Inter cellular adhesion molecule 1 is induced on isolated endocrine islet cells by cytokines but not by reovirus infection

(I interferon $\gamma$/tumor necrosis factor $\alpha$/human pancreatic beta cells/pancreatic delta cells)

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ABSTRACT The selective destruction of the pancreatic islet beta cells in type 1 diabetes mellitus is thought to be mediated by a cellular autoimmune process, possibly triggered by virus infection in genetically susceptible individuals. Because of the potentially important role of cell-cell adhesion in the immune response, we investigated whether cytokine products of mononuclear cells, or virus infection, induced the expression of intercellular adhesion molecule 1 (ICAM-1) on human endocrine islet cells. By flow cytometry, control islet cells did not express detectable ICAM-1. However, after a 72-hr exposure of islets to interferon $\gamma$ (IFN-$\gamma$) and/or tumor necrosis factor $\alpha$ (TNF-$\alpha$) (each at 250 units/ml), ICAM-1 was induced on >85% of islet cells. IFN-$\gamma$ was 50% more potent than TNF-$\alpha$; together, their effects were additive. Class I major histocompatibility complex (MHC) protein expression, detected on control islet cells, was also stimulated by IFN-$\gamma$ and/or TNF-$\alpha$. In contrast, infection with reovirus type 3 did not induce ICAM-1 on islet cells, although it stimulated the expression of class I MHC proteins. By double-label indirect immunofluorescence microscopy, ICAM-1 expression was identified on both beta (insulin-secreting) and delta (somatostatin-secreting) islet cells. Monoclonal antibody to ICAM-1 precipitated protein of $M_r$ 97,000 from $[^{35}]$methionine-labeled islets exposed to IFN-$\gamma$ and TNF-$\alpha$, but not from control islets. RNA blot analysis revealed a major species of 3.3 kilobases and a minor species of 2.2 kilobases induced in islets exposed to the cytokines. These findings have implications for the molecular mechanisms of beta-cell destruction in type 1 diabetes, in that expression of ICAM-1 by beta cells may facilitate adhesion of antigen-targeted immune cells.

Type 1 diabetes mellitus is thought to result mainly from the autoimmune destruction of the pancreatic islet beta cells, possibly triggered by virus infection in genetically susceptible individuals (1, 2). A number of lines of evidence indicate that T lymphocytes have a central role in the pathogenesis of beta-cell destruction. Thus, the islets of patients with type 1 diabetes of recent onset are infiltrated with mononuclear cells (3–5) and this so-called insulitis is also observed in the islets of pancreatic isografts between HLA-identical twins when the recipient develops recurrent diabetes (6). Immunocytochemical characterization of the insulitis lesion reveals a majority of the cells to be T cells, some of which are activated (4, 6). Indirect evidence for the role of T cells in the pathogenesis of type 1 diabetes also comes from recent immunotherapy trials using the drug cyclosporine, thought to act primarily by blocking T-cell lymphokine production, in which significant remissions from insulin dependence were achieved (7).

It is apparent that the activation and subsequent effector properties of T cells in response to antigen and major histocompatibility complex (MHC)-encoded proteins require cell contact and adhesion (8–10). The lymphocyte function-associated antigen 1 (LFA-1) plays an important role in this process (11, 12). The ligand for LFA-1-dependent T-cell adhesion has been identified as a 90-kDa cell surface glycoprotein designated intercellular adhesion molecule 1 (ICAM-1) (13, 14). ICAM-1 is expressed at low levels on resting peripheral blood leukocytes (15, 16) but is increased following activation. Antibodies to ICAM-1 have been shown to block T-cell adhesion to normal human endothelial cells (17) and to suppress activation induced by cell-cell contact between T and B lymphocytes (17) and monocytes (16). Furthermore, recent studies using L-cell transfectants coexpressing ICAM-1 with HLA-DR demonstrated a pivotal role for ICAM-1 in the induction of both antigen-specific and allospecific T-cell responses (18). ICAM-1 expression can also be induced by the cytokines interferon $\gamma$ (IFN-$\gamma$), interleukin 1 (IL-1), and tumor necrosis factor $\alpha$ (TNF-$\alpha$) on some nonhematopoietic cells such as fibroblasts and endothelial cells (15, 19). Cytokine-induced ICAM-1 expression at sites of inflammation may facilitate lymphocyte adhesion and responsiveness and may also have a role in regulating lymphocyte trafficking (16–20).

