Upsala J Med Sci 108: 37–50, 2003

Involvement of the proteasome in IL-1β induced suppression of islets of Langerhans in the rat

Johnny Sternesjö,[1] Allan E Karlsen[2] and Stellan Sandler[1]

[1]*Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden*
[2]*Steno Diabetes Center, Gentofte, Denmark*

ABSTRACT

The cytokine IL-1β suppresses rodent islets of Langerhans in vitro. Presently we used inhibitors of the proteasome to investigate if these compounds could counteract the suppressive effects of the cytokine. Thus, isolated rat islets were cultured and pre-treated with proteasome inhibitors and subsequently exposed for 48 h to 25 U/ml human IL-1β. After this period functional tests were carried out. The rate of glucose oxidation (pmol/ 10 islets x 90 min) was suppressed by IL-1β (115 ± 17 vs. control 380 ± 57). Pre-treatment with 10 μM of the proteasome inhibitor MG115 (N-carbobenzoxyl-leu-leu-norvalinal) and 100 μM of the calpain inhibitor norLEU (N-acetyl-leu-leu-norleucinal; known to affect proteasome activity) counteracted the suppressive effects (253 ± 17 and 262 ± 10 respectively). The calpain inhibitor alIMET (N-acetyl-leu-leu-methional) had no effect. MG115 (10 μM) and norLEU (100 μM) blocked nitric oxide formation induced by IL-1β, while alIMET was without effect. We also investigated if IL-1β could influence the expression of two inducible proteasome subunits, namely LMP2 and LMP7, and found that the cytokine increased the mRNA expression of the proteasome subunit LMP2 in islets, and that the proteasome inhibitor MG115 prevented this increase. In conclusion our study shows that IL-1β increases the transcription of the proteasome subunit LMP2, and that the proteasome is involved in IL-1β induced suppression of islet function. Moreover, the observation that inhibitors of the proteasome protect islets against IL-1β induced inhibition of glucose metabolism, suggests that these compounds might be worthwile to explore in future therapies against the development of type 1 diabetes.

INTRODUCTION

Proinflammatory cytokines have been proposed to be involved in the development of type 1 diabetes mellitus (1). lnterleukin-1b (IL-1β) stimulates the expression of

Received 23 January 2003
Accepted 10 February 2003

Abbreviations: NO, nitric oxide; IL-1β, interleukin-1β; iNOS, inducible nitric oxide synthase.

Key words: beta-cells, cytokine, interleukin-1β, nitric oxide, proteasome.

the inducible form of nitric oxide synthase, iNOS (2), thereby inducing nitric oxide (NO) formation and subsequent dysfunction of islet glucose metabolism and insulin secretion (3–5).

The proteasome is a multicatalytic protease complex important for generation of peptides for presentation on MHC class 1, as well as degradation of a number of regulatory peptides (6, 7). The transcription factor NF-KB is bound to its inhibitor I-KB and upon stimulation I-KB is degraded and NF-KB is activated, translocated to the nucleus where it binds to specific DNA sequences inducing transcription. Among the genes induced is iNOS. It has been shown in several cell systems that inhibition of the proteasome will inhibit I-KB degradation and subsequent activation of NF-KB indicating that the proteasome is responsible for I-KB degradation (8–11). Furthermore, inhibition of the proteasome has been shown in islets of Langerhans to abolish the production of NO induced by IL-1β (11). In another study with an immortalised mouse β-cell line, where a proteasome inhibitor was added 48 h after the cytokine IFN-γ, no protective effect of the inhibitor on insulin production was noted (12).

However, data regarding the protection of islet β-cell function against IL-1β induced suppression in conjunction with the inhibition of the proteasome are sparse. Moreover, if the proteasome is involved in cytokine induced damage of islet cells, a logic strategy would be to introduce the inhibitor before exposing cells to cytokines. Since NO induced by cytokines has been implicated in the process leading to type 1 diabetes and proteasome inhibitors have been suggested as a novel therapeutic tool against autolmmune diabetes, evaluation of the functional status of islets treated with proteasome inhibitors that abolish NO production is of great interest. Moreover, we also evaluated the effects of IL-1β on the transcription of two proteasome subunits, LMP2 and LMP7, previously known to be induced by IFN-γ (13).

MATERIALS AND METHODS

Islet isolation and culture

Pancreatic islets from male Sprague-Dawley rats, obtained from a local colony (Biomedical Centre, Uppsala, Sweden), were isolated by collagenase digestion (5). Groups of 150–200 islets were kept free-floating in culture medium RPM1 1640 (Sigma Chemicals, St Louis, MO, USA) supplemented with 10% (vol./vol.) fetal calf serum (Sigma), benzylpenicillin (100 U/ml) and streptomycin (0.1 mg/ml). The islets were cultured at 37°C in a gas phase of humidified air + 5% CO_2 for 7 days before use. The culture medium was changed every second day.

The inhibitors (Sigma) used in the study were norLEU (N-acetyl-leu-leu-norleucinal), alIMET (N-acetyl-leu-leu-methional) and MG115 (N-carboxybenzoxyl-leu-leu-norvalinal) in the concentration range 1–100 μM. DMSO was used as solvent for the stock solutions of the inhibitors (20 mM) resulting in final DMSO concentrations of 0.05–0.5% in the incubation media.

Islets were transferred to new culture dishes and preincubated with inhibitors for

30 min before exposure for 48 h to recombinant human IL-1β (25 U/ml; PeproTech, London, UK). We also performed experiments in which MG115 (10 μM) was added at different time points i.e. 0.5 before and 1, 3 and 6 h after adding IL-1β. Following exposure to IL-1β, islets were used for functional studies as described below.

