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Research Article

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Expression of an Insulin/Interleukin-1 Receptor Antagonist Hybrid Gene in Insulin-producing Cell Lines (HIT-T15 and NIT-1) Confers Resistance against Interleukin-1–induced Nitric Oxide Production

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Abstract

A hybrid gene consisting of the insulin gene enhancer/promoter region, the signal sequence, the insulin B- and C-chains, and the human interleukin-1 receptor antagonist (IL-1ra) gene was constructed. This hybrid gene was transfected together with the pSV2-neo construct into the insulin-producing cell lines HIT-T15 and NIT-1. One of the geneticin-selected clones, HITra2, expressed a 1.4-kb mRNA, which hybridized both to insulin and IL-1ra-cDNA in Northern blot analysis. Three proteins, with the mol wt 23, 17, and 14 kD, were immunoprecipitated with anti-IL-1ra antibodies from [^{35}S]methionine-labeled HITra2 cells. Both at a low and at a high glucose concentration, 4–5 ng of IL-1ra/10^6 cells (ELISA) was released from these cells. On the other hand, a high glucose concentration evoked a three-fold increase in the release of insulin, suggesting that IL-1ra was released constitutively. Measured by nitrite production, transfected HIT, and NIT-1 cells exhibited a more than 10-fold decrease in IL-1β sensitivity. Since the conditioned culture media from the HITra2 cells exhibited an anti-IL-1β activity of only 0.5 U/ml, and mixed culture of HITra2 cells and isolated rat islets prevented IL-1β induced inhibition of insulin release, it is likely that IL-1ra acts locally at the cell surface. It is concluded that expression of a hybrid insulin/IL-1ra gene confers resistence to IL-1 and that this technique may be used to elucidate the role of IL-1 in autoimmune disorders such as insulin-dependent diabetes mellitus. (*J. Clin. Invest.* 1995. 95:1717–1722.) Key words: interleukin-1β • interleukin-1 receptor antagonist • nitric oxide • insulin • gene therapy

Introduction

It has recently been established that binding of IL-1 to its receptor on rodent pancreatic β-cells activates a chain of events which leads to the induction of a nitric oxide synthase gene closely related to that characterized in activated macrophages (1). As a consequence, considerable amounts of nitric oxide are produced, which leads to inhibition of cell respiration and possibly also cell death (2–4). Therefore, a direct inhibitory and cytotoxic effect of IL-1 and other cytokines leading to β-cell destruction during the onset of insulin-dependent diabetes mellitus has been postulated (5, 6).

Although the effects of IL-1 on the pancreatic β-cell have been extensively characterized in vitro, little is known about the effects of IL-1 on insulin production in vivo. In a study by Jacobs et al. (7), it was found that administration of IL-1 in low doses to biobreeding rats accelerated the development of diabetes whereas high doses delayed the process. The picture is further complicated by the discovery of a naturally occuring IL-1 receptor antagonist (IL-1ra)[1] (8–10). This protein is homologous to IL-1, it is secreted by activated macrophages, and has been shown to counteract IL-1 effects such as hypotension, synthesis of acute phase proteins, and glucocorticoid production (8–10). Thus, the in vivo effects of IL-1 are counteracted and fine tuned by this naturally occuring antagonist. Today, attempts are being made to elucidate the role of IL-1/IL-1ra in immunological reactions, such as rejection of transplants and autoimmune destruction of pancreatic β-cells, by systemic administration of these agents (11, 12). However, these studies are difficult to interpret since high systemic doses of the cytokines over a prolonged period of time can modulate other finely tuned systems. To overcome this limitation, we have presently attempted to transfect insulin-producing cells with a gene construct consisting of the insulin gene enhancer/promoter region, the signal peptide, the insulin B- and C-chains, and the human IL-1ra gene. In this construct, we have preserved the dibasic amino acid cleavage signal between the C-chain of the insulin molecule and the IL-1ra protein. Using this strategy, we anticipated β-cell specific expression of the gene construct, transcriptional and translational control similar to that of insulin, targeting to the endoplasmic reticulum, and to the Golgi apparatus and secretory granules and, finally, cleavage of the IL-1ra protein from the insulin C-chain. Release of local high concentrations of IL-1ra from these cells would constitute an interesting model for the study of the role of IL-1 in autoimmune disease. In the present study, we show that insulin-producing cells transfected with this gene construct release proteins immunologically related to IL-1ra, leading to a decreased sensitivity of the β-cells to IL-1 in vitro.

