

# NFκB1 (p50)-deficient mice are not susceptible to multiple low-dose streptozotocin-induced diabetes

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## Abstract

Insulin-dependent diabetes mellitus (IDDM) is a disease characterized by the autoimmune destruction of the pancreatic β-cells, which requires the expression of a number of immune-related genes including major histocompatibility complex proteins, cytokines, chemokines, and cytotoxic enzymes, many of which are regulated by the transcription factor, NFκB. Inhibition of the entire NFκB family of transcription factors may be harmful, as these factors are involved in many normal physiological processes. However, identifying and targeting specific NFκB subunits critical for the pathogenesis of disease may prove to be valuable in designing new therapeutic strategies. To assess the potential role of the NFκB subunit, p50, in the development of IDDM, mice with gene disruption for *NFκB (p50)* were investigated for susceptibility to IDDM. We found that p50-deficient mice were fully resistant against multiple low-dose streptozotocin-induced dia-

betes, a model of diabetes with a strong autoimmune component. The site of involvement of NFκB (p50) lies at an early, critical juncture of immune activation and proinflammatory mediator production, because: (1) isolated islets of Langerhans from NFκB (p50)-deficient mice were not protected from the islet dysfunction induced by *in vitro* application of proinflammatory cytokines; (2) p50-deficient mice were not resistant to diabetes induced by a single high dose of streptozotocin, a model with a large oxidant component and no autoimmune involvement; and (3) diabetes induced up-regulation of nitric oxide and interleukin-12 was blocked in the p50-deficient mice. Our data suggest that NFκB (p50) has an essential role in the development of autoimmune diabetes. Selective therapeutic blockade of this subunit may be beneficial in preventing IDDM.

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## Introduction

Insulin-dependent diabetes mellitus (IDDM) is characterized by the selective destruction, via an autoimmune process, of the insulin-secreting β-cells in the pancreatic islets of Langerhans. Development of an autoimmune disease such as IDDM requires expression of a number of immune-related genes such as those which encode cytokines (tumour necrosis factor alpha (TNFα) and interleukin (IL)-1; Rabinovitch & Suarez-Pinzon 1998), chemokines (Bradley *et al.* 1999), adhesion molecules (vascular cell adhesion molecule-1 and intercellular cell adhesion molecule (ICAM; Bradley *et al.* 1999), costimulatory molecules (Karlsson *et al.* 2000) and cytotoxic enzymes (inducible nitric oxide synthase (iNOS) and cyclo-oxygenase (COX)-2; Eizirik *et al.* 1996). These proteins have crucial roles in the activation, migration and effector functions of inflammatory cells. Their expression is regulated by various transcription factor families, including the NFκB/Rel family.

The NFκB/Rel family of proteins consists of at least five members: NFκB1 (p50/p105), NFκB2 (p52/p100), Rel A (p65), Rel B and c-Rel (Tak & Firestein 2001). In unstimulated cells NFκB is primarily located in the cytoplasm as inactive homo- or heterodimeric proteins associated with an inhibitory protein termed IκB (Tak & Firestein 2001). Proinflammatory stimuli, cytokines, free radicals, and viral and bacterial products activate NFκB by phosphorylation and proteolytic degradation of the IκB subunit, leading to translocation of NFκB to the nucleus, where it binds to the κB sites of gene promoters (Chen *et al.* 1999, Janssen-Heininger *et al.* 2000). Immune-related gene promoter regions that contain the κB binding site include major histocompatibility complex molecules, proinflammatory cytokines (e.g. TNFα, IL-1β), proinflammatory enzymes such as iNOS and inducible COX, and apoptosis-related molecules (such as Fas and FasL) (Baeuerle & Henkel 1994, Barnes & Karin 1997).