Islet insulin and DNA content and glucose stimulated insulin release

After the culture period, samples were taken from the culture medium for insulin and nitrite determinations (see below). Triplicate groups of 10 islets each were transferred to sealed glass vials containing 0.25 mI Krebs-Ringer bicarbonate buffer (14) supplemented with 2 mg/ml BSA (Miles Laboratories, Slough, UK) and 10 mM HEPES (Sigma Chemicals), hereafter referred to as KRBH. During the first hour of incubation at 37°C (O_2:CO_2; 95:5) the KRBH medium contained 1.7 mM glucose. The medium was then gently removed and replaced by 0.25 ml KRBH supplemented with 16.7 mM glucose and the incubation continued for a second hour. The insulin concentration was measured by RIA (15). For the RIA, porcine insulin was used for the standard curve, ^{125}I-labelled insulin as tracer obtained from EuroDiagnostica (Malmö, Sweden) and an antibovine insulin antibody was from Biomakor (Rehovot, Israel).

After the incubation, islets were pooled and ultrasonically disrupted in 0.2 mI redistilled water. A 50 μl fraction of the aqueous homogenate was mixed with 125 μ1 acid ethanol (0.18 M HCl in 96% (vol./vol) ethanol) and the insulin was extracted overnight at 4°C. DNA was measured in another fraction of the water homogenate by means of a fluorophotometric method (16, 17).

Islet glucose oxidation

Triplicate groups of 10 islets each were transferred to glass vials containing 100 μ1 KRBH without BSA, but supplemented with D-[U-^{14}C]glucose (Amersham-Pharmacia Biotech, Amersham, UK) and nonradioactive glucose to a final concentration of 16.7 mM and a specific activity of 18.5 MBq/mM. The islet glucose oxidation rate was subsequently measured as previously described (18).

Nitrite determination and medium insulin accumulation

To aliquots of the culture medium (100 μl), 10 μl of 0.5% naphtylendiamine dihydrochloride together with 5% sulphanilamide and 25% concentrated H_3PO_4 was added (19). The reaction was carried out at 20°C for 5 min, and the absorbance was measured at 546 nm in a Beckman DU-62 spectrophotometer (Palo Alto, CA, USA) against a nitrite standard curve. Medium insulin accumulation was determined by RIA (15).

EMSA (Electrophoretic mobility shift assay)

Nuclear protein fractions were extracted from rat insulinoma cell line (RINm5F cells; 20) after the IL-1β exposure as described in detail elsewhere (21). Briefly, cells were washed with cold PBS, harvested, pelleted and resuspended in 50 μl buffer A. After 10 min, cells were pelleted, resuspended in 50 μl of the same buffer

and homogenised. Nuclei were pelleted and briefly sonicated in 50 µl buffer C. After sonication, the nuclear samples were kept on ice for 30 min.

For the EMSA a double-stranded 26 mer oligonucleotide containing the NF-KB binding site (5'AGCTTCAGAGGGGACTTTCCGAGAGG) was used. The oligonueleotide was labelled with [^{32}P]-dCTP using Megaprime labelling kit (Amersham Pharmacia) and extracted once with an equal volume of phenol/chloroform/isoamylalcohol (25/25/1, v/v). Before incubation with the oligonucleotide, nuclear fractions were denatured with 27% formamide. The fractions were then allowed to react with the nucleotide for 30 min at room temperature in a solution containing 10 mM Tris (pH 7.5), 0.2 deoxycholic acid, 40 mM NaCl, 1 mM EDTA 1 mM β-mercaptoethanol, 4% glycerol, 2 µg polydeoxyinosinicdeoxcytidylic acid and 0.1 ng DNA (14000 cpm). A 100-fold excess of non-labelled oligonucleotide was used as negative control. The samples were then separated on 5% non-denaturing polyacrylamide gel in 0.5 x TBE buffer, gel dried and exposed to X-ray film (Amersham Pharmacia).

RNA isolation and semi-quantitaive RT-PCR analysis:

Islets (200 for each condition) were cultured for 12 h with or without IL-1β (25 U/ml) before being snap-frozen and RNA extracted. In the groups where islets were exposed to MG115 (10 µM), the inhibitor was added 30 min before IL-1β. As a positive control for the proteasome subunits LMP2 and LMP7 islets treated with IFN-γ (1000 U/ml) for 12 h were used. Total RNA from the islets was extracted by a modification of the 8 M guanidine method (22) and cDNA (Invitrogen cDNA cycle Kit, CA, USA) prepared using oligo(dT) as primer (23). Semi-quantitative RT-PCR was done for 30 cycles as previously described (24) using dCTP as the ^{33}P-labeled nucleotide. Following separation by 6% PAGE, the transcription products were scanned and quantified on a PhosphorImager using the ImageQuant version 3.3 software (Molecular Dynamics, Sunnyvale, USA). To compensate for variations in cDNA concentration and PCR efficiency between tubes, an internal standard was included in each amplification for normalization. No contamination with genomic DNA was observed. As internal standard, SP-1 was used based on its linear amplification in the same range as for the mRNAs of interest (LMP2 and LMP7) and its unresponsiveness to the cytokine treatment. The primers used (GENEbank acc. # included) were:

LMP2: 5'gtagctggctgggaccaacgt, 5'gtgaccaggtagatgacac; #X97611
LMP7: 5'atgggactcggctctcggga, 5'tcttccttcatgtggtacat; #U17497/U22032
SPI: 5'gtatttgaccagaaccatcc, tacctcaaaggaacagagtgg; #D12768

Statistical analysis:

A mean was calculated from each triplicate group of islets and then considered as one separate observation. Furthermore, every observation represented different islet donors. Values are expressed as means ± SEM and groups of data were compared, using ANOVA with the Fisher's protected least square method or Dunnet's test.