Methods

Construction of insulin/IL-1ra gene. The rat insulin II gene was cleaved by BbvI yielding a fragment from position 3530 to 4450. This fragment

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1. *Abbreviations used in this paper:* HIT-T15, clonal hamster insulinoma cells; IL-1ra, IL-1 receptor antagonist; NIT-1, clonal NOD/Lt mouse insulinoma cells.

includes 450 bp upstream of the TATA box, 200 bp from the TATA box to the translational state site, and an additional 270 bp reaching just into the A chain. To the 3′ end was ligated a human IL-1ra gene (British Bio-technology Ltd., Oxford, United Kingdom) cleaved with EcoRI close to the NH_2-terminus, blunt end ligated using fill-in with T4 polymerase of the insulin gene fragment and Mung bean nuclease treatment of the IL-1ra gene. The construct was introduced into the vector pUC18, which was linearized before transfection.

Transfection of clonal hamster insulinoma (HIT-T15) and clonal nonobese diabetic/Lt mouse insulinoma (NIT-1) cells. HIT-T15 cells were kindly provided by Dr. S.H.J. Ashcroft, Nuffield Department of Clinical Biochemistry, Radcliffe Hospital, Oxford, United Kingdom. NIT-1 cells were obtained from American Type Culture Collection, Rockville, MD. The passage number of the HIT cells was 90–110 and of the NIT-1 cells 23–35. The insulin content of HIT cells, passage number 110, was 8 ng/10^6 cells and that of NIT-1 cells, passage number 35, 410 ng/10^6 cells. HIT-T15 cells were cultured in RPMI 1640 + 10% FCS and NIT-1 cells in Ham's F-12 + 10% FetalClone II (HyClone Laboratories Inc., Cramlington, United Kingdom). When reaching 50% confluency, the cells were transfected with the insulin/IL-1ra and the pSV2-neo constructs using the Lipofectin® method (GIBCO BRL Life Technologies, Inc., Gaithersburg, MD). 2 d after transfection, 400 or 100 μg/ml of geneticin was added to the the cell cultures, concentrations cytotoxic for HIT-T15 and NIT-1 cells, respectively, not expressing the pSV2-neo construct. After 7 d in the presence of geneticin, only cells having taken up the pSV2-neo construct were remaining and after additional 2 mo of culture with geneticin, two HIT-T15 cell colonies, HITra2 and HITra4, and one NIT-1 cell colony, NITra2, were isolated, subcloned, and characterized. Both nontransfected and transfected HIT-T15 and NIT-1 cells not expressing the insulin/IL-1ra construct were used as controls.

Northern blot analysis. Poly(A) RNA was extracted from 2 × 10^6 cells using the Micro-Fast Track™ kit (Invitrogen, San Diego, CA) according to the instructions of the manufacturer. Poly(A)RNA was quantified using Dip Stick™ (Invitrogen) and equal amounts (1–2 μg) were run on 1% agarose/formaldehyde gels (13). The RNA was blotted onto Genescreen membranes (NEN Research Products, Boston, MA) overnight (14) and cross-linked using ultraviolet illumination. The filters were hybridized to human synthetic IL-1ra, rat insulin II, and GAPDH (15) cDNA probes which were labeled using the Multiprime cDNA Labeling Kit and [^{32}P]dCTP (Amersham International, Little Chalfont, United Kingdom). The hybridizations were performed in hybridization solutions according to the manufacturers directions (Fast Hyb; Amersham International). After washing, the filters were exposed to Hyperfilm (Amersham International) at −70°C overnight.