Much of our information about the role of NFκB in diabetes and has come from *in vitro* experiments showing a

critical role of this factor in cytokine-mediated destruction of islet cells (Flodstrom *et al.* 1996, Giannoukakis *et al.* 2000). *In vivo*, however, it is more difficult to define the role of this ubiquitous transcription factor, as NFκB has a key role in many physiological functions. Immunosuppressive agents such as glucocorticoids, FK506 and cyclosporin A, used to prevent IDDM in animal models, have all been shown to inhibit NFκB activation, along with their other actions (Beauparlant & Hiscott 1996). It has been reported that an NFκB decoy oligodeoxynucleotide injected intravenously inhibits diabetogenesis and NFκB activation by alloxan in mice (Grankvist *et al.* 1981, Quan *et al.* 2001). The activation of NFκB and destruction of islet β-cells by alloxan is, however, likely to be a direct effect of alloxan, which is an oxygen free-radical generator, and to have no autoimmune component.

Therefore, in order to evaluate the specific role of NFκB1 (p50) in IDDM pathogenesis, we utilized NFκB (p50)-deficient mice (Sha *et al.* 1995) and determined their sensitivity to streptozotocin-induced diabetes. Streptozotocin is a specific β-cell toxin and can be used to induce diabetes chemically in rats and mice. Streptozotocin is taken up by the β-cells through the glucose transporter, Glut-2 (Schnedl *et al.* 1994, Elsner *et al.* 2000), where it decomposes intracellularly, causing DNA damage directly, by alkylation (Delaney *et al.* 1995, Elsner *et al.* 2000), and indirectly, via generation of nitric oxide (NO) (Turk *et al.* 1993), resulting in β-cell death by necrosis (Like *et al.* 1978). There are two streptozotocin-induced animal models of diabetes: administration of a single high dose of streptozotocin (SHDS) induces diabetes by directly destroying the β-cells, and administration of multiple low-dose streptozotocin (MLDS) induces diabetes progressively via an immune cell response directed towards the β-cells. In the MLDS model, hyperglycemia and diabetes are induced by several injections of subdiabetogenic doses, each injection resulting in a fraction of the β-cells being damaged (Kolb 1987, Kolb & Krönke 1993), eventually leading to a local inflammation and an insulinitis similar to that observed in patients with recent-onset IDDM (Like & Rossini 1976, Rossini *et al.* 1978).

The aims of this present study were to determine the sensitivity of NFκB (p50)-deficient mice to both the MLDS and SHDS models of diabetes, and to establish whether isolated islets of Langerhans from p50-deficient mice were resistant to the *in vitro* effects of cytokines – specifically, their induction of NOS and inhibitory effect on insulin secretion.

## Materials and Methods

### Animals

Six-to-eight-week-old (B6 × 129)F<sub>2</sub> (B6129) mice homozygous for NFκB1 mutation (Sha *et al.* 1995) and their littermate controls were purchased from The Jackson

Laboratory (Bar Harbor, ME, USA). The mice were housed at the Inotek Corporation animal facility for 1 week before the experimental procedure was begun. All animal experiments were carried out in accordance with the guidelines published by the National Institutes of Health (1985) and with the approval of the Inotek Corporation Institutional Animal Care and Use committee.

### Induction of diabetes

Two methods of inducing diabetes were utilized in this study. *NFκB1* (p50)<sup>-/-</sup> or *NFκB1* (p50)<sup>+/+</sup> mice were treated with either streptozotocin (40 mg/kg dissolved in citrate buffer, pH 4.5) or vehicle (citrate buffer) intraperitoneally (i.p.) for 5 consecutive days (MLDS model) (Flodstrom *et al.* 1999), or with streptozotocin (160 mg/kg) as a single i.p. injection (SHDS model) (Garcia Soriano *et al.* 2001). For the MLDS model, the blood glucose was monitored over the following 21 days using a one-touch blood glucose meter (Lifescan). Blood glucose was measured on days 1, 7, 14 and 21, from blood obtained from the tail vein. Mice treated with the single high dose of streptozotocin had their blood glucose measured daily for 4 days before being killed. Diabetes was defined as a non-fasting blood glucose concentration greater than 200 mg/dl.

### Pancreatic content of insulin

Pancreas samples from mice killed on day 21 (MLDS) or day 4 (SHDS) were weighed before being placed into 6 ml of acid ethanol (ethanol:H<sub>2</sub>O:HCl in the proportions 23:7:0.45) and homogenized. The pancreas was incubated for 72 h at 4 °C before being centrifuged (200 g for 10 min) and the insulin content of the supernatant was then determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit from Alpco (Windham, NH, USA). Insulin content was expressed as ng insulin/mg protein.