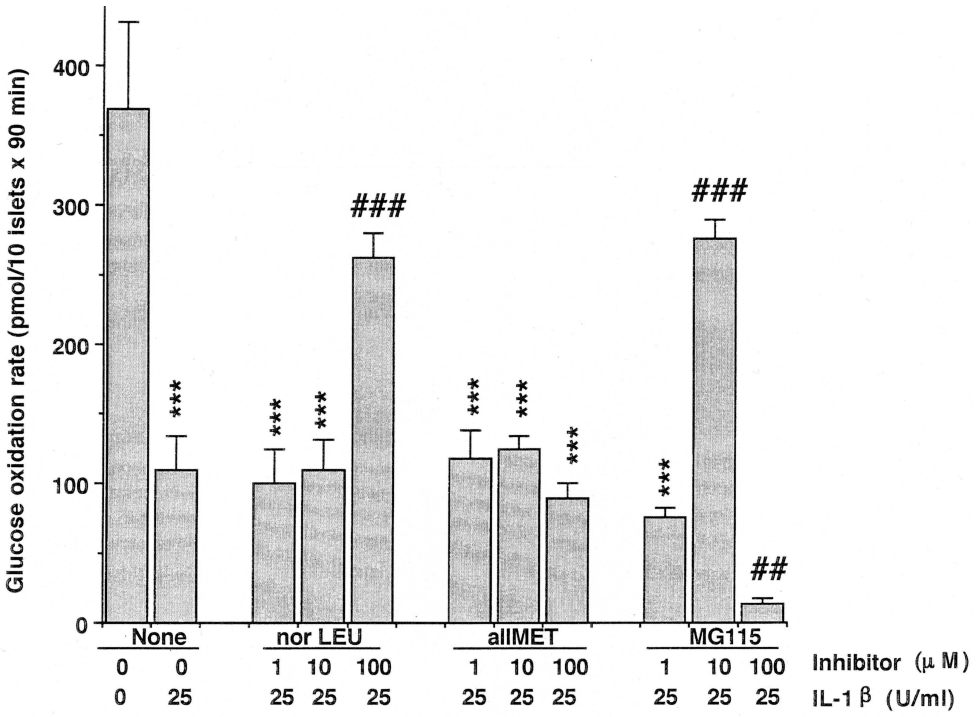

Fig. 1. Glucose oxidation rate of rat islets exposed to inhibitors at various concentrations as indicated. Islets were treated with the inhibitors for 30 min before IL-1β (25 U/ml) was added and then cultured for 48 h before functional tests were performed. Bars are means ± SEM (n = 6). *** denote P<0.001 compared with islets not exposed to any inhibitor and IL-1β, ## and ### denote p<0.05 and p<0.01, respectively, compared with IL-1β treated islets, using ANOVA with Fisher's PLSD test.

RESULTS

Glucose oxidation rate

Culture of rat islets for 48 h with IL-1β (25 U/m1) caused an inhibition in islet glucose oxidation rate, an effect that was counteracted by preculture with MG115 (10 μM) or norLEU (100 μM), but not by alIMET (Fig. 1). However, it should be noted that the presence of 100 μM MG115 led to inhibition of the glucose oxidation rate. In the time course study experiments, MG115 protected against IL-1β induced suppression when added up to 1 h after the cytokine (Fig. 2). Adding the inhibitor later (3 and 6 h) did not protect against inhibition of the glucose oxidation rate. In separate control experiments, the exposure of the islets to the solvent DMSO (0.5%) did not affect the suppressive effects of IL-1β (data not shown). MG115 (10 μM) alone did not induce an increase in glucose oxidation rate as compared to control islets, but rather had an inhibitory effect (213 ± 31 vs. 135 ± 52, pmol/10 islets x 90 min; n=7) although this was not statistically significant.

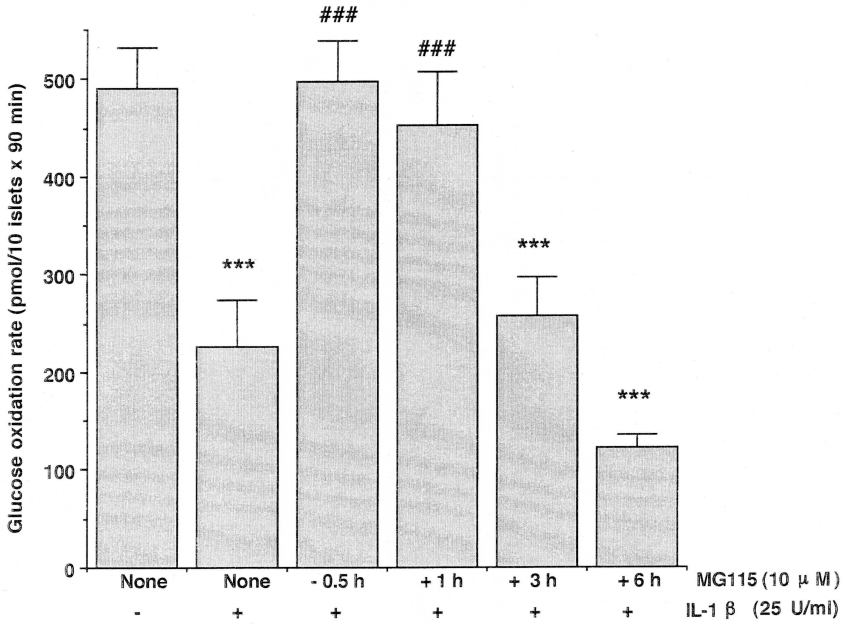

Fig. 2. Glucose oxidation rate of rat islets exposed to MG115 (10 μM) added at various time-points in relation to IL-1β addition as indicated. Islets were then cultured for 48 h before the experiment. Bars are means ± SEM (n = 6). *** denote p<0.001 compared with islets not exposed to MG115 and IL-1β and ### denote p<0.001 compared with IL-1β treated islets, using ANOVA with Fisher's PLSD test.

Table 1. Insulin release of rat pancreatic islets exposed to inhitors at various concentrations and IL-1β as indicated.

