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# Strain-dependent difference in inducible nitric oxide synthesis (iNOS) expression in rat pancreatic islets correlates with interferon regulating factor 1 (IRF-1) and heat shock protein 70 (HSP70) expression

## Abstract

Nitric oxide (NO) may be a necessary but not sufficient mediator of cytokine-mediated, selective beta-cell destruction. Previously, we have described a

difference in NO-dependent IL-1beta sensitivity in vivo and in vitro of pancreatic islets from two rat strains, Brown Norway (BN) and Wistar Kyoto (WK), the latter being the more sensitive strain. Here we investigated whether strain-dependent, differential islet iNOS expression was associated with differences in islet expression of the IL-1 receptor type 1(IL-1RI) or interferon regulating factor 1 (IRF-1), and/or caused differences in HSP70 expression, a marker of cell defence against oxidative stress. Methods: isolated islets from both rat strains were exposed to increasing concentrations of IL-1beta (0-150 pg/ml) for 24 hours or for varying culture periods (4-48 hours) to 15pg/ml of IL-1beta. Measurements: accumulated insulin and nitrite release into incubation medium, and islet mRNA and protein expression of iNOS, IL-1RI, IRF-1 and HSP70 by semi-quantitative RT-PCR and Western blotting. Results: Higher insulin and lower nitrite release into the incubation medium were seen for BN compared to WK rats islets in both dose- and time-response experiments. IRF-1 expression preceded iNOS expression and was more pronounced in WK than in BN islets. No strain differences were observed for islet expression of IL-1RI. A strain-dependent HSP70 expression in response to IL-1beta with the highest levels in WK rat islets following iNOS expression was seen. Conclusion: There was a strain-dependent difference in iNOS expression which was associated with IRF-1 and HSP70 expression.

## Full text

### INTRODUCTION

Insulin-dependent diabetes mellitus (IDDM) is the clinical result of selective pancreatic beta-cell destruction. *In vitro* experiments have shown that cytokines impair beta-cell function and cause beta-cell apoptosis and necrosis in both rodents and man. Beta-cells express IL-1 receptors, and *in vitro* exposure of rat islets to IL-1beta alone or together with TNF-alpha or IFN-gamma causes specific beta-cell destruction (for review see [1]). Several cytokines expressed in the islets of Langerhans (IL-1beta, TNF-alpha, IL-6, IL-12, IL-18) have been associated with the beta-cell destruction in animal models of spontaneous diabetes (NOD-mouse and BB-rat), and in syngeneic islet transplants in diabetic NOD-mice [2]. The mechanisms by which cytokines mediate beta-cell cytotoxicity are not known in detail. Cytokine-induced nitric oxide (NO) production in rodent islets, as a result of inducible nitric oxide synthesis (iNOS) expression, is associated with beta-cell destruction *in vitro* [3, 4]. However, IL-

1beta-mediated beta-cell toxicity may also involve NO-independent mechanisms [5], *e.g. via* MAPK-dependent caspase activation [6, 7].

Cytokine-induced iNOS transcription is tightly regulated. More than 30 putative transcription factor-binding motifs are present within the first 1.5 kb upstream of the transcription start site [8]. IL-1beta induces iNOS transcription in rat beta-cells mainly *via* the transcription factor nuclear factor-kappaB (NF-kappaB) [9, 10]. Although IL-1beta by itself can induce iNOS transcription and NO formation in rat islets, a mixture of IL-1beta, TNF-alpha, and IFN-gamma is required for iNOS mRNA expression in preparations of human islets [11]. It has been suggested that the combined activation of NF-kappaB and interferon regulating factor 1 (IRF-1) is required for iNOS mRNA expression, and that IL-1beta alone is sufficient for IRF-1 activation in rodent islets, whereas addition of IFN-gamma is needed in human islets to activate an IRF-1 response [12].

Differences in iNOS expression have been described between different chicken strains [13]. We have previously described different sensitivity to IL-1beta-induced transient diabetes between two rat strains, Brown Norway and Wistar Kyoto, which correlated to differences in pancreatic islet iNOS expression [14].

To further elucidate the molecular regulation of iNOS expression, in this study we aimed to investigate whether the iNOS expression (i) is associated with differences in islet expression of the IL-1 receptor type 1 (IL-1RI) or IRF-1 and (ii) caused differences in HSP70 expression - a marker of cell defence against oxidative stress.

## RESEARCH DESIGN AND METHODS

### *Animals*

Brown Norway and Wistar Kyoto rats were from Charles River (Sulzfeld, Germany) and Møllegården (Ll. Skensved, Denmark), respectively.

### *Materials*

Authentic recombinant human interleukin-1beta (rhIL-1beta) (Novo-Nordisk, Gentofte, Denmark) (batch C-O-4A, specific activity 400 U/ng in the mouse thymocyte assay [15]), was produced and purified as previously described [16].

The rhIL-1beta was diluted in endotoxin-free 0.9% NaCl + 0.1% rat serum (vehicle). RPMI 1640 and Hanks' balanced salt solution were purchased from Gibco (Paisly, UK). RPMI 1640 was supplemented with 20 mM HEPES buffer, 100,000 IU/l penicillin, and 100 mg streptomycin.