Immunoprecipitation of [^{35}S]methionine-labeled proteins. 2 × 10^6 control or HITra2 cells were labeled for 3 h in methionine-free RPMI 1640 supplemented with [^{35}S]methionine (0.1 mCi/ml). The culture medium was collected and concentrated using microconcentrators (Centricon; Amicon Corp., Beverly, MA). The cells were scraped off with a rubber policeman, washed in cold PBS, and homogenized in 150 μl of cold homogenization buffer containing 250 mM sucrose, 50 mM Tris, pH 7.5, 2 mM EDTA, 1 mM phenylmethylsulfonylfluoride, and 100 U/ml trasylol. Nuclei and large aggregates were removed by centrifugation at 12,000 *g* for 20 s. The remaining supernatants were centrifuged for 20 min at 160,000 *g* in an airfuge (Beckman Instruments, Inc., Fullerton, CA) yielding a membrane fraction and cytosol. Membranes were resupended in 2% Triton X-100, 0.1% SDS, 50 mM Tris, pH 7.5, 2 mM EDTA, and 150 mM NaCl and a polyclonal serum specific for human IL-1ra (αIRAP-1444) (16) was added. After a 1-h incubation period at 4°C, protein A sepharose was added and the incubation was continued for another 30 min. The cytosolic fractions and media samples were diluted in the immunoprecipitation buffer described above and immunoprecipitated likewise. The protein A sepharose pellets were washed three times with immunoprecipitation buffer and then boiled in SDS sample buffer before electrophoresis on SDS-polyacrylamide gels according to Laemmli (17). The polyacrylamide/Bis contents were 15/0.45%. After electrophoresis, gels were soaked in Amplify (Amersham International), dried, and then exposed to Hyperfilm at −70°C.

Nitrite determinations. HIT and NIT cells were seeded in multiwell plates and cultured to 50–70% confluency. On day 0, fresh medium (0.5 ml) was added to the cells and IL-1β was supplemented directly to the cells at the concentrations given in the figures. IL-1β was kindly provided by Dr. Kim Hejnaes (Novo-Nordisk, Bagsvaerd, Denmark). 24 h later, samples (80 μl) were taken in triplicates and nitrite contents were determined as described previously using the Greiss reagent (18). For IL-1ra ELISA determinations (19) and insulin media contents (20), samples were taken from the media of cells cultured for 1 d at 0.8 mM glucose, the second day at 11.1 mM glucose, and the third day at 0.8 mM glucose.

For determination of anti-IL-1β activity of HITra2-conditioned media, control and HITra2 cells (2–4 × 10^6) were cultured for 24 h in 5 ml of culture media. The conditioned culture media (0.5 ml) were then added to the rat insulinoma cell line RINm5F, which had been precultured in 24 well plates. IL-1β was then added that the concentrations given in the figure and nitrite accumulation in the medium was measured after 7 h.

Mixed culture of isolated rat pancreatic islets with control and HITra2 cells. Rat pancreatic islets were isolated using collagenase digestion and cultured in RPMI 1640 + 10% FCS supplemented with 2 mM glutamine and antibiotics (21). Trypsinized control HIT or HITra2 cells (2–5 × 10^6) were then added to groups of 200 islets and allowed to attach to the islets for 3 d. The islets that had been mixed with aggregates of cells were then hand picked and exposed to IL-1β (25 U/ml). After 24 h, the islets were incubated at 1.7 and 17 mM glucose for 60 min in KRB + 10 mM Hepes (pH 7.4) and the islet insulin release was determined by RIA (20).

Results

Northern blot analysis. Northern blot experiments demonstrated the expression of a 1.4-kb mRNA present in HITra2 cells, which hybridized to both a rat insulin cDNA probe (Fig. 1 *a*, lane *2*) and an IL-1ra cDNA probe (Fig. 1 *b*, lane *2*). In the HITra4 lane, a 2.0-kb band hybridized to insulin cDNA (Fig. 1 *a*, lane *3*). However, this band could not be detected when rehybridizing the same blot to the IL-1ra probe (Fig. 1 *b*, lane *3*). The 1.4- and 2.0-kb bands were not detected in control HIT cells (Fig. 1 *a* and *b*, lane *1*). The ratio between the 1.4/2.0-kb bands and the 0.6-kb band (insulin mRNA) was higher in HITra2 cells than in HITra4 cells (Fig. 1 *a*).

The effect of different glucose concentrations on the expression of the hybrid IL-1ra/insulin gene and the insulin gene (both normalized to GAPDH mRNA signals in the same blots) was studied in a separate series of experiments. A 24-h culture period at 11.1 mM glucose failed to increase the expression of insulin mRNA, in both control cells and in HITra2 cells, and the 1.4-kb transcript in HITra2 cells, as compared to culture at 0.8 mM glucose (Fig. 2). Interestingly, the insulin mRNA signal was clearly lower in HITra2 cells than in control cells (Fig. 2).

