatory responses have also revealed positive effects of rhIL-11. Thus, treatment with rhIL-11 reduced pro-inflammatory cytokine levels such as IFN-γ and TNF-α in a murine model of endotoxemia (Trepicchio *et al*., 1996). Furthermore, rhIL-11 reduced TNF-α levels and increased survival in a neutropenic rat model of sepsis (Redlich *et al*., 1996), and in a murine model of radiation-induced pulmonary injury (Opal *et al.,* 1998). In mice, rhIL-11 prevented acute graft versus host disease (GVHD) after allogeneic bone marrow transplantation, enhanced recipient survival and promoted T cell polarization from Th1-toward Th2-type responses (Hill *et al*., 1998). In NOD mice, rhIL-11 prevented development of spontaneous and cyclophosphamide-induced diabetes and down-regulated pro-inflammatory cytokine levels in the serum (Nicoletti *et al*., 1999). Recently, rhIL-11 therapy has been administered to patients with psoriasis in a phase I clinical dose-escalation trial. The treatment down-regulated Th1-type cytokines in psoriatic lesions and ameliorated disease signs (Trepicchio *et al.,* 1999).

Taken together, rhIL-11 has strong immuno-modulatory effects that can be applied in various therapeutic contexts. The diabetes-preventing effect of rhIL-11 in MLD-STZ diabetes has been accepted for publication (Lgssiar *et al*, in press) and will be presented in detail in my dissertation.

1.5.2 Biochemistry and function of the IL-11 receptor - IL-11R -

IL-11R mediates the action of IL-11. The α-subunit of the IL-11R, IL-11Rα, is required for high affinity binding of the ligand (Hilton *et al*., 1994; Chérel *et al*., 1995). The complex of IL-11 and IL-11R triggers the dimerization of gp130 (Yin *et al*., 1993) leading to activation of the Janus kinase (Jak) which phosphorylates tyrosine residues in the cytoplasmic domains of the gp130 subunit, which, in turn, are docking sites for members of a family of latent transcription factors, signal transducer and activator of transcription (STAT) (Lütticken *et al.*,1994). These recruited STAT3 proteins undergo tyrosine phosphorylation, which permits their dimerization and translocation to the nucleus, where they can activate gene expression (Darnell *et al*., 1997).

The cloned human IL-11R consists of 422 amino-acid protein containing a signal peptide followed by extracellular, trans-membrane and cytoplasmic domains. This receptor presents a 82% homology with the murine IL-11R (Chérel *et al*., 1995). The biological functions of IL-11R have been investigated through the generation of mice with a null mutation of the IL-11Rα gene (IL-11Rα -/-). IL-11Rα -/- mice are clinically healthy, however, female mice are infertile due to an impaired trophoblast implantation (Nandurkar *et al*., 1997; Bilinski *et al*., 1998; Robb *et al.*,1998). The results on the *ex vivo* reduction of IL-11R in pancreatic islets by MLD-STZ and the preventive effects of treatment with rhIL-11 has been accepted for publication (Lgssiar *et al*., in press) and will be presented in detail in my dissertation.

1.6 Zinc-ions -Zn^{2+}- and experimental diabetes

1.6.1 Biological functions of Zn^{2+}

Zn^{2+} is a trace element because of the low plasma concentration (12-16 µM). In the serum, Zn^{2+} is predominantly bound to albumin, α2-macroglobulin and transferrin (Scott and Bradwell, 1983). As a co-factor for more than 300 different enzymes (Coleman *et al*., 1992; Falchuk *et al.,* 1993), it plays a vital role in multiple biological processes such as DNA replication, RNA transcription, cell division, and cell activation. It is involved in carbohydrate, protein, fat, and energy metabolism and contributes to maintain immune responses (Cunningham, 1994). Zn^{2+} deficiency due to inappropriate or inadequate nutrition may result in immunological or autoimmune diseases (Rink and Gabriel, 2000). Furthermore, Zn^{2+} functions as an antioxidant, because it can induce metallothioneins (MTs), which are a group of ubiquitous, small, cystein-rich, metal-binding proteins involved in the regulation of cellular metal homeostasis and protection against toxicity of ROS (Sato *et al*., 2002). The Zn^{2+}-metallothionein-complex has been suggested to play an important role in the attenuation or amplification of signal transduction and in the regulation of NF-κB (Kim *et al*., 2003).

1.6.2 Zn^{2+} supplementation and prevention of experimental T1D

The cytokine-mediated induction of oxidative agents, particularly free radicals, is one of the mechanisms by which immune responses can damage pancreatic ß-cells. In our laboratory, Zn^{2+} has been shown to induce MT in pancreatic islets and to prevent MLD-STZ diabetes (Ohly *et al*., 2000). It is concluded that Zn^{2+}-induced MT may have protected ß-cells by scavenging deleterious $^{\bullet}OH$ generated by MLD-STZ. The protective effect of Zn^{2+} has also been observed in rats that had been injected with one single, toxic dose of STZ (Yang and Cherian, 1994). The authors have also attributed the protective effect to induced MT in pancreatic tissue that had scavenged $^{\bullet}OH$. As reported from our laboratory, Zn^{2+} also protected mice from alloxan-induced diabetes (Schulte im Walde *et al*., 2002). In trying to analyze the molecular mechanism(s) underlying the ß-cell protective effect of dietary Zn^{2+}, it has been reported that NF-κB

activation by one single dose of STZ was blocked by Zn^{2+} in pancreatic tissue of CD1 mice (Ho *et al*., 2001).

The effect of Zn^{2+} on NF-κB activity in pancreatic islets has been studied together with my colleagues. The results have been submitted in part for publication (Schott-Ohly *et al*., in press) and will be presented in detail in my thesis.

2. SCOPE OF THIS THESIS

MLD-STZ similarly up-regulate the pro-inflammatory Th1-type cytokines TNF-α and IFN-γ in pancreatic islets of both diabetes-susceptible male and diabetes-resistant female C57BL/6 mice. In contrast, MLD-STZ reduce the anti-inflammatory Th2-type cytokines IL-4 and IL-10 as well as the anti-inflammatory Th3-type cytokine TGF-ß1 in islets of male mice only and does not affect those in female mice. Presumably, it is the local reduction of the anti-inflammatory cytokines that may augment the ß-cell destructive potential of the Th1-type pro-inflammatory cytokines. These findings clearly indicate the impact of cytokines in promotion or inhibition of MLD-STZ diabetes. Since rhIL-11 also has a strong anti-inflammatory potential, its effect on MLD-STZ diabetes was studied male C57BL/6 mice.

The study focused effects of treatment with rhIL-11 on MLD-STZ-induced diabetes and ß-cell function *in vivo* (B, C) and *ex vivo* effects of rhIL-11 and Zn^{2+}-enriched drinking water in isolated islets of MLD-STZ-treated donors (A, C-H). The experi-ments were designed to answer questions as follows:

A: Does MLD-STZ affect the mRNA expression of IL-11 and its receptor IL-11R ?

B: Does rhIL-11 prevent MLD-STZ-induced hyperglycemia ?

C: Does rhIL-11 ameliorate oral glucose tolerance?

D: Does rhIL-11 affect the Th1-type and Th2-type cytokine profiles ?

E: Does rhIL-11 affect the mRNA expression of endogenous IL-11, IL-11R, and TGFß-1 ?

F: Does rhIL-11 affect the binding activities of the transcription factors NF-κB and AP-1 and the kinase activity of IKK-α ?

G: Does Zn^{2+} affect the binding activities of NF-κB, AP-1, and the kinase activity of IKK-α ?

H: Does Zn^{2+} affect the Th1-type and Th2-type cytokine profiles ?

3. MATERIAL AND METHODS

3.1 Materials

3.1.1 Mice

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, received rodent diet (Ssniff M, 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) as well as the current version of the German Law on the Protection of Animals.

3.1.2 Reagents

Substance	Supplier
Agarose	Roche (Mannheim, Germany)
Acrylamide	Serva (Heidelberg, Germany)
Antibodies (monoclonal)	
IFNγ-PE (clon: XMG 1.2; Rat IgG1)	PharMingen (Hamburg, Germany)
TNFα-PE (clon MPGXT22; Rat IgG1)	PharMingen (Hamburg, Germany)
IL-4-PE (clon BVD4-1D11; Rat IgG2b)	PharMingen (Hamburg, Germany)
IL-10-FITC (clon JE55-16E3; Rat IgG2b)	PharMingen (Hamburg, Germany)
CD4-PE (clon H129.19; Rat IgG2a)	PharMingen (Hamburg, Germany)
CD8-PE (clon 53-6.7; Rat IgG2a)	PharMingen (Hamburg, Germany)
Rat IgG1, κ-PE (clon R3-34)	PharMingen (Hamburg, Germany)
Rat IgG2a, κ-PE (clon R35-95)	PharMingen (Hamburg, Germany)
Rat IgG2b, κ-FITC (clon A95-1)	PharMingen (Hamburg, Germany)
(Each antibody was diluted in FACS-Buffer to a final concentration of 0.1 mg/ml)	
Acetic acid	Sigma (Deisenhofen, Germany)

Ammonium acetate	Sigma (Deisenhofen, Germany)
Benzylpenicillin	Gibco (Eggenstein, Germany)
Bouin´s solution	Merck (Darmstadt, Germany)
Bromphenol blue	Sigma (Deisenhofen, Germany)
BSA: bovine serum albumin	Sigma (Deisenhofen, Germany)
ß-glycerphosphate	Merck (Darmstadt, Germany)
Chloralhydrat	Merck (Darmstadt, Germany)
Chloroform	Merck (Darmstadt, Germany)
Collagenase V (2.3 U/mg)	Sigma (Deisenhofen, Germany)
DEPC: diethylpyrocarbonat	Sigma (Deisenhofen, Germany)
D-glucose	Sigma (Deisenhofen, Germany)
dNTP	Genecraft (Münster, Germany)
DTT: dithiothreitol	Gibco (Eggenstein, Germany)
EGTA: ethylene glycol-bis-(ß-amino-ethyl ether)N,N,N´,N´-tetra-acetic acid	Sigma (Deisenhofen, Germany)
Eosin	Merck (Darmstadt, Germany)
Ethanol	Merck (Darmstadt, Germany)
Ethidium bromide	ICN Biochemicals GmbH (Eschwege, Germany)
FBS: fetal bovine serum	Gibco (Eggenstein, Germany)
FCS: fetal calf serum	Gibco (Eggenstein, Germany)
Ficoll: lymphocyte separation medium	Biochrom KG (Berlin, Germany)
Glycerol	Merck (Darmstadt, Germany)
[γ-^{32}P] dATP	Hartmann Analytica (München, Germany)
Haematoxylin	Merck (Darmstadt, Germany)
Haemolysis solution (digitonin)	Roche (Mannheim, Germany)
HBSS: Hank´s balanced salt solution	Gibco (Eggenstein, Germany)
HCl: hydrogen chloride	Merck (Darmstadt, Germany)
HEPES: N-(2-hydroxyethyl)-piperazin-N´-2-ethansulfonic acid	Serva (Heidelberg, Germany)
IκB-α protein (1-317)	Santa Cruz (Santa Cruz, USA)

Isopropylalkohol	Merck (Darmstadt, Germany)
K-Al-sulphate: potassium aluminium sulfate	Sigma (Deisenhofen, Germany)
$MgCl_2$: magnesium chloride	Sigma (Deisenhofen, Germany)
Mineral oil	ICN Biochemicals GmbH (Eschwege, Germany)
Methyl benzoat	Merck (Darmstadt, Germany)
M-MLV reverse transcriptase	Roche (Mannheim, Germany)
Molecular weight marker	Roche (Mannheim, Germany)
NaCl: sodium chloride	Merck (Darmstadt, Germany)
$NaHCO_3$: sodium hydrogen carbonat	Merck (Darmstadt, Germany)
NaF: sodium fluoride	Sigma (Deisenhofen, Germany)
Na-jodate: sodium jodate	Merck (Darmstadt, Germany)
Na-orthovanadate: sodium orthovanadate	Sigma (Deisenhofen, Germany)
Nonidet-P40 (P-40)	Sigma (Deisenhofen, Germany)
Oligo $p(dT)_{12-18}$	Genecraft (Münster, Germany)
Oligo nucleotide primer for PCR	MWG Biotech GmbH (Ebersberg, Germany)
Oligo nucleotide primer for EMSA	Santa Cruz (Santa Cruz, USA)
Paraffin	Merck (Darmstadt, Germany)
PBS: phosphate buffered saline	Gibco (Eggenstein, Germany)
Poly (di-dc): polynucleotide (di-dc)	ICN (Costa Mesa, USA)
Protease inhibitor (tablet)	Boehringer Mannheim (Mannheim, Germany)
Protein A-sepharose	Sigma (St. Louis, USA)
RNasin: ribonuclease inhibitor	Serva (Heidelberg, Germany)
RPMI 1640	Gibco (Eggenstein, Germany)
SDS: sodium dodecyl sulfate	Merck (Darmstadt, Germany)
Spermidin	Sigma (Deisenhofen, Germany)
Spermin	Sigma (Deisenhofen, Germany)
STZ: streptozotocin	Roche (Mannheim, Germany)
Taq polymerase	Roche (Mannheim, Germany)
TRIS: Tris (hydroxylmethyl) aminomethan	Serva (Heidelberg, Germany)

Triton X-100	Serva (Heidelberg, Germany)
TRIzol™ reagent	Gibco (Eggenstein, Germany)
Trypsin	Sigma (Deisenhofen, Germany)
T4 polynucleotide kinase	Boehringer Mannheim (Mannheim, Germany
Zincsulfate (Zn^{2+})	Merck (Darmstadt, Germany)
Xylol	Merck (Darmstadt, Germany)

rhIL-11: a generous gift from Genetics Institute (Cambridge, MA, USA). It was purified from *Escherichia coli* and had a specific activity of 1.5 10^6 U/mg as determined by T10 proliferation assay.

3.1.3 Buffers and solutions

3.1.3.1 Buffers and solutions for treatments of mice

- STZ solution
40 mg STZ
10 ml sodium citrate buffer

- Sodium citrate buffer
4.5 g tri-sodium citrate-5,5-hydrat
ad 500 ml aqua steril (= 25 mmol/l; pH 4.0

- rhIL-11 solution
10 µg rhIL-11 diluted in 100 µl PBS

- Zn^{2+}-enriched drinking water
4.5 mg $ZnSO_4$
1,000 tap water (25 mmol/l)

3.1.3.2 Buffers and solutions for determination of plasma glucose

- Haemolysis solution
digitonin 0.04 mmol/l
maleinimid > 1.0 mmol/l

1 reagent tablet solved in 500 ml distilled water

3.1.3.3 Buffers and solutions for islet isolation

- Collagenase solution (digestion of pancreas)	2 mg collagenase V (2.3 U/mg) 1 ml HBSS
- HBSS medium	100 ml HBSS (10 x) 50 ml FCS (5%, inactive) 2.4 HEPES (10 mmol/l) ad 1000 ml water (steril) pH 7.2-7.4
- RPMI 1640 medium	10 g RPMI 1640 (+ L-glutamine; - D-glucose) 100 ml FBS [10%] 2 g $NaHCO_3$ 1.01 g D-glucose 20ml benzylpenicillin [100U/ml]/ streptomycin [0.1 mg/ml] ad 1000 ml aqua steril. pH 7.2 – 7.4
- PBS (wash and centrifugation buffer)	50 ml PBS (10 x) 450 ml aqua steril

3.1.3.4 Solution for separation of islets into single cells

- Trypsin solution	1.25 mg trypsin 0.5 ml HBSS

3.1.3.5 Buffers and solutions for RNA electrophoresis

- DEPC water (0.1 %)	10 ml DEPC 1,000 ml distilled water
- 50 x TAE buffer	0.04 mol/l Tris acetate 1 mmol/l EDTA concentrated solution: 242 g Tris base 57.1 ml acetic acide 100 ml 0.5 mol/l EDTA (pH 8.0)
- TE buffer	TrisCl (10 mmol/l) (pH 7.6) EDTA (1 mmol/l) (pH 8.0)
- Agarose gel (1.5 %)	600 mg agarose 40 ml TAE buffer (0.5 x) 1 µl ethidium bromide

3.1.3.6 Buffers and solutions for FACS-analysis

- BFA fixant (fixation and permeabilization of cells)	0.2 mg/ml Na_2HPO_4 1 mg/ml KH_2PO_4 45 % (vol/vol) formaldehyde 9.25 % (vol/vol) aceton 47.75 % (vol/vol) distilled water
- Saponin buffer (wash buffer)	5 g/l saponine (0.5%) 90 mg/l sodium azide (0.009%) 1,000 ml PBS

- FACS buffer
0.5 g/l sodium azide (0.1%)
5 ml BSA (0.1%)
500 ml PBS

3.1.3.7 Buffers and solutions for preparation of nuclear extracts

- Buffer A
20 mmol/l HEPES; pH 7.9
10 mmol/l NaCl
0.2 mmol/l EDTA
2 mmol/l DTT

Store at 4^{o}C until use. Before use add the protease inhibitor to buffer A (1 volume pretease stock solution: 24 volumes buffer A)

- Buffer C
20 mmol/l HEPES; pH 7.9
0.75 mmol/l spermidin
0.15 mmol/l spermin
420 mmol/l NaCl
0.2 mmol/l EDTA
2 mmol/l DTT
25% glycerol

Store at 4^{o}C until use. Before use add the protease inhibitor to buffer C (1 volume pretease stock solution: 24 volumes buffer C)

- Protease inhibitor
1 tablett in 5 ml distilled water

Aliquot the solution, store it at - 20^{o}C and add fresh to Buffer A and C.

3.1.3.8 Buffers and solutions for electrophoretic mobility shift assay

- 5 x gel shift binding buffer
25% glycerol
10 mmol/l $MgCl_2$
100 mmol/l HEPES pH 7.9
0.5 mmol/l EDTA

300 mmol/l KCl
2.5 mmol/l DTT
0.25 mg/ml poly (di-dc)

- 10 x TBE buffer 107.80 g Tris base
55.00 boric acid
7.44 g EDTA

Dissolve the components in deionized water and adjust the pH to 8.3. Adjust the final volume to 1l with deionised water.

- 4% nondenaturing polyacrylamide for analysis of DNA-protein complexes
2.5 ml 10 x TBE buffer
10.0 ml 30% acrylamide
37.0 ml deionized water
30 µl TEMED
500 µl 10% APS

- 10 x gel loading buffer 250 mmol/l Tris-HCl, pH 7.5
0.2% bromphenol blue
40% glycerol

3.1.3.9 Buffers and solutions for protein kinase assays

- Buffer L
20 mmol/l HEPES, pH 7.5
10 mmol/l EGTA
25 mmol/l $MgCl_2$
1 mmol/l DTT
20 mmol/l ß-glycerophosphate
2 mmol/l sodium ortho-vanadate
1% NP-40

Add protease inhibitors just before use.

- Triton X-100 lysis buffer 25 mmol/l HEPES, pH 7.5

0.3 mol/l NaCl
1.5 mmol/l $MgCl_2$
0.2 mmol/l EDTA
0.5 mmol/l DTT
20 mmol/l ß-glycerophosphate
0.1 mmol/l sodium ortho-vanadate
0.1% tritonX-100

Add protease inhibitors just before use.

- Kinase reaction buffer

12.5 mmol/l MOPS, pH 7.5
12.5 mmol/l glycerophosphate
7.5 mmol/l $MgCl_2$
0.5 mmol/l EGTA
0.5 mmol/l NaF
0.5 mmol/l vanadate

3.1.3.10 Buffers and solutions for histological examination

- Haematoxylin solution

1 g haematoxylin
1000 ml water
0.1 g Na-jodate
50 g K-Al-sulfate
50 g chloralhydrat
1 g acetic acid

Dissolve the haematoxylin in water. Dissolve the potassium alum in warm distilled water and bring to the boil. Remove from the heat and add the chloralhydrat and cool rapidly. Before use, add 1 g acetic acid to the haematoxylin solution, then filter the needed volume. The remainder of the solution is stored in a cool and dark place until use.

- Eosin solution

1 g eosin
100 ml water

Before use a few drops of 1-2% acetic acid in 100ml of the eosin solution usually suffices and the remainder of the solution is stored in a cool and dark place until use.

3.1.4 Technical equipment

Instrument	Supplier
Autoanalyser (Determination of plasma glucose)	Eppendorf APC 5040 (Hamburg, Germany)
Autoclave	Melag (Berlin, Germany)
Balance	Sartorius (Göttingen, Germany)
Blood glucose measuring instrument	Eppendorf EPOS Analyzer 5060 (Hamburg, Germany)
Binocularly microscope	Wild M8 (Heerbrug, Switzerland)
Centrifuge	Beckmann Typ GS-6KR (München, Germany)
Computer programs	Microsoft Word for Windows 98, Power-Point, Graphpad Prism
Densitometer	Millipore (Ann Arbor, MI, USA)
Electronic analytical balance	Sartorius Typ 2024 MPG (Göttingen, Germany)
Elektrophoresis chamber	Pharmacia Biotec (Freiburg, Germany)
Electrophoresis power supplier	Pharmacia Biotec (Freiburg, Germany)
FACScalibur flow cytometer	Becton Dikinson (Heidelberg, Germany)
Glass homogenisator (15ml)	Braun (Melsungen, Germany)
Incubator	Heraeus Typ B5060 EK/CO_2 (Osterode, Germany)
Lumi-Imager for PCR product quantification	Roche Diagnostics (Mannheim, Germany)
Magnet agitator	Ikamag RH; IKA Labortechnik

	(Staufen, Germany)
Microprocessor pH meter	Knick-Klees
	(Düsseldorf, Germany)
Mortar with pestle	Haldenwanger
	(Berlin, Germany)
Photo camera system	Polaroid (Offenbach, Germany)
Pipettes	Eppendorf (Hamburg, Germany)
Shaker	Laborbedarf Behr
	(Düsseldorf, Germany)
Spectrophotometer	Beckmann DU70; INV 3146
	(München, Germany)
Sterile work bench	Heraeus Lamin Air Modell HLB 2472
	(Osterode, Germany)
Thermocycler TRIO thermoblock	Biometra (Göttingen, Germany)
Table centrifuge	Heraeus Biofuge A
	(Osterode, Germany)
UV transluminator	Sigma (Deisenhofen, Germany)
X-ray films	Amersham-Buchler
(Biomax and X-Omat)	(Braunschweig, Germany)

3.1.5 One way material

Material	Supplier
Falcon centrifuge tubes	Becton Dickinson (Heidelberg,
(14 ml; 105 x 16 mm)	Germany)
Instant-Film Type 665	Polaroid Corporation
	(Cambridge, MA, USA)
Millex-GS filter	Millipore (Eschborn, Germany)
(O,22 µm Filter diameter)	
Petri dishes	Becton Dickinson (Heidelberg,
(60 x 15 mm,	Germany)
35 x 10 mm, 100 x 20 mm; steril)	

Pipettes (1 ml, 5 ml, 10 ml; steril)	Becton Dickinson (Heidelberg, Germany)
Pipette tips (51 mm lengh; 72 mm lengh)	Eppendorf (Hamburg, Germany)
Polystyren FACS tubes (12 x 75 mm)	Becton Dickinson (Heidelberg, Germany)
Reaction tubes (1.5 ml; 0.5 ml)	Sarstedt (Nürnberg, Germany)
Sterile needles (0,30 mm x 13 mm; 30 G x ½; steril 0,33 mm x 13 mm; 29 G x ½; steril 0,45 mm x 13 mm; 26 G x ½; steril	Becton Dickinson (Heidelberg, Germany)
Sterivex-GS Filter (0,22 µm Filter diameter)	Millipore (Eschborn, Germany)
Syringes (1 ml; 2 ml; 5 ml; 10 ml)	Amefa (Kriftel, Germany)
U40 insulin syringes	Becton Dickinson (Heidelberg, Germany)
UV-Cuvette	Sarstedt (Nürnberg, Germany)
0-10 µl pipette tips	Roth (Karlsruhe, Germany)

3.2 Methods

3.2.1 Treatment of mice

3.2.1.1 Induction of MLD-STZ diabetes

To induce diabetes, groups of 10 male C56BL/6 mice each were injected ip with STZ, 40 mg/kg body wt each on 5 consecutive days according to the MLD-STZ protocol as originally described (Like and Rossini, 1976). STZ was dissolved in sodium citrate buffer (25 mmol/l, pH 4.0) at a concentration of 0.4% and injected within 5 min after

preparation. Untreated mice served as controls. To evaluate an association between MLD-STZ-induced changes and diabetes, female mice received the same treatment as the male ones.

3.2.1.2 Treatment with rhIL-11

To analyze effects of rhIL-11 on MLD-STZ diabetes, groups of 10 male mice each received a total of 130 µg rhIL-11 injected ip at a dose of 10 µg on 13 subsequent days as follows: daily on the 4 days before the first STZ injection, 1 h before each of the five STZ injections, and daily on the 4 days after the last STZ injection. rhIL-11 was dissolved in sterile PBS, and its concentration was calculated, so that each mouse received 10 µg rhIL-11 in a final volume of 100 µl. Groups of mice that were injected ip with either rhIL-11 or MLD-STZ alone or left untreated served as controls.