### *Islet isolation and culture*

Islets from 5-7-day-old rats of both strains were isolated after collagenase digestion of the pancreata [17]. Islets were precultured for a period of 5-7 days in RPMI-1640 plus 10% foetal calf serum at 37° C. For dose-response experiments, 150 islets in 300 mul RPMI-1640 plus 0.5% human serum and 0-150 pg/ml IL-1beta, were incubated for 24 hours at 37° C in humidified atmospheric air, after which medium was sampled for insulin and nitrite measurements and the islets were snap frozen for RT-PCR analysis. For time-response experiments, islets were incubated as above but with a rhIL-1beta concentration of 15 pg/ml and culture periods ranging from 2-48 hours.

Islet culture using 450 islets in 900 mul of media were performed for selected dose- and time-conditions for protein level determination by Western blotting.

### *Supernatant insulin and nitrite analyses*

Insulin was measured by a recently developed competitive ELISA [18].

### *Nitrite*

The Griess reagent (1 part naphthylethylene-diamine-dihydrochloride and 1 part 1% sulfanilamide in 5% $H_3PO_4$) was added to culture media samples (150 mul), mixed and incubated for 10 min at room temperature [19], followed by measurement of absorbance at 550 nm in an immunoreader (Nippon InterMedK.K., Tokyo, Japan). Nitrite concentrations were determined by means of a sodium nitrite standard curve. The detection limit was 1 mumol/l, corresponding to 2 pmol/islet in our set-up.

### *Semi-quantitative RT-PCR analysis*

Total RNA was extracted from the snap-frozen islets using a chloroform-ethanol extraction method as previously described [20]. Owing to the small amounts of

tissue (150 islets of Langerhans from which the amount of total RNA was not detectable by spectrophotometry), cDNA was prepared on a fixed volume of total RNA (11.5 mul) using Oligo(dT) as primer, according to the manufacturer's instructions (Invitrogen, Leek, The Nederlands). Each RT-PCR reaction, in a total volume of 20 mul, was performed using dCTP as the $^{32}$P-labeled nucleotide and with a fixed volume of cDNA (3 mul). To compensate for varying amounts of cDNA between samples, data are semi-quantatively presented as fold amplification relative to different house-keeping genes included in each reaction. The house-keeping genes were selected to have a linear amplification in the same range as the mRNA to be tested: beta-glucoronidase (a lysosomal enzyme) was the house-keeping gene used, together with iNOS and IL-1R1, and glutamate dehydrogenase (GDH: converting L-glutamate to alpha-keto-gluterate, thereby degrading some amino acids through the citric acid cycle), was used with IRF-1, HSP70-1 and HSP70-2. Cytokine exposure did not alter the house-keeping gene transcript expression level and no differences were observed in expression levels between the two rat strains (data not shown). beta-glucoronidase/iNOS/IL-1R1 and GDH/IRF-1/HSP70-1/HSP70-2 were run for 27 and 28 cycles, respectively. The products were separated on a 6% polyacrylamide gel. The gel was dried and exposed to phosphoimager screens. The RT-PCR products were quantified on a Molecular Dynamics (Sunnyvale, CA, USA) Phosphoimager, Image Quant, Version 3.3. Reproducibility of the assay (coefficient of variance) was estimated by running the same sample in 6 independent PCR reactions and all products were subsequently run with 5 different gels: PCR to PCR variation was 35% and gel to gel variation was 7% on average.

### *Primer pairs for RT-PCR*

The primer pairs used are shown in Table 1. We tested for the expression of both genes that encode HSP-70. Primers were designed for the 3'UTR region where the two mRNAs can be distinguished.

### *Western Blot (WB) analysis*

The 450 islets used for WB analysis were snap frozen immediately after the experimental procedure. Equal amounts of protein (20 mug) from all samples were run on a 12% WB polyacrylamide gel, followed by electrophoresis overnight, semi-wet blotting onto nitrocellulose paper (2 hours), nonspecific blocking of

nitrocellulose paper with PBS plus 0.05% Tween 20 and 5% dried skimmed milk powder (blocking medium) for 60 min, gelatine 0.2% in PBS for 30 min, washing in blocking medium (three times each for 5 min) before exposure to primary antibody (IL-1RI plus NOS2 and HSP70 in Blotto A (1mug/ml, Santa Cruz)) for 120 min. After an additional washing in blocking medium, a horseradish peroxidase conjugated secondary antibody (1:10.000, BioRad) was added for 75 min, followed by three 5 min washing, in blocking medium before visualization using a chemiluminiscent reaction and X-ray film exposure. An equal amount of total protein from all samples was loaded onto all gels. A protein standard (IL-1beta-stimulated isolated islets from outbred Wistar Furth) served as an internal control for normalization of bands of the tested proteins between gels. The "Eagle eye II" (Stratagene) was used for converting the X-ray film into an electronic image and "Image Quant" was used for the final quantification of protein bands. Sensitivity: initial studies using out-bred Wistar Furth rat islets indicated that this method could detect a two-fold difference in iNOS, IRF-1, IL-1RI and HSP70 levels using 450 islets (equalling approximately 40 mug total protein) per condition. The amount of total protein was in the range 15 to 65 mug in the experiments with BN and WK rats. As a compromise, 20 mug of total protein was loaded onto all gels in order to include as many samples as possible. Unexpectedly, 20 mug of total protein were not enough for the IRF-1 antibody/secondary antibody system initially used (Santa Cruz/BioRad) to detect the IRF-1 protein. Hence, the residual lysates were pooled into 2-4 mixed-samples for each condition and were subsequently used for Western Blotting and quantified as described above using another secondary antibody (Santa Cruz).