### Serum cytokine concentrations

Blood samples were taken from mice treated with MLDS on day 21. ELISA kits that are specific against murine cytokines were used to determine cytokine concentrations in the serum. Concentrations of IL-12 (p40), IL-12 (p70) TNFα, IL-4 and IL-10 were measured using ELISA kits purchased from R & D systems (Minneapolis, MN, USA). Plates were read at 450 nm with a Spectramax 250 microplate reader from Molecular Devices (Sunnyvale, CA, USA). Assays were performed according to the manufacturers' instructions.

### Nitrite/nitrate concentrations

Serum or culture media nitrite/nitrate concentrations were determined by converting the nitrate to nitrite using

the enzyme nitrate reductase, followed by addition of Griess reagent to quantify the nitrite concentration colorimetrically. The serum was diluted 1:5 in PBS before a 25 µl aliquot was added to a mixture of 25 µl nitrate reductase (1 U/1.5 ml) and 25 µl NADPH (0.134 mg/ml) in 40 mM Tris, pH 7.6, and incubated at room temperature for 3 h. After this period, 100 µl of Griess reagent (1:1 mix of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl-ethylenediamine) was added and incubated for a further 10 min at room temperature, then the absorbency of the samples was read at 540 nm with a 650 nm reference (Green *et al.* 1982). The procedure was identical for the assay of nitrite/nitrate in the culture media, except that no dilution of the samples was required, the standards were prepared in the appropriate culture media, and the plate was incubated for only 2 h before addition of the Griess reagent. The concentration of nitrate was determined from a standard curve of sodium nitrate and calculated as µM nitrate.

#### Isolation and culture of islets of Langerhans

Islets of Langerhans from both the wild-type and NFκB1-deficient mice were isolated under aseptic conditions from collagenase-digested pancreata. Batches of 200 islets were cultured free-floating in RPMI 1640 medium containing 5.5 mM glucose, penicillin (50 U/ml), streptomycin (50 µg/ml) and 5% fetal calf serum (vol/vol) (Gibco Brl, Life Technologies, Frederick, MD, USA) for 48 h, before being assigned to experimental groups.

#### Cytokine treatment of the islets

Islets were treated with IL-1β, TNFα and interferon (IFN)-γ, either alone or in combination. Isolated islets were treated with human recombinant IL-1β (100 pM) and TNFα (100 pM) plus murine IFN-γ (10 U/ml), all obtained from R & D systems, for 24 h.

#### Measurement of glucose-stimulated insulin release

Islets were removed from culture and preincubated in Gey & Gey (1936) physiological buffer containing 2 mM glucose. The insulin secretory response was measured from groups of five islets incubated in 1 ml buffer containing 20 mM glucose, for 1 h (Green *et al.* 1993). At the end of the incubation, 0.4 ml was removed and assayed for insulin using a commercially available insulin ELISA kit (Alpco). Results were expressed as ng insulin/islet per h.

#### Statistical analysis

Data are presented as mean ± S.E.M. Statistical analysis was preformed using either  $\chi^2$  test or Student's *t*-test, as appropriate.