3.2.1.3 Treatment of mice with Zn^{2+}-enriched drinking water

To investigate effects of Zn^{2+}-enriched drinking water on *ex vivo* activities of the transcription factors NF-κB and AP-1 in pancreatic islets, groups of 10 male mice each were given free access to drinking water enriched with 25 mmol/l Zn^{2+} as previously described (Ohly *et al*., 2000). The treatment was started 1 week before the first STZ injection and conducted throughout the whole experimental period until the mice were killed. Groups of mice that had only received either Zn^{2+}-enriched drinking water or MLD-STZ served as controls. The Zn^{2+}-enriched drinking water was freshly prepared daily.

3.2.1.4 Oral glucose tolerance test (OGTT)

For the OGTT, an oral load of 2.0 g D-glucose/kg body wt was administered with an intubation tube after a fasting period of 16 h. Blood glucose concentrations were measured just before (0 min), at 15, 30, and 60 min after the glucose challenge. The OGTT was performed at week 4, 12, and 20 after the first of the five STZ injections and in age-matched control groups.

3.2.1.5 Determination of plasma glucose

At weekly intervals, blood samples were collected from the retro-orbital venous plexus of non-fasted animals between 09:00 and 11:00 am by using capillary glass tubes (20 µl). Glucose concentrations were determined by the hexokinase method using an autoanalyser. Diabetes was defined as a non-fasting blood glucose concentration greater than 11.1 mmol/l for 3 or more consecutive weeks.

3.2.2 Islet preparation

3.2.2.1 Isolation of islets

Islets were isolated from groups of 10 mice by collagenase digestion as described by Gotoh et al. (1985) with slight modifications (Zimny *et al*., 1993). After killing of mice by cervical dislocation, the abdominal cavity was opened and the common bile duct was prepared. The entrance of the common bile duct into the duodenum was clamped and cannulated with a 30 G 1/2 needle. Under a binocular microscope, 2 ml of cold and freshly prepared collagenase solution were carefully injected through the common bile duct to distend the pancreas for optimal digestion. The pancreas was removed and incubated in a petri dish for 30 min at 37°C and 5.5% CO_2. By addition of 10 ml cold HBSS, the collagenase activity was stopped. Digested tissue of pancreas was transferred into 15 ml Falcon centrifuge tubes and fragmented by mechanical shake. The tube was then centrifuged at 320 g for 10-15 sec. After washing twice with HBSS, the supernatant was rejected and the pellet was resuspended in 10 ml cold RPMI medium, and 2 ml of the resuspended pellet was carefully layered on 5 ml lymphocyte separation medium with a density of 1.077 g/l. The tubes were then centrifuged for 10 min at 800 g without brake. The islet-enriched fraction at the RPMI medium interface was collected with a pipette. Islets, free of connective tissue, were then hand-picked whith a pipette and transferred into fresh RPMI medium and incubated free-floating at 37°C and 5.5% CO_2. For the electrophoretic mobility shift assay (EMSA), kinase assay, and FACS analysis, islets were separated into single cells. For reverse transcriptase polymerase chain reaction (RT-PCR), islets were kept at - 80°C in RNase-free TE buffer.

3.2.2.2 Separation of islets into single cells

Isolated islets were transferred into a sterile microcentrifuge tube and centrifuged at 380 g for 5 min. The pellet was resuspended in 1.5 ml HBSS. For separation into single cells, 1.25 mg trypsin were solved in 0.5 ml HBSS and 50 µl of this solution were given into the reaction tube. After incubation at 37°C and 5.5% CO_2 for 6 min, the cell suspension was transferred into a second tube using a syringe (diameter: 0.33 mm) and centrifuged by 380 g for 5 min, the supernatant was rejected and the single cell-containing pellet was washed twice with HBSS.

3.2.2.3 Fixation of islet cells

After washing with HBSS (see 3.2.2.2), the islet cell suspension was fixed with 200 µl BFA under constant vibrating on a laboratory shaker for 10 sec; then, 1 ml of 4°C cold HBSS was added and the cells were centrifuged by 380 g at 4°C for 5 min. The supernatant was carefully aspirated with a pipette, the cell-containing pellet of approximately 200 µl was resuspended and stored at 4 °C for a maximum of 3 days until cytometric analysis.

3.2.2.4 Permeabilization of islet cells and staining with monoclonal antibodies

Fixed islet cell suspensions were washed once with saponin buffer. Saponin is a mild detergent used after the fixation step to increase the permeability of the cell membrane. After centrifugation by 225 g at 4°C, the pellet was resuspended in PBS and distributed on polystyrene centrifuge tubes. The cells were then incubated with fluorescence-labelled monoclonal antibodies with specifities for IFN-γ, TNF-α, IL-4, and IL-10) in a concentration of 1 µg/µl each (diluted in FACS buffer) for 30 min in the dark on ice. Cells incubated with fluorescence-labelled isotype antibodies against IgG1 and IgG2a served as control. After incubation, islet cells were washed twice with 3 ml cold PBS followed by centrifugation by 225 g at 4°C for 5 min to remove excess antibodies. The pellets were resuspended in 100 µl PBS and analyzed by FACScan for cytokine producing-cells.

3.2.3 FACS-analysis of cytokine-producing cells

Data acquisition was performed with a FACScalibur flow cytometer equipped with an argon-ion laser emitting at 488 nm in order to simultaneously excite fluorescein-isothiocyanate (FITC) and phycoerythrin (PE) pigments. All parameters were collected as logarithmic signals. The 488 nm laser was used to measure the forward angle light scatter (FSC, in the 488 nm band pass filter) and the side angle light scatter (SSC, in the 488 nm band pass). The acquisition threshold was set in the side scatter channel. The rate of events in the flow was generally below 3,000 events/sec. A total of 100,000 events was stored in list mode files. Isotype controls were used to guide quadrant setting and 10,000 cells were analyzed for cytokine production. Subsequent analyses were conducted using the CellQuest Software (Becton Dickinson).

3.2.4 RNA preparation

3.2.4.1 RNA isolation from islets

Total RNA was extracted from pools of all islets isolated from groups of 10 mice each, using the TRIzol reagent kit according to the manufacturer´s instruction. TRIzol is a mixture from phenole and guanidine isothiocyanate and has the capacity to break the cell membrane without destroying the RNA. One ml TRIzol was given to the thawed islets. Immediately after a short vortexing, 200 µl chloroform were added and the reaction mixture was incubated by room temperature for 3 min. After centrifugation for 15 min at 12,000 g and 4°C, the reaction mixture was separated in an organic phenol chloroform phase containing extra-cellular membranes, polysaccharides, and high molecular weight DNA, and a colorless upper phase containing total RNA. This phase was transferred into a new centrifuge tube. By addition of 500 ml isopropanol, the total RNA was precipitated. After incubation for 10 min by room temperature, the RNA was centrifuged for 10 min at 12,000 g and 4°C. The RNA pellet was washed with 100% ethanol in the first instance, then with 75%. The RNA sediment was dried at 56°C on a thermoblock for 5-10 min. Subsequently, the total RNA was resuspended in RNase-free TE buffer (pH.7.2).

3.2.4.2 Determination of RNA concentration

RNA samples were resuspended in RNAse-free TE buffer (pH. 7.2) and quantified spectrophotometrically at 260 and 280 nm. All RNA samples had an optical density $(OD)_{260}$: OD_{280} ratio between 1.8 and 2.0, indicating clean RNA isolates. Total RNA for each sample was calculated and 1 µg RNA was diluted in 4 µl TE buffer for cDNA synthesis. The concentrated RNA preparation was stored at -80°C until use.

3.2.5 Reverse transcription polymerase chain reaction (RT-PCR)

3.2.5.1 Principles of the RT-PCR method

RT-PCR is a highly sensitive and specific technique for mRNA detection and quantitation. The development of this technique resulted in an explosion of new techniques in molecular biology (Nobel Prize for Kary Mullins in 1993). Compared to the two other commonly used techniques for quantifying mRNA levels, Northern blot analysis and RNase protection assay, RT-PCR can be used to quantify specific RNA transcripts from much smaller samples and to detect rare transcripts as well as variation in mRNA expression levels under different experimental conditions. It includes a complementary DNA (cDNA) synthesis and the PCR steps. cDNA is a single-stranded copy of an RNA sequence synthesized by the retroviral enzyme reverse transcriptase and serves as template for PCR that is based on the DNA polymerization reaction. Two synthetic oligonucleotide primers, which are complementary to two regions of the target DNA to be amplified, are added to the target DNA, in the presence of Taq polymerase, a heat-stable DNA polymerase. In a series of temperature cycles, the target DNA is repeatedly denatured, annealed to the primers and a daughter strand extended from the primers. The daughter strands themselves act as templates for subsequent cycles.The DNA fragments matching both primers are amplified exponentially, rather than linearly. The reaction is performed in a thermocycler, which is a programmable heating block that will cycle between melting, annealing, and polymerization temperature.

3.2.5.2 Synthesis of cDNA

Messenger RNA can be copied by a retroviral enzyme, which catalyzes the synthesis of a cDNA strand from a single-stranded mRNA substrate. The first strand cDNA was made by mixing 1 µl of total RNA with 1 µl oligo $(dT)_{16}$ (1 µg/µl) and 4 µl DEPC water in a 0.2 ml microfuge tube. After incubation for 5 min at 60°C, 0.5 µl RNAsin, 12.0 µl 5 x reaction buffer (containing DTT), 4 µl dNTP (10 mmol/l), and 2.0 µl reverse transcriptase were added to the mixture and then incubated at 37 °C for 1 h to reversely transcribe the RNA. The reverse transcriptase was denatured at 72°C for 10 min and then cooled to 4°C.

3.2.5.3 PCR

The PCR was amplified using *Taq* polymerase. The primer used and the cycle numbers for each primer are listed in Table 1.

Table.1. Primer pairs and PCR conditions.

mRNA	Primer sequence (5´-Primer/3´-Primer)	Annealing temperature (°C)	Product length (bp)	Cycle number
IL-11	5´-TGCTGACAAGGCTTCGAGTAG-3´ 3´-CAGTCGAGTCTTTAACAACAGC-5´	56	319	35
IL-11R	5´-CTGATGAAGGCACTTATGTCTG-3´ 3´-CATCTGTTATCACTTCCTCCAAAG-5´	58	588	32
TGFß-1	5´-CTCCCACTCCCGTGGCTTCTAG-3´ 3´-GTTCACACCTCGTTGTACACCTTG-5´	55	472	30
ß-actin	5´-AAGTACCCCATTGAACATG-3´ 3´-AGGAGCAATGATCTTGATC-5´	55	795	30

The PCR amplifications were performed in a volume of 45 µl containing:

21.5 µl DEPC water
8.0 µl 10 x reaction buffer (containing $MgCl_2$)
8.0 µl dNTP (1.25 mmol/l)
2.5 µl 5´-primer (4 µM)
2.5 µl 3´-primer (4 µM)
2.5 µl Taq-polymerase (1 U/µl)

The reaction was started by addition of 5 µl cDNA and the amplifications were carried out in a programmable thermocycler in five steps as follows:

1) 94°C for 4 min to initially denature cDNA
2) 94°C for 1 min to denature cDNA
3) Primer annealing for 1 min, the annealing temperature depended on the primer used as indicated in Table. 1
4) 72°C for 1 min to extend the new cDNA strands
5) 72°C for 10 min for the final extention

The cycle numbers were chosen to be on the linear, i.e., exponential phase of the amplification of the four genes. To exclude the possibility of genomic DNA contamination during RNA preparation, negative controls were set up for each PCR amplification using purified RNA as template.

3.2.5.4 Separation of PCR products

For separation of the amplified PCR products, 8 µl of each, i.e., the target product and ß-actin were mixed with 3 µl loading buffer and were loaded on 1% agarose gels containing ethidium bromide (0.1 µg/ml). After electrophoresis at 120 V and 50 mA for 30 min, the bands were visualized using an UV-tansluminator.

3.2.5.5 Quantification of PCR products

The resulting bands were quantified with a Lumi-Imager. The ratio of the intensity integral of the target PCR products to that of ß-actin was calculated.

3.2.6 Electrophoretic mobility shift assay (EMSA)

3.2.6.1 Preparation of nuclear extracts

After separation of pancreatic islets into single cells, the cells were washed twice with PBS and lyzed in buffer A. The cell lysate was collected in microfuge tubes and incubated for 10 min at 4°C. Centrifugation in a bench top centrifuge at 15,000 rpm for 2 min pelleted the nuclei. The cytosolic fractions (supernatant) were stored at -80°C until use for determination of IKK-α activity. The pellet was resuspended in buffer C, incubated for 20 min at 4°C, and then centrifuged in a bench top centrifuge for 2 min at 4°C. The supernatant containing nuclear extracts was transferred into a microfuge tube and kept at -80°C until determination of NF-κB, AP-1, and Oct-1 activities.

3.2.6.2 End-labelling of oligonucleotides

Double-stranded oligonucleotide probes for NF-κB and AP-1 (5`-AGTTGAGGGGA-CTTTCCCAGGC-3` for NF-κB and 5`-CGCTTGATGAGTCAGCCGGAA-3` for AP-1) were commercially synthesized and end-labelled on their 5´hydroxyl terminus, in the form of γ-^{32}P [dATP]. This reaction is catalyzed by T4 polynucleotide kinase. The specifity of NF-κB and AP-1 signals was approved by using cold target competition. As an internal control for quality and quantity of cell extracts, Oct-1 DNA binding activity was also performed. The labelling of the oligonucleotides was made by mixing 1 µl of the desired oligonucleotide (1.75 pmol/µl), 1 µl of 10 x polynucleotide kinase buffer, and 1 µl polynucleotide kinase (5-10 U/µl) in a sterile microcentrifuge tube. The reaction mixture was incubated for 10 min at 37°C. After the incubation time, the reaction was stopped by addition of 1 µl of 0.5 mmol/l EDTA and the reaction volume was adjusted to 100 µl with TE buffer.

3.2.6.3 Removal of unincorporated label

To improve the quality of gel shifts, the labelled sample was mixed with 1/4 volume of 5 mmol/l ammonium acetate and 2 volumes of absolute ethanol. Following an incubation for 30 min at 20°C, the sample was centrifuged at 15,000 rpm for 15 min at 20°C and the supernatant was removed carefully by aspiration. The pellet was resuspended in 100 µl of 1 mmol/l ammonium acetate and 200 µl absolute ethanol and kept for 30 min at - 20°C. After centrifugation for 15 min at 15,000 rpm, the supernatant was removed and the pellet was allowed to dry under vacuum. The dried pellet was resuspended in 100 µl of TE buffer and stored at - 20°C until determination of NF-κB, AP-1, and Oct-1 activities.

3.2.6.4 DNA-binding reaction using nuclear extracts

DNA binding reaction was made by mixing 4 µg of the desired nuclear extract with 2 µl of 5 x gel shift binding buffer in a sterile microfuge tube. The total volume was adjusted to 9 µl with nuclease-free water. After incubation of the reaction for 10 min at room temperature, 1 µl of [γ-^{32}P] ATP (50,000-200,000 cpm)-labelled oligonu-cleotide was added followed by an incubation for further 20 min at room temperature.Then, the sample was mixed with 1 µl of 10 x gel loading buffer and analyzed on 6% polyacrylamide native gel.

3.2.6.5 NF-kB and AP-1 activity by EMSA

DNA-protein complexes were analyzed on a 14 x 22 cm, native, 6% acrylamide (30:1 acrylamide: bis acrylamide) gel, 0.75 mm thick. Before loading the sample, the gel was allowed to pre-run for 30 min in 0.5 x TBE buffer at 100 V. After loading the samples, the gel was allowed to run at 100 V at RT in 0.5 x TBE buffer for approximately 3 h. At the end of the run the gel was wrapped in plastic wrap and exposed to X-ray film overnight at - 70°C with an intensifying screen. The films were scanned and band intensity was quantified using TINA 2.09 d quantification software (Raytest).

3.2.7 Measurement of IKK-a activity

The test applied is able to detect the activity of individual protein kinases in immune complexes bound to protein A-sepharose. In principal, IKK-α activity was determined through phosphorylation of IκB as described (Rolli-Derkinderen *et al*., 2003). Briefly, stored cytosolic fractions of islet cells were thawed and mixed with lysis buffer containing a complete protease inhibitor cocktail set and the anti-IKK-α antibody sc-7182, and this mixture was kept overnight at 4°C. IKK-α antigen-antibody immune complexes were recovered with protein A-sepharose beads and washed three times with lysis buffer and twice with kinase buffer. 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) as substrate for 30 min at 37°C. The reactions were terminated by adding 15 µl of 2 x SDS-PAGE loading buffer and then this mixture was boiled for 5 min. Samples were resolved by 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).

3.2.8 Histological examination

For histology, groups of 5 mice each injected with rhIL-11 and/or MLD-STZ were sacrified by cervical dislocation on day 12 after the first STZ injection. Mice treated with rhIL-11 only or left untreated served as controls. The pancreata were removed and fixed in Bouin´s solution for 24 h, dehydrated in ethanol, incubated in methyl benzoate for 24 h, and then embedded in paraffin. This paraffin wax method is considered to be the most suitable for routine preparation, for sectioning, staining, and subsequent storage of large numbers of tissue samples. Serialsections, 5 µm thick at a distance of 50 µm, were prepared from each paraffin-blocked pancreas, collected on histology slides, and stained with hematoxylin-eosin. Before staining, the paraffin was removed and the pancreas sections were rehydrated. Hematoxylin-eosin stain is a simple method for general histology. Basophilic nuclei are stained "blue" with hematoxylin, eosinophilic cytoplasm, connective, and all other tissues are counterstained "red" with eosin.

The protocol for paraffin removal and rehydratation of sections on histology slides was as follows:

Reagent	Incubation time (min)
Xylol (solves the paraffin)	10
Isopropanol (solves the xylol)	2
96% ethanol	2
80% ethanol	2
70% ethanol	2
Water	0.5

After rehydration, the sections were stained and dehydrated according to the following protocol:

Reagent	Incubation time (min)
Hematoxylin	10
Running water	15
Distilled water (pH > 7)	0.5
Eosin	0.5
70% ethanol	0.5
80% ethanol	0.5
96% ethanol	0.5
100% ethanol	0.5
Xylol	10

After this procedure, the pancreas sections were enclosed using Euparal, coded, and examined independently for the presence of infiltrates with mononuclear cells at both islet-pole and intra-islet sites by a coworker and myself. The degree of intra-islet infiltrates (insulitis) was scored as follows: 0 = no infiltrate; 1+ = mild infiltrate (**#**30% of islet cells are mononuclear cells); 2+ = moderate infiltrate (>30 to **#**75% of islet cells are mononuclear cells); 3+ = severe infiltrate (>75% islet cells are mononuclear cells). Perivascular and/or periductular sites at islet poles were examined for absence or

presence of infiltrates.

3.2.9 Data analysis

All data of molecular analyses are means ± SE of three independent experiments. For statistical analysis, the unpaired Student´s *t* test was used. $P < 0.05$ was considered statistically significant.

4. RESULTS

4.1 MLD-STZ reduce the mRNA expression of IL-11 and IL-11R in pancreatic islets of male C57BL/6 mice

To investigate whether an effect on the gene expression of IL-11 and its receptor IL-11R is associated with MLD-STZ diabetes, C57BL/6 mice of both sexes were injected with MLD-STZ or the solvent of STZ as controls. Islets were isolated on day 1 and 3 after the last injection and used for semiquantitative RT-PCR analyses. Primers for IL-11, the α-subunit of IL-11R, and ß-actin as internal control were used and the resulting products showed the expected bands of 319, 588, and 795 bp, respectively. Since IL-11R mediates the activity of IL-11 and since IL-11Rα is required for the high affinity binding of IL-11, a primer with specifity for the α-subunit was selected. The ratio of the mRNA expression of the target genes over ß-actin was calculated.

As illustrated in Fig. 5A, MLD-STZ significantly decreased ($P<0.001$) the levels of IL-11 mRNA expression in islets of male mice. The mean ± SE was already reduced to 0.044 ± 0.010 on day 1 after the last STZ injection and was even lower with 0.007 ± 0.007 on day 3 thereafter compared with 0.142 ± 0.0025 in control islets. As shown in Fig. 5B, MLD-STZ also gradually reduced the level of mRNA expression of IL-11R in islets of male mice. The mean ± SE was significantly reduced ($P<0.001$) to 0.106 ± 0.006 on day 3 after the last STZ injection compared with 0.44 ± 0.026 in controls. This significant reduction, again, was observed in islets of male mice only. In islets of female mice, in contrast, MLD-STZ did not significantly change the levels of the mRNA expression of IL-11 and IL-11R.

Based on these data, the effect of the anti-inflammatory cytokine rhIL-11 on MLD-STZ-induced hyperglycemia was tested.

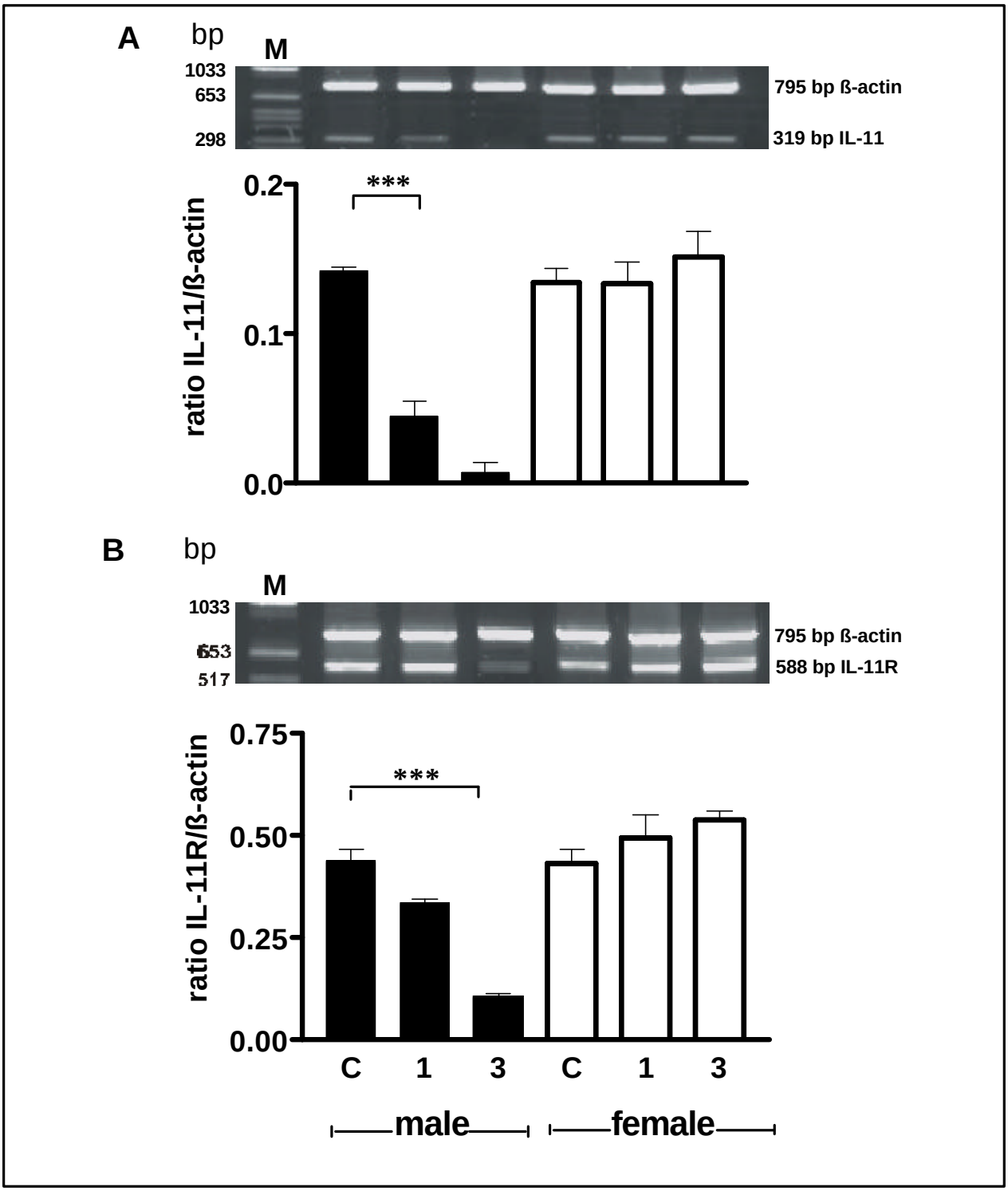

Fig. 5. MLD-STZ reduce mRNA levels of IL-11 and IL-11R in male but not female mice.

RT-PCR determination of IL-11 (**A**), IL-11R (**B**), and ß-actin mRNA in pancreatic islets isolated from C57BL/6 mice of both sexes that had been injected with 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. Means ± SE of the ratio of IL-11 and IL-11R to ß-actin mRNA were calculated for three independent experiments.*******P<0.001 comparing MLD-STZ-treated vs untreated controls. M, marker. bp, base pairs.