Immunoprecipitation of IL-1ra. IL-1ra-specific immunoreactivity was not detected in the cytosolic fractions from HITra2 and control cells (results not shown). However, in the membrane fraction, one 23-kD band could be observed in HITra2 cells which was not present in control lanes (Fig. 3, lanes *1* and *2*). Three proteins with mol wt 14, 17, and 23 kD were specifically immunoprecipitated in media samples from HITra2 cells (Fig. 3, lanes *3* and *4*).

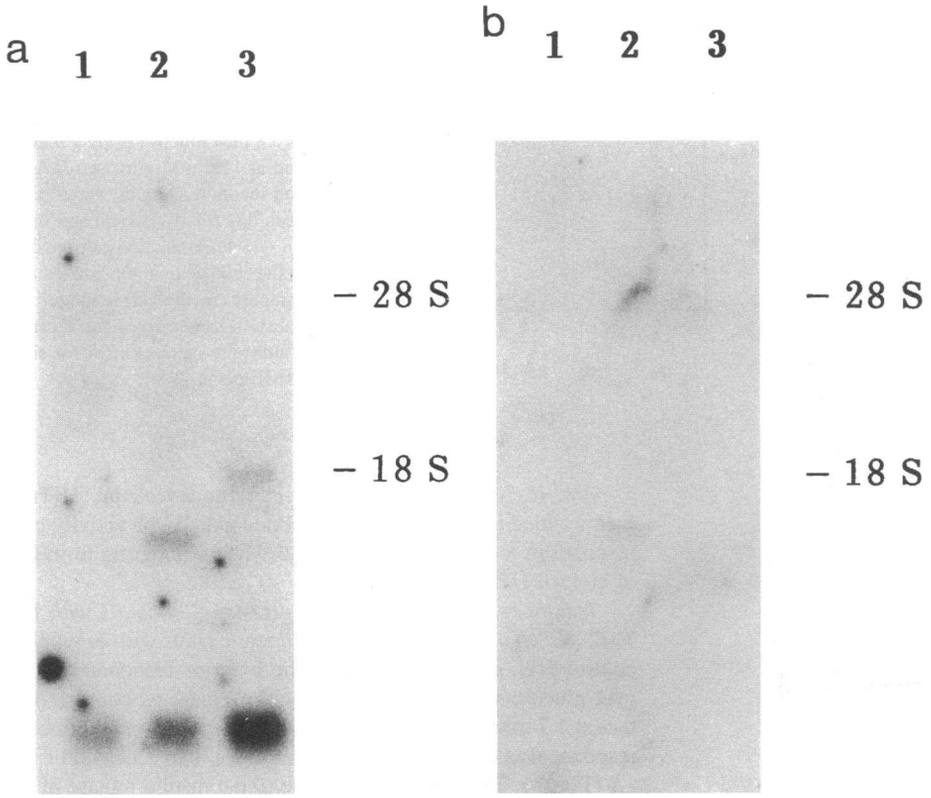

Figure 1. Northern blot analysis of insulin and IL-1ra mRNA. RNA from control HIT (lane *1*), HITra2 (lane *2*), and HITra4 (lane *3*) cells (5×10^6) was isolated, electrophoresed, and transferred to nylon filters. The filters were hybridized to labeled insulin (*a*) and IL-1ra (*b*) cDNA probes. Positions of 28 and 18 S rRNA markers are given on the right.

Release and contents of IL-1ra and insulin in HITra2 cells. The amount of IL-1ra released, as determined by ELISA, during a 24-h culture period by HITra2 cells ranged between 4 and 5 ng/10^6 cells (Fig. 4 *a*). The release of IL-1ra was not affected by the glucose concentration in the media (Fig. 4 *a*). The IL-1ra content of the HITra2 cells was 3 ng/10^6 cells (Fig. 4 *a*). IL-1ra could not be detected in media or homogenates from control HIT cells (results not shown). In contrast to IL-ra, the release of insulin was increased more than threefold by 11.1 mM glucose as compared to 0.8 mM glucose (Fig. 4 *b*).

IL-1β-induced nitrite production in HITra2 and NITra2 cells. Testing for IL-1 sensitivity, and using nitrite production as an indicator of nitric oxide formation, it was found that control HIT and NIT cells reached their maximal IL-1 induced nitrite production at 10–25 U/ml of IL-1β (Fig. 5, *a* and *b*). HITra2 and NITra2 cells, however, remained completely protected at IL-1β concentrations at or below 100–125 U/ml (Fig. 5, *a* and *b*). At 250 U/ml of IL-1β, however, both HITra2 and NITra2 cells released considerable amounts of nitrite (Fig. 5, *a* and *b*).