Cytokines produced by lymphocytes and monocytes in the insulitis lesion are likely to play an important role in regulating immune responsiveness to the islet beta cell in type 1 diabetes. IFN-$\gamma$ and TNF-$\alpha$ have been shown to upregulate the expression of the MHC proteins on beta cells in vitro (21–23) and to induce IL-6 production by islet cells (24). In addition, IFN-$\gamma$, TNF-$\alpha$, and IL-1 alone or in combination directly impair beta-cell function and viability in vitro (25, 26). In view of the apparent importance of cellular autoimmune in the pathogenesis of type 1 diabetes and cell surface adhesion in the induction, expression, and regulation of the immune response, our aim was to determine whether isolated human endocrine islet cells, in particular beta cells, could be induced to express ICAM-1 by exposure to cytokines or reovirus infection. The rationale for testing reovirus was based on the observation that this virus induces pancreatic autoimmune diabetes in mice (27) and upregulates class I MHC protein expression by beta cells in vitro (28).

MATERIALS AND METHODS

Islet Isolation and Culture. Human pancreatic islets were isolated by a ductal collagenase digestion technique (29) from pancreata (obtained with the consent of relatives) of three organ donors. Donor 1 was a woman, aged 57 yr, with tissue type A2 A3 B7 B37 DR2. Donor 2 was a man, aged 45, with tissue type A2 A3 B60 B18 DR3. Donor 3 was a woman, aged 45.

Abbreviations: ICAM-1, intercellular adhesion molecule 1; LFA-1, lymphocyte function-associated antigen 1; IFN-$\gamma$, interferon $\gamma$; TNF-$\alpha$, tumor necrosis factor $\alpha$; IL-$\beta$, interleukin $\beta$; MHC, major histocompatibility complex; FBS, fetal bovine serum; BSA, bovine serum albumin; PBS, phosphate-buffered saline.

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aged 55, with tissue type A3 A29 B49 B57 DR1,7. Pancreas procurement and all subsequent procedures were performed according to protocols approved by Institutional Ethics Committees. To ensure purity, following Ficoll density gradient centrifugation individual islets were handpicked with the aid of a dissection microscope and cultured free-floating in plastic Petri dishes (Sterilin Middlesex, Middlesex, U.K.). Groups of ≈500 islets were cultured in 7 ml of RPMI-1640 with 5% fetal bovine serum (FBS) and incubated at 37°C in an atmosphere of 5% CO₂ in air. Medium was changed twice weekly. Experiments were commenced by the addition of fresh medium with or without IFN-γ and/or TNF-α (each at 250 units/ml) and islets were cultured for a further 3 days. Half the supernatant medium was removed daily and replaced with an equal volume of fresh medium with or without cytokine(s). Recombinant human IFN-γ (2.7 × 10⁵ units/mg) and TNF-α (5.02 × 10⁵ units/mg) were kindly provided by H. Michael Shepard (Genentech). For virus infection studies, reovirus type 3 (Dearing strain) was grown, passaged, and used to infect the human islets as described (28).

Preparation of Islet-Cell Suspensions. Islets were collected and washed twice in ice-cold Ca²⁺/Mg²⁺-free Hanks’ balanced salts solution containing EGTA (2 mM) and bovine serum albumin (BSA, 2 mg/ml), resuspended in 1.0 ml of this buffer containing 0.05% trypsin, and then incubated at 37°C for 10 min. The islets were dispersed by brief aspiration and exposure through a pipette and the resultant cells, after washing, were incubated for 60 min at 37°C in culture medium to allow for recovery.

Monoclonal Antibodies. Murine antibody W-CAM-1 (IgG2b) directed against ICAM-1 was prepared and characterized as described (17). Two murine monoclonal antibodies were purchased from Serotec: W6/32 (IgG2a) to a framework determinant on human class I MHC proteins; OK-6 (IgG1) to rat class II MHC proteins, used as a control for nonspecific binding in cell surface immunofluorescence studies. Murine antibody 9BG5 (IgG2a) to the hemagglutinin protein of reovirus 3 (30) was used as a control in the immunoprecipitation experiments.