4.2 rhIL-11 prevents MLD-STZ-induced hyperglycemia in male C57BL/6 mice

As expected, MLD-STZ induced diabetes in male C57BL/6 mice. About 2 weeks after the first STZ injection, the mice started to develop a persisting hyperglycemia with a mean blood glucose concentration of 12.59 mmol/l ± 0.29 SE (Fig. 6A) over 18 weeks. However, co-treatment with rhIL-11 significantly prevented (P<0.001) high blood glucose levels induced by MLD-STZ. The protective effect of rhIL-11 persisted for the observation period of 18 weeks with a mean of the blood glucose levels of 10.3 mmol/l ± 0.18 SE that slightly and only transiently exceeded the euglycemic threshold of 11.1 mmol/l. Yet, the curve of blood glucose levels of these mice still remained significantly higher (P<0.001) compared with the values measured in the two control groups. There were no significant differences in the kinetics of blood glucose levels between the two control groups that had either been injected with the solvent of STZ or rhIL-11 only.

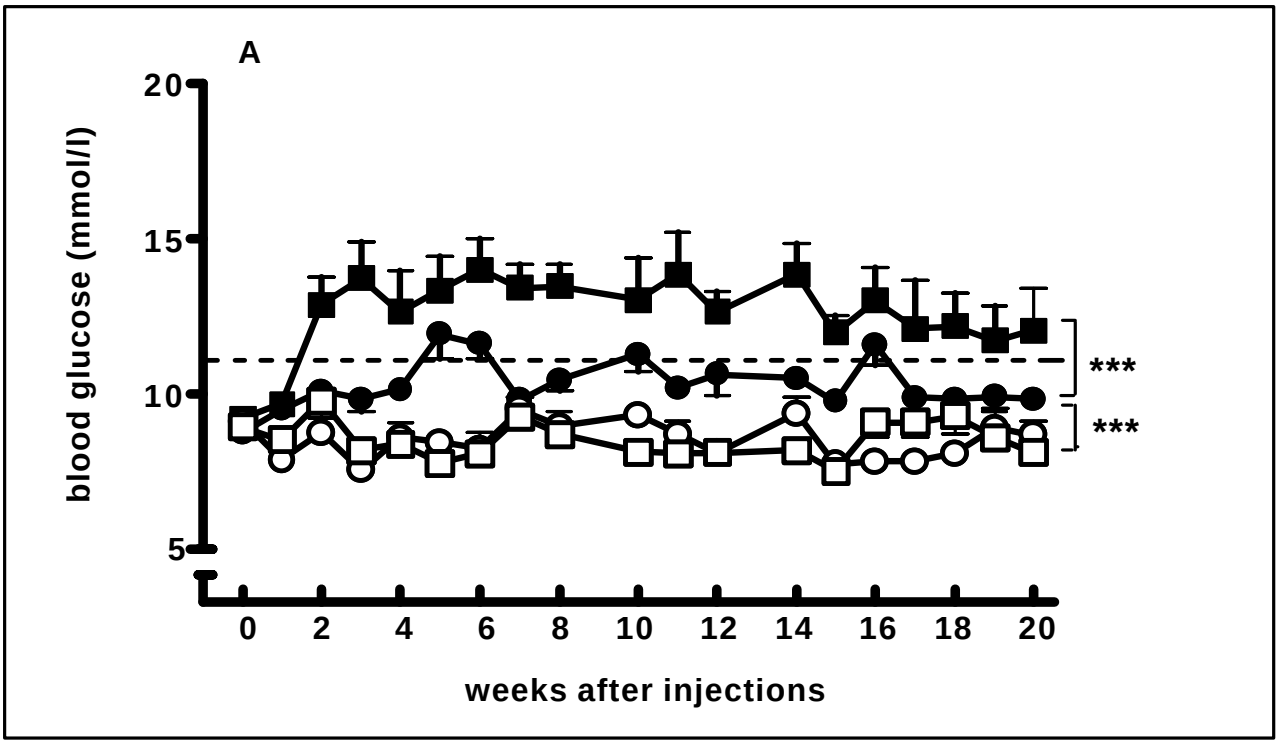

Fig. 6A. rhIL-11 prevents MLD-STZ-induced hyperglycemia.
Effect of rhIL-11 on diabetes induced with MLD-STZ in male C57BL/6 mice. blood glucose concentrations (means ± SE) over time in weeks. Mice were injected with MLD-STZ - either alone (■) or in addition to rhIL-11, i.e., 13 injections of 10 µg each (●) - or with rhIL-11 (❍) or remained untreated (□). Each group injected with MLD-STZ consisted of 10 mice and for the control groups 6 mice each were used. *******P<0.001 comparing the areas under the curves.

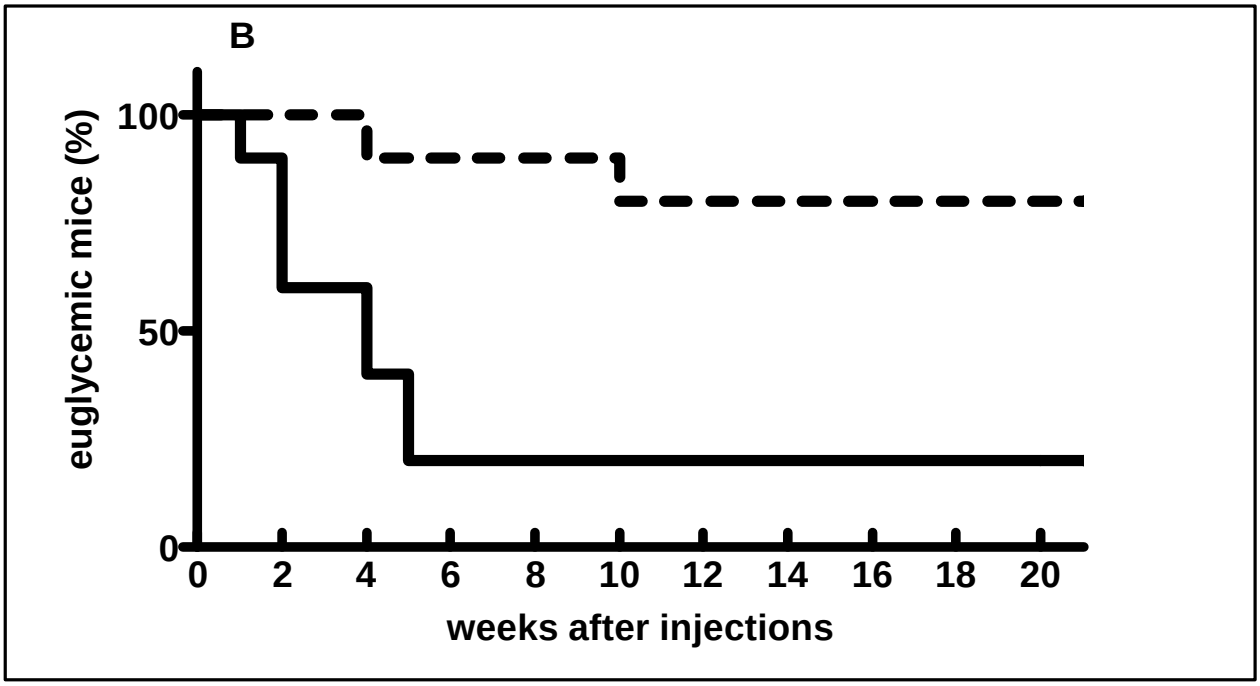

Fig. 6B. Percentage of mice with euglycemia in the MLD-STZ-injected group (*solid line*) and the group injected with both MLD-STZ and rhIL-11 (*dotted line*).

As illustrated in Fig. 6B, in the MLD-STZ group, 11 out of 14 (80%) developed diabetes and only 3 (20%) remained non-diabetic. In contrast, in the group treated with rhIL-11 in addition only 3 out of 13 (20%) developed persistent hyperglycemia, whereas 10 (80%) remained euglycemic.

4.3 rhIL-11 ameliorates ß-cell function in MLD-STZ-injected male mice

OGTT measures the ability of ß-cells to respond to a glucose challenge with insulin *in vivo*. The effect of rhIL-11 on the OGTT in male C57BL/6 mice was investigated. The mice had been injected with MLD-STZ - either alone or in addition to rhIL-11 - or the solvent of STZ or rhIL-11 alone as controls. In general, the results correlated with those shown in Fig. 6.

As expected, the group of mice injected with MLD-STZ showed a severe glucose intolerance at week 4 (Fig. 7A), week 12 (Fig. 7B), and week 20 (Fig. 7C). These mice had high blood glucose levels at 15, 30, and 60 min after the oral administration of

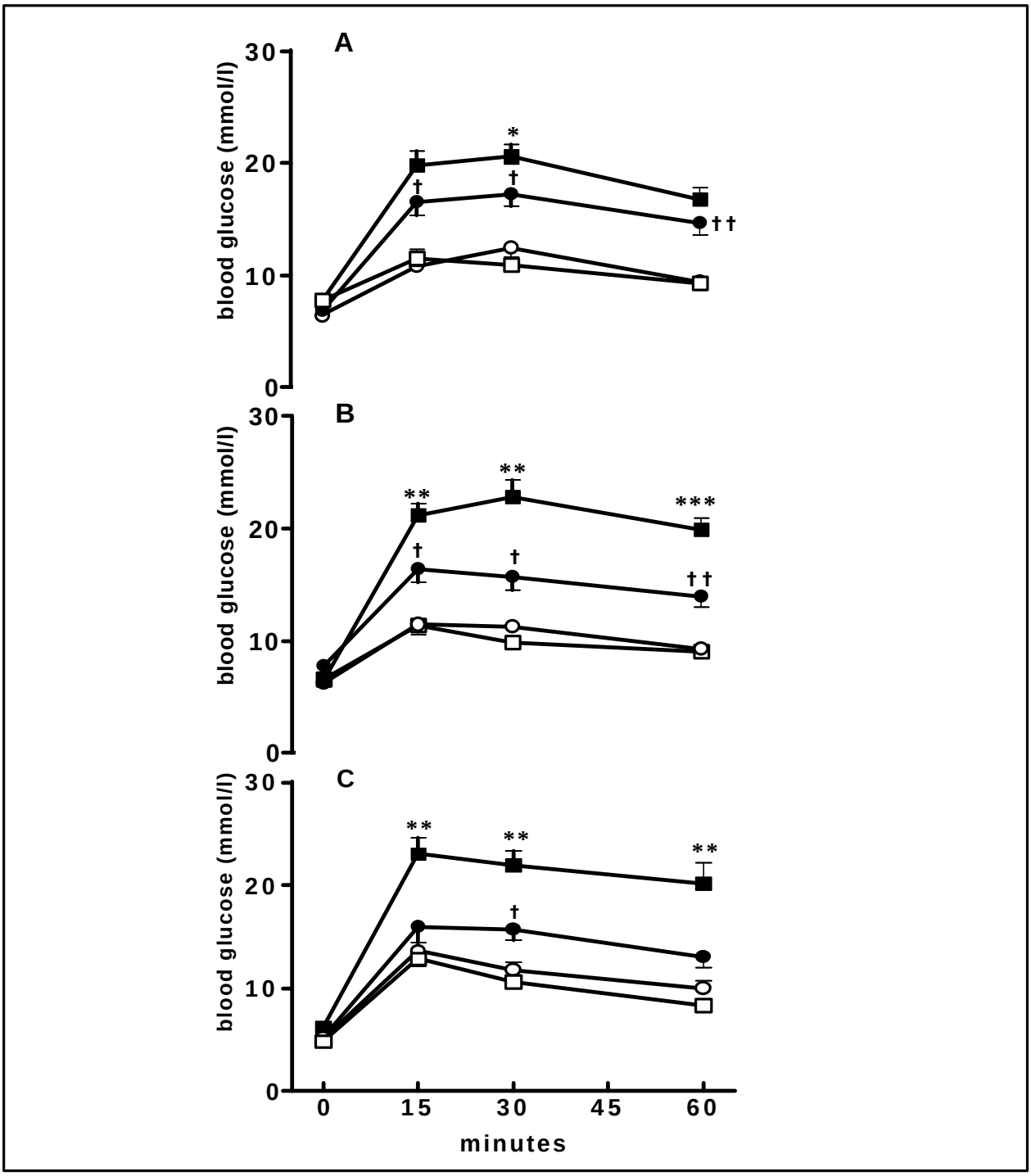

Fig. 7. rhIL-11 ameliorates intolerance induced with MLD-STZ.

Blood glucose values (means ± SE) before (0), at 15, 30, and 60 min after a glucose load of 2.0 g/kg body wt at weeks 4 (**A)**, 12 (**B)**, and 20 (**C)** after the first STZ injection. Male C57BL/6 mice were treated with MLD-STZ - either alone (■) or in addition to 130 µg (13 injections of 10 µg each) rhIL-11 (●), or with rhIL-11 alone (❍) or remained untreated (□). Each group injected with MLD-STZ consisted of 10 mice and for the control groups 6 mice each were used. *****P<0.05, ******P<0.01, and ******* P<0.001 comparing MLD-STZ-treated vs MLD-STZ- plus rhIL-11-treated groups; **†** P<0.05 and **††** P<0.01 comparing MLD-STZ- plus rhIL-11-treated vs control groups.

glucose (given at time 0) compared with the two control groups. The deteriorated glucose tolerance persisted on a similar level over 20 weeks. In contrast, treatment with rhIL-11 in addition to MLD-STZ significantly ameliorated the glucose intolerance. Yet, the response to the glucose challenge was still significantly impaired compared with the responses obtained in the two control groups. Interestingly, rhIL-11 treatment continuously improved the glucose tolerance with time to near normal at week 20. Apparently, rhIL-11 had a long-lasting effect in protecting ß-cell function from MLD-STZ damage.

4.4 rhIL-11 stimulates Th2-type cytokine responses in islets of MLD-STZ-injected male mice

As described above, MLD-STZ reduce the anti-inflammatory Th2-type cytokines IL-4 and IL-10, the anti-inflammatory Th3-type cytokine TGF-ß1 as well as IL-11 and IL-11R in islets of diabetes-susceptible male, but not diabetes-resistant female, C57BL/6 mice, whereas the pro-inflammatory cytokines TNF-α and IFN-γ are similarly upregulated in islets of both sexes.

As shown in Figs. 8 and 9, treatment of mice with MLD-STZ alone markedly increased the number of the pro-inflammatory cytokine-producing cells. The percentage for TNF-α- and IFN-γ-producing cells was 25% and 22.8% of the gated islet cells, respectively, compared with 11% and 10.9% measured in the solvent-injected control group. Again, MLD-STZ decreased the number of cells producing the anti-inflammatory cytokines. The percentage of IL-4- and IL-10-producing cells was only 8% and 2.5%, respectively, compared with 11.9% and 4.9% in the control group. Treatment of mice with rhIL-11 in addition to MLD-STZ completely reversed this profile. An increment of the anti-inflammatory cytokines IL-4 and IL-10 was evident, and, in particular, the production of IL-4 was more pronounced than of IL-10. However, the increment of IL-4 was not significant compared with the value obtained in solvent-injected control. The percentage was 23.5% and 5.1% for IL-4- and IL-10-producing cells, respectively, compared with 8% and 2.5% observed in islet cells of mice treated with MLD-STZ alone. Furthermore,

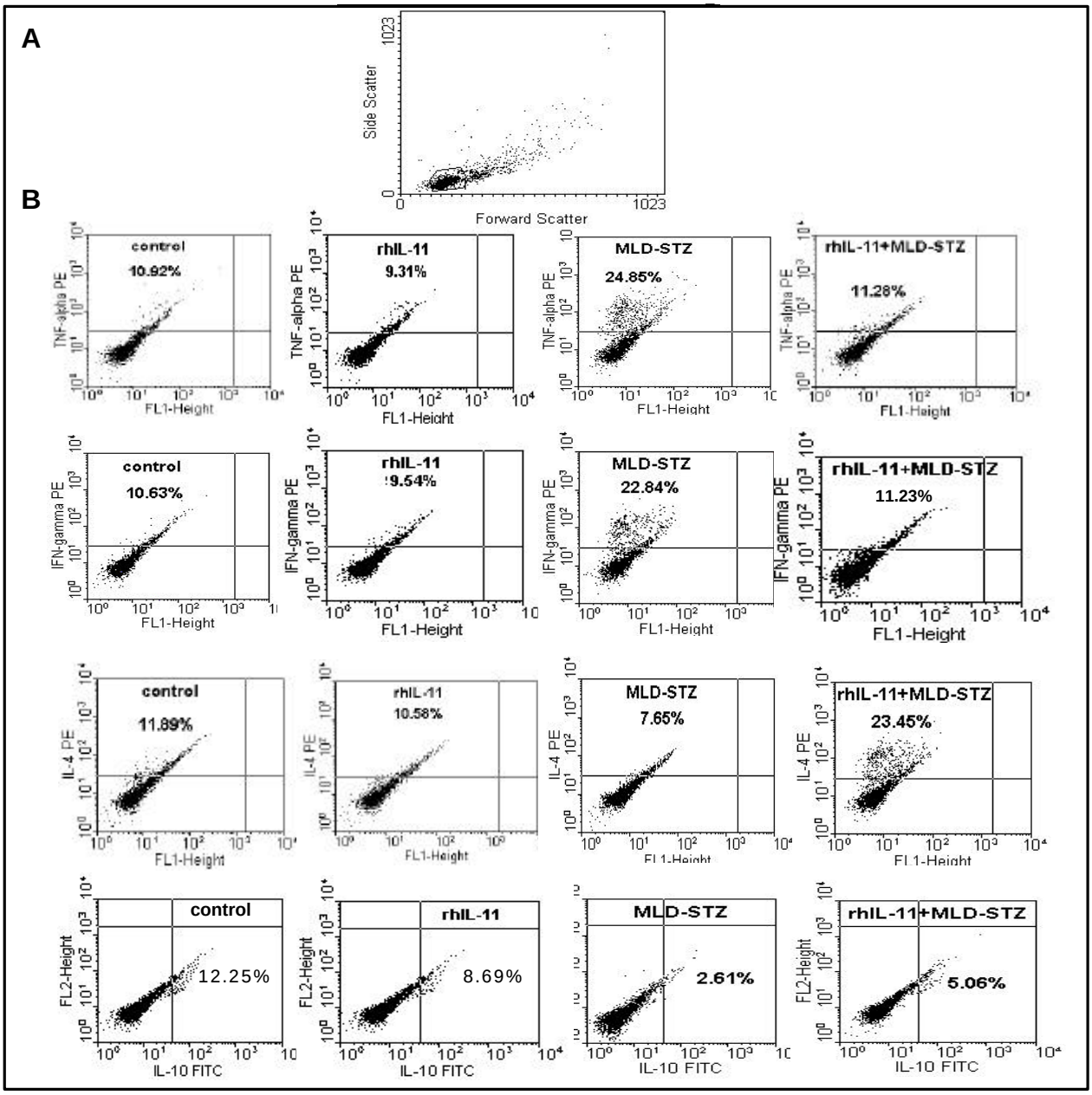

Fig. 8. FACS-analysis of cytokine-producing cells in the pancreatic islets.
A. Gate setting on the population of cells present in the pancreatic islets using forward scatter (FSC-H) and side scatter (SSC-H). The light scatter were used to exclude dead cells, cell aggregates, and debris from the fluorescence data, large and very granular cells can be readily differentiated from the smaller, less granular islet cells. **B**. Dot blot representations of flow cytometry data from pancreatic islet cells isolated from male C57BL/6 mice that had been injected with MLD-STZ - either alone or in addition to rhIL-11 - or treated with rhIL-11 alone or the solvent of STZ. The PE-conjugated antibodies against TNF-α, IFN-γ, and IL-4 fluoresce in the FL2 chanel and the FITC-conjugated antibodies against IL-10 fluoresce in the FL1 chanel. This mode of analysis allowes to find exactly what percentages of the cells are single-positive for each cytokine. Fluorescence settings are adjusted for each group of samples by using negative control cells incubated with an irrelevant, isotype-matched monoclonal antibody.

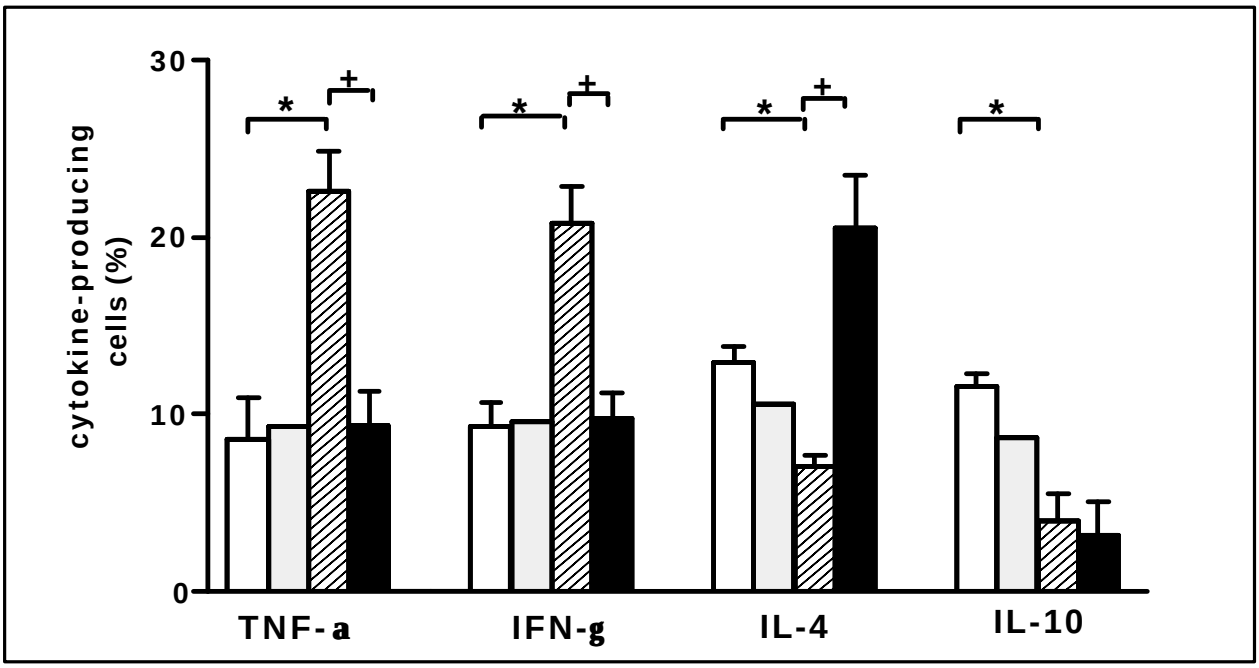

Fig. 9. rhIL-11 shifts MLD-STZ responses toward anti-inflammatory reactions. Effect of rhIL-11 on the percentage of cytokine producing-cells in islets isolated from male C57BL/6 mice that had been injected with MLD-STZ - either alone (▨) or in addition to rhIL-11 (■) - or with rhIL-11 alone (⁘) or with the solvent of STZ (□) as controls. Islets were isolated on day 3 after the fifth STZ injection and from age-matched controls**.** Since rhIL-11 alone had no effect, this control experiment was only conducted once. Means ± SE of three independent experiments are given. *P<0.05 comparing MLD-STZ-treated vs untreated control mice. $^{+}$P<0.05 comparing MLD-STZ-treated vs MLD-STZ- plus rhIL-11-treated groups.

treatment with rhIL-11 decreased the percentage of TNF-α- and IFN-γ-producing cells being 11.2% and 9.7%, respectively, compared with 25% and 22.8% in mice treated with MLD-STZ alone. The percentage of cells producing TNF-α and IFN-γ remained close to the constitutive levels found in solvent-treated control mice. Moreover, rhIL-11 by itself did not alter the constitutive cytokine profiles found in the groups of mice injected with the solvent of STZ or with rhIL-11 alone.