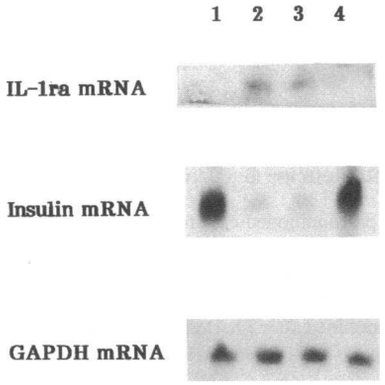

Figure 2. Northern blot analysis of IL-1ra, insulin, and GAPDH mRNA. Control HIT (lanes *1* and *4*) and HITra2 (lanes *2* and *3*) cells (5×10^6) were incubated for 24 h at 0.8 (lanes *1* and *2*) or 11.1 mM glucose (lanes *3* and *4*). RNA was isolated, electrophoresed, and transferred to nylon filters. The filters were hybridized to labeled insulin, GAPDH, or IL-1ra cDNA probes. The figure is representative for three separate experiments.

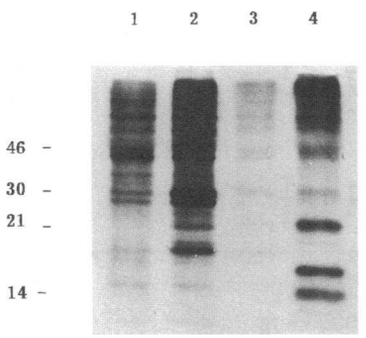

Figure 3. Immunoprecipitation of [^{35}S]-methionine-labeled membrane-associated and medium proteins with anti-IL-1ra serum. Control HIT (lanes *1* and *3*) and HITra2 cells (lanes *2* and *4*) were labeled with [^{35}S]-methionine, homogenized, and subcellularly fractionated. The membrane fractions (lanes *1* and *2*) were suspended in immunoprecipitation buffer. Media from the labeled cells were collected, concentrated, and diluted into immunoprecipitation buffer. The different fractions were immunoprecipitated with anti-IL-1ra serum (16) and run on 15% polyacrylamide gels. Positions of the molecular weight markers are given to the left. Position of three bands present in the media fraction (23-kD protein also present in HITra2 membrane fraction) of HITra2 cells are indicated to the right. The figure is representative for two separate experiments.

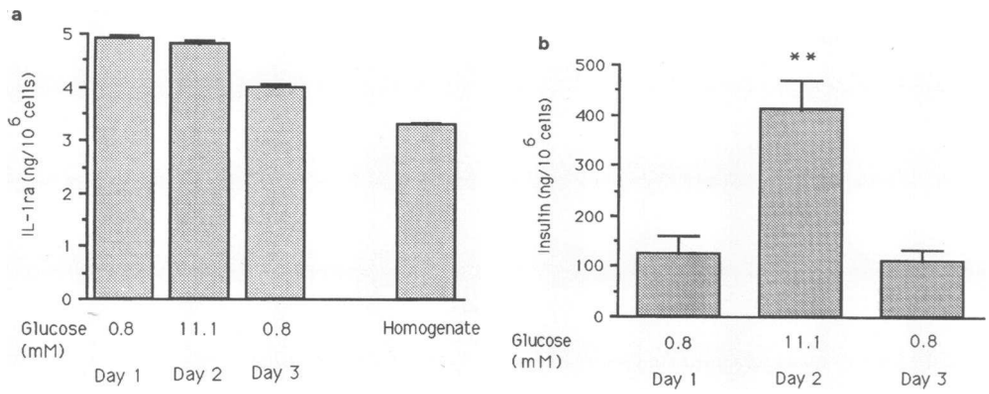

Figure 4. IL-1ra and insulin levels in medium samples and homogenates from HITra2 cells. HITra2 cells ($5-10 \times 10^5$) were cultured at 0.8 mM glucose (*Day 1 and 3*) and at 11.1 mM glucose (*Day 2*) and medium samples were taken each day for IL-1ra (*a*) and insulin (*b*) determinations. After day 3, the cells were homogenized and samples (media and homogenates) were analyzed for IL-1ra. Values are means±SEM for six experiments.

