TNF-α Regulated MAdCAM-1 Expression in Pancreatic Microvessel Endothelium: A Possible Role for MAdCAM-1 in Pancreatitis?

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Abstract: The Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1) is associated with active inflammatory bowel disease; however MAdCAM-1 expression in the pancreas has not been previously documented. This study investigated MAdCAM-1 expression during cerulein-induced pancreatitis, and examined MAdCAM-1 expression, regulation and function in cytokine-treated pancreatic endothelial cells. Acute pancreatitis was induced by serial injections of cerulein over 10h, at which point tissue samples were collected. Pancreas endothelial cells (PMEC) were prepared from H-2Kb-tsA58 (Immortomouse) mice. MAdCAM-1 expression was examined by immunoblotting after cytokine (TNF-α, IL-β or IFN-γ) stimulation. 2nd messengers regulating MAdCAM-1 were studied by adding pharmacological blockers prior to cytokines. MAdCAM-1 dependent lymphocyte adhesion was monitored using α4β7-integrin expressing lymphocytes. In cerulein-pancreatitis, MAdCAM-1 was upregulated by 10h, and closely correlated with histopathology. In PMEC, only TNF-α induced MAdCAM-1 dose (0-20ng/ml) and time (≥12h-48h) dependently. MAdCAM-1 expression was PKC, tyrosine kinase, p38 MAPK, and NF-κB-PARP dependent. PMEC-lymphocyte adhesion was increased by TNF-α, and significantly reduced by MAdCAM-1 antibody. MAdCAM-1 is induced in the inflamed pancreas, and in TNF-α stimulated PMEC, and may represent a novel determinant of pancreatic-lymphocyte recruitment in inflammation. PMEC cells might provide a useful system to study mechanisms related to both acute and chronic pancreatitis.

Keywords: Pancreatitis, inflammation, cytokine.

INTRODUCTION

Acute pancreatitis (AP) is a disease with very high morbidity and high mortality (up to 20-30% in severe AP) with no specific therapy beyond palliative supportive treatment [1]. The severe necrotizing form of AP is considered to be a multi-stage disease, where the pathological events beginning at the acinar cell level, are paralleled by an exaggerated local but also systemic inflammatory response [2]. As a result, AP is frequently associated with remote injury to the lung that presents clinically as ‘adult respiratory distress’ or ‘ARDS’ [3, 4]. Generally, AP is thought to result from the premature and/or excessive intra-acinar zymogen activation by several stimuli including an exaggerated and inappropriate secretion of toxic oxidants, enzymes and bacteriostatic/bactericidal factors by several classes of activated leukocytes [5]. After the initiation of acinar cell injury, inflammatory cells penetrate the pancreatic interstitium first by adhering to activated endothelium, and then extravasating into the tissues. Leukocytes adhere to endothelial cells during inflammation after exposure to a variety of chemical mediators and inflammatory cytokines released at sites of tissue damage. These

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cytokine therapies may be effective avenues of treatment for AP.

**MATERIALS AND METHODS**

**Isolation of Pancreatic Endothelial Cells**

PMEC were isolated by using a modification of the technique reported by Jat et al. [25]. Mice, homozygous for a temperature-sensitive SV40 large T antigen (ImmortalMice; CBA/ca X C57Bl/10 hybrid; Charles River Laboratories, Wilmington, MA), were sacrificed by ketamine/xylazine overdose. Pancreas tissues were minced into small (1 mm) fragments in 1ml HBSS and digested in 0.25% type IV collagenase (Sigma Chemical Co., St. Louis, MO), at 4°C for 24h. Tissues were then immersed in a 37°C water bath for 1 h with intermittent shaking every 15 minutes. Vessel fragments were filtered across 100 μm nylon mesh into a 50ml polypropylene centrifuge tube; microvessel fragments were captured on 20μm nylon screening. The filtered fractions were centrifuged at 1500 RPM for 5 min at 10°C. The supernatant was discarded, and the pellet was resuspended in MEM D-valine (Promo Cell, Heidelberg, Germany) supplemented with 10% fetal bovine serum, 2-mM L-glutamine, nonessential amino acids, MEM-vitamin solution and 1% antibiotic/antimycotic (Life Technologies, Inc., Rockville, MD). Cells were plated onto 25-cm² flasks (coated previously with 2% gelatin). Twenty-four hours later, the medium was discarded, and fresh 10% DMEM containing 10 units/ml IFN-γ (Pierce Biotechnology Inc., Rockford, IL) was added. The addition of IFN-γ was used to activate the MHC H-2Kb class I promoter, to induce the expression of large T antigen in 'Immortomouse' derived cell cultures [25]. Primary endothelial cultures were incubated at 33°C in 7.5% carbon dioxide, and media replaced twice weekly. Primary isolates were grown to confluence (~10–20 days), and medium was changed to MEM D-valine supplemented with 10% fetal bovine serum, 2-mM L-glutamine, non-essential amino acids, MEM-vitamin solution and 1% antibiotic/antimycotic also containing 10 units/ml IFN-γ. Cells were grown on gelatin-coated T-25 flasks or gelatin-coated glass coverslips. After cultures reached confluence, they were maintained in this medium and sub-cultured weekly.