### *Statistical analysis*

Data are presented as means ± SD. Two-way ANOVA was used for comparisons between groups testing the effect of strain and different IL-1beta concentrations or exposure time. Significant differences between groups were determined by Wilcoxon's non-parametric test. JMP (SAS Inst.) was used as statistical software.

## RESULTS

To establish and validate the experimental model system, we first ensured that we could confirm our previous IL-1beta dose-response experiments for WK and

BN islets with regard to strain-related differences in islet insulin and nitrite release, and iNOS mRNA levels. The insulin data are expressed as percentage of control, as the basal insulin release was different in the two strains (see Figure 1a, legend). Increasing IL-1beta concentrations (i) initially increased the insulin release from BN islets, followed by inhibition, whereas only the inhibitory phase was seen for WK islets, and (ii) strain-dependently increased the nitrite release and iNOS mRNA content with the highest nitrite release and iNOS mRNA levels seen for WK islets (Figure 1 and Table 2). Based on these dose-finding studies, we proceeded to further characterize the exposure time-dependence of the islet response to IL1-beta from the two rat strains and to measure levels of IL-1RI, IRF-1 and HSP70 expression with 15 pg/ml of IL-1beta as under this condition insulin release was stimulated in BN islets and inhibited in WK islets (Figure 1a).

### *Insulin and nitrite release into culture medium*

Insulin-release from WK islets was increased to a maximum of 185 ± 70.9% of the control with a short exposure time (6 hours), and inhibition (38.9 ± 22.1%) with a long exposure time (48 hours), using 15 pg/ml IL-1beta (Figure 2). No inhibition of the insulin release was seen for BN islets in a parallel set-up. Nitrite release into the medium was not significantly detectable for the first 9 hours of IL-1beta incubation in either BN or WK islets. After 12 hours of IL-1beta exposure, nitrite release was only detectable from islets of WK, but after an exposure time of 24 hours nitrite release was demonstrated from both strains, with significantly higher levels from WK (7.7 ± 1.4 pmol/islet/24 hours) compared to that from BN islets (5.1 ± 2.2 pmol/islet/ 24 hours), ($p < 0.008$) (Figure 2).

### *iNOS expression*

iNOS mRNA and protein increased for both strains - highest in WK - with increasing IL-1beta concentrations and exposure time (Tables 2 and 3). Strain-dependent differences were seen for iNOS mRNA in both the IL-1beta concentration- and exposure time-response experiments (Tables 2 and 3), whereas iNOS protein level differences between the strains was only observed with increasing concentrations of IL-1beta (Table 2).

### *IL-1RI expression*

Increasing IL-1beta concentrations or exposure time did not affect IL-1RI expression at the mRNA or protein level (Tables 2 and 3). A strain-dependent difference in the kinetics of IL-1RI expression with the highest levels in BN rats was observed at the mRNA but not at the protein level (Table 3).

### *IRF-1 expression*

IRF-1 mRNA expression increased in islets from both strains with increasing IL-1beta concentration and exposure time - highest in WK rats (Table 2 and 3). Strain-dependent differences were found for IRF-1 mRNA in both the IL-1beta concentration- and exposure time-response experiments (Tables 2 and 3). The protein data from the pooled lysates showed higher IRF-1 protein levels in WK compared to BN islets in both dose- and time-response experiments (Tables 2 and 3), except in the unstimulated condition.

### *HSP-70 expression*

HSP70-1 and 2 mRNA, and protein increased in islets from both strains, with increasing IL-1beta concentration and exposure time, with WK showing highest levels (Tables 2 and 3). Strain-dependent differences were observed for islet HSP70-1 and -2 mRNA expression in the IL-1beta concentration-response experiments (Table 2), and for HSP70-1 in the exposure time-response experiments (Table 3). At the protein level, an increase in HSP70 was observed with increasing IL-1beta exposure time (Table 3).

## DISCUSSION

This study has confirmed and extended our previously observed strain-dependent differences in insulin and nitrite release responses to IL-1beta and demonstrate that these differences are associated with iNOS expression in both IL-1beta concentration and time exposure response experiments.

Our data strongly suggest that IRF-1 is associated with the strain-related difference in iNOS expression, as the rapid increase in IRF-1 mRNA in WK islets preceeded the rise in iNOS mRNA in response to IL-1beta exposure.