Inhibitor (μM)	IL-1β (U/ml)	Insulin release 1.7 mM glucose	16.7 mM glucose
0	0	14.7 ± 2.2	50.2 ± 2.4
0	25	8.0 ± 1.4*	13.3 ± 0.8***
norLEU (1)	25	8.4 ± 0.4*	14.2 ± 2.0***
norLEU (10)	25	7.1 ± 0.9*	16.5 ± 5.5***
norLEU (100)	25	12.9 ± 1.8	12.5 ± 1.2***
allMET (1)	25	8.9 ± 1.3*	11.0 ± 0.8***
allMET (10)	25	9.8 ± 1.3	12.5 ± 1.2***
allMET (100)	25	8.7 ± 1.1*	16.5 ± 4.8***
MG115 (1)	25	10.7 ± 0.9	14.2 ± 0.8***
MG115 (10)	25	14.7 ± 0.9##	22.7 ± 2.7** ##
MG115 (100)	25	31.6 ± 4.9**	60.4 ± 11##

Islets were treated with the inhibitors for 30 min before IL-1β (25 U/ml) was added, and then cultured for 48 h before the experiment. Islets were first incubated for 60 min in 1.7 mM glucose, the medium replaced with 16.7 mM glucose and the islets incubated for another 60 min. Values are means ± SEM (n = 6). *, ** and *** denote P<0.05, P<0.01 and P<0.001 respectively, compared with islets incubated at the same glucose concentration, but not exposed to inhibitor or IL-1β. ## denote P<0.01 compared with islets incubated at the same glucose concentration and IL-1β, but not exposed to inhibitor, using ANOVA with Fisher's PLSD test.

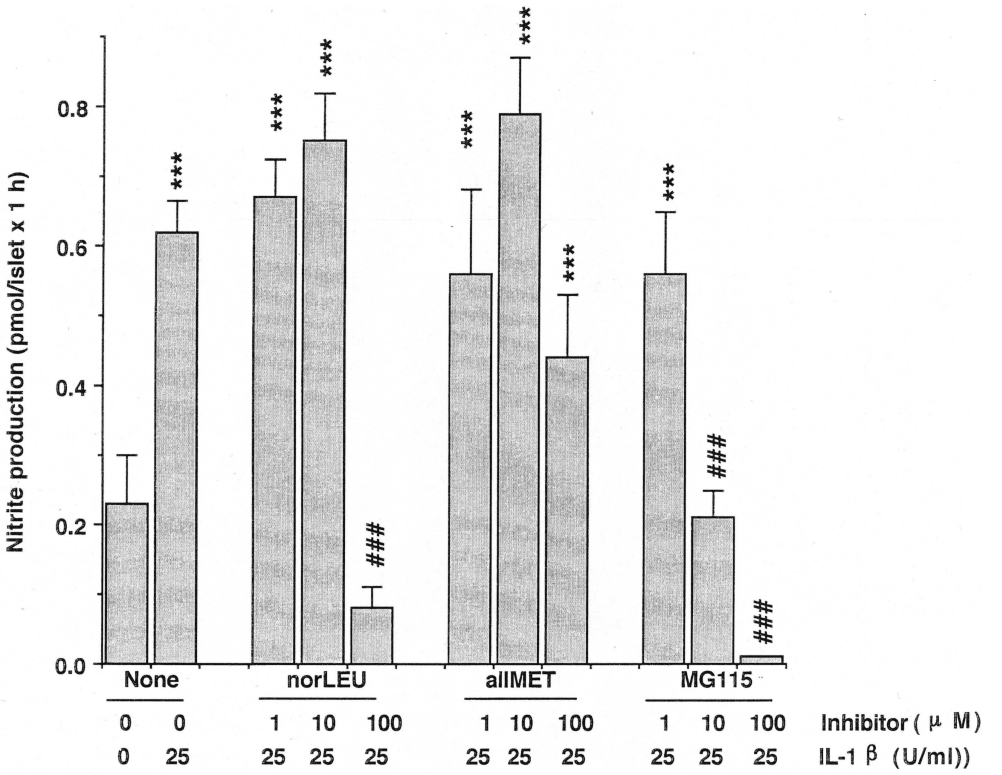

Fig. 3. Nitrite production of rat islets exposed to inhibitors at various concentrations as indicated. Islets were treated with the inhibitors for 30 min before IL-1β (25 U/ml) was added and then cultured for 48 h before nitrite in the culture medium was measured. Bars are means ± SEM (n = 6). *** denote P<0.001 compared with controls not exposed to MG115 and IL-1β, and ### denote P<0.001 compared with IL-1β treated islets, using ANOVA and Fishers's PLSD test.

Glucose stimulated insulin release

Insulin release was suppressed by culture with IL-1β both at basal and high glucose concentrations (Table 1). Neither norLEU (10–100 μM) nor allMET (10–100 μM) affected this inhibition. With MG115 there was an increased release of insulin with the two highest concentrations of the inhibitor. This was observed at both high and low glucose concentrations. It should be noted that the protective effect of 10 μM MG115 against IL-1β induced inhibition of insulin release at high glucose was only partial. In control experiments using the inhibitor alone, 100 μM of MG115 did not affect glucose stimulated insulin release (data not shown).

Nitrite accumulation

IL-1β induced a 2–3 fold increase in medium nitrite accumulation as compared to control islets (Fig. 3). MG115 (10 μm) or norLEU (100 μm) completely blocked this effect, while allMET did not affect the elevated nitrite accumulation (Fig 4).