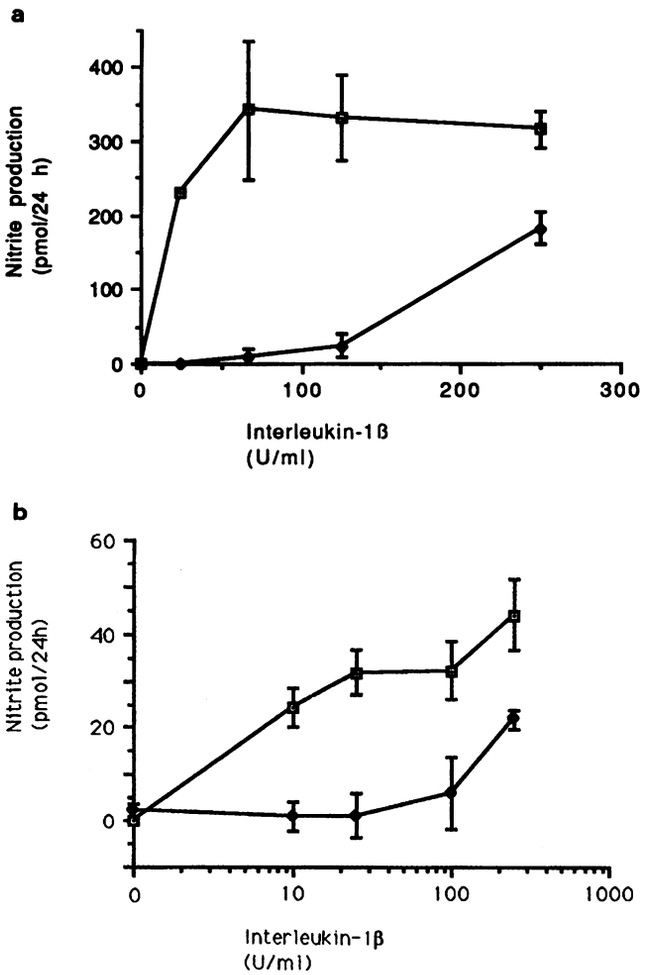

Figure 5. Nitrite production from HITra2 and NITra2 cells exposed to IL-1β. HITra2 (Fig. 5 *a*) and NITra2 (Fig. 5 *b*) cells (5×10^5 cells) were seeded in multiwell plates and exposed to IL-1β in fresh medium at the concentrations given in the figure. After 24 h, medium samples were taken and nitrite accumulation was determined using the Greiss reagent. Results are means±SEM for three separate experiments. (*a*) —⊡—, control cells; —♦—, HITra2 cells. (*b*) —⊡—, control cells; —♦—, NITra2 cells.

Anti-IL-1β activity of HITra2-conditioned media. HITra2 media added to RINm5F cells exhibited anti-IL-1β activity corresponding to ~ 0.5 U/ml of IL-1β when comparing to media of control HIT cells (Fig. 6).

Insulin release of rat pancreatic islets "coated" with HITra2 and HIT cells. Isolated rat pancreatic islets with or without control HIT cells "coated" on the exterior responded to 17 mM glucose with an increased insulin release as compared to that at 1.7 mM glucose (Fig. 7). Furthermore, the high glucose response was diminished by a 24-h exposure to 25 U/ml of IL-1β (Fig. 7). There was no decrease in the insulin release of rat islets coated with HITra2 cells and exposed to 25 U/ml of IL-1β (Fig. 7).

Discussion

This investigation demonstrates that one HIT cell clone, HITra2, which was obtained by transfection with the hybrid insulin/IL-1ra gene, expressed an mRNA which hybridized to both insulin

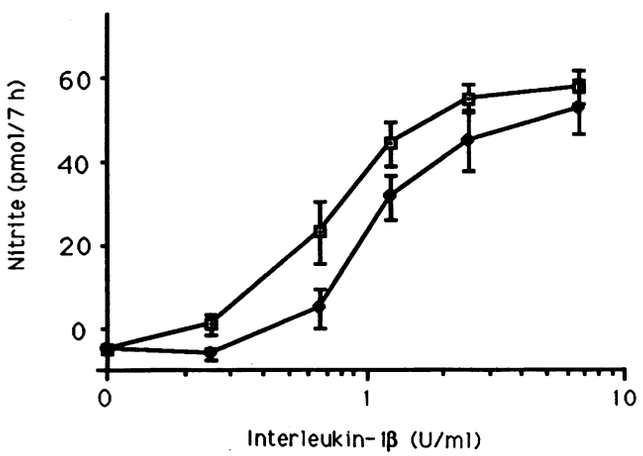

Figure 6. Effects of HITra2 preconditioned culture medium on IL-1β-induced nitrite production from RINm5F cells. To RINm5F cells was added culture medium preconditioned with control HIT cells or with HITra2 cells. IL-1β was added at the concentrations given in the figure and nitrite production was determined after 7 h. Results are means±SEM for three separate experiments. —⊡—, control HIT cells; —♦—, HITra2 cells.