Cytokine-mediated IRF-1 expression is associated with recruitment and activation of STAT-1 at the intracellular level of the cytokine receptors [21]

leading to increased IRF-1 synthesis. IRF-1 is weakly expressed in resting cells and because of its short half-life (approximately 30 min for both mRNA and protein), IRF-1 is synthesised *de novo* upon cytokine stimulation [22]. Once the IRF-1 protein is synthesized, it rapidly enters the nucleus and binds to specific DNA motifs (IRF-E) [23]. Sequential binding of IRF-1 to the iNOS promoter to form a dimeric complex is essential for full activation of the iNOS gene in vascular smooth muscle cells [24]. In beta-cells, full cytokine-induced iNOS expression has been shown to be dependent upon NF-kappaB and IRF-1 expression [10, 12]. Recently, it was shown that NF-kappaB and IRF-1 interact *in vivo*, and that this interaction physically bends the iNOS promoter DNA [25], facilitating transcription of the gene. This complex interaction between IRF-1 and NF-kappaB is further demonstrated by the finding of an NF-kappaB binding motif in the IRF-1 promoter [26]. As we have not measured NF-kappaB translocation from the cytosol to the nucleus, we cannot evaluate the kinetics of NF-kappaB and the NF-kappaB/IRF-1 interaction. It seems reasonable to assume however, that the NF-kappaB/IRF-1 interaction takes place, as IL-1beta-mediated NF-kappaB activation and translocation to the nucleus is a rapid event, and IL-1beta-exposed RIN cells have been shown to express IRF-1 mRNA after 60 min [12]. Further studies are needed to confirm this proposed direct effect of IL-1beta stimulation leading to differential IRF-1 expression in these strains.

The complex interaction between IRF-1 and NF-kappaB might explain the different IL-1beta susceptibility between rat and human islets, as IL-1beta alone induces NF-kappaB in both islet species, but barely induces IRF-1 in human beta-cells [12]. The contribution of IRF-1 to cytokine-mediated beta-cell destruction and death was recently demonstrated using IRF-1 knock-out (IRF-$1^{-/-}$) mice. Despite similar increases in nitrite accumulation and iNOS expression following exposure of wild type and IRF-$1^{-/-}$ FACS purified beta-cells to IL-1beta and IFN-gamma, isolated pancreatic islets from IRF-$1^{-/-}$ mice presented a 30-50% reduction in medium nitrite accumulation and iNOS expression. Thus, it was suggested that the IRF-1-mediated deleterious effects were related to non-endocrine cells in the islet, *e.g.* NO production from ductal cells or macrophages present in the islets [27, 28]. Our data show an association between differential IRF-1 expression and the difference in iNOS expression between the two strains, and indicate a role for IRF-1 in IL-1beta-mediated iNOS expression in rat islets of Langerhans. The study design did not allow a conclusion of any causal relation between IRF-1 and iNOS expression, but it is speculated that polymorphisms in the IRF-1 gene, as well as quantitative differences in transcriptional regulation,

could be involved. We have previously tested one polymorphism within the human IRF-1 gene in a Danish population, without demonstrating an association or linkage to type 1 diabetes [29]. Finally, IRF-1 has been suggested to be involved in cytokine-mediated IL-1-converting enzyme (ICE) [7] and serine protease inhibitor 3 (SPI-3) [27] expression, potentially contributing to the observed difference in the sensitivity of IL-1beta-mediated beta-cell destruction between the two strains, as these two proteins represent a pro-apoptotic protein and a natural protease inhibitor, respectively.

Apart from differences in the level of IRF-1 putatively controlling iNOS gene transcription, structural differences in the iNOS promoter region could account for a differentiated level of transcription of this gene. Within the rat iNOS promoter region, a polymorphic region has been identified, and differences seen between BN and WK rat strains [30, 31]. No functional data have so far been published.

Cytokine-resistant insulinoma cells were found to have decreased levels of the IL-1RI mRNA [32]. Our data do not support a difference in IL-1RI levels as an explanation for the observed strain differences in IL-1beta sensitivity. On the contrary, the increased concentration of IL-1RI mRNA in BN islets in time exposure experiments compared to WK would be expected to confer a stronger effect of IL-1beta which was not observed. At the protein level no differences were identified.

The observed HSP70 expression may be secondary to the deleterious effects of NO production. That HSP70 may be induced by NO production is in agreement with the finding in human islets [33], neonatal rat islets [34] and in islets from iNOS knock-out mice, showing that NO is required for cytokine-induced HSP70 mRNA expression [35]. On the other hand, HSP70 over-expression enhanced cytokine-induced iNOS gene expression [36], which may rather be due to extra-cellular stimulation of the islets cells with HSP70 [37]. Taken together, the increased level of HSP70 in WK rat islets compared to BN rat islets could facilitate a higher cytokine-mediated iNOS response if HSP70 was liberated to the exterior or could merely be a result of a higher oxidative stress in WK compared to BN islets.

Strain-dependent differences in iNOS expression are not restricted to islets of Langerhans. LPS induced different levels of iNOS transcription and expression in

macrophages from different chicken strains [13]. Furthermore, iNOS expression levels have also been demonstrated to be dependent upon beta-cell and intestinal epithelial cell differentiation [18, 38] and age [39, 40].