4.5 Effects of treatment with rhIL-11 on mRNA expression of endogenous IL-11, IL-11R, and TGFß-1

To determine, whether the ß-cell-protective activity of rhIL-11 was also mediated

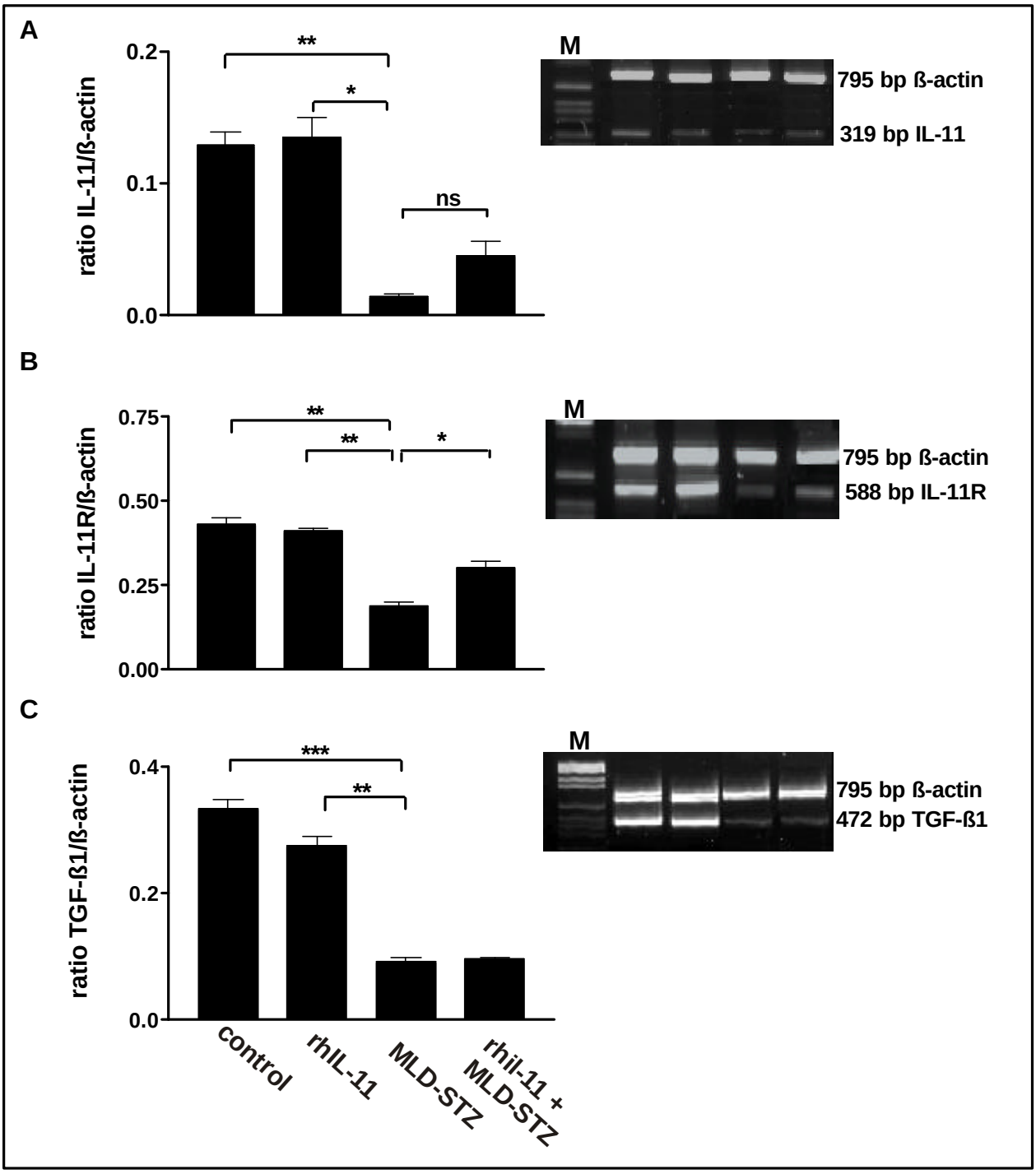

Fig. 10. rhIL-11 increases the mRNA expression of IL-11R only .
Effect of rhIL-11 on levels of mRNA expression of IL-11 (**A**), IL-11R (**B**), and TGF-ß1 (**C**) in islets isolated from male C57BL/6 mice that had been injected with MLD-STZ - either alone or in addition to rhIL-11 - or with the solvent of STZ or had received rhIL-11 alone as controls. Islets were isolated on day 3 after the last STZ injection. Means ± SE of the ratio of the target gene over ß-actin were calculated. Means ± SE of three independent experiments are given. ns, not significant; *****P<0.05; ******P<0.01; *******P<0.001.

through increment of the endogenous anti-inflammatory cytokines IL-11 and TGF-ß1 in addition to IL-4, the effect of rhIL-11 on the mRNA expression of IL-11, its receptor IL-11R, and TGFß-1 was examined. Mice were treated with MLD-STZ alone oder in addition to rhIL-11. Islets were isolated on day 3 after the last STZ injection and the mRNA expression of IL-11, IL-11R, and TGFß-1 was analyzed by RT-PCR. The results are illustrated in Fig. 10.

In this set of experiments, treatment with MLD-STZ alone significantly reduced ($P<0.01$) the mRNA expression of endogenous IL-11 and IL-11R. These results confirm those obtained in Fig. 5A and 5B. In the group of mice treated with rhIL-11 in addition to MLD-STZ, only a slight, but not significant, increase of endogenous IL-11 was observed (Fig. 10A). However, additional treatment with rhIL-11 significantly increased ($P<0.05$) the mRNA expression of IL-11R. The mRNA level of IL-11R over ß-actin increased from 0.19 ± 0.01 detected in the MLD-STZ-treated group to 0.30 ± 0.02 on day 3 after the last STZ injection (Fig. 10B). Moreover, treatment with MLD-STZ alone significantly reduced ($P<0.01$) the expression of TGFß-1 in pancreatic islets. These data confirm those obtained in our laboratory (Müller *et al*., 2002). The MLD-STZ-induced reduction of the mRNA level of TGFß-1 remained unchanged by rhIL-11 (Fig. 10C). Additionally, rhIL-11 by itself did not alter the constitutive mRNA expression of the three genes analyzed.

4.6 MLD-STZ stimulate NF-κB and AP-1 activity

Since the local cytokine profile in pancreatic islets in MLD-STZ diabetes of C57BL/6 mice is relatively dominated by a Th1-type response, it was questioned, whether the transcription factors NF-κB and AP-1 are involved, since they regulate the gene activation of pro-inflammatory cytokines. To characterize their kinetics, analyses on different time intervals were performed. Therefore, mice were sacrificed on day 1 after the third and on day 1 and 3 after the fifth STZ injection. Nuclear extracts were prepared from islet cells of male and female C57BL/6 mice. The female mice were included as controls to evaluate an association between changes and diabetes susceptibility. NF-κB and AP-1 binding activities were analyzed *ex vivo* using EMSA.

To test for the specifity of shifted bands for NF-κB and AP-1, specific competition tests were run. Nuclear extracts of islet cells isolated on day 3 after the last STZ injection from male C57BL/6 mice and incubated with radioactive-labelled oligonucleotide probes, which contain the specific recognition sequence for either NF-κB or AP-1, displayed intense shifted bands for NF-κB and AP-1 compared with the bands of solvent-injected control groups. Cold non-labelled NF-κB and AP-1 oligonucleotide sequences prevented signals indicating the specificity of the observed signals for NF-κB and AP-1 (Fig. 11).

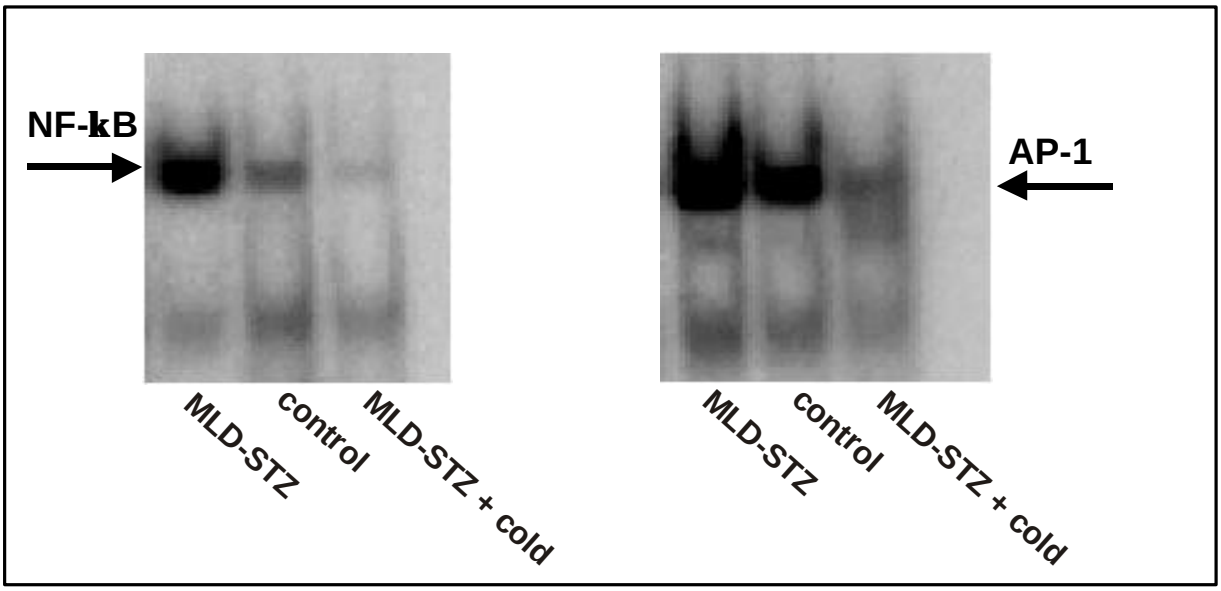

Fig. 11. NF-kB and AP-1 signals are specific.
NF-κB and AP-1 activity in islets of C57BL/6 male mice that had received five injections of STZ or the solvent of STZ as control. The islets were isolated on day 3 after the last injection.

As illustrated in Fig. 12, treatment with MLD-STZ significantly increased ($P<0.001$) the activity of NF-κB on day 3 after the last STZ injection in islets isolated from male C57BL/6 mice, the NF-κB activity was by 169 ± 9.776% compared with 100% of the control islet cells. On day 1 after 3 and 5 injections with STZ, no changes of NF-κB activity was measurable compared with the values obtained in islets from the control mice (Fig. 12A). 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, the activity decreased from 100% of the control islet cells to 47.17 ± 14.05%. On day 1 after the

third and day 3 after the fifth STZ injections, the mean activities remained below those of the control values (Fig. 12B). The activity of the ubiquitous transcription factor Octamere-1 (Oct-1) as internal control remained unchanged by the different treatments.

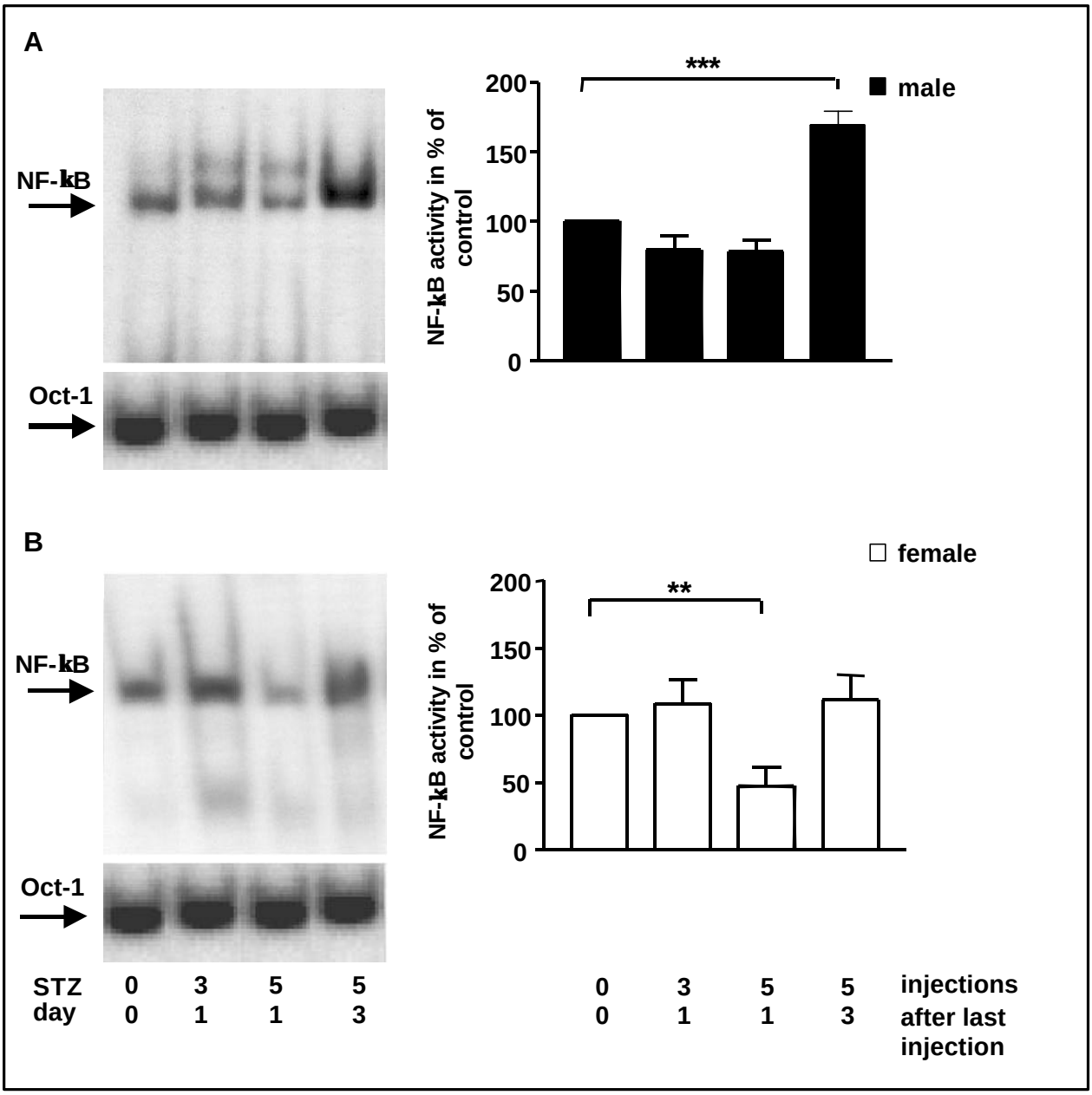

Fig. 12. MLD-STZ increase the *ex vivo* activity of NF-kB in islets isolated from male C57BL/6 mice on day 3 after the last STZ injection.

NF-κB activity in islets of C57BL/6 mice of male (**A**) and female (**B**) that had received three or five injections of STZ or five injections of the solvent of STZ (0) as control. Islets were isolated on day 1 and/or day 3 after the last of the five STZ injections. As an internal control for quality and quantity of cell extracts, Oct-1 DNA binding activity was also monitored by EMSA. Means ± SE of three independent experiments. ******P<0.01; *******P<0.001.

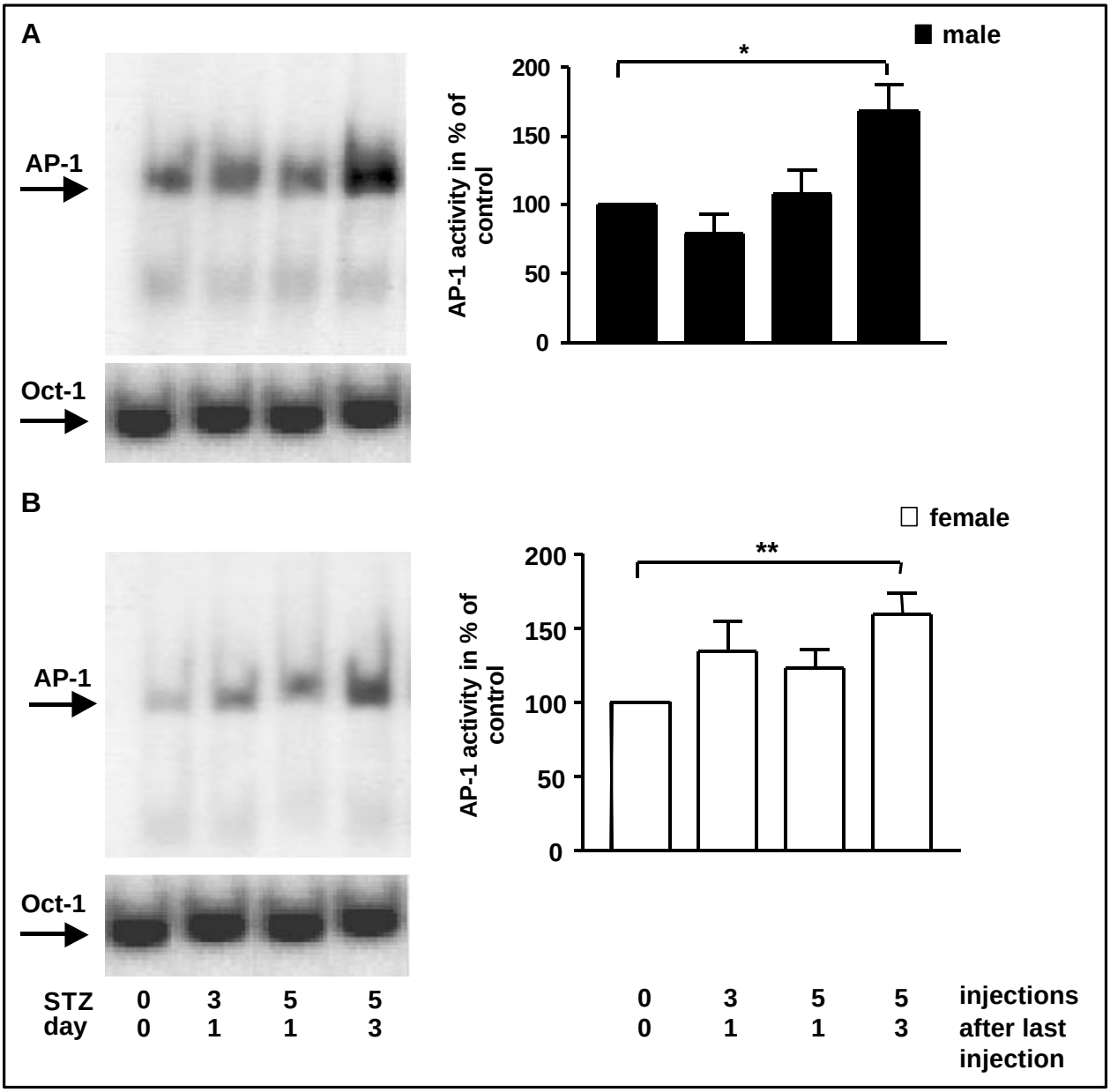

Fig. 13. MLD-STZ increase the *ex vivo* activity of AP-1 in islets isolated from male and female C57BL/6 mice on day 3 after the last STZ injection.
AP-1 activity in islets of C57BL/6 mice of male (**A**) and female (**B**) that had received three or five injections of STZ or five injections of the solvent of STZ (0) as control. Islets were isolated on day 1 and/or day 3 after the last of the five STZ injections. As an internal control for quality and quantity of cell extracts, Oct-1 DNA binding activity was also monitored by EMSA. Means ± SE of three independent experiments. *****P<0.05; ******P<0.01.

The AP-1 activities are shown in Fig. 13. Treatment with MLD-STZ significantly induced (P<0.001) the AP-1 activities in both sexes. The AP-1 activity was by 168.1 ± 19.64% on day 3 after the last STZ injection compared with 100% of the control islet cells (Fig. 13A), in female mice by 159.1 ± 14.8% on day 3 after the last STZ injection compared

with 100% of the control islet cells (Fig. 13B). On day 1 after the third and day 3 after the fifth STZ injection, the mean activities remained below those of the control values in both sexes. The activity of Oct-1 as internal control remained unchanged by the different treatments.

4.7 rhIL-11 inhibits activation by MLD-STZ of NF-κB and AP-1

Since gene activation of pro-inflammatory cytokines is regulated by the transcription factors NF-κB and AP-1, the ability of rhIL-11 to inhibit their binding activities was investigated.

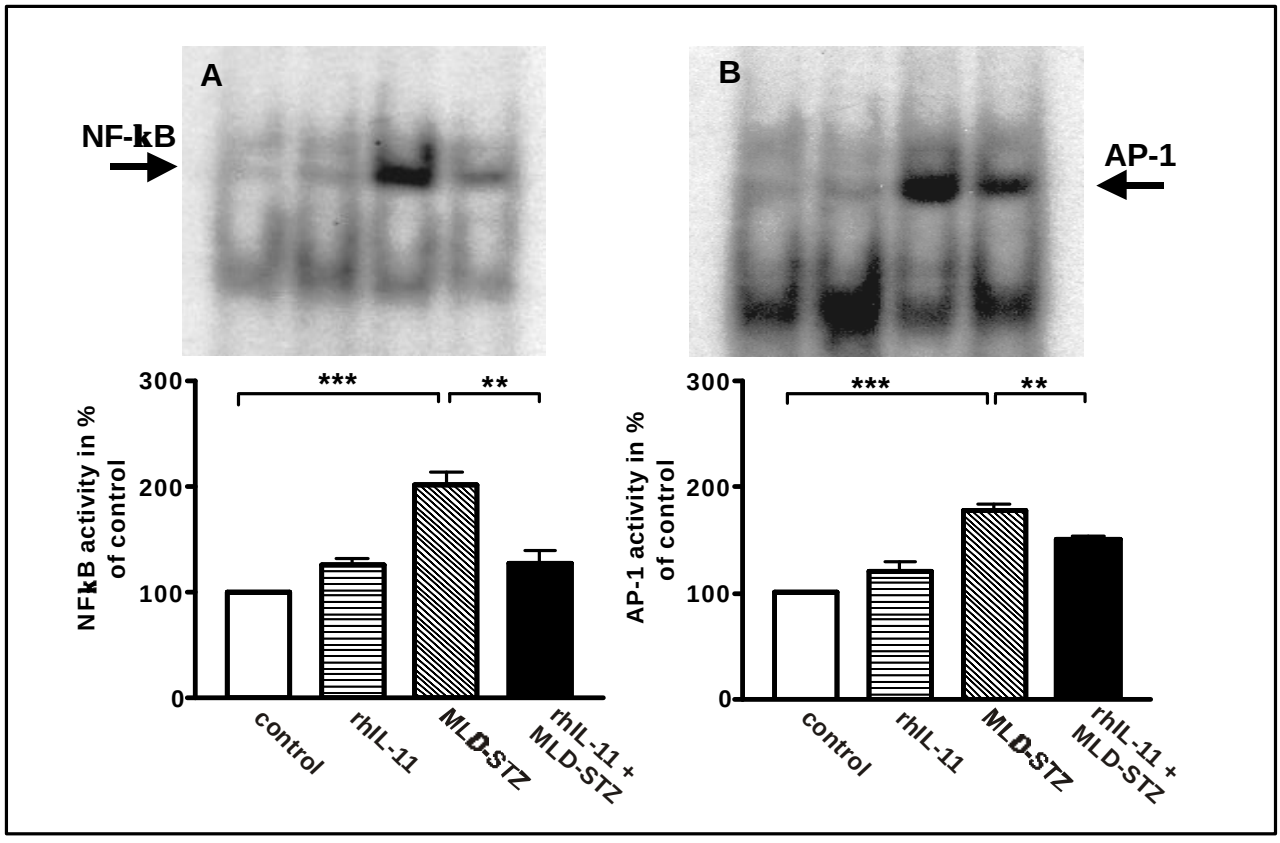

Fig. 14. rhIL-11 prevents stimulation by MLD-STZ of NF-κB and AP-1 activities in islets isolated from male C57BL/6 mice.
Effect of rhIL-11 on NF-κB (**A**) and AP-1 (**B**) activity in islets isolated from male C57BL/6 mice that had been injected with MLD-STZ - either alone or in addition to rhIL-11 - 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. Means ± SE of three independent experiments. ******P<0.01; *******P<0.001.

As shown above in Figs. 12 and 13, the up-regulation of NF-κB and AP-1 binding activities were most pronounced on day 3 after the last STZ injection in islets of male C57BL/6 mice and therefore, the effect of rhIL-11 on both activities was examined at this time point only.

As illustrated in Fig. 14, treatment with MLD-STZ alone significantly induced ($P<0.001$) the binding activities of both NF-κB and AP-1 on day 3 after the last STZ injection. In this set of experiments, the means ± SE activities of NF-κB and AP-1 were respectively by 201 ± 12.28% and 178 ± 4.957% compared with 100% of the solvent-injected control mice. Treatment of male mice with rhIL-11 in addition to MLD-STZ inhibited ($P < 0.01$) activation of both transcription factors. The activities of NF-κB and AP-1 were reduced to 127 ± 12.62% and to 151 ± 3.164%, respectively. rhIL-11 by itself did not alter the constitutive activities measured in solvent-treated controls.

4.8 rhIL-11 inhibits stimulation by MLD-STZ of IKK-a activity in islets of male mice

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 the IKK-α activity.

As illustrated in Fig. 15, MLD-STZ alone stimulated IKK-α activity ($P < 0.001$) on day 3 after the last STZ injection. The mean value ± SE of this stimulation was 194 ± 5.54% compared with 100% of untreated control mice. Additional treatment with rhIL-11 attenuated ($P < 0.01$) stimulation of the IKK-α activity, the mean value ± SE was only 125.5 ± 5.11%.