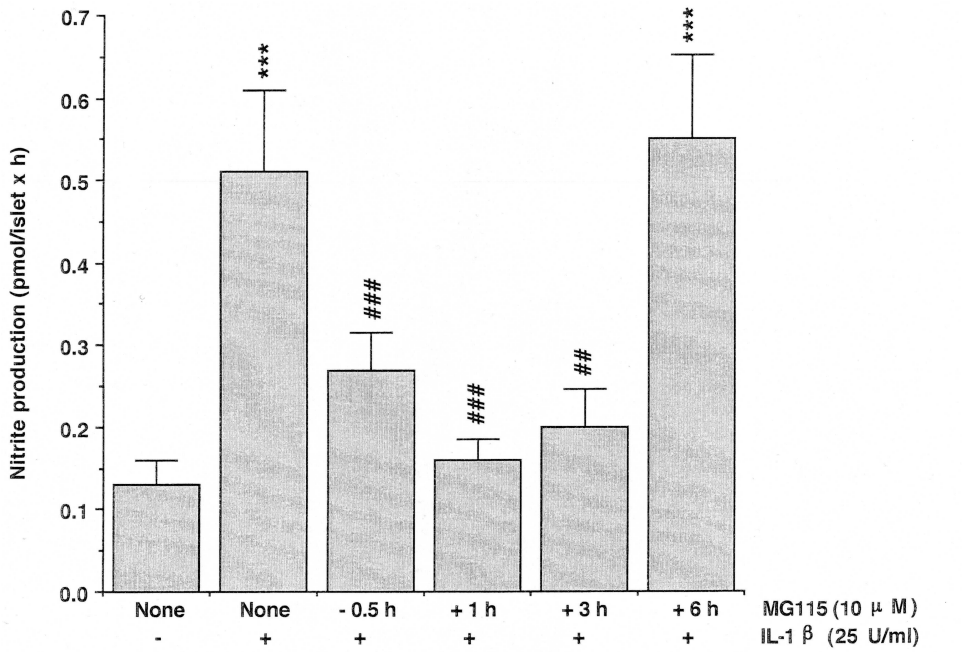

Fig. 4. Nitrite production of rat islets exposed to MG115 (10 μM) added at various time points in relation to IL-1β as indicated. Islets were then cultured for 48 h before nitrite in culture medium was measured. Bars are means ± SEM (n = 6). *** denote P<0.001 compared with islets not exposed to MG115 and IL-1β, and ### and ## denote P<0.001 and P<0.01, respectively, compared with IL-1β treated islets, using ANOVA with Fisher's PLSD test.

Table 2. DNA and insulin content of islets exposed to inhibitors at various concentrations and IL-1β as indicated.

Inhibitor (μM)	IL-1β (U/ml)	DNA content (μg/10 islets)	Insulin content (ng/10 islets)	Insulin/DNA (ng/μg)
0	0	0.19 ± 0.01	483 ± 61	2566 ± 463
0	25	0.15 ± 0.01	402 ± 36	2332 ± 554
norLEU (1)	25	0.17 ± 0.02	452 ± 54	2667 ± 379
norLEU (10)	25	0.18 ± 0.01	386 ± 40	2137 ± 207
norLEU (100)	25	0.19 ± 0.01	372 ± 50	2004 ± 328
allMET (1)	25	0.16 ± 0.01	476 ± 60	2974 ± 450
allMET (10)	25	0.16 ± 0.01	448 ± 38	2696 ± 327
allMET (100)	25	0.19 ± 0.01	448 ± 79	2464 ± 565
MG115 (1)	25	0.19 ± 0.02	485 ± 65	2813 ± 649
MG115 (10)	25	0.18 ± 0.01	386 ± 40	2137 ± 207
MG115 (100)	25	0.24 ± 0.03	679 ± 148	3153 ± 518

DNA and insulin content of islets treated with IL-1β and proteasome inhibitors. DNA and insulin content was measured as described in METHODS. Islets were treated with the inhibitors for 30 min before IL-1β and the cultured for 48 h. Data are expressed as means ± SEM for 5–6 independent experiments.

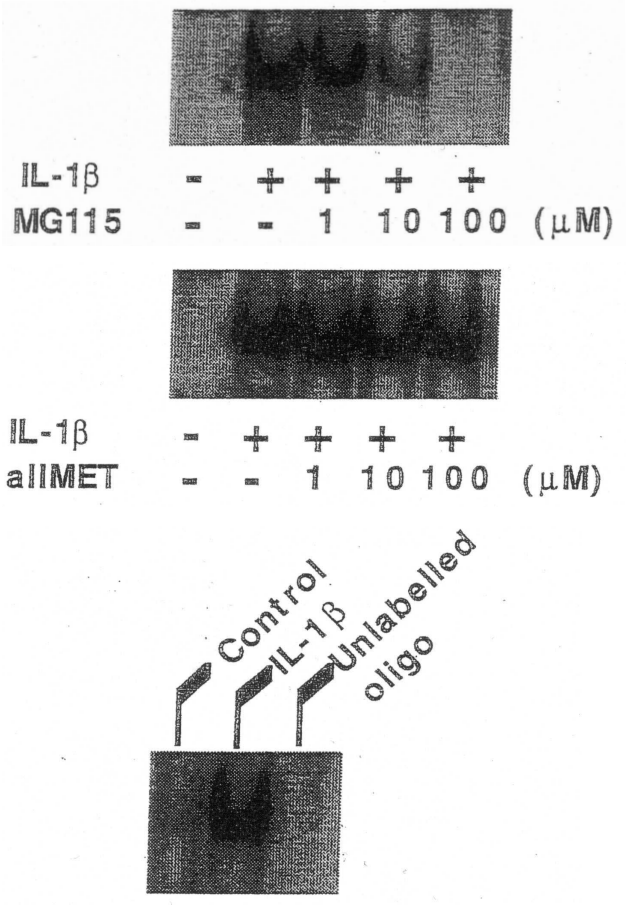

Fig. 5. EMSA of nuclear extracts of RINm5F cells treated with IL-1β (25 U/ml) for 20 min. The inhibitors MG115 (upper panel) and alIMET (middle panel) were added 30 min before IL-1β. As a negative control a 100-fold excess unlabelled oligonucleotide was used (lower panel). Representative for 2–3 experiments.