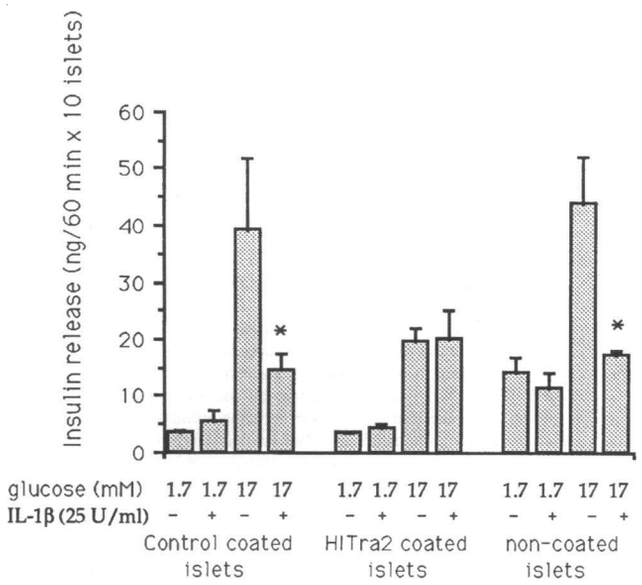

Figure 7. Effects of HITra2 cells on IL-1β-induced inhibition of rat islet insulin release. Rat pancreatic islets were isolated and precultured for 3 d. Trypsinized control HIT cells or HITra2 cells were added to the islets and allowed to attach for 3 d. Islets with or without attached HIT or HITra cells were then hand picked and exposed to 25 U/ml of IL-1β for 24 h as given in the figure. Islet insulin release was determined at 1.7 and 17 mM glucose during a 60-min incubation period. Values are means±SEM for three separate experiments. $*P < 0.05$ vs 17 mM glucose only using Student's t test.

and IL-1ra cDNA probes. Moreover, it released a membrane fraction–associated protein which is immunologically related to and size matched with IL-ra. Since the cells were analyzed several months and passages after the transfection event, it is highly probable that the gene coding for the insulin/IL-1ra hybrid mRNA is stably integrated into the chromosomal genome. The size of the IL-1ra/insulin mRNA band was higher than that anticipated; 1.4 kb vs 1.0 kb (nucleotide difference between transcriptional start site and end of the IL-1ra gene including a poly(A) tail). The reason for this discrepancy is unclear, however, the integrated gene may use a polyA site downstream of the IL-1ra sequences. The nature of the 2.0-kb transcript observed in HITra4 cells is not known. A recombination event could have occurred explaining the absence of any hybridization of this mRNA to IL-1ra cDNA.

It was previously reported that HIT cells respond to increasing glucose concentrations with an enhanced expression of the insulin gene (22). Therefore, we presently studied the effect of glucose on insulin mRNA levels in control and HITra2 cells to see whether the transfected gene was under the same transcriptional control as the native insulin gene. Unfortunately, neither control cells nor the transfected cells contained more insulin mRNA, or insulin/IL-1ra mRNA, after culture at a high glucose concentration. This is in line with a recent report showing that late passages of HIT cells lose their glucose-sensitive insulin gene transcription, an effect which may be mediated by a decreased expression of the transcription factor STF-1 (23). Surprisingly, we observed that HITra2 cells contained considerably less insulin mRNA than control cells. This may reflect promoter competition, i.e., the extra insulin promoter(s) transfected into the genome will bind the specific transcription factors involved in insulin gene transcription so that fewer are available for the native insulin gene. On the other hand, it may also be explained by altered characteristics of the transfected clone.