**Immunofluorescent Staining for VCAM-1 and Dil-Ac-LDL in PMEC**

At confluence, PMEC cultured on gelatin-coated glass coverslips were fixed in 95% ethanol for 30 minutes (on ice), and extracted in 100% acetone (for 1 minute) and air dried. Primary antibody for mouse anti-VCAM-1 (1: 250) (Endogen, Rockford, IL) in 0.1% milk powder in PBS was incubated with coverslips for 1 hour at 37°C. Coverslips were washed with 0.1% milk/PBS solution for 5 minutes 3 times each. Secondary antibody (donkey-anti-mouse FITC diluted 1: 200 in 0.1% milk/PBS, Jackson Immunoresearch Laboratories, West grove, PA) was incubated at 37°C for 1 hour. Coverslips were washed with 0.1% milk/PBS solution for 5 minutes (3 times), mounted in glycerol-PBS and sealed with nail polish.

The receptor mediated endocytosis of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate acetylated low-density lipoprotein (Dil-Ac-LDL) (Biomedical Technologies Inc., Stoughton, MA) was investigated in PMEC. Dil-Ac-LDL (10μg/ml) was added to the culture dish and was incubated for 4 hours. The dishes were washed three times in medium and were visualized immediately by fluorescence microscopy.

**Treatment of PMEC Cells with Cytokines**

To determine how PMEC respond to cytokines, confluent monolayers were treated with either TNF-α, IL-1β or IFN-γ in complete cell culture medium for different amounts of time (up to 48 hours), and at different concentrations (IL-1β 0-100 ng/ml; IFN-γ, 0-5000 U/ml). Bioactivity of the 3 cytokines used in this study were confirmed in parallel adhesion molecule expression studies (data not shown). In some experiments, PMEC cells were pre-treated with pharmacological blockers for 1h prior to addition of cytokines to investigate which 2nd messages mediate cytokine induced MAdCAM-1 protein transcription/translation in PMEC. Similar PMEC stimulation was also carried out in lymphocyte adhesion studies. In adhesion studies, stimulating cytokines were removed in prior addition of labeled lymphocytes.

**Western Analysis of Cell Lysates**

Western blotting was performed as previously described. Briefly, protein samples (75 μg each) were separated on 7.5% SDS-PAGE gels and were electro-blotted to nitrocellulose membranes. Equal protein content per lane was confirmed by reversibly staining the nitrocellulose blots with Ponceau Red-S dye (Sigma, St. Louis, MO) at 37°C for 15 min. The cells were washed twice with ice-cold HBSS, to remove unincorporated fluorescence and rescein diacetate (FDA) per ml (Sigma-Aldrich, St. Louis, MO) at 37°C for 15 min. The cells were washed twice with ice-cold HBSS, to remove unincorporated fluorescence and suspended in HBSS. The lymphocyte cell line used in this assay expresses high levels of the α4β7 integrin, which can interact with multiple ligands including mucosal addressin-1 (MAdCAM-1), but also VCAM-1, L-selectin and fibronectin [27, 28]. In this system, TNF-α stimulated TK-1 adhesion to endothelial cells was at least 50% MAdCAM-1 mediated.