CONCLUSION

To summarize, this investigation confirmed and extended evidence for strain dependent differences in sensitivity to IL-1beta exposure regarding iNOS, IRF-1 and HSP70 expression, between islets from WK and BN rats. Furthermore, the different iNOS expression levels were associated with differences in the expression of IRF-1 and HSP70.

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## Figures

**Figure 1a**
**Insulin release (percentage of control, 24 hours exposure time) to the incubation medium in response to increasing IL-1**beta **concentrations (pg/ml).**

Absolute insulin release for WK and BN in control conditions: 1,097 ± 272 and 718 ± 226 nM/150 islet/24 hours, respectively ($p = 0.008$), as seen in lower panel. ANOVA for IL-1beta concentration and strain: $p < 0.0001$ for both.

**Figure 1b**
**Nitrite release into the incubation medium in response to increasing IL-1**beta **concentrations.**

Measurements below the detection limit (2 pmol/islet/24 hours) are given the value of 1 pmol/islet/24 hours as values below the detection limit are considered to follow "the normal distribution" with 1 as mean. ANOVA for IL-1beta concentration: $p < 0.001$, and for strain: $p = 0.04$. As 15 pg/ml of IL-1beta was the only concentration where stimulation in BN rat islets and inhibition in WK rat islets was observed as regards insulin release to the incubation medium, this condition was tested separately in the nitrite response: * $p = 0.005$ between strains.

Open bars: Wistar Kyoto (n = 9) and closed bars: Brown Norway (n = 9). All values are given as means ± SD.

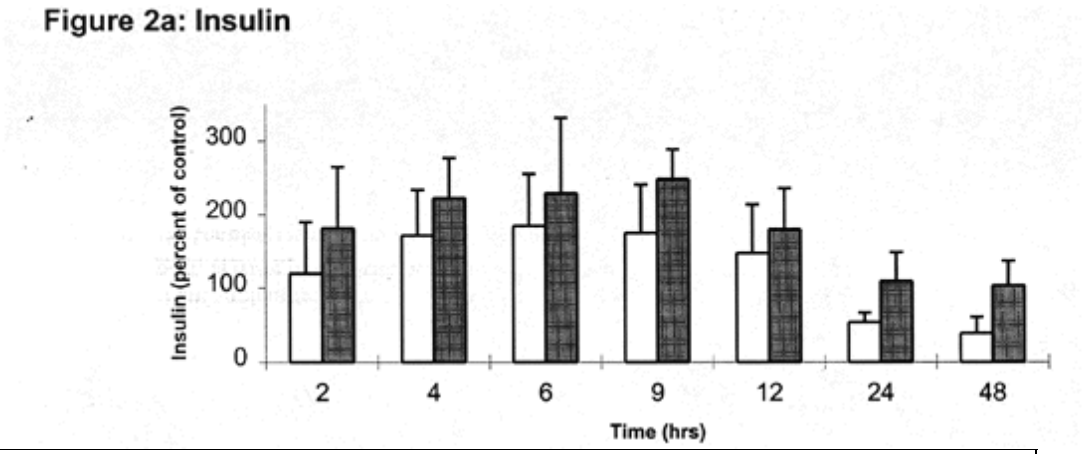



**Figure 2a**
**Accumulated insulin release for islets exposed to 15 pg/ml of IL-1**beta **as a percentage of the control.**

Control values for accumulated insulin were in the range 78 to 3,700 fmol/islet depending on the exposure time. ANOVA for exposure time and strain: $p < 0.0001$.