MG115 at 100 μM essentially abolished all nitrite formation. In time course experiments, nitrite accumulation could be blocked by adding MG115 up to 3 h after IL-1β, but not after 6 h (Fig. 4).

Islet insulin and DNA content

Islet insulin or DNA contents were not significantly affected by IL-1β or the inhibitors, either alone (data not shown) or in combination with IL-1β (Table 2).

EMSA

The proteasome inhibitor MG115 dose-dependently inhibited NF-KB activation induced by IL-1β (Fig. 5 upper panel), while the structurally related calpain

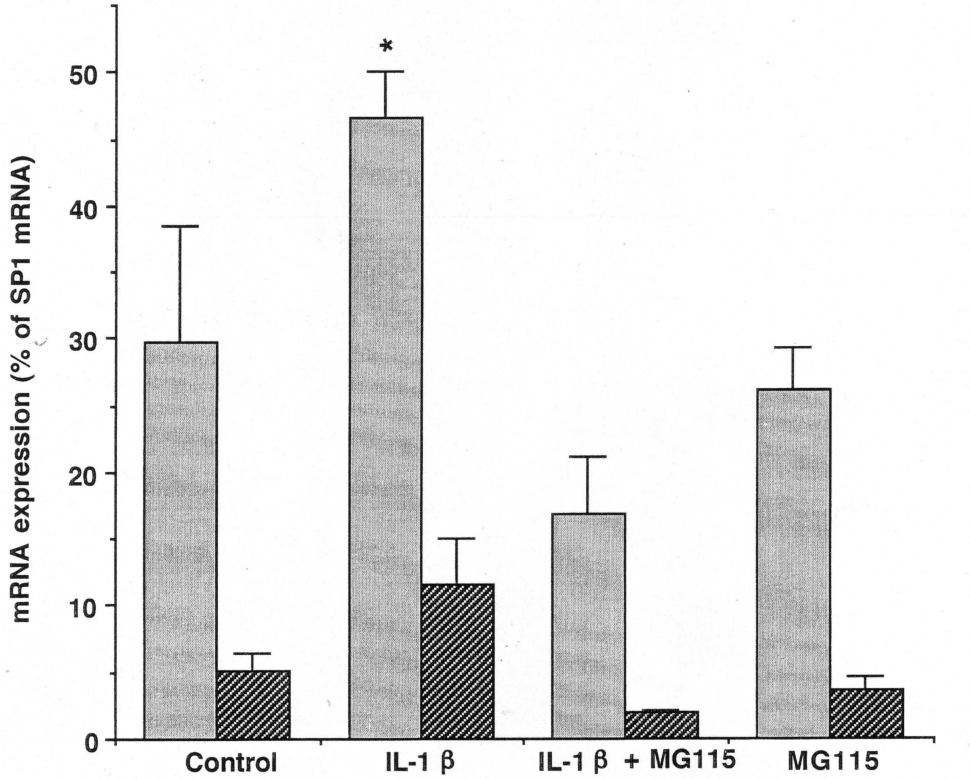

Fig. 6. mRNA for LMP2 (grey bars) and LMP7 (hatched bars) from islets treated with 25 U/ml IL-1β for 12 h. The inhibitor MG115 10 μM was added 30 min before IL-1β. Bars are means ± SEM (n = 4–6).* denotes $P<0.05$ compared with corresponding control islets not exposed to MG115 and IL-1β, using ANOVA with Dunnet's test.

inhibitor allMET did not have such an effect (Fig. 5 middle panel). As a negative control, 100-fold excess oligonucleotide was used (Fig. 5 lower panel).

RT-PCR of proteasome subunits after cytokine treatment

Treating islets with IL-1β for 12 h resulted in an increase in message for the inducible subunit LMP2 (Fig. 6). There was an apparent increase also in the mRNA expression for LMP7, however, this did not attain statistical significance (Fig. 6). The mRNA for LMP2 and LMP7 in IFN-γ treated islets, the positive control group, was increased 2–3 fold (data not shown). The proteasome inhibitor MG115 counteracted IL-1β mediated upregulation of mRNA for LMP2.

DISCUSSION

We demonstrate in this study that inhibition of the proteasome will partly protect isolated islets of Langerhans against the deleterious effects of the cytokine IL-1β in

vitro. Glucose metabolism measured as islet glucose oxidation rate was suppressed by IL-1β as shown earlier (3, 5), and we show that this can partly be restored by the proteasome inhibitor MG115 (10 μM), as well as by the combined calpain/proteasome inhibitor norLEU (100 μM). The calpain inhibitor alIMET, previously shown to have only a weak inhibitory effect on proteasomes (25), did not protect islets against the inhibitory effects of IL-1β. This argues against the involvement of calpain, another cell proteolytic system, because alIMET and norLEU inhibit this system with similar potencies (25).

Another crucial islet function, insulin release is also well known to be affected by IL-1β. A suppression of this function by IL-1β can be seen at both low and high glucose concentrations. This was unaffected by allMET and norLEU at all concentrations tested. MG115 caused an increased medium insulin secretion at high glucose giving the impression of restoration of function, but this increase was also seen at low glucose. This may indicate that the highest concentration of MG115 was impairing β-cell function leading to leakage of insulin. Interestingly, Kwon and colleagues (11) reported that in their initial studies a restoration of insulin secretion was seen with another proteasome inhibitor, MG132. The proteasome inhibitor MG132 was also reported to have a suppressive effect on insulin production (defined as insulin secretion + insulin content) of two immortalised mouse β-cell lines, βTC3 and βTC6-F7 (12). However, glucose stimulated insulin secretion was not tested specifically in that study, since the glucose concentration during the 2 h test period was the same as during the 3-day culture period. In the present study, we did not find a decreased insulin content with any of our inhibitors. This might be due to differences in sensitivity or differences in the regulation of insulin production between less differentiated cell lines and primary islets of Langerhans.