**TK-1 Lymphocyte Adhesion Assay**

Mouse lymphocytes (Mouse CD8+ T cell lymphoma tyrosine kinase (TK)-1 cells) that constitutively express integrin α4β7 were a generous donation by Dr. Eugene Butcher (Stanford Univ., Stanford, CA) [26]. These cells were cultured in RPMI medium supplemented with 10% FCS, 2 mM L-glutamine, and 0.05 mM 2-mercaptoethanol. TK-1 cells, suspended in culture medium, were fluorescence labeled by incubating 2 × 10⁶ cells/ml with 0.02 mg fluoresein diacetate (FDA) per ml (Sigma-Aldrich, St. Louis, MO) at 37°C for 15 min. The cells were washed twice with ice-cold HBSS, to remove unincorporated fluorescence and suspended in HBSS. The lymphocyte cell line used in this assay expresses high levels of the α4β7 integrin, which can interact with multiple ligands including mucosal addressin-1 (MAdCAM-1), but also VCAM-1, L-selectin and fibronectin [27, 28]. In this system, TNF-α stimulated TK-1 adhesion to endothelial cells was at least 50% MAdCAM-1 mediated.
injected with cerulein (50 μg/kg) 1 X every hour for 8 hours. Mice were sacrificed 1 hour after the last cerulein injection. Pancreas tissue was frozen at -20ºC in OCT embedding medium and frozen sections (10 μm) were cut. Sections were immunostained with MAdCAM-1 (MECA-367) mAb. We found that unlike VCAM-1 (see Fig. 1C), MAdCAM-1 was not constitutively expressed on unstimulated PMEC, but was potently induced by TNF-α (20 ng/ml; Fig. 2A). Interestingly, MAdCAM-1 was not expressed in the absence of treatment with either IL-1β (10 ng/ml; Fig. 2A) or IFN-γ (1000 U/ml; Fig. 2A). Bioactivity of IFN-γ was confirmed by cell ELISA.

Fig. (1). (A) Phase contrast image of pancreatic microvessel endothelial cells (PMEC). (B) PMEC receptor mediated endocytosis of Dil-AC-LDL. (C) PMEC VCAM-1 immunofluorescence. (200X magnification).

2nd Messages Involved in TNF-α-Induced MAdCAM-1

G-132 was purchased from Biomol (Plymouth Meeting, PA) [29]. GPI-6150, a poly-ADP-ribose polymerase (PARP) inhibitor, was obtained from Guilford Pharmaceuticals (Perryton, PA) [29]. PMEC were pretreated with either proteosome inhibitor (MG-132), poly-ADP ribose polymerase inhibitor (GPI-6150), tyrosine kinase inhibitor (genistein), mitogen/extracellular signal-regulated kinase (MEK)-1 inhibitor (PD-98059), a PKG inhibitor (KT-5823) or a cytochrome P450 inhibitor (itraconazole, Ortho biotech Products, Raritan, NJ). These drugs were added to PMEC 1 hour before stimulation with TNF-α to induce MAdCAM-1 protein expression.

Cerulein Model of Pancreatitis

C57BL/6J mice were purchased from Jackson Labs (Bar Harbor, Maine). Cerulein was purchased from Bachem, Inc. (Torrance, CA), and cerulein induced pancreatitis was induced as described by Ethridge et al. [32]. Briefly, mice (under ketamine/xylazine anesthesia) were intraperitoneally injected with cerulein (50 μg/kg) 1 X every hour for 8 hours. Mice were sacrificed 1 hour after the last cerulein injection. Pancreas tissue was frozen at -20ºC in OCT embedding compound and 10 μm frozen sections stained for MAdCAM-1 immunofluorescence. This study was reviewed and approved by the LSUHSC-Shreveport Animal Care and Use Committee.

Statistical Analysis

All values are expressed as mean ± SD. Data were analyzed using One-way Analysis of Variance (ANOVA) with Dunnett’s post-testing. Probability (P) values of < 0.05 were considered significant.

RESULTS

Pancreatic Endothelial Cell Line

We established the pancreatic endothelial cell line ‘PMEC’ from ‘Immortomouse’ (H-2Kb-tsA58) mice in which all cells express the transgene for a temperature-sensitive SV40 large T antigen. Fig. (1A) shows a phase-contrast image of this cell line, which shows a uniform cobblestone appearance. These cells also demonstrated endocytosis of Dil-AC-LDL (Fig 1B). These cells also positively immunostained for m-VCAM-1 (Fig. 1C) and expressed MAdCAM-1 following TNF-α stimulation. These properties confirm that these cells have an endothelial origin. Histopathological staining showed that in passage 8 cultures there were up to 4.5% desmin-positive cells.
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...occurred at 20 ng/mL. TNF-α (20ng/ml) for 8, 24 or 48h also caused a time-dependent increase in MAdCAM-1; maximal

Fig. (2). Immunoblotting for MAdCAM-1 in PMEC. PMEC were incubated with TNF-α (20ng/ml), IL-1β (10ng/ml) or IFN-γ (1000U/ml) for 24h. Immunoblotting of total cellular protein extracted from PMEC was performed. *p<0.001 vs TNF-α.

MAdCAM-1 expression occurred at 48h (Fig. 4). Both Figs. (3,4) show approximately a 10-fold increase in MAdCAM-1 expression following TNF-α treatment (compared to untreated controls). On the other hand, MAdCAM-1 was not induced in PMEC by incubation for up to 24 hours with either IL-1β or IFN-γ Fig. (3B)).