Cell Surface Immunofluorescence and Flow Cytometry. Aliquots of cell suspensions (∼5 × 10⁵ cells) were stained by standard indirect immunofluorescence methods. In brief, cells were incubated at 4°C for 30 min with a saturating concentration of monoclonal antibody. After washing, cells were incubated with 30 μl of fluorescein-conjugated antiserum immunoglobulin (Dakopatts, Copenhagen) at 4°C for 30 min. For flow cytfluorimetry, ∼10,000 stained cells were analyzed in a FACS II (Becton Dickinson).

Double Indirect Immunofluorescence and Microscopy. To identify ICAM-1 expression by specific islet cells, surface-stained cells were fixed in phosphate-buffered saline (PBS: 0.149 M NaCl/16 mM Na₂HPO₄/4 mM NaH₂PO₄, pH 7.3) containing 1% formalin and 0.1% BSA for 30 min at 4°C. After washing in PBS containing 0.1% BSA, cells were centrifuged onto glass slides, postfixed in 70% ethanol, washed, and incubated with either guinea pig anti-insulin serum (diluted 1:50 in PBS/BSA; Miles) or a monoclonal antibody that recognizes somatostatin (SOM 018; Novo BioLabs, Bagsvaerd, Denmark). The cells were then washed and incubated with rhodamine-conjugated second antibody: anti-guinea pig immunoglobulin (Commonwealth Serum Laboratories, Melbourne, Australia) or anti-mouse immunoglobulin (Silenus, Melbourne, Australia), respectively. Staining was visualized through a fluorescence microscope (Axiopt model; Zeiss) with appropriate filters for fluorescein and rhodamine.

[S]³Methionine Labeling and Cell Lysis. Groups of ∼5000 islets were cultured in 7 ml of Dulbecco’s modified Eagle’s medium (DMEM) containing 5% FBS with or without IFN-γ and TNF-α (each at 250 units/ml) for 8 hr. After 8 hr, islets were collected, washed once in methionine-free DMEM, and transferred to corresponding cultures with 5 ml of methionine-free DMEM containing 5% FBS and 500 μCi (18.5 MBq) of [³⁵S]methionine (New England Nuclear) with or without IFN-γ and TNF-α. Cultures were maintained at 37°C for a further 16 hr in an atmosphere of 5% CO₂ in air. The islets then were washed once in complete DMEM containing 5% FBS and twice in PBS containing 0.1% BSA, then centrifuged at 1500 × g for 1 min. The islet pellet was placed on ice prior to solubilization in 1.2 ml of ice-cold lysis buffer [50 mM Hepes, pH 7.5/150 mM NaCl/1.5% (vol/vol) Triton X-100/10% (vol/vol) glycerol/1.5 mM MgCl₂/1 mM EDTA/1 mM phenylmethylsulfonyl fluoride containing 1000 kcal-kilimk-inhibitory units of aprotonin and 10 μg of leupeptin per ml] for 2 hr at 4°C. The lysate was centrifuged at 20,000 × g for 30 min and the pellet was discarded.

Immunoprecipitation. Lysates were precleared for 1 hr at 4°C with 200 μl of protein A-Sepharose CL-4B (Pharmacia) that had previously been incubated with monoclonal antibody 9BG5. The immunoadsorbent was removed by centrifugation and 200-μl aliquots of the supernatant, containing 32 × 10⁵ cpm in trichloroacetic acid-precipitable protein, were incubated for 2 hr at 4°C with 40 μl of protein A-Sepharose CL-4B that had been preincubated with monoclonal antibody 9BG5, W6/32, or W-CAM-1. The precipitates were washed once in a high-salt buffer (PBS/0.5 M NaCl with 0.2% Triton X-100) and twice in low-salt buffer (PBS with 0.2% Triton X-100). Bound proteins were eluted from the immunoadsorbent by boiling for 3 min in sample buffer (62.5 mM Tris-HCl, pH 6.8/2% NaDodSO₄/10% glycerol/100 mM dithiothreitol/0.01% bromophenol blue) and then subjected to NaDodSO₄/7.5% PAGE (31) and autoradiography.