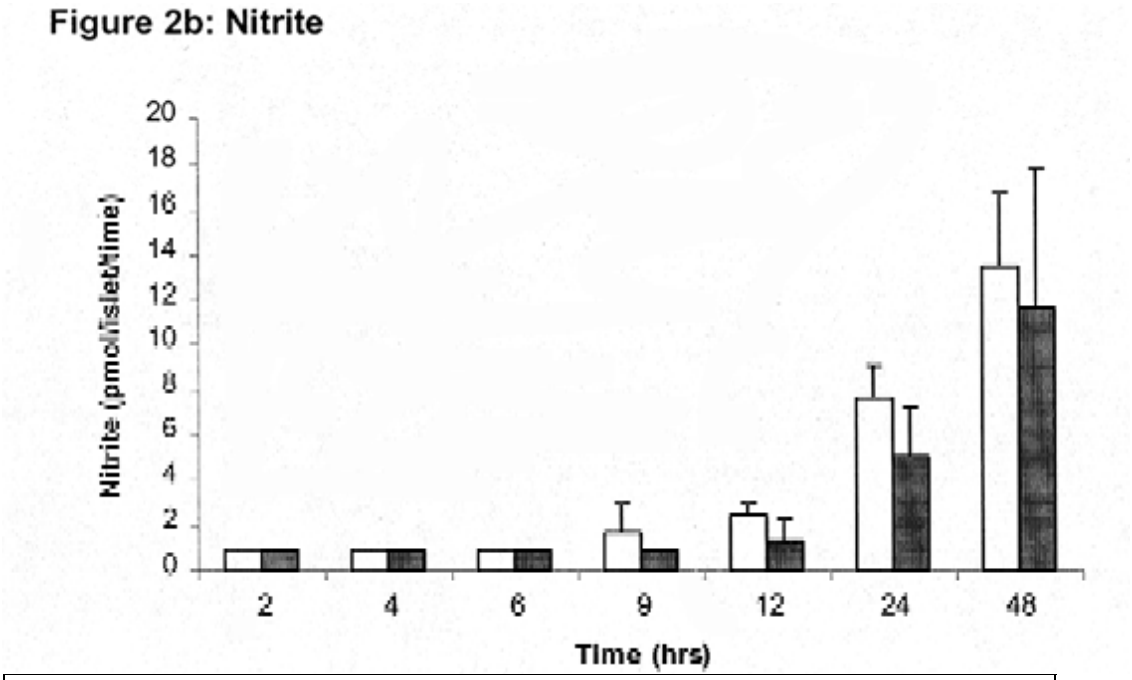



**Figure 2b**

**Accumulated nitrite release with increasing IL-1**beta **exposure time.**

Measurements below the detection limit (2 pmol/islet/24 hours) are given the value of 1 pmol/islet/24 hours as values below the detection limit are considered to follow "the normal distribution" with 1 as mean. ANOVA for exposure time: $p < 0.001$, and for strain: $p = 0.02$.

Open bars: Wistar Kyoto (n = 10-13) and closed bars: Brown Norway (6-8). All values are given as means ± SD.

**Table 1**
**Primer design**

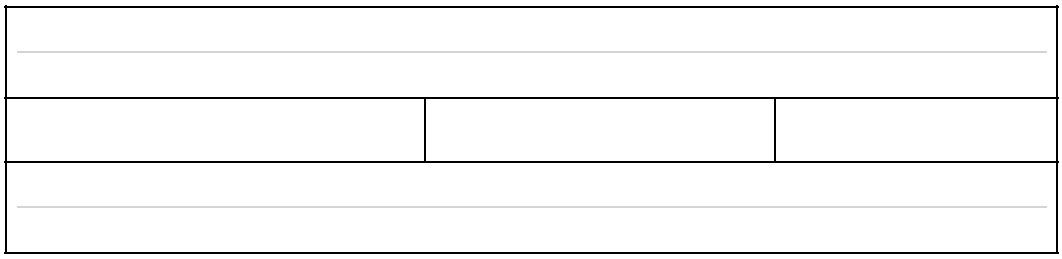
| Product | | Primers | PCR length | GenBank accession |
|---|---|---|---|---|
| beta-Glucoronidase | FP | 5'TCT GCT CCA TAC TCG CTG TG 3' | 290 | M13962 |
| | RP | 5' GTG ATG TGG TCT GTG GCC AA 3' | | |
| GDH | FP | 5' AGT GTG CAG TGG TTG ATG TGC 3' | 154 | X14223 |
| | RP | 5' AGG CAC ATC AAT GCC AGG ACC 3' | | |
| IL-1R1 | FP | 5' ACG GAT AGC GAT AAA ACT GGC 3' | 318 | M95578 |
| | RP | 5' GGA AGC AAT ATC CCG TCA CAC 3' | | |
| IRF-1 | FP | 5' GGT TAG TGT GAG GCA GAG GAC 3' | 309 | M34253 |
| | RP | 5' GCA GGG AGT GAG GCA GAA 3' | | |
| iNOS | FP | 5' CAG CAA TGG GCA GAC TCT 3' | 240 | U26686 |
| | RP | 5' CAC AGG CTG CCC GGA AGG TTT G 3' | | |
| HSP70-1 | FP | 5' GTT TCC TTC CTG CGA ACA CCT 3' | 209 | X77207 |
| | RP | 5' CCC CAT TTC CCC GTC ATC 3' | | |
| HSP70-2 | FP | 5' GCT TCT CCC GTT TGA CAC TCT 3' | 253 | X77208 |
| | RP | 5' CAG ACA CAA GAC CTG GCA AGT | | |

**Table 2**

**IL-1beta dose-response: expression levels of iNOS, IRF-1, HSP70 and IL-1RI**

Expression levels of mRNA and protein from 150 and 450 islets, respectively, exposed to increasing concentrations of IL-1beta for 24 hours. Levels of mRNA transcripts are expressed relative to a co-amplified house-holding transcript. There was no effect of cytokines, or differences between rat strains at the house-keeping transcript (data not shown). Protein expression levels are relative to a co-electrophoresed standard on each gel. All data are given as means ± SD. No statistical analyses were performed for IRF-1 protein data due the small number of experiments.