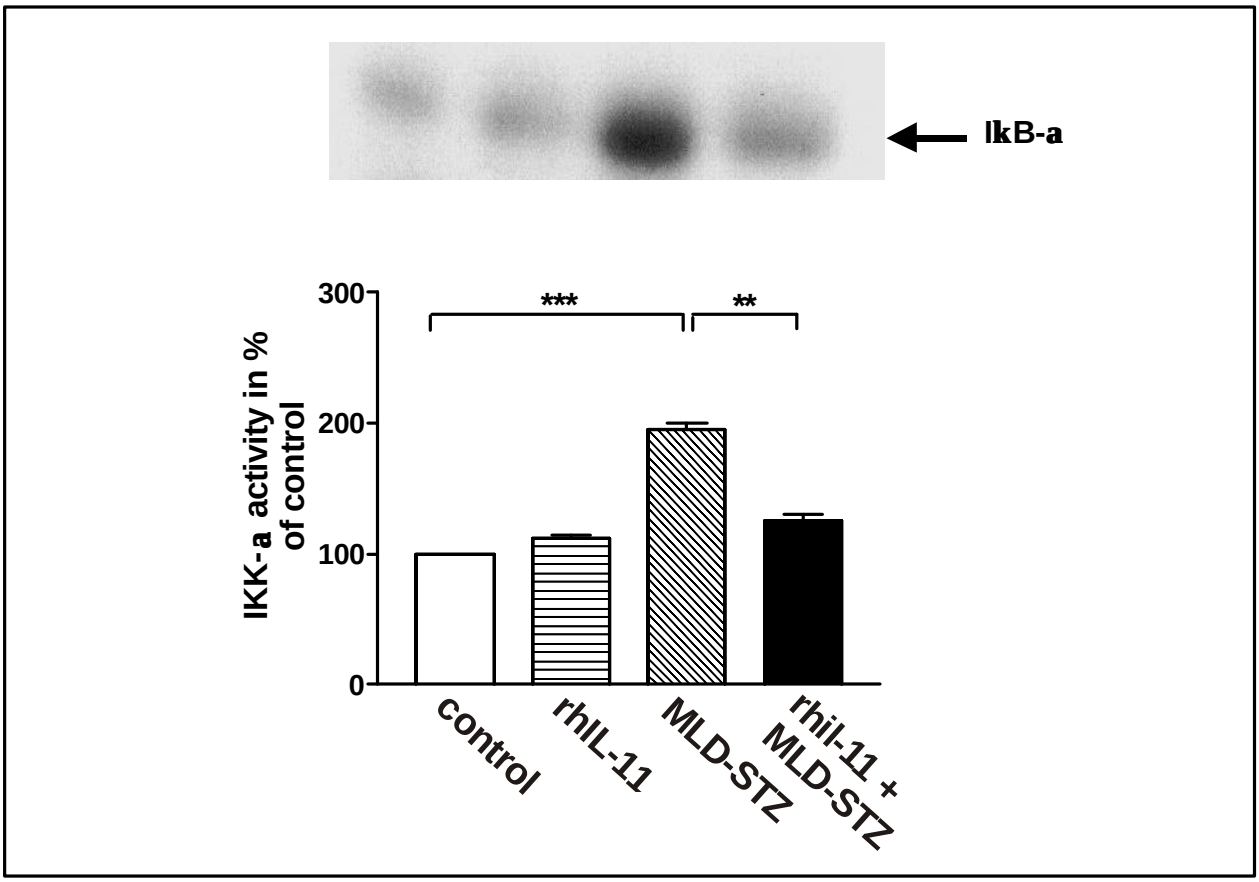

Fig. 15. rhIL-11 prevents stimulation by MLD-STZ of IKK-a activity in islets isolated from male C57BL/6 mice.
Effect of rhIL-11 on IKK-α activity in islets isolated from male C57BL/6 mice that had been injected with MLD-STZ - either alone or in addition to rhIL-11 - or with the solvent of STZ or rhIL-11 alone as controls. Islets were isolated on day 3 after the last of five STZ injections. IKK-α activities were measured by an *in vitro* kinase assay using IκB-α (1-317) as a substrate. Means ± SE of two independent experiments. ******P<0.01; *******P<0.001.

4.9 rhIL-11 does not change insulitis

Histological examination of pancreatic sections were done to test, whether treatment of diabetes-susceptible male C57BL/6 mice with rhIL-11 in addition to MLD-STZ prevent the infiltration of islets with mononuclear cells. Mice were killed on day 12 after the first STZ injection, because the islet infiltration was most pronounced at this time point (Like and Rossini, 1976). The degree of insulitis was similar in the mice injected with MLD-STZ alone and those receiving rhIL-11 in addition to MLD-STZ (Table 1). As measured against a scoring system from 0 to 3+, the percentage of islets without infiltrates or with 1+, 2+, or 3+ insulitis of mice treated with rhIL-11 in addition to MLD-

STZ was compared with that of donors treated with MLD-STZ only. Treatment with rhIL-11 failed to essentially change the degree of insulitis (Fig.16).

Table 1. Effect of treatment with MLD-STZ and rhIL-11 on infiltrates with mononuclear cells in pancreatic islet of male C57BL/6 mice.

Treatment of mice	Islets with mononuclear cell infiltrates, n (%)					
	At islet poles		At intra-islet sites			
	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 plus MLD-STZ	34.3	65.7	85.2	8.3	4.4	2.1

Data were obtained from at least 300 islets per group.

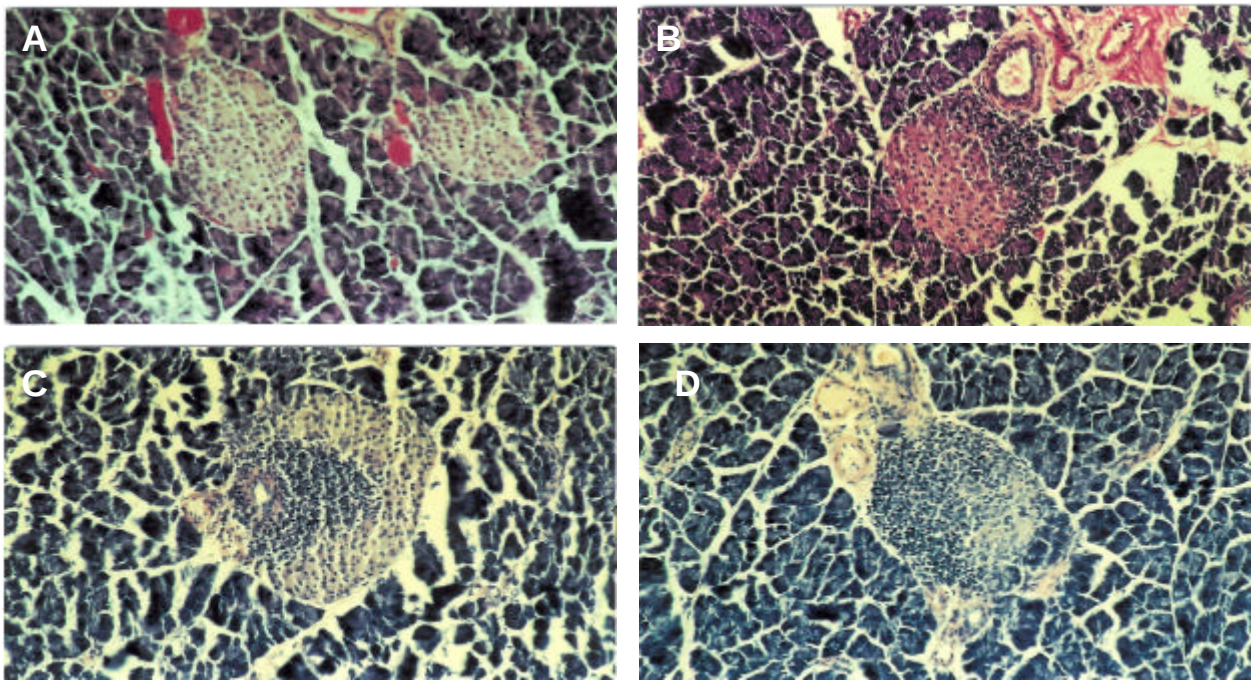

Fig.16. Representative histology of pancreatic sections prepared from C57BL/6 male mice.
A: no infiltrates with mononuclear cells in islets of untreated control mice.
B, **C**, and **D**: intra-islet infiltrates scored as 1+, 2+, and 3+ of MLD-STZ-treated mice 12 days after the first injection of MLD-STZ, respectively. Original magnification x 200. Haematoxylin and eosin stain.

Insulitis was absent in untreated mice and the minor infiltrates in rhIL-11-injected controls could have resulted from a non-specific reaction to the injections.

4.10 Zn^{2+}-enriched drinking water inhibits *ex vivo* stimulation by MLD-STZ of NF-κB and AP-1 activities in islets of male mice

Previous data of my colleagues have demonstrated that treatment with Zn^{2+}-enriched drinking water prevented MLD-STZ diabetes in male C57BL/6 mice. Then, our laboratory has observed that the MLD-STZ-induced diabetes is associated with activation of NF-κB and AP-1 in islets (present data). Based on these findings, the effect of Zn^{2+} on NF-κB and AP-1 activities was analyzed in islets that were isolated from both male and female C57BL/6 mice. The question was, whether Zn^{2+}-enriched drinking water affects MLD-STZ-induced activation of the transcription factors.

As illustrated in Fig. 17A, treatment of male C57BL/6 mice with MLD-STZ alone significantly ($P<0.001$) induced the NF-κB activity on day 3 after the last STZ injection, the mean value ± SE was 169.4 ± 9.89% compared with 100% of solvent-treated control mice. This activation was significantly prevented ($P<0.01$) by Zn^{2+} and the mean value ± SE activity remained by 113.2 ± 5.1% comparable to the activity of the control group. In female C57BL/6 mice (Fig. 17B), in contrast, a transient reduction of the NF-κB activity was only induced on day 1 after the fifth STZ injection, but no significant changes were observed in the other experimental groups. Zn^{2+}-treatment alone failed to alter the NF-κB activity and the activity of the ubiquitous transcription factor Oct-1 as internal control remained unchanged by the different treatments.

As shown in Fig. 18A, in male C57BL/6 mice, MLD-STZ significantly induced ($P<0.01$) the AP-1 activity in islets *ex vivo* on day 3 after the last STZ injection. The mean value ± SE was 168.1 ± 19.64% compared with 100% of solvent-treated control mice. Additional treatment with Zn^{2+} significantly inhibited ($P<0.05$) the AP-1 activity, the mean value ± SE was 125.3 ± 10.21% comparable to the activity of the control group. In female C57BL/6 mice (Fig. 18B), only a significant ($P<0.01$) up-regulation of AP-1 activity was

detected on day 3 after the last STZ injection. The mean value ± SE was 159.3 ± 14.8% compared with 100% of solvent-treated control mice. Additional treatment with Zn^{2+} to MLD-STZ did not affect the AP-1 activity. No changes in the activity were observed, when the mice were treated with Zn^{2+} alone. The Oct-1 activity remained unchanged in islets isolated from different experimental groups.

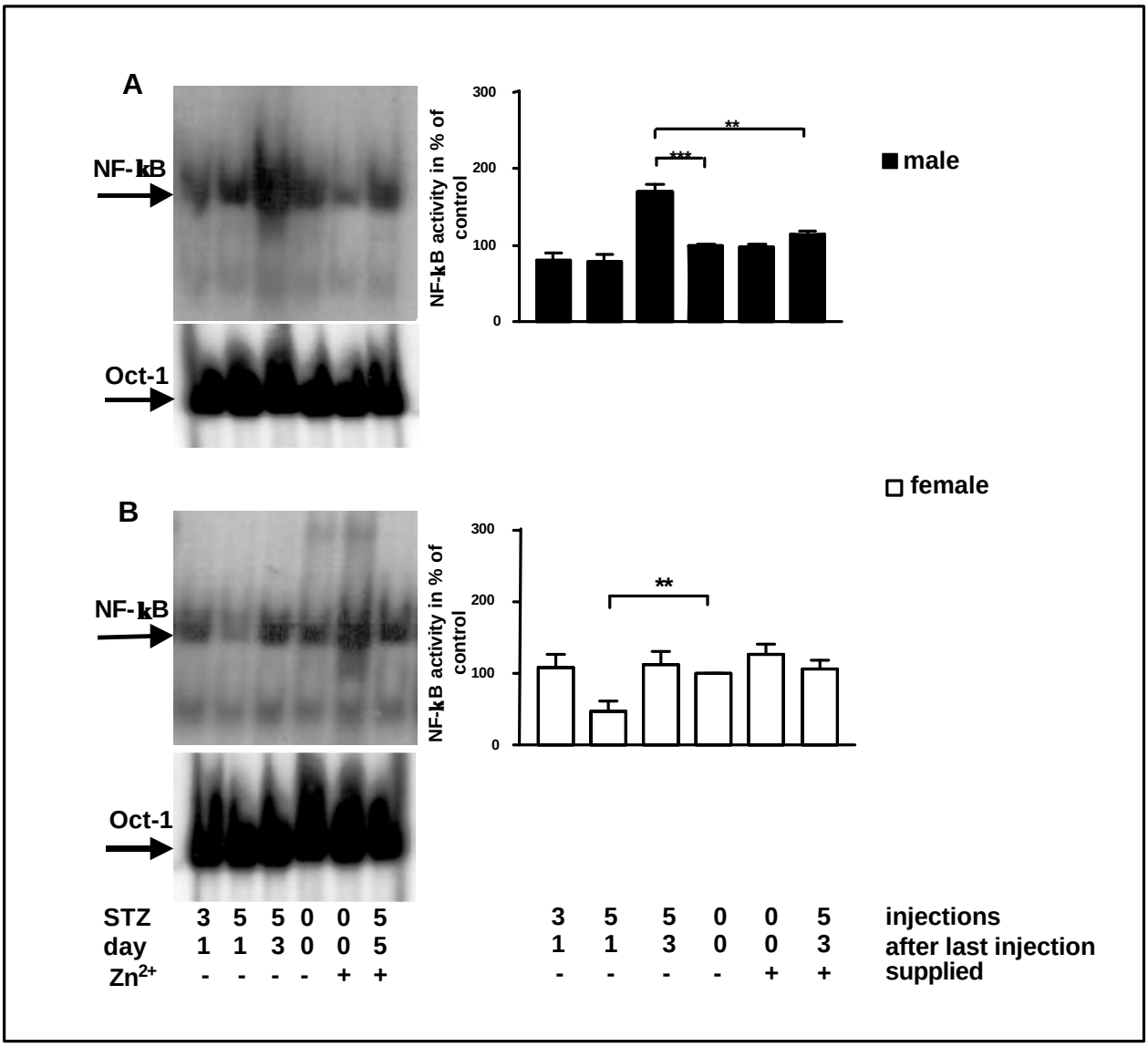

Fig. 17. Zn^{2+} reduces stimulation by MLD-STZ of the NF-kB *ex vivo* activity in islets isolated from male C56BL/6 mice.

Ex vivo activity of NF-κB and Oct-1 in islets of male (**A**) and female C57BL/6 (**B**) mice that had received three or five injections of STZ or five injections of the solvent of STZ (0) as control - either alone or in addition to Zn^{2+}. Islets were isolated on day 1 and/or day 3 after the last of the five STZ injections. Means ± SE of three independent experiments. ******P<0.01; *******P<0.001.

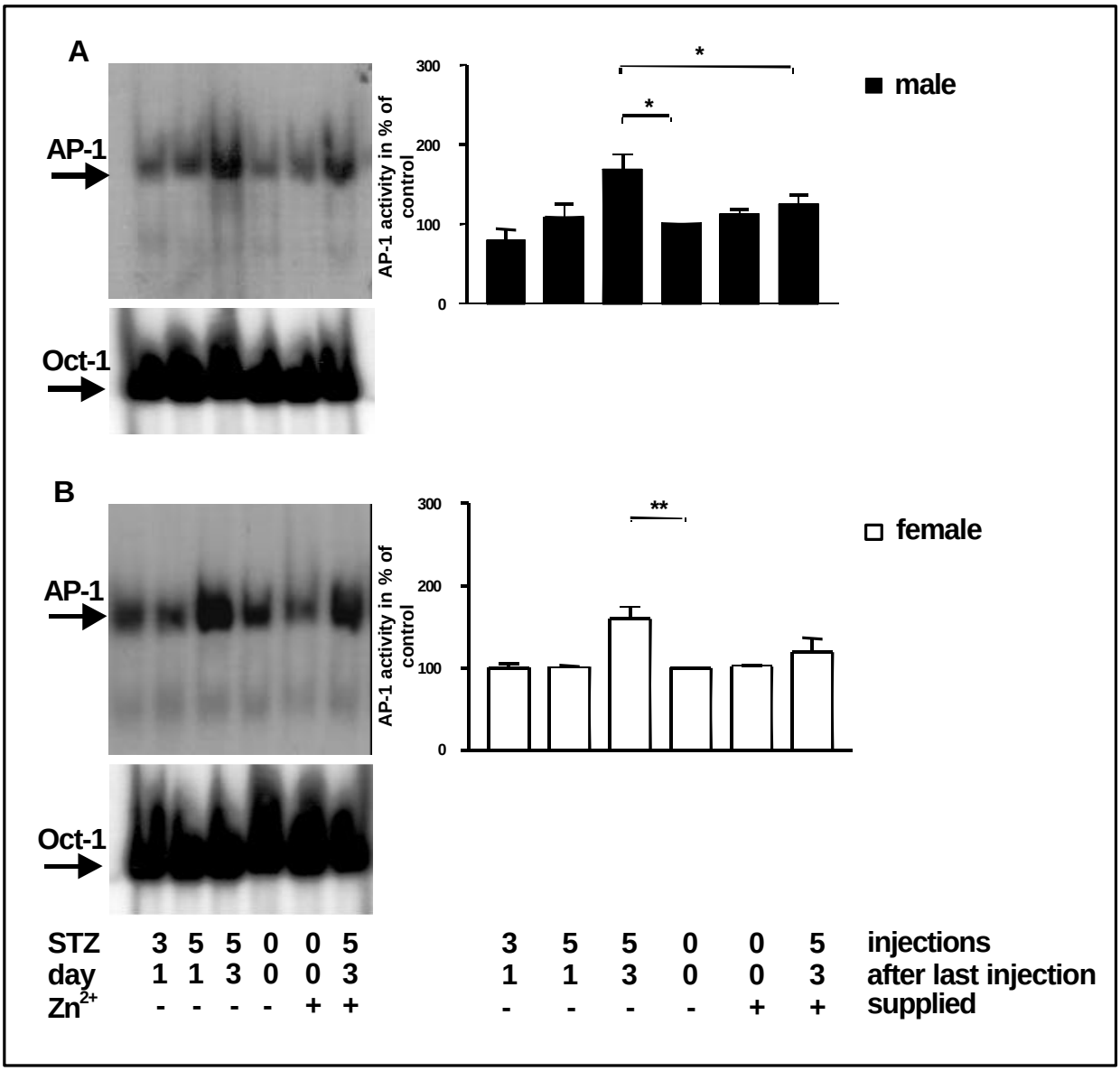

Fig. 18. Zn^{2+} reduces stimulation by MLD-STZ of the AP-1 *ex vivo* activity in islets isolated from male C56BL/6 mice.

Ex vivo activity of AP-1 and Oct-1 in islets of male (**A**) and female C57BL/6 (**B**) mice that had received three or five injections of STZ or five injections of the solvent of STZ (0) as control - either alone or in addition to Zn^{2+}. Islets were isolated on day 1 and/or day 3 after the last of the five STZ injections. Means ± SE of three independent experiments. *****P<0.05; ******P<0.01.

4.11 Zn^{2+}-enriched drinking water prevents stimulation by MLD-STZ of the IKK-a activity in islets of male mice

As illustrated in Fig.19, MLD-STZ alone stimulated IKK-α activity ($P < 0.01$) on day 3 after the last STZ injection. The mean value ± SE of this stimulation was 193 ± 6.99%

compared with 100% of control mice. Additional treatment of mice with Zn^{2+}-enriched drinking water attenuated ($P<0.05$) this stimulation, the mean value ± SE was only 123.8 ± 2.21%.

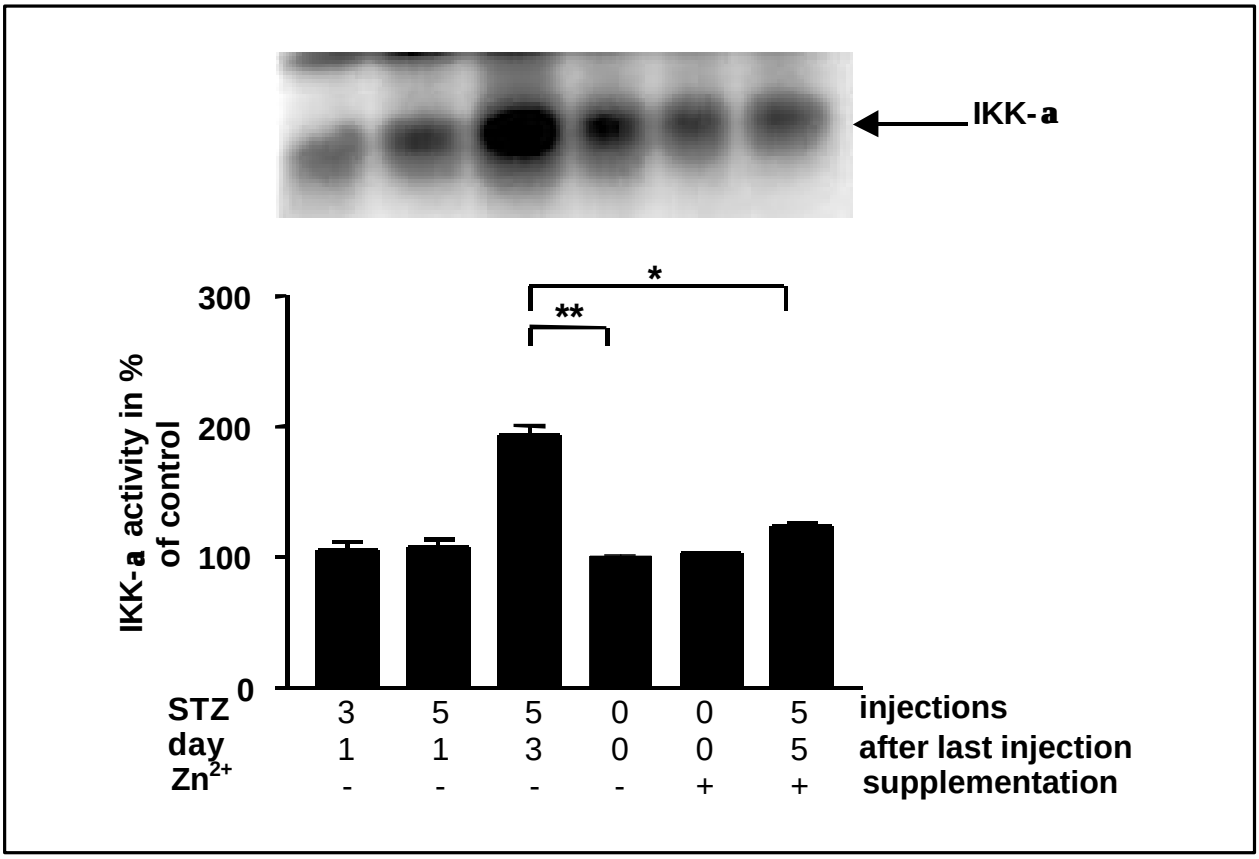

Fig. 19. Zn^{2+} inhibits stimulation of the IKK-a activity by MLD-STZ in islets isolated from male C57BL/6 mice.
Effect of Zn^{2+}-enriched drinking water on IKK-α activity in islets isolated from male C57BL/6 mice that had been treated with MLD-STZ - either alone or in addition to - Zn^{2+} - or with the solvent of STZ or Zn^{2+} alone as controls. Islets were isolated on day 3 after the last injection. IKK-α activities were measured by an *in vitro* kinase assay using IκB-α (1-317) as a substrate. Means ± SE of two independent experiments. *****$P<0.05$; ******$P<0.01$.

4.12 Zn^{2+} enriched drinking water shifts MLD-STZ- induced cytokine responses from Th1-type to Th2-type in islets of male mice

Since treatment with Zn^{2+}-enriched drinking water reduced the *ex vivo* binding activities of NF-κB and AP-1, a possible effect on MLD-STZ- induced local cytokine profiles in islets of male mice was investigated.