Our study is in agreement with earlier findings (11) that proteasome inhibitors abolish nitrite production induced by IL-1β in islets of Langerhans, probably via inhibition of the transcription factor NF-KB. The latter is also known to be important in mediating NO formation induced by cytokine combinations in human islets (26). The protective effect conferred by the inhibitor on glucose oxidation rate in our study can be explained by this phenomenon since NO has been shown to inhibit the Krebs cycle enzyme aconitase (3). It is well established that inhibition of iNOS restores glucose stimulated insulin secretion suppressed by IL-1β (27). In the current study we observed inhibition of NO formation, but no protective effect on insulin secretion using MG115. Since the proteasome is involved in many cellular processes, we speculate that inhibition of the proteasome might perturb other processes that are necessary for normal insulin secretion.

In the time course experiments it was found that addition of the inhibitor up to 3 h after IL-1β decreased nitrite accumulation. Adding the inhibitor 6 h after the cytokine resulted in nitrite levels comparable to when IL-1β was present alone. This suggests that the inhibitor did not affect NO formation by directly interfering with the transcript or the iNOS enzyme itself because in β-cells at least 4–6 h was required after IL-1β exposure for iNOS mRNA formation (2), and in another study

the iNOS protein could first be detected after 5 h after adding IL-1β to islets (28) and FACS purified rat β-cells (29).

In the RT-PCR experiments we found that message for the proteasome subunit LMP2 was upregulated by IL-1β, and to the best of our knowledge this is the first time an upregulation of this subunit has been shown with IL-1β. This might have an effect on which antigens are expressed on the surface of islet cells since proteasomes containing LMP2 and/or LMP7 have different peptide cleaving properties compared to proteasomes that do not contain these subunits (30, 31). There was presently an increase in message for LMP7 in IL-1β treated islets, but this did not attain statistical significance. It should be noted, however, that the transcript for LMP7 is expressed at a lower level than for LMP2 in our system, making it more difficult to detect and quantitate. Our data do not rule out the possibility that LMP7 might also be upregulated by IL-1β. Longer exposure to IL-1β or higher concentrations might give a more pronounced upregulation. Using purified β-cells, Cardozo et al. showed that IL-1β induced a number of genes, including proteasome subunits (32). In a study where the pancreata from four deceased patients with clinically manifest type 1 diabetes were investigated, no upregulation of LMP2 or LMP7 could be found (33), but this could be different in patients where the disease is developing and β-cells are still present. Interestingly, in NOD mice, lymphocytes have been reported to have a deficient LMP2 expression caused by a mutation in the promoter region (34).

We conclude that IL-1β can enhance the transcription of the proteasome subunit LMP2 and that the proteasome is important for IL-1β induced suppression of islet function. If intervention with proteasome activity will influence the development of type 1 diabetes in experimental models requires further investigation.

ACKNOWLEDGEMENTS

The skilled technical assistance of Susanne Munch, Astrid Nordin, Margareta Engkvist, and Eva Forsbeck (†) is gratefully acknowledged. The study was supported by grants from the Swedish Medical Research Council (72X-8273), the Swedish Diabetes Association, the Swedish Society of Medicine, the Family Ernfors Fund, Svenska Barndiabetesfonden, Swedish Society for Medical Research, the Juvenile Diabetes Foundation International and the Wallenberg Foundation.

REFERENCES

1. Mandrup-Poulsen T (1996). The role of interleukin-1 in the pathogenesis of IDDM. Diabetologia 39: 1005–1029.
2. Eizirik DL, Cagliero E, Björklund A, Welsh N (1992). Interleukin-1β induces the expression of an isoform of nitric oxide synthase in insulin-producing cells, which is similar to that observed in activated macrophages. FEBS Lett 308: 249–252.
3. Welsh N, Eizirik DL, Bendtzen K, Sandler S (1991). Interleukin-1β-induced nitric oxide production in isolated rat pancreatic islets requires gene transcription and may lead to inhibition of the Krebs cycle enzyme aconitas. Endocrinology 129: 3167–3173.