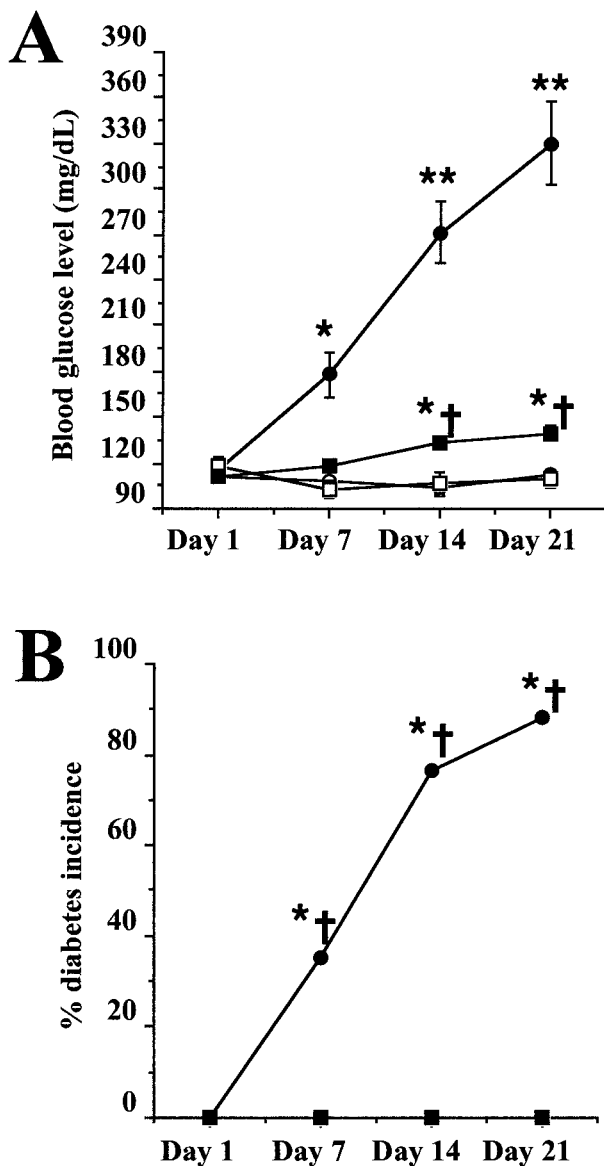
## Results

### Studies in mice treated with MLDS

In response to MLDS, the wild-type mice responded with progressive hyperglycemia and an increased incidence of diabetes (Fig. 1), whereas the NFκB (p50)-deficient mice were fully resistant to the development of diabetes (Fig. 1) over the 21-day period of observation. The degree of insulin depletion in the pancreata of the NFκB (p50)-deficient mice was significantly attenuated compared with the response in the wild-type mice. The insulin content in wild-type mice decreased by 71% with MLDS treatment (from  $73 \pm 18.8$  to  $21 \pm 4$  ng insulin/mg protein), whereas in NFκB (p50)-deficient mice the decrease was 49% (from  $72 \pm 11$  to  $37 \pm 5$  ng insulin/mg protein), indicating that p50 deficiency protects against the immune-cell-mediated islet cell destruction. MLDS-treated wild-type mice had an approximately twofold increase in serum nitrite/nitrate concentrations, indicative of increased formation of NO, whereas NFκB (p50)-deficient mice exhibited no increase in nitrite/nitrate concentrations (Fig. 2a). To investigate further the mechanism of protection by *NFκB (p50)* gene disruption, serum concentrations of inflammatory cytokines in mice subjected to MLDS were determined. In wild-type mice, there was an increase in serum IL-12 (p40) concentrations (Fig. 2b), but no detectable effect on IL-10 concentrations (Fig. 2c). Serum concentrations of IL-12 (p70), TNFα and IL-4 were undetectable using the available ELISA kits (not shown). In the NFκB (p50)-deficient mice treated with streptozotocin, the serum NO and IL-12 (p40) responses were abolished (Figs 2a and 2b). However, the basal concentrations of IL-12 (p40) found in the serum of p50-deficient mice were sixfold greater than those measured in the wild-type mice (Fig. 2b). Interestingly, we found, in contrast, that the basal serum concentrations of IL-10 in p50-deficient mice were half those found in wild-type mice (Fig. 2c), and that MLDS treatment had no effect on serum concentrations of IL-10.