HITra2 cells contained a 23-kD protein and released three proteins with mol wt 14, 17, and 23 kD recognized by a polyclonal antiserum specific for human IL-1ra. It is likely that the 23-kD protein represents a hybrid of human IL-1ra (17 kD) and the rat insulin B- and C-chains (6 kD). That the anti–IL-1ra-precipitated proteins could only be observed in the membrane fraction is consistent with the notion that the synthesis of the proteins is directed to the endoplasmic reticulum by the signal sequence of preproinsulin. The subsequent fate of the nascent polypeptide is more uncertain. Since the A-chain of insulin is missing, the disulfide bridges necessary for the correct folding of the B- and C-chain cannot be formed. Unfolded proteins are often degraded rather than transported to the Golgi system (24). Another possibility is that the hybrid protein is directed to the constitutive pathway for its release. Finally, the hybrid protein may be properly processed and released via the regulated pathway. However, the present findings that considerable amounts of IL-1ra were released from HITra2 cells and that this release was not regulated by glucose, in contrast to insulin, suggests that the hybrid protein is secreted via the constitutive, and not the regulated pathway. To what extent the hybrid gene product is processed is unclear since the lower molecular weight IL-1ra proteins in culture media from HITra2 cells (17 and 13 kD) may represent nonspecific degradation products rather than processing products. Indeed, IL-1ra is known to be thermolabile (16) which could explain the relatively low accumulation of the protein in the culture medium.

HITra2 cells produced no nitrite when challenged with IL-1β, at concentrations $\leq$ 125 U/ml. That the lower sensitivity of the HITra2 cells is due to release of IL-1ra and not due to an altered response to IL-1 receptor activation is supported by the high nitrite levels produced at the highest IL-1β concentration (250 U/ml). This notion is further supported by the finding that the mouse insulinoma cell line NIT-1 transfected with the same IL-1ra/insulin construct also exhibited a similar loss of sensitivity to IL-1β. This indicates that the HITra2 and NITra2 cells release a protein with IL-1ra activity, which competitively inhibits IL-1 activation of the β-cell IL-1 receptor. HIT cells have previously been shown to express the mRNA for type I and typ II IL-1 receptors (25).

It appears as if the HITra2 cells secrete enough anti-IL-1 activity to neutralize 125 U/ml of IL-1. Since 125 U/ml corresponds to 25 ng/ml, the IL-1ra medium accumulation of 5 ng/ml of IL-1ra appears far from sufficient. Indeed, the anti-IL-1β activity of HITra2-conditioned medium was only 0.5 U/ml. However, the concentration of IL-1ra may be considerably higher in the vicinity of a cell secreting the inhibitor than in the medium. This notion is supported by the experiments in which isolated rat pancreatic islets were mixed cultured with either control HIT cells or with HITra2 cells. Since isolated islets secrete 50–100 times more insulin than HIT cells (results not shown), the present results reflect IL-1β effects on islet β-cells and not on the insulinoma cells. Therefore, the lack of inhibitory effect of 25 U/ml of IL-1β on HITra2-coated islets suggests that the release of IL-1ra by cells in close contact with the primary β-cells is enough to completely block the IL-1β effect. It should be pointed out, however, that islets coated with

HITra2 cells tended to release less insulin than control islets. Therefore, we cannot exclude that some of the IL-1β effect may have been masked by this decrease.

To our knowledge, this study is the first to use gene transfection to manipulate β-cell susceptibility to cytokines. Although the present work was performed on an immortalized β-cell line and not with primary β-cells, rapid progress is now taking place in the field of gene transfer. For example, in vivo and ex vivo transduction of primary cells are now being performed using different viral vectors (26). This opens interesting possibilities for ex vivo transfection of islet cells before transplantation and even in vivo β-cell transfections of prediabetic individuals. One major advantage of this approach is the possibility of cell-specific expression of the gene construct mediated by tissue specific enhancers/promoters. This leads to local production of the gene construct with high levels in the target area with little or no systemic effects. This might be beneficial during autoimmune β-cell destruction induced by macrophages invading the pancreatic islet (27). High local levels of IL-1ra would counteract the action of the cytokine released by activated macrophages by blocking IL-1 receptor activation. This might also counteract macrophage-induced activation of T lymphocytes involved in cell-mediated β-cell destruction (28). The gene transfection model may also be of interest in studies on the role of cytokines in other situations. For example, attempts to treat rheumatoid arthitis in an animal model with gene transfection using the IL-1ra gene have shown promising results (29). These studies have initiated plans to start clinical trials using the same gene transfection model (30).

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