Preparation and Blot Hybridization Analysis of Total Cytoplasmic RNA. Approximately 1000 islets previously exposed to IFN-γ and/or TNF-α for 24 hr were washed twice with Heps-buffered Krebs-Ringer bicarbonate buffer containing 0.1% BSA, placed on ice, and lysed with 750 μl of homogenization buffer (10 mM Tris-HCl, pH 7.6/10 mM EDTA/5 M guanidinium thiocyanate/5% 2-mercaptoethanol) by aspiration and expulsion through a needle and syringe; 0.15 g of CsCl and 30 μl of sarcosyl (N-lauroylsarcosine) were then added and the homogenization was repeated. The homogenate was layered over 0.3 ml of 5 M CsCl and ultracentrifuged (100,000 × g, 16 hr, 18°C). The pellet was resuspended in 100 μl of diethyl pyrocarbonate-treated water and the RNA was recovered by ethanol precipitation. RNA (10 μg per lane) was fractionated in 1% agarose/6.6% formaldehyde minigel slices and transferred to Biotrace RP nylon membrane (Gelman). A cDNA probe for ICAM-1 (500-base-pair internal Smu I fragment; ref. 32) was labeled by nick-translation with [α-³²P]ATP (Amersham) to a specific activity of 8 × 10⁶ cpm/μg.

RESULTS

IFN-γ and TNF-α Induce Surface Expression of ICAM-1 by Islet Cells. Islet cells from control cultures stained for ICAM-1 did not show significant fluorescence above background levels as judged by flow cytfluorimetry (Fig. 1). In contrast, following exposure to IFN-γ and/or TNF-α, a pronounced shift to the right in the fluorescence profile was seen, the order of potency being IFN-γ plus TNF-α > IFN-γ > TNF-α. In the experiment shown, exposure of islets to IFN-γ plus TNF-α resulted in >85% of the cells being stained for ICAM-1, with a 7-fold increase in the mean fluorescence intensity. Staining for class I MHC proteins, present on control cells, increased significantly after exposure of the islets to cytokines, with IFN-γ plus TNF-α > IFN-γ > TNF-α (Fig. 1).

Islets infected with reovirus 3 did not show any increase in fluorescence staining for ICAM-1 above control levels (Fig. 2). In contrast, staining for class I MHC proteins increased 2-fold on reovirus 3-infected cells compared with uninfected cells (Fig. 2).
Indirect double-label immunofluorescence was used to determine whether the expression of ICAM-1 was a property of the islet beta (insulin) cell or also occurred on islet delta (somatostatin) cells. After exposure to IFN-γ both beta and delta cells stained brightly for ICAM-1 (Fig. 3); fluorescent staining for ICAM-1 was not observed on control cells.

**IFN-γ and TNF-α Induce Islet-Cell Synthesis of ICAM-1.** Control islets and islets exposed to IFN-γ and TNF-α were metabolically labeled with [35S]methionine. Monoclonal antibody W-CAM-1 (anti-ICAM-1) precipitated a protein of Mr 97,000 from lysates of cytokine-treated cells, but not from control lysates (Fig. 4). Monoclonal antibody W6/32 (anti-MHC class I) precipitated a protein of Mr 45,000 that was significantly increased in islets exposed to IFN-γ and TNF-α. W6/32 also precipitated a protein of Mr 97,000 from islets exposed to IFN-γ and TNF-α. No proteins were specifically precipitated by monoclonal antibody 9BG5 (anti-reovirus 3).

**IFN-γ and TNF-α Induce Islet-Cell mRNA for ICAM-1.** In blot analysis of RNA from islets exposed to IFN-γ and TNF-α, the ICAM-1 cDNA probe hybridized to an mRNA species of 3.4 kilobases (kb) (Fig. 5). The intensity of the 3.4-kb species was significantly increased in islet cells exposed to IFN-γ plus TNF-α, and after prolonged autoradiography, a minor species of 2.2 kb was also observed. In Raji cells (Burkitt lymphoma line, used as a positive control) the probe hybridized to a major mRNA species of 3.9 kb and a minor species of 2.2 kb.

**DISCUSSION**

ICAM-1 belongs to a family of cell adhesion molecules that includes neural cell adhesion molecule (NCAM) and myelin-associated glycoprotein (MAG) (33). In general these cell surface adhesion molecules are thought to specify cell–cell interactions in embryogenesis and histogenesis. The apparent absence of ICAM-1 on normal adult endocrine islet cells and its induction by the immunoinflammatory mediators IFN-γ and TNF-α suggests that one role for ICAM-1 is to facilitate interaction between cells of the immune and endocrine systems. Specifically, ICAM-1 expression on endocrine cells such as the beta cell may lead to the binding of LFA-1+ T and B cells and monocytes. Our findings are therefore relevant to understanding the molecular processes associated with beta-cell destruction in autoimmune type 1 diabetes.