### Studies in mice treated with SHDS

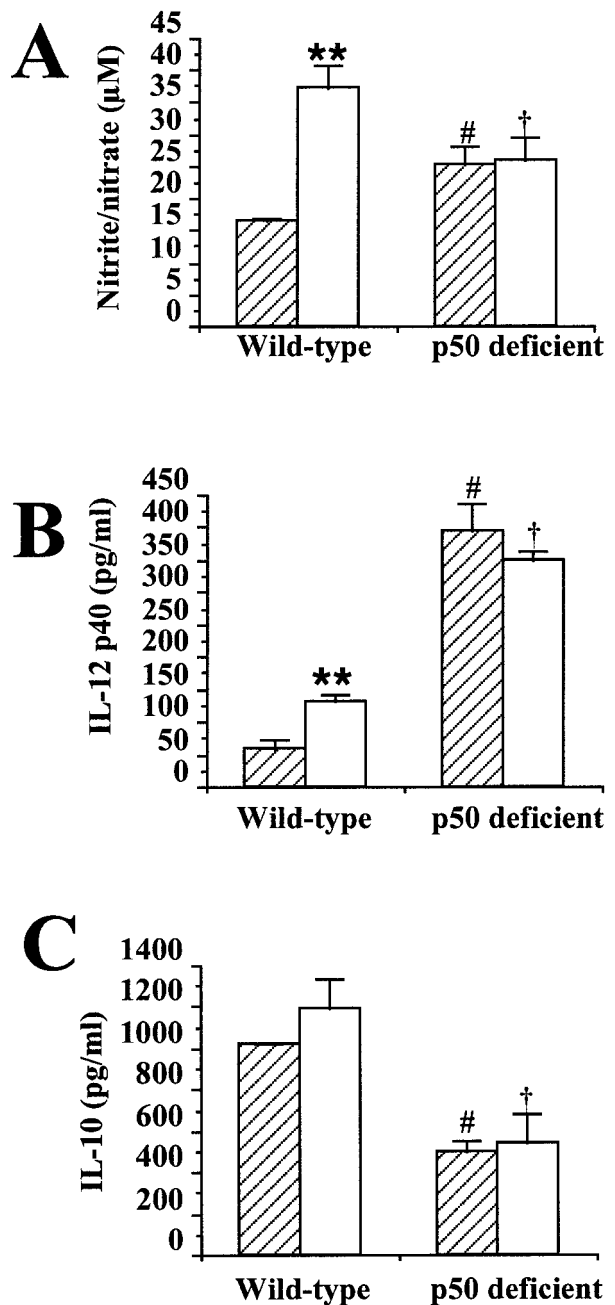
Both wild-type and NFκB (p50)-deficient mice developed severe diabetes. Blood glucose concentrations, incidence of diabetes and pancreatic insulin depletion were  $360 \pm 11$  mg/dl, 100% and 94% ( $68 \pm 8$  to  $4 \pm 2$  ng insulin/mg protein) in the wild-type mice, and  $305 \pm 14$  mg/dl, 100% and 97% ( $73 \pm 6$  to  $2 \pm 1$  ng insulin/mg protein) in the NFκB (p50)-deficient mice, at 4 days after administration of streptozotocin ( $n=12$  per group). There was no effect of SHDS on serum nitrite/nitrate concentrations in either the wild-type or the NFκB (p50)-deficient mice (data not shown).



**Figure 1** *NFκB* (*p50*) gene disruption prevents multiple low-dose streptozotocin (MLDS)-induced diabetes. Blood glucose concentrations (A) and incidence of diabetes (B) in wild-type and *NFκB* (*p50*)-deficient mice with or without MLDS treatment. ○, Vehicle-treated wild-type mice; ●, MLDS-treated wild-type mice; □, vehicle-treated *p50*-knockout mice; ■, MLDS-treated *p50*-knockout mice. Glycemia is expressed as mg glucose/dl and plotted as mean ± s.e.m. ( $n=6$  for untreated mice and  $n=17$  for MLDS-treated mice for each respective experimental group). The incidence of diabetes is expressed as the cumulative percentage of mice that have a blood glucose concentration greater than 200 mg/dl. \* $P<0.05$ , \*\* $P<0.01$  compared with untreated mice; † $P<0.05$  compared with *NFκB*<sup>+/+</sup> mice treated with MLDS.