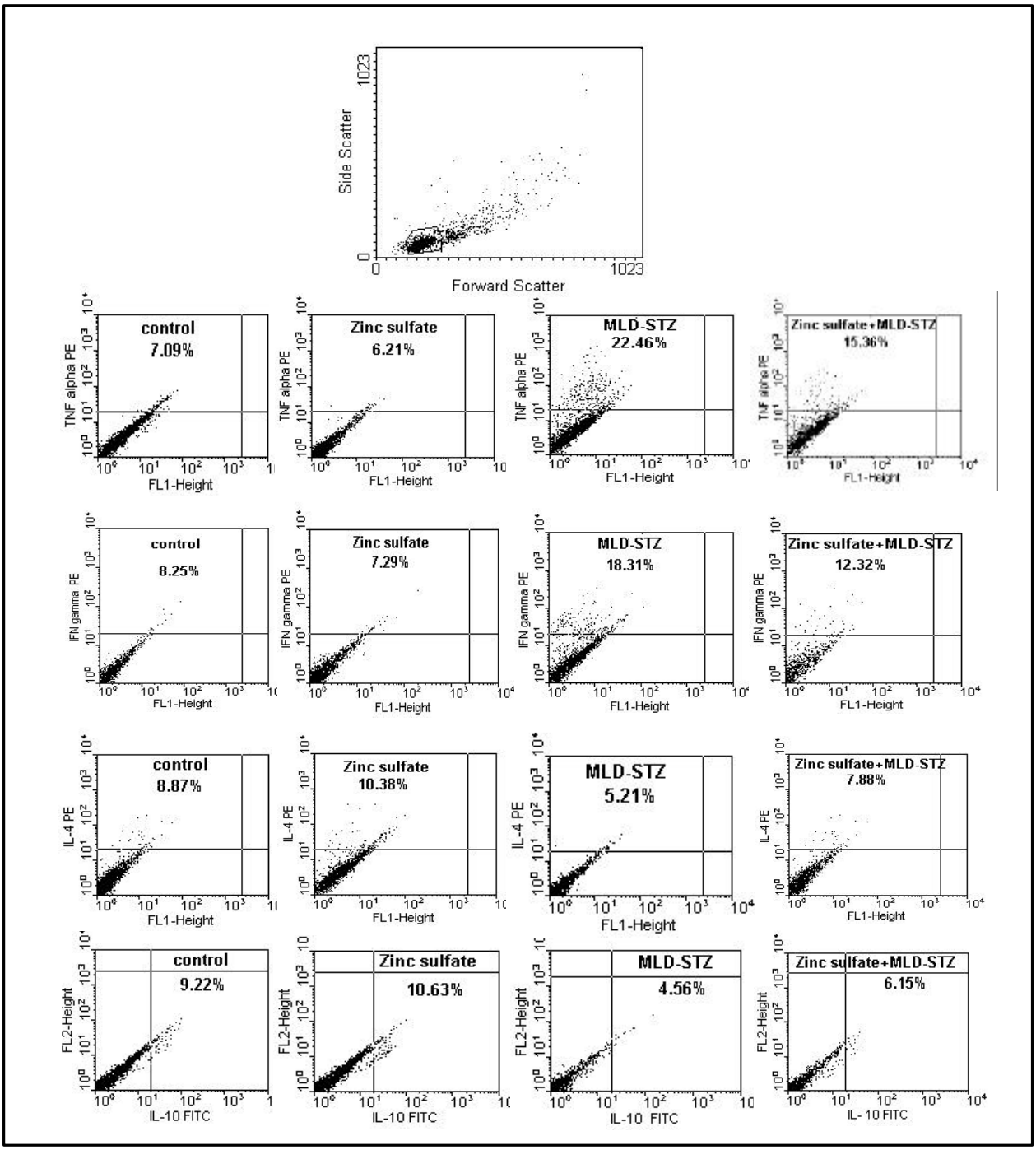

Fig. 20. FACS-analysis of cytokine-producing cells in pancreatic islets.
A. Gate setting on the population of cells present in the pancreatic islets using forward scatter (FSC-H) and side scatter (SSC-H). **B.** Dot blot representations of flow cytometry data from the pancreatic islet cells isolated from male C57BL/6 mice that had been treated with MLD-STZ - either alone or in addition to Zn^{2+} - or treated with Zn^{2+} or the solvent of STZ. The PE-conjugated antibodies against TNF-α, IFN-γ and IL-4 fluoresce in the FL2 chanel and the FITC -conjugated antibodies against IL-10 fluoresce in the FL1 chanel. This mode of analysis allowes to find exactly what percentages of the cells are single-positive for each cytokine. Fluorescence settings are adjusted for each group of samples by using negative control cells incubated with an irrelevant isotype-matched monoclonal antibody.

As illustrated in Figs. 20 and 21, treatment with MLD-STZ alone, as expected, markedly increased the production of pro-inflammatory cytokines, 22.46% and 18.31 % of the gated islet cell population produced TNF-α and IFN-γ, respectively, compared with 7.09% and 8.25% measured in the control groups. Again, MLD-STZ decreased the production of the anti-inflammatory cytokines IL-4 and IL-10, since only 5.21% and 4.56% of cells were positive for IL-4 and IL-10, respectively, in islets of MLD-STZ treated mice compared with the respective results of 8.87% and 9.22% in control mice. Additional treatment of mice with Zn^{2+}-enriched drinking water decreased the percentage of TNF-α- and IFN-γ-producing cells, only 15.36% and 12.32% of islet cells were positive for TNF-α and IFN-γ, respectively, compared with the corresponding results of 22.46% and 18.31% measured in MLD-STZ-treated mice. Treatment with Zn^{2+} did not affect the IL-4 and IL-10 production and Zn^{2+} by itself did not alter the constitutive cytokine profiles found in controls groups. The experiment was performed once only and does not permit any conclusion on significant Zn^{2+} effects.

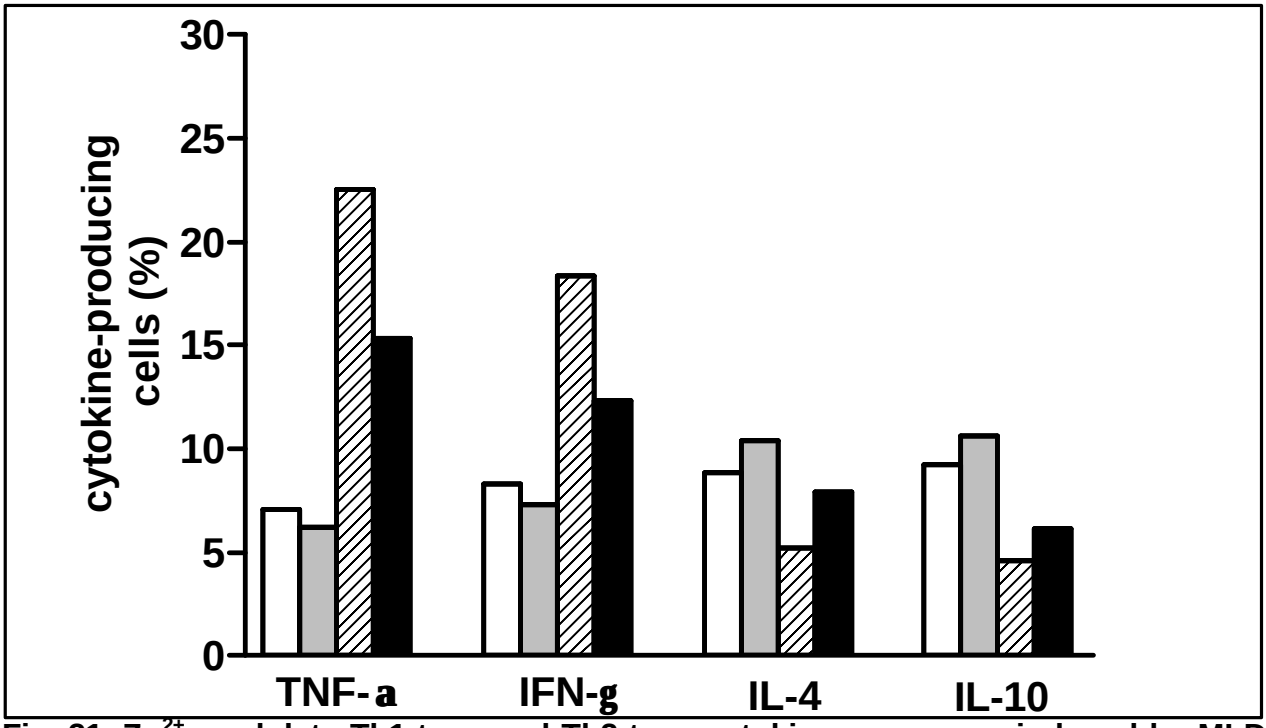

Fig. 21. Zn^{2+} modulats Th1-type and Th2-type cytokine responses induced by MLD-STZ in pancreatic islets of male C57BL/6 mice.

Effect of Zn^{2+} on the percentage of cytokine producing-cells in islets isolated from male C57BL/6 mice that had been treated with MLD-STZ - either alone (▨) or in addition to Zn^{2+} (■) - or treated with Zn^{2+} alone (⁝)- or remained untreated (□) as control . Islets were isolated on day 3 after the fifth STZ injection and from age-matched controls**.** Since this experiment was performed only once, the results do not permit conclusions on significances.

5. DISCUSSION

The present investigations demonstrate for the first time that treatment with the multifunctional anti-inflammatory cytokine rhIL-11 prevents diabetes induced with MLD-STZ in male C57BL/6 mice. The question, whether IL-11 may be involved in MLD-STZ-induced diabetes was derived from the observation that the level of mRNA expression of IL-11 and its receptor IL-11R were reduced in the pancreatic islets of diabetes-susceptible male C57BL/6 mice, but not of the diabetes-resistant female mice. It is demonstrated that the MLD-STZ-induced decrement of IL-11 and IL-11R levels progressed with time after the last STZ injection. Consequently, this data anticipated that IL-11 could play a pivotal role in the pathogenesis of MLD-STZ diabetes.

A total of 130 µg rhIL-11 injected ip at equal doses for 13 subsequent days prevented MLD-STZ-induced hyperglycemia. The anti-diabetic effect of rhIL-11 was long-lasting, since the euglycemic state persisted for an observation period of 20 weeks after starting treatment with MLD-STZ. The potential of rhIL-11 to protect mice from MLD-STZ-induced diabetes is in accordance with previous studies, in which prolonged administration with rhIL-11 prevented, yet transiently only, development of spontanous diabetes in NOD mice (Nicoletti *et al*., 1999).

Consistent with the beneficial effects of rhIL-11 on MLD-STZ-induced hyperglycemia are the data obtained with a test challenging ß-cell function, i.e., the OGTT. The markedly increased blood glucose levels upon glucose challenge in MLD-STZ-injected mice were significantly reduced by rhIL-11 treatment and gradually decreased to near normal values during the weeks after treatment.

Cytokine functions are characterized by redundancy and pleiotropism, and the endogeneous cytokine network is tightly regulated *in vivo*. It is well known that the systemic administration of cytokines, both in humans and rodents, can lead to a general modulation of the immune system (Gherardi *et al*., 2001, Rabinovitch and Saurez-Pinzon, 2003). Therefore, the cytokine profile that develops in the pancreatic islets after MLD-STZ treatment alone and in addition to rhIL-11 was analyzed. Colleagues from our

laboratory (Müller *et al*., 2002) have recently demonstrated that MLD-STZ diabetes is associated with up-regulation of the pro-inflammatory cytokines TNF-α and IFN-γ and down-regulation of the anti-inflammatory cytokines IL-4 and IL-10. The present data clearly indicate that treatment with rhIL-11 completely reversed the MLD-STZ-induced cytokine responses: rhIL-11 inhibited pro-inflammatory reactivities and stimulated 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 the Th2-type cytokine IL-4. Moreover, the percentage of IL-4-producing cells surmounted, although not significantly, those in the islets of control mice. There were no detectable changes in IL-10-producing cells. Treatment of mice with rhIL-11 alone did not affect the constitutive cytokine levels of solvent-injected mice. Therefore, the mechanism by which rhIL-11 treatment prevents MLD-STZ diabetes may be due to its ability to shift the Th1-type pro-inflammatory cytokine responses induced by MLD-STZ to those of Th2-type anti-inflammatory reactivities.

To further understand the molecular mechanism, through which rhIL-11 attenuates the inflammatory response, the effects of treatment with MLD-STZ alone or in combination with rhIL-11 on the transcription factors NF-κB and AP-1 were analyzed. Analyses of these transcription factors were selected, because they not only regulate the activation of genes involved in inflammatory immune responses (Gottschalk *et al.*, 1993; Rao, 1994) but also in the transcription of many pro-inflammatory immediate-early genes such as TNF-α and IL-6 (Ghosh *et al*., 1998).Therefore, these transcription factors were suspected to also play a pivotal role in the immune-mediated ß-cell destruction by MLD-STZ. Indeed, treatment with MLD-STZ alone induced the activation of NF-κB and AP-1 in male C57BL/6 mice. In contrast, co-treatment with rhIL-11 significantly inhibited the activation of both transcription factors. Thus, the results indicate that rhIL-11 can inhibit the production of pro-inflammatory cytokines by 1) suppressing the transcription of pro-inflammatory cytokine genes and 2) by deviating the cytokine profile in the pancreatic islets from pro-inflammatory toward anti-inflammatory reactions. The latter is assumed to be secondary to an enhancement of the activity of NF-κB inhibitors, which bind NF-κB in the cytosolic fraction and prevent its nuclear translocation. Treatment with rhIL-11 coincides with its ability to elevate the kinase activity of IKK-α, a member of a kinase

family that phosphorylates and thereby activates the inhibitory protein of NF-κB, IκB-α. The present data demonstrating that the stimulation of IKK-α activity by MLD-STZ was attenuated by co-treatment with rhIL-11 further substantiate the role of rhIL-11 in the transcriptional regulation of pro-inflammatory cytokine genes. The critical role of NF-κB in the pathogenesis of MLD-STZ-induced diabetes is supported by recent studies demonstrating that mice possessing a gene disruption for NF-κB (p50) were resistant against MLD-STZ diabetes (Mabley *et al*., 2002). However, NF-κB (p50)-deficient mice succumbed to diabetes induced with one single high toxic dose of STZ. These observations clearly indicate that immediate, direct ß-cell toxicity and destruction causes diabetes induced with one toxic dose of STZ without involving NF-κB-regulated immune responses, and strengthens the impact of NF-κB-regulated T cell-mediated inflammatory immune reactions in the pathogenesis of MLD-STZ diabetes. The failure of treatment with rhIL-11 to inhibit insulitis is in accordance with previous findings from our laboratory, in which neither treatment of mice with 5-thio-D-glucose (Wang *et al*., 1995) nor with Zn^{2+}-enriched drinking water (Ohly *et al*., 2000) prevented the infiltration of ß-cells with mononuclear cells. In the present study, the infiltration detected in the group of mice treated with rhIL-11 was non-specific and remained functionally inert. Therefore, the preventive effect of rhIL-11 against MLD-STZ diabetes could be attributed to the inhibition of NF-κB and AP-1 bindings activities and the following induction of protective Th2-type cytokine responses. The ability of rhIL-11 to markedly augment the IL-4 production in islet cells is comparable with data obtained in a mouse model of graft-versus-host disease (Hill *et al*., 1998). The authors have observed a 10-fold increase in IL-4 production in concanavalin A-stimulated spleen cells prepared from rhIL-11-treated mice. The mechanism underlying this increase in IL-4 remains unknown. The present data of failure of rhIL-11 to stimulate IL-10 production and to enhance the transcription rates of TGF-ß1 mRNA expression are in line with *in vitro* studies by Trepicchio et al. (1996), in which incubation with rhIL-11 also had no effect on IL-10 and TGF-ß1 cytokine production from primary murine peritoneal macrophages.

It is well documented that pancreatic ß-cells are particularly vulnerable to damage caused by ROS (Tiedge *et al*., 1998; Kubisch *et al*., 1997). STZ enters the cytoplasm via

the facilitative GLUT2. Although the GLUT2 is also present in the membrane of liver and kidney cells, these organ, however, are relatively resistant to STZ damage. The causes for the different sensitivity of ß-cells for ROS and other tissues, most likely, is sequelae of different levels of anti-oxidative enzymes such as glutathione peroxidase (Gpx), catalase, and superoxiddismutase (SOD), which are low in ß-cells (Tiedge *et al*., 1997). Therefore, ß-cells are unable to inactivate ROS and to interrupt their deadly mission. The reduced levels of intracellular anti-oxidants observed in islets of male C57BL/6 mice may be one of the multifactorial causes for MLD-STZ diabetes susceptibility, whereas the high levels in female mice may contribute to resistance (Friesen *et al*., 2004, in press).

Aiming at elucidating the complex molecular mechanism by which MLD-STZ induce the diabetogenic pathway, two different STZ effects are proposed that are possibly initiated by ROS: 1) direct toxicity on the essential ß-cell molecule GLUT2 (Wang and Gleichmann, 1998) and 2) T cell-dependent inflammatory immune reactions (Nakamura *et al*., 1984; Klinkhammer *et al*., 1988) with stimulation of the pro-inflammatory cytokines TNF-α and INF-γ (Müller *et al*., 2002) and the activation of NF-κB and AP-1 (present data). Both of these effects are required, since the prevention of either the toxicity with 5-thio-D-glucose or glucose (Wang *et al*., 1993; Wang and Gleichmann, 1998; Gai *et al*., 2004) or the immune reaction with anti-lymphocyte serum (Rossini *et al.*, 1978) abrogates the development of diabetes. There is strong evidence for the two initial effects by ROS, as recent *in vitro* studies have demonstrated that the generation of ROS, in particular the most toxic $^{\bullet}OH$ was stimulated in islets by STZ (Gille *et al*., 2002). Above that, the generation of ROS, namely H_2O_2, was also found in islets *ex vivo* isolated from MLD-STZ-treated male, but not female C57BL/6, mice (Friesen *et al*., 2004, in press). Furthermore, previous studies from this laboratory have demonstrated that STZ is an antigen for T cells *in vivo* (Klinkhammer *et al*., 1988) and activation of T cells may be triggered by ROS (Los *et al*., 1995). This T cell activation results in Th1-type cytokine production, which together with other immune cells may transiently produce low levels of ROS which are a potent activator of NF-κB (Schulze-Osthoff *et al*., 1995). In turn, NF-κB translocates to the nucleus and may activate the gene transcription of pro-inflammatory cytokines which induce formation of ROS again. This regula-

tory cycle favors and sustains local inflammatory reactivities, down-regulates Th2-type anti-inflammatory cytokine responses, exhausts anti-oxidative enzymes, and may finally destruct ß-cell function. Although MLD-STZ similarly up-regulated Th1-type cytokines in islets of both sexes, it remains unclear, whether or not the production of pro-inflammatory cytokines in male mice may exceed that of female mice beyond day 3 after the last STZ injection. If this was the case, the absolute instead of the relative imbalance between Th1- and Th2-type cytokines may be decisive for diabetogenesis. However, due to the method applied, analyses at later time points are hindered by loss of ß-cells.

The present data shows that rhIL-11 is capable of preventing diabetes induced with MLD-STZ by antagonizing Th1-type cytokines through stimulation of IL-4 production. Further work is required to investigate the molecular mechanisms of rhIL-11. The inhibition of endogeneous IL-11 with specific inhibitors would be required to clarify the role of endogeneous IL-11 in the pathogenesis of MLD-STZ diabetes. It is also necessary to define the cell(s) targeted by rhIL-11.

In the present work, the inhibiting effect of rhIL-11 on the activity of NF-κB and AP-1 is obviously in part selective, because the activity of the transcription factor Oct-1 as internal control remained unaffected. Moreover, it cannot be excluded that rhIL-11 affects other transcription factors such as the signal transducer and activator of transcription (STAT), the nuclear factor controlling IL-6 gene expression (NF-IL-6), and cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), all of which have already been shown to be involved in the regulation of immune responses (Akira, 1999; Zhang *et al*., 1995; Wang *et al*., 2003). To determine an effect of rhIL-11 on these transcription factors requires further investigations.

The present study also demonstrates that the induction of IKK-α activity parallels with the increased NF-κB activity found in islets obtained from MLD-STZ- treated mice. IKK-α phosphorylates IκB-α which leads to its degradation and results in the translocation of NF-κB to the nucleus. Yet, the significant inhibition of NF-κB and IKK-α activities by rhIL-11 does not rule out the possibility that rhIL-11 may also affect other protein kinase pathways, which lead to the phosphorylation and degradation of IκB. Furthermore, it

would also be necessary to determine effects of rhIL-11 on other inhibitory proteins of NF-κB such as IκB-ß, since *in vitro,* rhIL-11 can enhance IκB-ß at both the protein and mRNA levels in activated macrophages (Trepicchio *et al*., 1997). Recent studies have indicated that the cytokine production is negatively regulated by members of the suppressor of cytokine signalling family (SOCS). Among these inhibitory proteins, SOCS-1 appears to have the most potent inhibitory effect on cytokines including IL-4 and IFN-γ *in vivo* (Naka *et al*., 1999; Krebs *et al*., 2001). Since SOCS-1 plays a regulatory role in both Th1 and Th2 polarization (Fujimoto *et al*., 2002) and since overexpression of SOCS-1 targeted to ß-cells markedly reduced the incidence of diabetes in NOD mice (Flodström-Tullberg *et al*., 2003), the role of SOCS-1 in MLD-STZ diabetes and in the preventive effect of rhIL-11 should be investigated. Such informations may contribute to establish schedules for monitoring treatment with rhIL-11 in clinical settings. The various possibilities of IL-11 interactions with cytokines or transcription factors as postulated above are illustrated in Fig. 22.

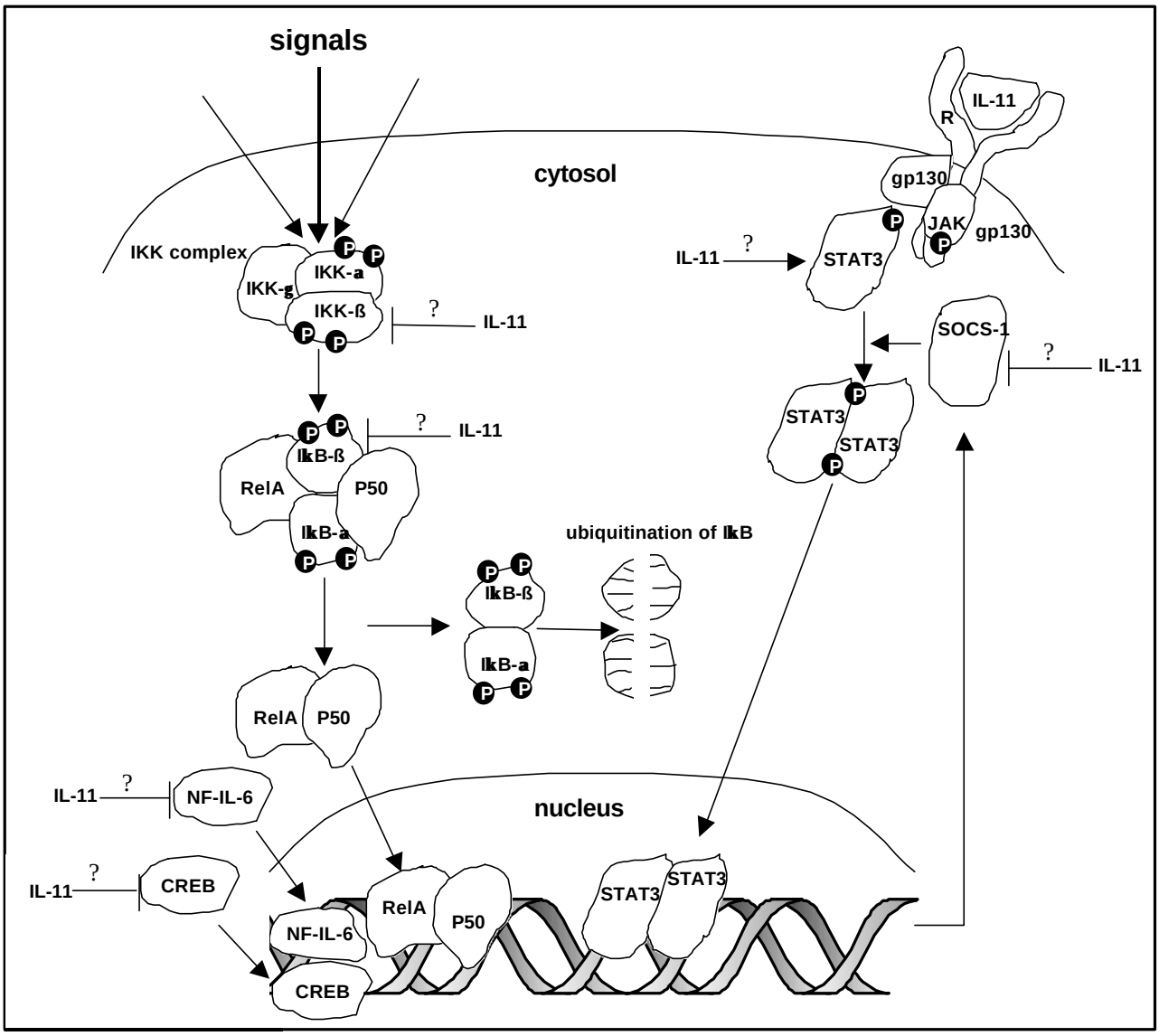

Fig. 22. Scheme depicting future approaches to define the precise mechanism of IL-11 on NF-κB signalling in pancreatic islets: 1) IL-11 may inhibit the IKK-ß kinase activity or the expression of IκB-ß so that NF-κB can not be translocated to the nucleus; 2) IL-11 may suppress the inhibitory effect of SOCS-1 involved in the IL-11-induced signalling pathway, so that STAT 3 can translocate into the nucleus and promote the gene activation of endogenous IL-11; 3) IL-11 may inhibit the binding activities of NF-IL-6 and CREB and stimulate the activation of STAT 3.

rhIL-11 has been shown to have favorable modulating effects on immuno-inflammatory diseases such as psoriasis by reducing the cutaneous inflammation. This reduced inflammation is associated with decreased expression of pro-inflammatory cytokines and increased expression of endogeneous IL-11 (Trepicchio *et al*., 1999). rhIL-11 treatment has also promising effects on rheumatoid arthritis (Walmsley *et al*., 1998), sepsis (Opal *et al*., 1998), and in patients with Crohn´s disease (Sands *et al*., 1999). In the current study, rhIL-11 has been applied with success in a mouse model of induced T1D, although it does not bind efficiently to the murine IL-11R (Barton *et al*., 1996). Therefore, it is possible that murine IL-11 may exert an anti-diabetogenic effect being even superior to rhIL-11 and, conversely, that rhIL-11 may prevent diabetes development in human more powerfully than in NOD mice as a model of spontanous diabetes (Nicoletti *et al*., 1999) and in the present induced model. Furthermore, the mice studied may have produced antibodies to rhIL-11 that could have reduced its bioactivity (Nicoletti *et al*., 1999). Since treatment of patients with rhIL-11 has so far not been accompanied by serious side effects (Trepicchio *et al*., 1999), and since rhIL-11 has already 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 may be considered.