| | | 0 | 15 (pg/ml) | 25 (pg/ml) | 40 (pg/ml) | 50 (pg/ml) | 100 (pg/ml) | 150 (pg/ml) | Two-way Anova |
|---|---|---|---|---|---|---|---|---|---|
| **iNOS** | | | | | | | | | |
| mRNA | WK (n: 5-9) | 0 ± 0 | 1.7 ± 1.3 | 2.3 ± 2.1 | 12.1 ± 6.1 | 10.1 ± 9.1 | 14.4 ± 2.6 | 10.3 ± 8.0 | Dose: p < 0.0001 |
| | BN (n: 7-8) | 0 ± 0 | 1.3 ± 1.2 | 2.5 ± 1.8 | 4.2 ± 3.2 | 4.7 ± 2.2 | 7.3 ± 6.6 | 6.8 ± 5.5 | Strain: p = 0.0001 |
| Protein | WK (n: 4-6) | 0 ± 0 | 2.0 ± 1.1 | | | | | 2.9 ± 2.1 | Dose: p < 0.001 |
| | BN (n: 5-7) | 0 ± 0 | 1.3 ± 0.8 | | | | | 1.2 ± 0.9 | Strain: p = 0.046 |
| **IRF-1** | | | | | | | | | |
| mRNA | WK (n: 5-9) | 0.7 ± 1.2 | 5.9 ± 5.2 | 4.9 ± 5.3 | 7.4 ± 5.2 | 8.7 ± 5.9 | 13.7 ± 8.6 | 14.9 ± 11.3 | Dose: p = 0.0003 |
| | BN (n: 7-8) | 0.3 ± 0.3 | 2.6 ± 1.9 | 4.6 ± 4.8 | 5.6 ± 5.4 | 9.0 ± 9.1 | 4.7 ± 3.2 | 7.5 ± 8.8 | Strain: p = 0.02 |
| Protein | WK (n: 2-3) | 4.3 ± 1.3 | 4.8 ± 3.8 | | | | | 6.3 ± 8.2 | Not applicable |
| | BN (n: 2) | 7.5 ± 0.3 | 0.9 ± 0.4 | | | | | 5.3 ± 4.0 | Not applicable |
| **HSP70** | | | | | | | | | |
| HSP70-1 mRNA | WK (n: 5-9) | 0.1 ± 0.1 | 0.6 ± 0.9 | 1.6 ± 2.0 | 1.1 ± 0.6 | 2.2 ± 1.9 | 2.7 ± 1.5 | 2.9 ± 2.8 | Dose: p = 0.001 |
| | BN (n: 7-8) | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.4 ± 0.6 | 0.4 ± 0.4 | 0.6 ± 0.6 | 0.8 ± 0.6 | 0.5 ± 0.5 | Strain: p < 0.0001 |
| HSP70-2 mRNA | WK (n: 5-9) | 0 ± 0 | 0.2 ± 0.3 | 0.6 ± 0.6 | 0.6 ± 0.5 | 1.5 ± 2.3 | 2.0 ± 1.4 | 1.8 ± 2.1 | Dose: p = 0.003 |
| | BN (n: 7-8) | 0 ± 0 | 0.1 ± 0.2 | 0.4 ± 0.6 | 0.4 ± 0.3 | 0.5 ± 0.6 | 0.5 ± 0.4 | 0.6 ± 0.5 | Strain: p = 0.002 |
| Protein | WK (n: 4-6) | 0.7 ± 0.3 | 1.2 ± 0.9 | | | | | 1.8 ± 0.8 | Dose: p = 0.047 |
| | BN (n: 5-7) | 0.5 ± 0.3 | 0.9 ± 0.6 | | | | | 1.1 ± 0.9 | Strain: p = 0.13 |
| **IL-1R I** | | | | | | | | | |
| mRNA | WK (n: 6-9) | 1.2 ± 2.1 | 1.1 ± 1.2 | 0.8 ± 0.7 | 2.4 ± 1.7 | 3.3 ± 2.8 | 3.3 ± 4.8 | 0.8 ± 1.1 | Dose: NS |
| | BN (n: 7-8) | 1.3 ± 1.8 | 4.4 ± 7.7 | 3.1 ± 3.3 | 1.7 ± 1.8 | 2.7 ± 3.3 | 2.8 ± 2.6 | 1.8 ± 2.2 | Strain: NS |
| Protein | WK (n: 4-6) | 1.4 ± 0.6 | 1.4 ± 0.7 | | | | | 1.7 ± 0.8 | Dose: NS |
| | BN (n: 5-7) | 1.2 ± 0.4 | 1.3 ± 0.9 | | | | | 0.3 ± 0.3 | Strain: NS |



## Authors

J. Johannesen, F. Pociot, A. E. Karlsen, T. Mandrup-Poulsen, J. Nerup

Steno Diabetes Center, Niels Steensensvej 2, DK-2820 Gentofte, Denmark.

## In this issue

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virus, cytokines, signal transduction, immune evasion, tumor growth.

## Safety, pharmacokinetics and pharmacodynamics of recombinant human tumour necrosis factor-binding protein-1 (Onercept) injected by intravenous, intramuscular and subcutaneous routes into healthy volunteers

I. Trinchard-Lugan, Q. Ho-Nguyen, W.M. Bilham, M. Buraglio, A. Ythier, A. Munafo
recombinant human tumour necrosis factor-binding protein-1 (r-hTBP-1), safety, pharmacokinetics, pharmacodynamics.

## Direct effects of tumor necrosis factor alpha (TNF-alpha) on murine skeletal muscle cell lines. Bimodal effects on protein metabolism

B. Alvarez, L. S. Quinn, S. Busquets, F. J. López-Soriano, J. M. Argilés
cytokines, myotubes, cachexia, protein turnover.