*Response of islets of Langerhans exposed to cytokine treatment*

Islets isolated from both mouse phenotypes were exposed to a mixture of proinflammatory cytokines IL-1β



**Figure 2** *NFκB* (*p50*) gene disruption reduces the ability of diabetes to induce NO and IL-12 formation. Serum concentrations of (A) nitrite/nitrate, (B) IL-12 and (C) IL-10 in control animals and at 21 days of diabetes are shown as mean ± s.e.m. of nine untreated (vehicle-treated) mice (cross-hatched bars) or 17 MLDS-treated mice (open bars) per group. \*\* $P<0.01$  compared with untreated mice; # $P<0.05$  compared with untreated *NFκB*<sup>+/+</sup> mice; † $P<0.05$  compared with *NFκB*<sup>+/+</sup> mice treated with streptozotocin.

(100 pM), TNFα (100 pM) and IFN-γ (10 U/ml) for 24 h. Cytokine-treated islets from wild-type mice had a compromised insulin secretory response to glucose

stimulation, the insulin secretion in response to 20 mM glucose being reduced from  $2.6 \pm 0.4$  to  $1.2 \pm 0.2$  ng insulin/islet per h ( $P < 0.05$ ). Similar results were obtained from cytokine-treated islets obtained from NFκB (p50)-deficient mice, with a reduction in glucose-stimulated insulin release from  $2.2 \pm 0.4$  to  $0.96 \pm 0.2$  ng insulin/islet per h ( $P < 0.05$ ). Cytokine treatment of islets isolated from wild-type mice resulted in increased NO formation: from  $46 \pm 0.8$  to  $69 \pm 3.4$  pmol nitrite+nitrate/islet per 24 h ( $P < 0.05$ ). Cytokine treatment of islets from NFκB (p50)-deficient mice also significantly increased NO formation, from  $39 \pm 2.6$  to  $49 \pm 2.5$  pmol/islet per 24 h ( $P < 0.05$ ); however, this effect was reduced by 56% compared with the formation of NO in wild-type islets. Exposure of islets from either mouse phenotype to individual cytokines had no effect on either glucose-stimulated insulin secretion or NO formation (data not shown).

## Discussion

IDDM results from the selective destruction of the β-cell. Data obtained from animal models and diabetic patients have suggested that this destruction is mediated by the immune system (Yoon *et al.* 1998). The results presented here indicate that the transcription factor, NFκB, has a vital role in the cascade of events culminating in MLDS-induced diabetes in mice; more specifically, it implicates the p50 subunit as the major member of this transcription factor family responsible for development of diabetes. Our findings have indicated that the protection of p50-deficient mice from MLDS is due to an immune-system-related mechanism, rather than a direct protection of islet β-cells from damage or destruction by either streptozotocin or inflammatory cell mediators such as cytokines.

It has been reported previously that the induction of iNOS and the subsequent formation of NO mediate destruction of the β-cells in diabetes. The sources of NO include immune cells of the insulinitis lesion, such as macrophages, which produce NO and can directly destroy pancreatic β-cells, plus indirect formation of NO from pancreatic islet cells exposed to cytokines. Islet endothelial cells (Steiner *et al.* 1997), duct cells (Pavlovic *et al.* 1999) and β-cells (Southern *et al.* 1990, Mabley *et al.* 1997) all produce NO after cytokine stimulation. Inhibition of NO formation using either a specific iNOS inhibitor (Suarez-Pinzon *et al.* 2001) or iNOS gene disruption (Flodstrom *et al.* 1999) renders mice more resistant to diabetes. Unlike the wild-type mice, NFκB (p50)-deficient mice show no increase in serum nitrite/nitrate concentrations, indicative of NO formation, in response to MLDS. The lack of NO formation in response to MLDS may be one reason why the p50-deficient mice are protected from developing diabetes. Interestingly, the basal concentrations of serum nitrite/nitrate were significantly greater in the knockout

mice as compared with the wild-type mice, probably reflecting an increased basal production of NO. Though it is not possible to determine which cells are responsible for this NO production, these knockout mice have in their serum a depressed basal concentration of IL-10 – a cytokine known to inhibit production of NO (Cunha *et al.* 1992, Corraliza *et al.* 1995) by both inhibiting expression of the enzyme and reducing the cellular concentrations of the substrate, arginine. Therefore, it may be that some cell types of the NFκB (p50)-deficient mouse are less able to regulate their NO production, resulting in an increased basal production.