4. Mandrup-Poulsen T, Bendtzen K, Nerup J, Egeberg J, Nielsen JH (1986). Mechanisms of pancreatic islet cell destruction. Dose-dependent cytotoxic effect of soluble blood mononuelear cell mediators on isolated islets of Langerhans. Allergy 41: 250–259.
5. Sandler S, Andersson A, Hellerström C (1987). Inhibitory effects of interleukin 1 on insulin secretion, insulin biosynthesis, and oxidative metabolism of isolated rat pancreatic islets. Endocrinology 121: 1424–1431
6. Groettrup M, Soza A, Kuckelkorn U, Kloetzel PM (1996). Peptide antigen production by the proteasome: complexity provides efficiency. Immunol Today 17: 429–435.
7. Hershko A, Ciechanover A (1998). The ubiquitin system. Annu Rev Biochem 67: 425–479.
8. Traenckner EB, Wilk S, Baeuerle PA (1994). A proteasome inhibitor prevents activation of NF-KB and stabilizes a newly phosphorylated form of I-KB-α that is still bound to NF-KB. EMBO J 13 5433–5441.
9. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T (1994). The ubiquitin-proteasome pathway is required for processing the NF-KB1 precursor protein and the activation of NF-KB. Cell 78: 773–785.
10. Palombella VJ, Conner EM, Fuseler JW, Destree A, Davis JM, Laroux FS, Wolf RE, Huang J, Brand S, Elliot PJ, Lazarus D, McCormack T, Parent L, Stein R, Adams J, Grisham MB (1998). Role of the proteasome and NF-KB in streptococcal cell wall-induced polyarthritis. Proc Natl Acad Sci USA 95: 15671–15676.
11. Kwon G, Corbett JA, Hauser S, Hill JR, Turk J, McDaniel ML (1998). Evidence for involvement of the proteasome complex (26S) and NF-KB in IL-1β-induced nitric oxide and prostaglandin production by rat islets and RINm5F cells. Diabetes 47: 583–591.
12. Baldeon ME, Neece DJ, Nandi D, Monaco JJ, Gaskins HR (1997). Interferon-γ independently activates the MHC class 1 antigen processing pathway and diminishes glucose responsiveness in pancreatic β-cell lines. Diabetes 46: 770–778.
13. Yang Y, Waters JB, Fruh K, Peterson PA (1992). Proteasomes are regulated by interferon-g: implications for antigen processing. Proc Natl Acad Sci USA 89: 4928–4932.
14. Krebs HA, Henseleit K (1932). Untersuchungen über die Harnstoffbildung im Tierkorper. Hoppe-Seylers Z Physiol Chem 210: 33–66.
15. Heding LG (1972). Determination of total serum insulin (IRI) in insulin treated diabetic patients. Diabetologia 8: 260–266.
16. Kissane JM, Robins E (1958). The fluorometric mesurement of deoxyribonueleic acid in animal tissues with special reference to the central nervous system. J Biol Chem 233: 184–188.
17. Hinegardner RT (1971). An improved fluorometric assay for DNA. Anal Biochem 39: 197–201.
18. Andersson A, Sandler S (1983). Viability tests of cryopreserved endocrine pancreatic cells. Cryobiology 20: 161–168.
19. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS Tannenbaum SR (1982). Analysis of nitrate, nitrite, and [15]nitrate in biological fluids. Anal Biochem 126: 131–138.
20. Gazdar AF, Chick WL, Oie HK, Sims HL, King DL, Weir GC, Lauris V (1980). Continuous , clonal insulin- and somatostatin-secreting cell lines established from a rat transplantable rat islet cell tumor. Proc Natl Acad Sci USA 77. 3519–3523.
21. Saldeen J & Welsh N (1994). Interleukin-1β induced activation of NF-KB in insulin producing RINm5F cells is prevented by the protease inhibitor N-α-p-tosyl-L-lysine chloromethylketone. Biochem Biophys Rese Commun 203: 149–155.
22. Karlsen AE, Hagopian WA, Grubin CE, Dube S, Disteche CM, Adler DA, Bärmeier H, Matheewes S, Grant FVJ, Foster D, Lernmark Å (1991). Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. Proc Natl Acad Sci USA 88: 8337–8341.
23. Wadt KA, Larsen CM, Andersen HU, Nielsen K, Karlsen AE, Mandrup-Poulsen T (1998). Ciliary neurotrophic factor potentiates the β-cell inhibitory effect of IL-1β in rat pancreatic islets associated with increased nitric oxide synthesis and increased expression of inducible nitric oxide synthase. Diabetes 47:1602–1608.
24. Jensen J, Serup P, Karlsen Q Nielsen TF, Madsen OD (1996). mRNA profiling of rat islet tumors reveals nkx 6.1 as a β-cell-specific homeodomain transcription factor. J Biol Chem 271: 18749–18758.
25. Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, Hwang D, Goldberg AL (1994).

Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class 1 molecules. Cell 78: 761–771.
26. Flodström M, Welsh N, Eizirik DL (1996). Cytokines activate the nuclear factor KB (NF-KB) and induce nitric oxide production in human pancreatic islets. FEBS Lett 385: 4–6.
27. Southern C, Schulster D, Green IC (1990). Inhibition of insulin secretion by interleukin-lβ and tumour necrosis factor-α via an L-arginine-dependent nitric oxide generating mechanism. FEBS Lett 276: 42–44.
28. Corbett JA, Kwon G, Turk J, McDaniel ML (1993). IL-1b induces the coexpression of both nitric oxide synthase and cyclooxygenase by islets of Langerhans: activation of cyclooxygenase by nitric oxide. Biochemistry 32:13767–13770.
29. Corbett JA, McDaniel ML (1995). Intraislet release of interleukin 1 inhibits β-cell function by inducing β-cell expression of inducible nitric oxide synthase. J Exp Med 181: 559–568.
30. Gaczynska M, Rock KL, Spies T, Goldberg AL (1994). Peptidase activities of proteasomes are differentially regulated by the major hi sto compatibility complex-encoded genes for LMP2 and LMP7. Proc Natl Acad Sci USA 91: 9213–9227.
31. Kuckelkorn U, Frentzel S, Kraft R, Kostka S, Groettrup M, Kloetzel PM (1995). Incorporation of major histocompatibility complex-encoded subunits LMP2 and LMP7 changes the quality of the 20S proteasome polypeptide processing products independent of interferon-g. Eur J Immunol 25: 2605–2611.
32. Cardozo AK, Kruhoffer M, Leeman R, Ørntoft T, Eizirik DL (2001). Identification of novel cytokine-induced genes in pancreatic b-cells by high density oligonucleotide arrays. Diabetes 50: 909–920.
33. Vives-Pi M, Vargas F, James RF, Trowsdale J, Costa M, Sospedra M, Somoza N, Obiols G, Tampe R, Pujol-Borrell R (1997). Proteasome subunits, low-molecular-mass polypeptides 2 and 7 are hyperexpressed by target cells in autoimmune thyroid disease but not in insulin-dependent diabetes mellitus: implications for autoimmunity. Tissue Antigens 50:153–163.
34. Yan G, Fu Y, Faustman DL (1997). Reduced expression of Tapl, and Lmp2 antigen-proces sing genes in the nonobese diabetic (NOD) mouse due to a mutation in their shared bidirectional promoter. J Immunol 159: 3068–3080.

Address correspondence to: Dr Johnny Sternesjö
Department of Medical Cell Biology, Biomedicum,
P.O. Box 571, SE-751 23 Uppsala, Sweden.
Telephone: +46 (0)18 4714430
FAX: +46 (0)18 556401
E-mail: johnny.sternesjo@spray.se