Returning to the ß-cell vulnerability to ROS attacks, experimental work of my colleagues is of particular interest. They found that treatment of mice with Zn^{2+}-enriched drinking water induces MT in pancreatic islets and protects against MLD-STZ diabetes (Ohly *et al*. 2000) and alloxan-induced diabetes (Schulte im Walde *et al*., 2003). In a subsequent study, together with my colleagues, Zn^{2+}-enriched drinking water also protected NOD mice from spontaneous diabetes (Ohly *et al*., submitted). Most likely, Zn^{2+}-induced MT, which is a potent scavenger of $^{\cdot}OH$ of the group of ROS, rescued ß-cells from ROS-mediated damage. This assumption is supported by the observation that overexpression of MT targeted to ß-cells reduces STZ-induced diabetes (Chen H *et al*., 2001). Based on these observations, the effect of Zn^{2+} on the ROS-sensitive transcription factors NF-κB and AP-1 was investigated. Similar to the effects of rhIL-11, Zn^{2+} also inhibited the MLD-STZ-induced activation of NF-κB and AP-1 in pancreatic islets of male C57BL/6 mice. In these mice, MT may have scavenged $^{\bullet}OH$ induced by MLD-STZ and thereby may have

inhibited the binding activity of NF-κB. Since the IKK-α activity was also reduced in these mice, the up-regulated MT may have exerted a regulatory effect in NF-κB cell signalling. By this mechanism, IκB-α remains inactivated and sequesters NF-κB in the cytoplasm. This mechanistic assumption is supported by results demonstrating the negative regulatory role of MT on NF-κB activity *in vitro* using MT null cells. The NF-κB binding activity in MT-expressing cells established from MT-/- cells by transfection with the murine MT-1 gene was significantly lower than that in MT-/- cells (Sakurai *et al*., 1999). Thus, further sudies are required to clarify the mechanisms by which MT regulates NF-κB activity. However, the inhibition of the NF-κB pathway may prove to be an important criterion for choosing nutritional strategies for T1D prevention.

Finally, as stated above, the prevention of MLD-STZ diabetes in male C57BL/6 mice by treatment with either rhIL-11 or Zn^{2+}-enriched drinking water was in so far incomplete as the curves of blood glucose levels still remained above those of untreated control mice. In order to elucidate, whether these two treatments could have synergistic effects, male C57BL/6 mice received both Zn^{2+}-enriched drinking water and rhIL-11. However, this combination did not improve the effect of either treatment alone on MLD-STZ-induced hyperglycemia (data not shown). Therefore, it remains to be investigated, whether Zn^{2+}-enriched drinking water or rhIL-11 in combination with alternative compounds could have synergistic effects.

6. SUMMARY

Multiple low doses of streptozotocin (MLD-STZ) can induce diabetes in male C57BL/6 mice, whereas female mice are resistant. The pathogenic pathway is similar to that of human type 1 diabetes (T1D). This laboratory has reported that MLD-STZ similarly up-regulate the T helper (Th)1-type pro-inflammatory cytokines tumor necro-sis factor (TNF)-α and interferon (IFN)-γ in islets of both sexes, however, down-re-gulate 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)-ß1 in islets of male, but not female, mice. Presumably, this reduction results in a relative preponderance of local pro-inflammatory cytokines that may augment their ß-cell de-structive potential. Furthermore, treatment with zinc ions (Zn^{2+})-enriched drinking wa-ter prevented MLD-STZ diabetes. Based on these data, in the present study effects were analyzed of 1) MLD-STZ on the anti-inflammatory cytokine IL-11, the transcription factors nuclear factor (NF)-κB and activator protein (AP)-1, which are involved in gene activation of pro-inflammatory cytokines, and on the cytosolic kinase (IKK-α) of NF-κB inhibitor (IκB), 2) recombinant human (rh)IL-11 on MLD-STZ diabetes, insulitis, cytokine profiles, IKK-α, NF-κB, and AP-1, and 3) Zn^{2+} on the MLD-STZ-induced activity of IKK-α, NF-κB, and AP-1.

The data are the first to demonstrate that rhIL-11 prevented diabetes without affect-ing insulitis, shifted MLD-STZ-induced Th-1 type cytokine responses toward Th2-type reactions, and inhibited activation by MLD-STZ of IKK-α, NF-κB, and AP-1. Zn^{2+} also inhibited activation by MLD-STZ of IKK-α, NF-κB, and AP-1. The results demonstrate the potential of rhIL-11 in preventing MLD-STZ diabetes through enhancement of an-ti-inflammatory responses in islets. In this process, the transcription factors NF-κB and AP-1 may play a key role. The effect of Zn^{2+} to prevent MLD-STZ diabetes may also result from inhibiting activation of IKK-α, NF-κB, and AP-1.

Since successful treatment of patients with rhIL-11 with psoriatic lesions has not been accompanied by serious side effects and since rhIL-11 ameliorates disease signs in animal models of inflammatory diseases including spontaneous diabetes in NOD mice by modulating inflammatory reactivities, further clinical evaluation of rhIL-11 is warranted for intervention in individuals at high risk for T1D.

7. ZUSAMMENFASSUNG

In männlichen C57BL/6-Mäusen kann mit multiplen, niedrigen Dosen Streptozotocin (MLD-STZ) ein Diabetes induziert werden, der pathogenetisch dem humanen, autoimmunen Typ-1-Diabetes (T1D) vergleichbar ist. Weibliche C57BL/6-Mäuse hingegen entwickeln keinen MLD-STZ Diabetes und sind somit eine Kontrolle, um Assoziationen zwischen MLD-STZ-Effekten und Diabetes zu analysieren. In unserem Labor wurde bereits gezeigt, dass MLD-STZ die T-Helfer (Th)1-typischen, pro-inflammatorischen Zytokine Interferon (IFN)-α und Tumor Nekrose Faktor (TNF)-γ in Inseln beiderlei Geschlechts vergleichbar induziert, jedoch die anti-inflammatorischen Th2-typischen Zytokine Interleukin (IL)-4 und IL-10 sowie das anti-inflammatorische Th3-typische Zytokin Transformierender Wachstumsfaktor (TGF)-ß1 nur in Inseln männlicher, jedoch nicht weiblicher Mäuse reduziert. Es entsteht ein Ungleichgewicht zugunsten der pro-inflammatorischen Zytokine, deren Potential, ß-Zellen zu zer-stören, erhöht wird. Weiterhin wurde mit Zink-Ionen (Zn^{2+})-angereichertem Trink-wasser der MLD-STZ-Diabetes verhindert.

In der vorliegenden Arbeit wurde der Effekt von 1) MLD-STZ auf das anti-inflammatorische Zytokin IL-11, die Transkriptionsfaktoren nukleärer Faktor (NF)-κB und Aktivator Protein (AP)-1, die an der Genaktivierung pro-inflammatorischer Zytokine beteiligt sind, und die cytosolische Kinase (IKK-α) des NF-κB Inhibitors (IκB) in Inseln, 2) rekombinantem, humanem (rh)IL-11 auf den MLD-STZ-Diabetes, die Insulitis, Zytokinprofile, IKK-α, NF-κB und AP-1 und 3) Zn^{2+} auf IKK-α, NF-κB und AP-1 untersucht. Es wird erstmal gezeigt, dass die Behandlung mit rhIL-11 1) einen MLD-STZ-Diabetes in C57BL/6-Mäusen verhindert ohne Veränderung der Insulitis, 2) die Produktion von IL-4 stimuliert und 3) eine Th1-typische in eine Th2-typische Immunantwort moduliert und Aktivitäten von IKK-α, NF-κB und AP-1 inhibiert. Auch Zn^{2+} verhindert die Aktivierung durch MLD-STZ von IKK-α, NF-κB und AP-1. Die Ergebnisse belegen das anti-inflammatorische Potential von rhIL-11, einen MLD-STZ-induzierten Diabetes durch Stimulierung der anti-inflammatorischen Antwort in Inseln zu verhindern. In diesem Prozess könnten die Transkriptions-faktoren NF-κB und AP-1 eine zentrale Rolle spielen. Da rhIL-11 in Patienten vertretbare Nebenwirkungen auslöst, sind weitere klinische Evaluierungen gerechtfertigt, um es bei Personen mit erhöhten Risiko für einen T1D einzusetzen.

8. REFERENCES

Angel,P,Karin,M: The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochim.Biophys. Acta*. 10: 129-157, 1991

Atkinson,MA, McLaren,NK.: The pathogenesis of insulindependent diabetes mellitus. *New England Journal of Medicine* 331 1428–1436.1994

Akira,S: Functional roles of STAT family proteins: lessons from knockout mice. *Stem Cells.* 17(3):138-146. 1999

Alberti,KG, Zimmet,PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet.Med.* 15:539-553, 1998

Baldwin,A: The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu. Rev. Immunol*. 14:649-683, 1996

Baeuerle,PA, Baltimore,D: I kappa B: a specific inhibitor of the NF-kappa B transcription factor. *Science*. 28: 540-546, 1988

Baeuerle,PA, Henkel,T: Function and activation of NF-κB in the immune system. *Annu.Rev.Immunol.* 12:141-179, 1994

Barnes,PJ, Adcock,IM: Transcription factors and asthma. *Eur.Respir.J.* 12:221-234, 1998

Barton,BE, Shortall,J, Jackson,JV: Interleukins 6 and 11 protect mice from mortality in a staphylococcal enterotoxin-induced toxic shock model. *Infect.Immun.* 64:714-718, 1996

Bilinski,P, Roopenian,D, Gossler,A: Maternal IL-11R-α function is required for normal decidualization and fetoplacental development in mice. *Genes Dev.* 12:2234-2243, 1998

Cameron,MJ, Arreaza,GA, Waldhauser,L, Gauldie,J, Delovitch,TL: Immunotherapy of spontaneous type 1 diabetes in nonobese diabetic mice by systemic interleukin-4 treatment employing adenovirus vector-mediated gene transfer. *Gene Ther.* 7:1840-1846, 2000

Cantor,SB, Elting,LS, Hudson,DV Jr, Rubenstein,EB: Pharmacoeconomic analysis of oprelvekin (recombinant human interleukin-11) for secondary prophylaxis of thrombocytopenia in solid tumor patients receiving chemotherapy. *Cancer.* 15;97(12):3099-106, 2003

Chen,F, Castranova,V,Shi,X,Demers,L: New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. Clin Chem. 45(1):7-17, 1999

Chen,H, Carlson,EC, Pellet,L, Moritz,JT, Epstein,PN: Overexpression of metallothionein in pancreatic β-cells reduces streptozotocin-induced DNA damage and diabetes. *Diabetes* 50:2040-2046, 2001

Chen,Y, Kuchroo,VK, Inobe,J, Hafler,DA, Weiner,HL: Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science* 265:1237-1240, 1994

Cherel,M, Sorel,M, Lebeau,B, Dubois,S, Moreau,JF, Bataille,R, Minvielle,S, Jacques,Y: Molecular cloning of two isoforms of a receptor for the human hematopoietic cytokine interleukin-11. *Blood* 86:2534-2540, 1995

Chiu,R, Boyle,WJ, Meek,J, Smeal,T, Hunter,T, Karin,M: The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. *Cell* 54:541-552, 1988

Coleman,JE: Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annu.Rev.Biochem.* 61:897-946, 1992

Crisa,L, Mordes,JP, Rossini,AA: Autoimmune diabetes mellitus in the BB rat. *Diabetes Metab Rev.* 8:4-37, 1992

Cunningham,JJ, Fu,A, Mearkle,PL, Brown,RG: Hyperzincuria in individuals with insulin-dependent diabetes mellitus: concurrent zinc status and the effect of high-dose zinc supplementation. *Metabolism* 43:1558-1562, 1994

Curran,T, Franza,B-RJ: Fos and Jun: the AP-1 connection. *Cell* 55:395-397, 1988

Darnell,J-EJ: STATs and gene regulation. *Science* 277:1630-1635, 1997

Du,XX, Everett,ET, Wang,G, Lee,WH, Yang,Z, Williams,DA: Murine interleukin-11 (IL-11) is expressed at high levels in the hippocampus and expression is developmentally regulated in the testis. *J.Cell Physiol* 168:362-372, 1996

Du,XX, Williams,DA: Interleukin-11: a multifunctional growth factor derived from the hematopoietic microenvironment. *Blood* 83:2023-2030, 1994

Elenkov,IJ, Chrousos,GP: Stress, cytokine patterns and susceptibility to disease. *Baillieres Best.Pract.Res.Clin Endocrinol.Metab* 13:583-595, 1999

Falchuk,KH: Zinc in developmental biology: the role of metal dependent transcription regulation. *Prog.Clin Biol.Res.* 380:91-111, 1993

Fearon,DT, Locksley,RM: The instructive role of innate immunity in the acquired immune response. *Science* 272:50-53, 1996

Fiorentino,DF, Bond,MW, Mosmann,TR: Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J.Exp Med.* 170:2081-2095, 1989

Fitch,FW, McKisic,MD, Lancki,DW, Gajewski,TF: Differential regulation of murine T lymphocyte subsets. *Annu.Rev.Immunol.* 11:29-48, 1993

Flodström-Tullberg,M, Yadav,D, Hagerkvist,R, Tsai,D, Secrest,P, Stotland,A, Sarvetnick,N: Target cell expression of suppressor of cytokine signaling-1 prevents diabetes in the NOD mouse. *Diabetes*. 52(11):2696-2700, 2003

Foulis,AK, Farquharson,MA, Meager,A: Immunoreactive α-interferon in insulin-secreting beta cells in type 1 diabetes mellitus. *Lancet* 2:1423-1427, 1987

Foulis,AK, McGill,M, Farquharson,MA: Insulitis in type 1 (insulin-dependent) diabetes mellitus in man--macrophages, lymphocytes, and interferon-γ containing cells. *J.Pathol.* 165:97-103, 1991

Foxwell,B, Browne,K, Bondeson,J, Clarke,C, de Martin,R, Brennan,F, Feldmann,M: Efficient adenoviral infection with IκB-α reveals that macrophage tumor necrosis factor alpha production in rheumatoid arthritis is NF-κB dependent. *Proc.Natl.Acad.Sci.U.S.A* 95:8211-8215, 1998

Friesen,NTE, Büchau,AS, Schott-Ohly,P, Lgssiar,A, Gleichmann,H: Generation of hydrogen peroxide and failure of antioxidative responses in pancreatic islets is associated with diabetes induced with multiple low doses of streptozotocin in C57BL/6 male mice. *Diabetologia*, 2004 (in press)

Fujimoto,M, Tsutsui,H, Yumikura-Futatsugi,S, Ueda,H, Xingshou,O, Abe,T, Kawase,I, Nakanishi,K, Kishimoto,T, Naka,T: A regulatory role for suppressor of cytokine signaling-1 in T(h) polarization in vivo. *Int.Immunol*. 14(11):1343-1350, 2002

Gai,W, Schott-Ohly,P, Schulte im Walde,S, Gleichmann,H: Differential target molecules for toxicity induced by streptozotocin and alloxan in pancreatic islets of mice in vitro. Exp.Clin.Endocrinol.Diabetes. 112(1):29-37, 2004

Gajewski,TF, Schell,SR, Nau,G, Fitch,FW: Regulation of T-cell activation: differences among T-cell subsets. *Immunol.Rev.* 111:79-110, 1989

Gherardi,MM, Ramirez,JC, Esteban,M: Towards a new generation of vaccines: the cytokine IL-12 as an adjuvant to enhance cellular immune responses to pathogens during prime-booster vaccination regimens. *Histol. Histopathol*. 16(2):655-667, 2001

Ghosh,S, May,MJ, Kopp,EB: NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu.Rev.Immunol.* 16:225-260, 1998

Gille,L, Schott-Ohly,P, Friesen,N, Schulte-im-Walde,S, Udilova,N, Nowl,H, Gleichmann,H: Generation of hydroxyl radicals mediated by streptozotocin in pancreatic islets of mice in vitro. *Pharmacol.Toxicol.* 90:317-326, 2002

Gotoh,M, Maki,T, Kiyoizumi,T, Satomi,S, Monaco,AP: An improved method for isolation of mouse pancreatic islets. *Transplantation* 40:437-438, 1985

Gottschalk,LR, Giannola,DM, Emerson,SG: Molecular regulation of the human IL-3 gene: inducible T cell-restricted expression requires intact AP-1 and Elf-1 nuclear protein binding sites. *J.Exp Med.* 178:1681-1692, 1993

Halstead,J, Kemp,K, Ignotz,RA: Evidence for involvement of phosphatidylcholine-phospholipase C and protein kinase C in transforming growth factor-β signaling. *J.Biol.Chem.* 270:13600-13603, 1995

Helgason,T, Jonasson,MR: Evidence for a food additive as a cause of ketosis-prone diabetes. *Lancet II*:716–720. 1981

Hill,GR, Cooke,KR, Teshima,T, Crawford,JM, Keith,J-CJ, Brinson,YS, Bungard,D, Ferrara,JL: Interleukin-11 promotes T cell polarization and prevents acute graft-versus-host disease after allogeneic bone marrow transplantation. *J.Clin Invest* 102:115-123, 1998

Hilton,DJ, Hilton,AA, Raicevic,A, Rakar,S, Harrison-Smith,M, Gough,NM, Begley,CG, Metcalf,D, Nicola,NA, Willson,TA: Cloning of a murine IL-11 receptor α-chain; requirement for gp130 for high affinity binding and signal transduction. *EMBO J.* 13:4765-4775, 1994

Hirai,H, Kaino,Y, Ito,T, Kida,K: Analysis of cytokine mRNA expression in pancreatic islets of nonobese diabetic mice. *J.Pediatr.Endocrinol.Metab* 13:91-98, 2000

Ho,E, Quan,N, Tsai,YH, Lai,W, Bray,TM: Dietary zinc supplementation inhibits NF-κB activation and protects against chemically induced diabetes in CD1 mice. *Exp Biol.Med.(Maywood.)* 226:103-111, 2001

Huang,X, Yuang,J, Goddard,A, Foulis,A, James,RF, Lernmark,A, Pujol-Borrell,R, Rabinovitch,A, Somoza,N, Stewart,TA: Interferon expression in the pancreases of patients with type I diabetes. *Diabetes* 44:658-664, 1995

Im Walde,SS, Dohle,C, Schott-Ohly,P, Gleichmann,H: Molecular target structures in alloxan-induced diabetes in mice. Life Sci. 71(14):1681-1694, 2002

Katz,JD, Benoist,C, Mathis,D: T helper cell subsets in insulin-dependent diabetes. *Science* 268:1185-1188, 1995

Keith,J-CJ, Albert,L, Sonis,ST, Pfeiffer,CJ, Schaub,RG: IL-11, a pleiotropic cytokine: exciting new effects of IL-11 on gastrointestinal mucosal biology. *Stem Cells* 12 Suppl 1:79-89, 1994

Kim,CH, Kim,JH, Lee,J, Hsu,CY, Ahn,YS: Thiol antioxidant reversal of pyrrolidine dithiocarbamate-induced reciprocal regulation of AP-1 and NF-κB. *Biol.Chem.* 384:143-150, 2003

King,C, Davies,J, Mueller,R, Lee,MS, Krahl,T, Yeung,B, O'Connor,E, Sarvetnick,N: TGF-β1 alters APC preference, polarizing islet antigen responses toward a Th2 phenotype. *Immunity.* 8:601-613, 1998

Klinkhammer,C, Popowa,P, Gleichmann,H: Specific immunity to streptozocin. Cellular requirements for induction of lymphoproliferation. *Diabetes* 37:74-80, 1988

Krebs,DL, Hilton,DJ: SOCS proteins: negative regulators of cytokine signaling. *Stem Cells* 19:378-387, 2001

Kröncke,KD, Fehsel,K, Sommer,A, Rodriguez,ML, Kolb-Bachofen,V: Nitric oxide generation during cellular metabolization of the diabetogenic N-methyl-N-nitroso-urea streptozotozin contributes to islet cell DNA damage. *Biol.Chem.Hoppe Seyler* 376:179-185, 1995

Kubisch,HM, Wang,J, Bray,TM, Phillips,JP: Targeted overexpression of Cu/Zn superoxide dismutase protects pancreatic beta-cells against oxidative stress. *Diabetes* 46(10):1563-1566, 1997

Lgssiar,A, Hassan,M, Schott-ohly,P, Friesen,N, Nicoletti,F, Trepicchio,WL,Gleichmann, H: Interleukin-11 inhibits NF-κB and AP-1 activation in islets and prevents diabetes-induced with streptozotocin in Mice. *Exp. Biol. Med*. 299:000-000, 2004 (in press)

Leng,SX, Elias,JA: Interleukin-11 inhibits macrophage interleukin-12 production. *J.Immunol.* 159:2161-2168, 1997

Lewis,C, Barbiers,AR: Streptozotocin, a new antibiotic *In vitro* and *in vivo* evaluation. *Antibiot. Ann*. 22:247-254, 1960

Liblau,RS, Singer,SM, McDevitt,HO: Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol.Today* 16:34-38, 1995

Like,AA, Rossini,AA: Streptozotocin-induced pancreatic insulitis: new model of diabetes mellitus. *Science* 193:415-417, 1976

Los,M, Droge,W, Stricker,K, Baeuerle,PA, Schulze-Osthoff,K: Hydrogen peroxide as a potent activator of T lymphocyte functions. *Eur.J.Immunol.* 25:159-165, 1995

Lütticken,C, Wegenka,UM, Yuan,J, Buschmann,J, Schindler,C, Ziemiecki,A, Harpur,AG, Wilks,AF, Yasukawa,K, Taga,T: Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science* 263:89-92, 1994

Mabley,JG, Hasko,G, Liaudet,L, Soriano,F, Southan,GJ, Salzman,AL, Szabo,C: NF-κB (p50)-deficient mice are not susceptible to multiple low-dose streptozotocin-induced diabetes. *J.Endocrinol.* 173:457-464, 2002

MacDonald,TT: T cell immunity to oral allergens. *Curr.Opin.Immunol.* 10:620-627, 1998

MacDonald,TT: Effector and regulatory lymphoid cells and cytokines in mucosal sites. *Curr.Top.Microbiol.Immunol.* 236:113-135, 1999

Makino,S, Kunimoto,K, Muraoka,Y, Mizushima,Y, Katagiri,K, Tochino,Y: Breeding of a non-obese, diabetic strain of mice. *Jikken Dobutsu* 29:1-13, 1980

Mellits,KH, Hay,RT, Goodbourn,S: Proteolytic degradation of MAD3 (IκB-α) and enhanced processing of the NF-κB precursor p105 are obligatory steps in the activation of NF-κB. *Nucleic Acids Res.* 21:5059-5066, 1993

Mosmann,TR, Coffman,RL: Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu.Rev.Immunol.* 7:145-173, 1989

Mosmann,TR, Sad,S: The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol.Today* 17:138-146, 1996

Müller,A, Schott-Ohly,P, Dohle,C, Gleichmann,H: Differential regulation of Th1-type and Th2-type cytokine profiles in pancreatic islets of C57BL/6 and BALB/c mice by multiple low doses of streptozotocin. *Immunobiology* 205:35-50, 2002

Naka,T, Tsutsui,H, Fujimoto,M, Kawazoe,Y, Kohzaki,H, Morita,Y, Nakagawa,R, Narazaki,M, Adachi,K, Yoshimoto,T, Nakanishi,K, Kishimoto,T: SOCS-1/SSI-1-deficient NKT cells participate in severe hepatitis through dysregulated cross-talk inhibition of IFN-gamma and IL-4 signaling in vivo. *Immunity*. 14(5):535-545, 2001

Nakamura,M, Nagafuchi,S, Yamaguchi,K, Takaki,R: The role of thymic immunity and insulitis in the development of streptozocin-induced diabetes in mice. *Diabetes* 33:894-900, 1984

Nandurkar,HH, Robb,L, Tarlinton,D, Barnett,L, Kontgen,F, Begley,CG: Adult mice with targeted mutation of the interleukin-11 receptor (IL11Ra) display normal hematopoiesis. *Blood* 90:2148-2159, 1997

Nathan,CF, Tsunawaki,S: Secretion of toxic oxygen products by macrophages: Regulatory cytokines and their effects on the oxidase. *Ciba Found Symp* 118:211–230, 1986

Neurath,MF, Fuss,I, Schurmann,G, Pettersson,S, Arnold,K, Muller-Lobeck,H, Strober,W, Herfarth,C, Buschenfelde,KH: Cytokine gene transcription by NF-κB family members in patients with inflammatory bowel disease. *Ann.N.Y.Acad.Sci.* 859:149-159, 1998