*In vitro* data have implicated NO in the cytokine-mediated inhibitory effects on isolated rat (Southern *et al.* 1990, Mabley *et al.* 1997), mouse (Yamada *et al.* 1993) and human (Corbett *et al.* 1993, Giannoukakis *et al.* 2000) islets of Langerhans. NFκB has been reported to be an essential transcription factor for the induction of NO synthase in β-cells (Flodstrom *et al.* 1996) and other cell types (Forstermann & Kleinert 1995). These observations, coupled with *in vivo* data, may lead to the suggestion that the NFκB (p50) mouse is essentially an iNOS-deficient mouse. However, isolated islets from NFκB (p50)-deficient mice treated with cytokines produce NO, as do macrophages isolated from NFκB (p50)-deficient mice treated with lipopolysaccharide (G Haskó & C Szabó, unpublished observations), indicating that cells lacking the p50 subunit are still capable of expressing iNOS. Our data do suggest that NFκB (p50) may potentiate induction of NOS in islets of Langerhans, as NO formation was less in cytokine-treated p50-deficient mouse islets than in wild-type islets. This reduction in NO formation had no effect on the degree of inhibition of insulin secretion observed after cytokine treatment of p50-deficient islets – an effect which may be partly due to a recently reported NO-independent pathway of cytokine-mediated inhibition of insulin secretion (Andersson *et al.* 2001).

NFκB (p50)-deficient mice had no resistance to the SHDS model of diabetes, indicating that, unlike alloxan (Quan *et al.* 2001), streptozotocin's mechanism of direct β-cell damage and destruction is not mediated by NFκB. The decrease in the insulin content of the pancreas in p50-deficient mice treated with MLDS indicates that even subdiabetogenic doses of streptozotocin cause β-cell destruction. These observations, plus the lack of protection of islets from immune-cell mediators such as cytokines, suggest that the mechanism by which NFκB (p50)-deficient mice are protected from MLDS-induced diabetes involves inhibition of activation of the immune system and subsequent β-cell functional inhibition and destruction.

It has been reported that NFκB (p50)-deficient mice have an impaired immune system and antibody responses (Sha *et al.* 1995). In the collagen-induced animal model of arthritis, it was noted that NFκB (p50)-deficient mice did not produce IgM antibodies and had markedly reduced IgG antibodies in response to an injection of collagen, and

this was proposed as the mechanism by which p50-deficient mice were completely protected from arthritis (Campbell *et al.* 2000). It was also found that p50-deficient mice had a reduced T-cell proliferative response to collagen (Campbell *et al.* 2000), which is consistent with previous findings of a diminished response to mitogens and altered production of cytokines by T-cells of p50-deficient mice (Sha *et al.* 1995, Hilliard *et al.* 1999). It may be that, in the absence of NFκB (p50), autoreactive T-cells may not be fully activated, with a compromised differentiation into T helper (Th) 1- or Th2-type cells, which in turn may inhibit development of an autoimmune disease such as diabetes. However, although none of the NFκB (p50)-deficient mice developed overt diabetes – that is, a blood glucose concentration greater than 200 mg/dl – four of 17 of these mice had a blood glucose concentration greater than 150 mg/dl on day 21 after MLDS treatment. Indeed, two of these mice had blood glucose concentrations greater than 170 mg/dl, and this was coupled with both mice having serum nitrite/nitrate concentrations double those found in non-diabetic mice, indicating an immune-cell-mediated attack, possibly via cytokine production on the islet β-cells, inhibiting their function. Therefore, the absence of NFκB (p50) is not absolutely protective against MLDS-induced hyperglycemia, suggesting that the immune cell defects of the NFκB (p50)-deficient mice do not fully explain the protection against IDDM, and that we must also consider other possible mechanisms.