Molecular characterization of the cytokine-induced ICAM-1 synthesized by islet cells revealed a protein with Mr 97,000. This compares with reported (15) ICAM-1 Mr values of 100,000 for fibroblasts and 114,000 for phorbol ester-stimulated U-937 (monocyte-like) cells. ICAM-1 thus displays molecular weight heterogeneity in different cell types, the basis of which appears to be the result of differential glycosylation of a common protein (15). In addition to the Mr 45,000 heavy chain of the MHC class I protein, monoclonal antibody W6/32 also precipitated a protein of Mr 97,000 from islet cells exposed to IFN-γ and TNF-α. It is tempting to speculate that this represents ICAM-1 coprecipitated by W6/32 because of its proximity to or even association with the MHC class I protein. The MHC class I proteins have been reported to associate with a number of other proteins on the
FIG. 3. Immunofluorescence localization of ICAM-1 expression by human beta and delta cells. Isolated islets were cultured as described in Fig. 1. ICAM-1 expression (a-c) was visualized on beta cells stained for insulin (d and e) or on delta cells stained for somatostatin (f) by double indirect immunofluorescence photomicroscopy. Stained cells were derived from control (a, d) or IFN-γ-plus-TNF-α-treated (b, e and c, f) islets. Corresponding cells are indicated by arrows. (×700.)

cell surface, including insulin (34) and epidermal growth factor (35) receptors and the CD8 molecule (36).

The induction of islet-cell surface-expressed ICAM-1 by IFN-γ and/or TNF-α was mirrored by a corresponding increase in the expression of 3.3-kb mRNA for ICAM-1. This suggests that the induction of ICAM-1 in islets by cytokines is a transcriptional event. The sizes of the heavy (3.3-kb) and light (2.2-kb) mRNA species for ICAM-1 were similar to those for ICAM-1 in Raji cells, and in a variety of other cell types (32, 33).

The present findings add to the growing list of beta-cell functions modulated by IFN-γ and TNF-α. These include induction of MHC protein expression (21, 23), IL-6 production (24), and impairment of insulin secretion and viability (26). The specificity of beta-cell destruction in type 1 diabetes, in contrast to the pleiotropic effects of IFN-γ and TNF-α, implies that cytokines might be delivered directly from effector mononuclear cells into the beta cell (37, 38), as described for the interaction between helper T cells and B cells (39). The finding that beta cells express the lymphocyte recognition/adhesion protein ICAM-1 adds further strength to this hypothesis.

It is of interest that reovirus 3 infection of islet cells does not lead to induction of ICAM-1 expression. In previous studies (28), confirmed here, reovirus 3 infection significantly enhanced the expression of class I MHC proteins by beta cells. Stimulation of class I MHC protein can therefore be dissociated from induction of ICAM-1 on beta cells. Further, the lack of ICAM-1 induction in reovirus 3-infected islet cells suggests that ICAM-1 expression is not a nonspecific response to
injury. We cannot rule out the possibility that viruses other than reovirus 3 could induce ICAM-1 upon infection of islet cells. In this regard, induction of ICAM-1 expression has been observed for dermal fibroblasts and bone marrow stromal cells after transformation with the large tumor antigen of simian virus 40 (J. R. Novotny, personal communication).

Presumably, targeting to a beta-cell autoantigen accounts for the cellular specificity of the immune reaction in type 1 diabetes, although identification of the definitive autoantigen is lacking. It has become clear that the beta cell, in response to cytokines such as IFN-γ and TNF-α, can be induced to express many of the properties of more classical antigen-presenting cells. The expression of ICAM-1 and class I and class II MHC proteins (21, 23) and the production of IL-6 (24) constitute a triad of acquired properties that we hypothesize could equip the beta cell to (i) bind lymphocytes and/or monocytes (ICAM-1), (ii) present autoantigen (MHC), and (iii) provide costimulation for immune activation (IL-6). Although islet cells other than beta cells can, as shown here, be induced to express ICAM-1 and could presumably bind lymphocytes and monocytes, such binding in the absence of class II MHC protein (4, 40) and a target autoantigen might not elicit immuneocyte activation and consequent target-cell destruction.

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