Nicoletti,F, Zaccone,P, Conget,I, Gomis,R, Moller,C, Meroni,PL, Bendtzen,K, Trepicchio,W, Sandler,S: Early prophylaxis with recombinant human interleukin-11 prevents spontaneous diabetes in NOD mice. *Diabetes*. 48(12): 2333-9, 1999

Oberley LW*:* Free radicals and diabetes. *Free Rad Med* 5:113-124, 1988

Ohly,P, Dohle,C, Abel,J, Seissler,J, Gleichmann,H: Zinc sulphate induces metallothionein in pancreatic islets of mice and protects against diabetes induced by multiple low doses of streptozotocin. *Diabetologia* 43:1020-1030, 2000

Opal,SM, Jhung,JW, Keith,J-CJ, Palardy,JE, Parejo,NA, Young,LD, Bhattacharjee,A: Recombinant human interleukin-11 in experimental Pseudomonas aeruginosa sepsis in immunocompromised animals. *J.Infect.Dis.* 178:1205-1208, 1998

Pak,CY, Eun,HM, McArthur,RG, Yoon,JW: Association of cytomegalovirus infection with autoimmune type 1 diabetes. *Lancet* 2:1-4, 1988

Papaccio,G, Pisanti,FA, Latronico,MV, Ammendola,E, Galdieri,M: Multiple low-dose and single high-dose treatments with streptozotocin do not generate nitric *oxide. J. Cell Biochem*. 77(1): 82-91, 2000

Pato,E, Cour,MI, Gonzalez-Cuadrado,S, Gonzalez-Gomez,C, Munoz,JJ, Figueredo,A: Coxsackie B4 and cytomegalovirus in patients with insulin-dependent diabetes. *An.Med.Interna* 9:30-32, 1992

Paul,SR, Bennett,F, Calvetti,JA, Kelleher,K, Wood,CR, O'Hara,R-MJ, Leary,AC, Sibley,B, Clark,SC, Williams,DA: Molecular cloning of a cDNA encoding interleukin 11, a stromal cell-derived lymphopoietic and hematopoietic cytokine. *Proc.Natl.Acad.Sci.U.S.A* 87:7512-7516, 1990

Pennline,KJ, Roque-Gaffney,E, Monahan,M: Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *Clin Immunol.Immunopathol.* 71:169-175, 1994

Peterson,RL, Wang,L, Albert,L, Keith,J-CJ, Dorner,AJ: Molecular effects of recombinant human interleukin-11 in the HLA-B27 rat model of inflammatory bowel disease. *Lab Invest* 78:1503-1512, 1998

Powrie,F, Coffman,RL: Cytokine regulation of T-cell function: potential for therapeutic intervention. *Trends Pharmacol.Sci.* 14:164-168, 1993

Rabinovitch,A, Sumoski,W, Rajotte,RV, Warnock,GL: Cytotoxic effects of cytokines on human pancreatic islet cells in monolayer culture. *J.Clin Endocrinol.Metab* 71:152-156, 1990

Rabinovitch,A: Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM. Therapeutic intervention by immunostimulation? *Diabetes* 43:613-621, 1994

Rabinovitch,A, Suarez-Pinzon,WL, Sorensen,O, Bleackley,RC, Power,RF, Rajotte,RV: Combined therapy with interleukin-4 and interleukin-10 inhibits autoimmune diabetes recurrence in syngeneic islet-transplanted nonobese diabetic mice. Analysis of cytokine mRNA expression in the graft. *Transplantation* 60:368-374, 1995

Rabinovitch,A, Suarez-Pinzon,WL, Strynadka,K, Lakey,JR, Rajotte,RV: Human pancreatic islet beta-cell destruction by cytokines involves oxygen free radicals and aldehyde production. *J. Clin. Endocrinol. Metab*. 81(9):3197-202, 1996

Rabinovitch,A: An update on cytokines in the pathogenesis of insulin-dependent diabetes mellitus. *Diabetes Metab Rev.* 14:129-151, 1998

Rabinovitch,A, Suarez-Pinzon,WL: Cytokines and their roles in pancreatic islet β-cell destruction and insulin-dependent diabetes mellitus. *Biochem.Pharmacol.* 55:1139-1149, 1998

Rabinovitch,A, Suarez-Pinzon,WL: Role of cytokines in the pathogenesis of autoimmune diabetes mellitus. *Rev.Endocr.Metab.Disord*. 4(3):291-299. 2003

Rao,A: NF-ATp: a transcription factor required for the co-ordinate induction of several cytokine genes. *Immunol.Today* 15:274-281, 1994

Redlich,CA, Gao,X, Rockwell,S, Kelley,M, Elias,JA: IL-11 enhances survival and decreases TNF production after radiation-induced thoracic injury. *J.Immunol.* 157:1705-1710, 1996

Rink,L, Gabriel,P: Zinc and the immune system. *Proc.Nutr.Soc.* 59:541-552, 2000

Robb,L, Li,R, Hartley,L, Nandurkar,HH, Koentgen,F, Begley,CG: Infertility in female mice lacking the receptor for interleukin 11 is due to a defective uterine response to implantation. *Nat.Med.* 4:303-308, 1998

Rolli-Derkinderen,M, Machavoine,F, Baraban,JM, Grolleau,A, Beretta,L, Dy,M: ERK and p38 inhibit the expression of 4E-BP1 repressor of translation through induction of Egr-1. *J.Biol.Chem*. 278(21):18859-18867, 2003

Romagnani,S: Human Th1 and Th2 subsets: regulation of differentiation and role in protection and immunopathology. *Int.Arch.Allergy Immunol.* 98:279-285, 1992

Rossini,AA, Like,AA, Chick,WL, Appel,MC, Cahill,G-FJ: Studies of streptozotocin-induced insulitis and diabetes. *Proc.Natl.Acad.Sci.U.S.A* 74:2485-2489, 1977

Rossini,AA, Williams,RM, Appel,MC, Like,AA: Complete protection from low-dose streptozotocin-induced diabetes in mice. *Nature* 276:182-184, 1978

Rossini,AA, Williams,RM, Appel,MC, Like,AA: Sex differences in the multiple-dose streptozotocin model of diabetes. *Endocrinology* 103:1518-1520, 1978

Sakurai,A, Hara,S, Okano,N, Kondo,Y, Inoue,J, Imura,N: Regulatory role of metallothionein in NF-κB activation. *FEBS Lett.* 455:55-58, 1999

Sands,BE, Bank,S, Sninsky,CA, Robinson,M, Katz,S, Singleton,JW, Miner,PB, Safdi,MA, Galandiuk,S, Hanauer,SB, Varilek,GW, Buchman,AL, Rodgers,VD, Salzberg,B, Cai,B, Loewy,J, DeBruin,MF, Rogge,H, Shapiro,M, Schwertschlag,US: Preliminary evaluation of safety and activity of recombinant human interleukin 11 in patients with active Crohn's disease. *Gastroenterology* 117:58-64, 1999

Sato,M, Kondoh,M: Recent studies on metallothionein: protection against toxicity of heavy metals and oxygen free radicals. *Tohoku J.Exp Med.* 196:9-22, 2002

Schernthaner,G, Banatvala,JE, Scherbaum,W, Bryant,J, Borkenstein,M, Schober,E, Mayr,WR: Coxsackie-B-virus-specific IgM responses, complement-fixing islet-cell antibodies, HLA DR antigens, and C-peptide secretion in insulin-dependent diabetes mellitus. *Lancet* 21, 2(8456):630-632, 1985

Schnedl,WJ, Ferber,S, Johnson,JH, Newgard,CB: STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. *Diabetes* 43:1326-1333, 1994

Schott-Ohly,P, Lgssiar,A, Partke,HJ, Hassan,M,Friesen,M, Gleichmann,H: Zn^{2+}-enriched drinking water activates nuclear factor κB in islets and prevents diabetes in NOD mice. (in press)

Schulze-Osthoff,K, Los,M, Baeuerle,PA: Redox signalling by transcription factors NF-κB and AP-1 in lymphocytes. *Biochem.Pharmacol.* 50:735-741, 1995

Schwertschlag,US, Trepicchio,WL, Dykstra,KH, Keith,JC, Turner,KJ, Dorner,AJ: Hematopoietic, immunomodulatory and epithelial effects of interleukin-11. *Leukemia* 13:1307-1315, 1999

Scott,BJ, Bradwell,AR: Identification of the serum binding proteins for iron, zinc, cadmium, nickel, and calcium. *Clin Chem.* 29:629-633, 1983

Sen,R, Baltimore,D: Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 46:705-716, 1986

Shreedhar,V, Giese,T, Sung,VW, Ullrich,SE: A cytokine cascade including prostaglandin E2, IL-4, and IL-10 is responsible for UV-induced systemic immune suppression. *J.Immunol.* 160:3783-3789, 1998

Shaulian,E, Karin,M: AP-1 as a regulator of cell life and death. *Nat. Cell Biol*. 4(5): 131-136, 2002

Somoza,N, Vargas,F, Roura-Mir,C, Vives-Pi,M, Fernandez-Figueras,MT, Ariza,A, Gomis,R, Bragado,R, Marti,M, Jaraquemada,D: Pancreas in recent onset insulin-dependent diabetes mellitus. Changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor Vβ usage, and cytokine profile. *J.Immunol.* 153:1360-1377, 1994

Sternesjö,J, Welsh,N, Sandler,S: S-methyl-L-thiocitrulline counteracts interleukin 1 beta induced suppression of pancreatic islet function in vitro, but does not protect against multiple low-dose streptozotocin-induced diabetes in vivo. *Cytokine*. 9(5):352-9, 1997

Sun,SC, Maggirwar,SB, Harhaj,E: Activation of NF-κB by phosphatase inhibitors involves the phosphorylation of IκB alpha at phosphatase 2A-sensitive sites. *J.Biol.Chem.* 270:18347-18351, 1995

Taga,T, Kishimoto,T: Gp130 and the interleukin-6 family of cytokines. *Annu.Rev.Immunol.* 15:797-819, 1997

Tan,X, Sun,X, Gonzalez-Crussi,FX, Gonzalez-Crussi,F, Hsueh,W: PAF and TNF increase the precursor of NF-κB p50 mRNA in mouse intestine: quantitative analysis by competitive PCR. *Biochim.Biophys.Acta* 1215:157-162, 1994

Thorpe,R, Wadhwa,M, Bird,CR, Mire-Sluis,AR: Detection and measurement of cytokines. *Blood Rev.* 6:133-148, 1992

Tiedge,M, Lortz,S, Drinkgern,J, Lenzen,S: Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. *Diabetes*. 46(11):1733-42. 1997

Tiedge,M, Lortz,S, Munday,R, Lenzen,S: Complementary action of antioxidant enzymes in the protection of bioengineered insulin-producing RINm5F cells against the toxicity of reactive oxygen species. *Diabetes*. 47(10):1578-1585, 1998

Trepicchio,WL, Bozza,M, Pedneault,G, Dorner,AJ: Recombinant human IL-11 attenuates the inflammatory response through down-regulation of proinflammatory cytokine release and nitric oxide production. *J.Immunol.* 157:3627-3634, 1996

Trepicchio,WL, Wang,L, Bozza,M, Dorner,AJ: IL-11 regulates macrophage effector function through the inhibition of nuclear factor-κB. *J.Immunol.* 159:5661-5670, 1997

Trepicchio,WL, Dorner,AJ: Interleukin-11. A gp130 cytokine. *Ann.N.Y.Acad.Sci.* 856:12-21, 1998

Trepicchio,WL, Ozawa,M, Walters,IB, Kikuchi,T, Gilleaudeau,P, Bliss,JL, Schwertschlag,U, Dorner,AJ, Krueger,JG: Interleukin-11 therapy selectively downregulates type I cytokine proinflammatory pathways in psoriasis lesions. *J.Clin Invest* 104:1527-1537, 1999

Trepicchio,WL, Bozza,M, Bouchard,P, Dorner,AJ: Protective effect of rhIL-11 in a murine model of acetaminophen-induced hepatotoxicity. Toxicol. Pathol. 29(2):242-249, 2001

Trinchieri,G: Natural killer cells wear different hats: effector cells of innate resistance and regulatory cells of adaptive immunity and of hematopoiesis. *Semin.Immunol.* 7:83-88, 1995

Turk,J, Corbett,JA, Ramanadham,S, Bohrer,A, McDaniel,ML: Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets. *Biochem.Biophys.Res.Commun.* 197:1458-1464, 1993

Walmsley,M, Butler,DM, Marinova-Mutafchieva,L, Feldmann,M: An anti-inflammatory role for interleukin-11 in established murine collagen-induced arthritis. *Immunology* 95:31-37, 1998

Wang,Z, Dohle,C, Friemann,J, Green,BS, Gleichmann,H: Prevention of high- and low-dose STZ-induced diabetes with D-glucose and 5-thio-D-glucose. *Diabetes* 42:420-428, 1993

Wang,Z, Gleichmann,H: Glucose transporter 2 expression: prevention of streptozotocin-induced reduction in beta-cells with 5-thio-D-glucose*. Exp. Clin. Endocrinol. Diabetes*.103 Suppl 2:83-97, 1995

Wang,Z, Gleichmann,H: GLUT2 in pancreatic islets: crucial target molecule in diabetes induced with multiple low doses of streptozotocin in mice. *Diabetes* 47:50-56, 1998

Wang,Z, Zhang,B, Wang,M, Carr,BI: Persistent ERK phosphorylation negatively regulates cAMP response element-binding protein (CREB) activity via recruitment of CREB-binding protein to pp90RSK. *J. Biol. Chem*. 278(13):11138-11144. 2003

Wilson,GL, Leiter,EH: Streptozotocin interactions with pancreatic beta cells and the induction of insulin-dependent diabetes. *Curr.Top.Microbiol.Immunol.* 156:27-54, 1990

Yamagata,K, Nakajima,H, Tomita,K, Itoh,N, Miyagawa,J, Hamaguchi,T, Namba,M, Tamura,S, Kawata,S, Kono,N, Kuwajima,M, Noguchi,T, Hanafusa,T, Matsuzawa,Y: Dominant TCR alpha-chain clonotypes and interferon-gamma are expressed in the pancreas of patients with recent-onset insulin-dependent diabetes mellitus. *Diabetes Res.Clin Pract.* 34:37-46, 1996

Yang,J, Cherian,MG: Protective effects of metallothionein on streptozotocin-induced diabetes in rats. *Life Sci*.55(1):43-51, 1994

Yin,T, Taga,T, Tsang,ML, Yasukawa,K, Kishimoto,T, Yang,YC: Involvement of IL-6 signal transducer gp130 in IL-11-mediated signal transduction. *J. Immunol*. 151(5):2555-2561, 1993

Zhang,Y, Broser,M, Rom,W: Activation of the interleukin 6 gene by Mycobacterium tuberculosis or lipopolysaccharide is mediated by nuclear factors NF IL 6 and NF-kappa B. *Proc. Natl. Acad. Sci. U S A*. 92(8):3632, 1995

Zimny,S, Gogolin,F, Abel,J, Gleichmann,H: Metallothionein in isolated pancreatic islets of mice: Induction by zinc and streptozotocin, a narurally occuring diabetogen. *Arch. Toxicol.* 67:61-65, 1993

9. ACKNOWLEDGMENTS

This work was financially 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.

First, I would like to express my sincere gratitude to my supervisor, Prof. Dr. med. Helga Gleichmann for introducing me to the exciting field of diabetes research, and for the continous encouragements. Thanks for never-ending support and guidance. Thanks for good advices, constructive criticism, for letting me work independently and for teaching me patiently the art of scientifically precise writting.

I would like to thank Prof. Dr. rer. nat. Frank Wunderlich for having accepted the responsibility as supervisor and referee of my dissertation.

I thank Prof. Dr. med. Werner Scherbaum for giving me the opportunity to work at the german Diabetes Research Institute.

My appreciation also goes to my colleagues, Patricia Schott-Ohly, Nadira Friesen, and Amanda Büchau, for creating a friendly atmosphere, for discussions, and for powerful assistance.

Many thanks to Dr. Mohamed Hassan for fruitful collaboration and interest in my work.

I also thank my parents, brothers, and sisters for their continuous support.

Finally, very warm thanks to my friend Lisa for listening with patience to my detailed reports on the progress of my laboratory work.

VERÖFFENTLICHUNGEN

1. **Lgssiar A**, Hassan M, Schott-Ohly P, Friesen N, Nicoletti F, Trepicchio WL, Gleichmann H
   Interleukin-11 inhibits NF-κB and AP-1 activation in islets and prevents diabetes induced with streptozotocin in mice
   Exp Biol Med, 229(5): 425-436, 2004

2. Friesen NTE, Büchau AS, Schott-Ohly P, **Lgssiar A**, Gleichmann H
   Generation of hydrogen peroxide and failure of antioxidative responses in pancreatic islets is associated with diabetes induced with multiple low doses of streptozotocin in C57BL/6 male mice
   Diabetologia, 47: 676-685, 2004

3. Schott-Ohly P, **Lgssiar A**, Partke HJ, Hassan M, Friesen N, Gleichmann H Zn^{2+}-Enriched Drinking Water Prevents Spontaneous Diabetes in NOD Mice
   Diabetes (submitted)

4. Friesen NTE, Schott-Ohly P, **Lgssiar A**, Gleichmann H
   Dietary selenium deficiency aggravates hyperglycemia induced with multiple low doses of streptozotocin in male C57BL/6 mice
   European journal of nutrition (submitted)

5. Hassan M, **Lgssiar A**, Ghozlan H, Ola A, Megahed M
   Activation of c-Jun NH_2 terminal kinase (JNK) signaling pathway by hepatitis C Virus (HCV) non-structural protein 3 (NS3) mediates proliferation of HepG2 cells
   (submitted)

ABSTRACTS

1. **Lgssiar A**, Müller A, Gleichmann H
   Reduction of the anti-inflammatory cytokines IL-4, IL-10, and IL-11 in pancreatic islets is associated with severe diabetes induced with multiple low doses of streptozotocin
   7. Inselworkshop der Deutschen-Diabetes-Gesellschaft, Münster, 14.-15.12. **2001**

2. Friesen N, Schott-Ohly P, **Lgssiar A**, Gleichmann H
   STZ-induced H_2O_2 generation in pancreatic islets of mice in vitro
   Diabetes und Stoffwechsel 11 (Suppl): 56, **2002**

3. **Lgssiar A**, Schott-Ohly P, Friesen N, Gleichmann H
   Reduction of IL-4, IL-10, and IL-11 in pancreatic islets is associated with diabetes induced with multiple low doses of streptozotocin
   Diabetes und Stoffwechsel 11 (Suppl): 55, **2002**

4. **Lgssiar A**, Müller A, Gleichmann H
   Reduction of IL-4, IL-10, and IL-11 in pancreatic islets is associated with diabetes induced with multiple low doses of streptozotocin
   Diabetes 51: (suppl 2) A15, **2002**

5. **Lgssiar A**, Hassan M, Schott-Ohly P, Friesen N, Gleichmann H
   Zn^{2+} upregulates metallothionein and reduces NF-6B and AP-1 activity in pancreatic islets ex vivo and prevents diabetes induced with multiple low doses of streptozotocin in mice
   11^{th} International Symposium on Trace Elements in Man and Animal, Berkeley, 02.-06.06.**2002**

6. **Lgssiar A**, Hassan M, Friesen N, Büchau A, Schott-Ohly P, Gleichmann H
   Susceptibility of immune diabetes induced with multiple low doses of streptozotocin (MLD-STZ) is associated with down-regulation of anti-inflammatory cytokines, generation of reactive oxygen species, and activation of NF-6B and AP-1
   Immunobiol, 206, 121, I.8, **2002**

7. **Lgssiar A**, Hassan M, Schott-Ohly P, Friesen NTE, Büchau AS, Nicoletti F, Gleichmann H
   Recombinant human interleukin-11 inhibits NF-6B and AP-1 activation in pancreatic islets and prevents diabetes induced with multiple low doses of streptozotocin
   Diabetes und Stoffwechsel 12: V55 (Suppl), **2003**

8. Büchau AS, Friesen NTE, Schott-Ohly P, **Lgssiar A**, Gleichmann H
   Generation of H_2O_2 and failure of antioxidative enzymes in pancreatic islets of C57BL/6 mice is associated with susceptibility for diabetes induced with multiple low doses of streptozotocin
   Diabetes und Stoffwechsel 12: P135 (Suppl), **2003**

9. Schott-Ohly P, Partke HJ, **Lgssiar A**, Friesen N, Büchau A, Gleichmann H
   Zn^{2+}-enriched drinking water prevented spontaneous diabetes in NOD mice
   Diabetes und Stoffwechsel 12: P133 (Suppl), **2003**

10. **Lgssiar A**, Hassan M, Schott-Ohly P, Friesen NTE, Nicoletti F, Gleichmann H
    Recombinant human interleukin-11 inhibits NF-6B and AP-1 activation in pancreatic islets and prevents diabetes induced with multiple low doses of streptozotocin
    Diabetes 52: A66 (suppl 1), **2003**

11. **Lgssiar A**, Hassan M, Schott-Ohly P, Friesen NTE, Büchau AS, Nicoletti F, Gleichmann H
    Recombinant human interleukin-11 inhibits NF-6B and AP-1 activation in pancreatic islets and prevents diabetes induced with multiple low doses of streptozotocin
    Diabetologia 46: A9 (suppl 2), **2003**

12. Friesen NTE, Büchau AS, Schott-Ohly P, **Lgssiar A**, Gleichmann H
    Diabetes induced with multiple low doses of streptozotocin is associated with generation of H_2O_2 and deficient antioxidant enzyme responses in pancreatic islets of C57BL/6 mice
    Diabetologia 46: A180 (suppl 2), **2003**

13. Schott-Ohly P, Partke HJ, **Lgssiar A**, Friesen NTE, Büchau AS, Gleichmann H
Zn^{2+}-enriched drinking water prevented spontaneous diabetes in NOD mice
Diabetologia 46: A181 (suppl 2), **2003**

ORALE PRÄSENTATIONEN AUF WISSENSCHAFTLICHEN KONGRESSEN

1. Reduction of Th2-type cytokines in murine pancreatic islets by multiple low doses of streptozotocin is associated with diabetes
36. Jahrestagung der Deutschen-Diabetes-Gesellschaft, Aachen, 25.05.2001

2. Reduction of the anti-inflammatory cytokines IL-4,IL-10, and IL-11 in pancreatic islets is associated with severe diabetes induced with multiple low doses of streptozotocin
7. Inselworkshop der Deutschen-Diabetes-Gesellschaft, Münster, 14.12.2001

3. Zn^{2+} upregulates metallothionein and reduces NF-6B and AP-1 activity in pancreatic islets ex vivo and prevents diabetes induced with multiple low doses of streptozotocin in mice
TEMA-11: Trace Elements in Man and Animals. Berkeley, California, June 2-6, 2002
Einarbeitung und Organisation der Präsentation von A. Lgssiar, vorgetragen von Prof. Dr. med. H. Gleichmann

4. Reduction of IL-4, IL-10, and IL-11 in pancreatic islets is associated with diabetes induced with multiple low doses of streptozotocin
62nd Annual Scientific Meeting of the American Diabetes Association, San Francisco, CA, USA, June 14th, 2002
Einarbeitung und Organisation der Präsentation von A. Lgssiar, vorgetragen von Prof. Dr. med. H. Gleichmann

5. Recombinant human interleukin-11 inhibits NF-6B and AP-1 activation in pancreatic islets and prevents diabetes induced with multiple low doses of streptozotocin
38. Jahrestagung der Deutschen Diabetes-Gesellschaft, Bremen, 30. Mai, 2003

6. Recombinant human interleukin-11 inhibits NF-6B and AP-1 activation in pancreatic islets and prevents diabetes induced with multiple low doses of streptozotocin
63rd Annual Scientific Meeting of the American Diabetes Association, New Orleans, TX, USA, June, 16th, 2003
Einarbeitung und Organisation der Präsentation von A. Lgssiar, vorgetragen von Prof. Dr. med. H. Gleichmann

7. Recombinant human interleukin-11 inhibits NF-6B and AP-1 activation in pancreatic islets and prevents diabetes induced with multiple low doses of streptozotocin
   18th International Diabetes Federation Congress, Paris, Frankreich, August, 25th, 2003

POSTER PRÄSENTATIONEN AUF WISSENSCHAFTLICHEN KONGRESSEN

1. Reduction of IL-4, IL-10, and IL-11 in pancreatic islets is associated with diabetes induced with multiple low doses of streptozotocin
   37. Jahrestagung der Deutschen Diabetes-Gesellschaft. Dresden, 09-11.05.2002

2. Susceptibility of immune diabetes induced with multiple low doses of streptozotocin (MLD-STZ) is associated with down-regulation of anti-inflammatory cytokines, generation of reactive oxygen species and activation of NF-6B and AP-1
   Jahrestagung der Deutschen Gesellschaft für Immunologie. Marburg, 26-28.09.2002

Erklärung:

Hiermit erkläre ich, die vorliegende Arbeit selbstständig angefertigt und keine anderen als die angegebenen Hilfsmittel verwendet zu haben.

Düsseldorf, den 23. März 2004