The increased basal concentrations of the p40 subunit of IL-12 in the NFκB (p50)-knockout mice may provide an explanation as to why the immune system is not activated by MLDS treatment. IL-12 is a 75 kDa heterodimeric cytokine produced from macrophages and other antigen-expressing cells. The p35 subunit is ubiquitously expressed in many cell types, whereas the p40 subunit is inducible (Trinchieri 1994). The production of biologically active recombinant IL-12 requires the expression of both the p35 and p40 subunits (Burunda 1994). The p40 subunit, however, has been shown to form a homodimer that antagonizes IL-12 activities *in vitro*, and hence may reflect a natural suppressor of Th1 activation *in vivo* (Mattner *et al.* 1993, Gillessen *et al.* 1995). Previous work has indicated that treatment of the spontaneous animal model of IDDM (the non-obese diabetic (NOD) mouse) with the IL-12 antagonist IL-12 (p40)<sub>2</sub> suppressed the development of diabetes, and this protection was associated with a decreased pancreatic expression of mRNA for both iNOS and IFN-γ (Rothe *et al.* 1997). The intra-islet infiltration by immune cells was also reduced. Interestingly, Rothe *et al.* (1997) also reported that the administration of IL-12 (p40)<sub>2</sub> down-regulated expression of the IL-12 (p35) subunit. Therefore, it was proposed that the IL-12 p40 homodimer suppressed the development of diabetes by reducing islet infiltration by selective down-regulation of Th1-type responses (Rothe *et al.* 1997). The dramati-

cally increased serum concentrations of IL-12 (p40) in the NFκB (p50)-deficient mice may act as a natural antagonist to IL-12 and limit the Th1 cell response to β-cell antigens released after damage to the β-cells by streptozotocin treatment. This increased expression of IL-12 (p40) in NFκB (p50)-deficient mice may be explained by the fact that, although most of the NFκB dimers are activators of transcription, the p50/p50 and p52/p52 homodimers repress the transcription of their target genes (Ghosh *et al.* 1998, Karin & Ben-Neriah 2000). The *IL-12 (p40)* gene may be such a target.

The significantly lower basal concentrations of IL-10 in the NFκB-deficient mice are probably a result of the increased IL-12 (p40) concentrations, as these two cytokines have been shown to be reciprocally regulated, with increased concentrations of either one decreasing the concentrations of the other (Hasko & Szabo 1999). The decreased concentration of IL-10, however, seems to have no effect on the development of diabetes in these mice, even though IL-10 has been shown to be an important regulator of the development of diabetes (Wogensen *et al.* 1994). Recently, adeno-associated virus vector-mediated delivery of IL-10 to female NOD mice was found to prevent the development of diabetes (Goudy *et al.* 2001). The authors speculated that the mechanism for this protection was that IL-10 inhibited the synthesis of a number of proinflammatory cytokines, including IFN-γ, IL-2 and TNF. It is likely that the protective effects of removing the *NFκB (p50)* gene override any increased susceptibility caused by the resulting decrease in basal concentrations of IL-10.

The above is only one hypothesis, and recently reported data have indicated a primary role for NFκB (p50) in the expression of many genes in cytokine-treated rat islet β-cells, including such genes as *ICAM*, various apoptosis-related genes, *COX-2*, *FAS* and *iNOS* (Cardozo *et al.* 2001, Heimberg *et al.* 2001). Although we were unable to show any protective effect of *NFκB (p50)* gene disruption on isolated islets exposed to cytokine combinations *in vitro*, we assessed the functionality of the β-cells only by insulin secretory responsiveness, and did not assess viability or β-cell death. *In vivo*, where the concentrations of cytokines to which the β-cell is exposed may be lower, a more pronounced effect on β-cell functionality and survival may be observed after disruption of the *NFκB (p50)* gene.

## Conclusion

We have presented evidence suggesting a pivotal role for the p50 subunit of NFκB in the pathogenesis of IDDM in the MLDS model. Other autoimmune diseases have also been shown to be dependent on NFκB (p50), including encephalomyelitis (Hilliard *et al.* 1999) and rheumatoid arthritis (Campbell *et al.* 2000). To date, NFκB blockade with NFκB decoy oligonucleotides has been shown to

inhibit arthritis (Tomita *et al.* 1999) and inflammatory bowel disease (Neurath *et al.* 1996) in animal models. Thus the feasibility and efficacy of such strategies has already been proved. Specific blockade of NF- $\kappa$ B (p50) subunit may potentially be an effective treatment with which to prevent human IDDM.

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