## Receptor-like properties of the 26 kDa transmembrane form of TNF

A. Domonkos, A. Udvardy, L. László, T. Nagy, E. Duda
cytokine, TNF family, receptor, signaling, reverse-signaling.

## Local administration of interleukin-1beta for the treatment of lung abscesses induces neutrophil activation and changes in proinflammation cytokine production

A. Simbirtsev, E. Variouchina, V. Konusova, A. Kotov, S. Ketlinsky, A. Salamatov, L. Bisenkov
recombinant human interleukin-1beta, lung inflammation, abscess, neutrophils, cytokines.

## Effects of interleukin-2 therapy on the proliferation and differentiation of CD4/CD25 positive and CD4/CD25 negative cells in HIV+ patients

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## Tumor necrosis factor-alpha and nitric oxide in vertically HIV-1-infected children: implications for pathogenesis

J. González-Nicolás, S. Resino, J. L. Jiménez, S. Alvarez, M. Fresno, M. A. Muñoz-Fernández
tumor necrosis factor, nitric oxide, HIV-infection, immunological and virological markers.

## Serum levels of angiogenic cytokines in systemic lupus erythematosus and their correlation with disease activity

E. Robak, A. Wozniacka, A. Sysa-Jedrzejowska, H. Stepien, T. Robak
systemic lupus erythematosus, disease activity, angiogenesis, platelets, hepatocyte growth factor, vascular endothelial growth factor, basic fibroblast growth factor, transforming growth factor beta.

## Modulation of the allergen-induced human IgE response in Hu-SCID mice: inhibitory effect of human recombinant IFN-gamma and allergen-derived lipopeptide

C. Duez, H. Gras-Masse, H. Hammad, H. Akoum, A. Didierlaurent, C. André, A.-B. Tonnel, J. Pestel
SCID mouse, IgE, IFN-gamma, lipopeptide, house dust mite.

## Cytokine-inducing activity of a proline-rich polypeptide complex (PRP) from ovine colostrum and its active nonapeptide fragment analogs

A. Zablocka, M. Janusz, K. Rybka, I. Wirkus-Romanowska, G. Kupryszewski, J. Lisowski
ovine colostrum, proline-rich polypeptide complex (PRP), nonapeptide (NP) and hexapeptide (HP) from PRP, oligomers of NP and HP, cytokines.

## Quantitation of chemokines (MDC, TARC) expression in mucosa from Crohn's disease and ulcerative colitis

F. Jugde, M. Alizadeh, C. Boissier, D. Chantry, L. Siproudhis, S. Corbinais, E. Quelvennec, F. Dyard, J.-P. Campion, M. Gosselin, J.-F. Bretagne, G. Sémana, D. Heresbach
real-time TR-PCR, chemokines, Crohn’s disease, ulcerative colitis.

## Activation of caspase-3 by interferon alpha causes interleukin-16 secretion but fails to modulate activation induced cell death

O. Ludwiczek, A. Kaser, R. O. Koch, W. Vogel, W. W. Cruikshank, H. Tilg
interleukin-16, interferon-alpha, caspase-3.

## Cytokine deviation induced by intrathymic injection of staphylococcal enterotoxin B (SEB)

P. Goettelfinger, F. Lecerf, S. Berrih-Aknin, M. German-Fattal
SEB, intrathymic injection, anergy, cytokine deviation, quantitative RT-PCR.

## Strain-dependent difference in inducible nitric oxide synthesis (iNOS) expression in rat pancreatic islets correlates with interferon regulating factor 1 (IRF-1) and heat shock protein 70 (HSP70) expression

J. Johannesen, F. Pociot, A. E. Karlsen, T. Mandrup-Poulsen, J. Nerup
type 1 diabetes, islets of Langerhans, beta-cells, cytokines, interleukin-1beta, interleukin-1beta receptor type I, nitric oxide, inducible nitric oxide synthase, heat shock protein 70, interferon regulating factor 1.

## Neutrophil apoptosis in autoimmune Fas-defective MRL lpr/lpr mice

E. Del Giudice, A. Ciaramella, N. Balestro, D. Neumann, P. G. Romano, M. P. Cesaroni, G. Maurizi, P. Ruggiero, D. Boraschi, P. Bossù
neutrophils, apoptosis, autoimmunity.

## A role for interferon-gamma in the hypermetabolic response to murine toxoplasmosis

D. Arsenijevic, F. de Bilbao, P. Giannakopoulos, L. Girardier, S. Samec, D. Richard
toxoplasma, hypermetabolism, reactive oxygen species, lipid peroxidation, UCP1, interferon-gamma.

## Protection of mice from malaria after co-administration of recombinant mouse granulocyte-macrophage colony-stimulating factor and methionine-enkephalin

P. Pal Singh, S. Singh
Plasmodium berghei, protection, rmGM-CSF, met-enkephalin.

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D. Kube, D. Schmidt, M. Mörmann, A.C. Uhlemann, J. Tomiuk, H. Tesch, P.G. Kremsner

microsatellites, interleukin-10 promoter, multiplex-PCR, Caucasian, African.

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