Effect of Signal Transduction Blockers on MAdCAM-1 Expression

To determine the cytoplasmic signals mediating TNF-α-induced MAdCAM-1 protein expression, immunoblotting was performed with pharmacological pretreatments against specific signal pathways. Preincubation of PMEC with the proteosomal inhibitor MG-132 (5 μM) significantly decreased TNF-α-induced MAdCAM-1 protein expression (Fig. 5). TNF-α-induced MAdCAM-1 protein induction was also blocked by the classical-PKC blocker, GO6976 (100 nM), a p38 MAPK blocker, SB-202190 (10 μM), a non-isotype selective tyrosine kinase inhibitor, genistein (30 μM) as well as by the poly-ADP-ribose polymerase inhibitor, GPI-6150 (60 μM). MAdCAM-1 induction by TNF-α was not affected by either a protein kinase G inhibitor, KT-5823 (0.5 μM) or a p42/44 MAP kinase inhibitor PD-98059 (20 μM). MAdCAM-1 was also significantly attenuated by the CYP450 antagonist itraconazole (5 μM) (Fig. 5). None of the tested inhibitors induced expression of MAdCAM-1 in the absence of TNF-α (data not shown).

MAdCAM-1 Expressed on the Surface of Pancreatic Endothelial Cells is Functional

Having established a role for several second message signals in cytokine induced MAdCAM-1 expression by PMEC, we next examined the adhesion of the mouse lymphocyte cell line TK-1 which expresses α4β7, a MAdCAM-1 ligand, to TNF-α-treated PMEC. Fig. (6) shows that control adhesion of TK-1 cell adhesion increased 154% by TNF-α; this adhesion was inhibited by preincubation of PMEC cells with anti-MAdCAM-1 antibody (10 μg/ml), (P < 0.05 vs TNF-α treatment). We found that 73.2% of the TNF-α-induced TK-1 adhesion was MAdCAM-1 dependent. Importantly, we also found that an isotype matched IgG did not reduce TK-1 adhesion as MAdCAM-1 antibody did.

MAdCAM-1 Expression in Cerulein Pancreatitis

Our in vitro findings paralleled injury in the cerulein-induced model of pancreatitis. We examined MAdCAM-1 expression in PMEC treated with cerulein. As shown in Fig. 7, cerulein treatment induced a time-dependent increase in MAdCAM-1 expression. This induction was blocked by the proteosomal inhibitor MG-132 (5 μM) and the classical-PKC blocker, GO6976 (100 nM), but not by the p38 MAPK blocker, SB-202190 (10 μM), indicating that the proteosomal pathway is necessary for cerulein-induced MAdCAM-1 expression.
model of pancreatitis in vivo. We observed visible differences in pancreatic structure between control tissues and those from mice receiving consecutive injections of cerulein (Fig. 7). Cerulein treatment promoted cell swelling and detachment from their neighbors at foci of leukocyte infiltration; (control Fig. (7A) vs Fig. (7B) visualized by hematoxylin/eosin). The inset in Fig. (7B) shows a mass of infiltrated leukocytes obliterating other structures in the inflamed pancreas (arrow); this is in contrast to the inflamed but still structurally recognizable tissues seen in Fig. (7B). Similarly, MAdCAM-1 staining was not seen in control tissues, but was observed in cerulein treated pancreas tissue (Fig. 7C vs Fig. 7D).

**DISCUSSION**

**Fig. (4).** Time-dependent TNF-α-induced MAdCAM-1 expression. Confluent endothelia were incubated with 20 ng/mL TNF-α for the time periods indicated. Expression of MAdCAM-1 protein increased at 12, 24 and 48 h. (Maximum expression [100%] in this set of experiments was set as the scan density at 48 hours). In each graph, the t=0 time point represents non-stimulated pancreatic endothelial cells. Each value represents the mean ± s.e.; each group (n=3). *P < 0.001, **P < 0.05 vs TNF-α treatment.

**Fig. (5).** Effect of signal transduction blockers on MAdCAM-1 induction by TNF-α in PMEC. Cells were pretreated with Go 6976(100nM), SB-202190 (10 μM), genistein (30 μM), PD-98059 (20 μM), MG-132 (5 μM), GPI-6150 (60 μM) or KT-5823 (0.5 μM) and then exposed to TNF-α (20 ng/ml) stimulation in the continued presence of inhibitors. MAdCAM-1 expression is significantly increased after TNF-α. Go 6976, SB-20219, genistein, MG-132 or GPI-6150 blocked the increase of MAdCAM-1 induced by TNF-α. *=p<0.001, **=p<0.01, significance vs TNF-α. Each value represents the mean ± s.e.; each group (n=6). *P < 0.05 vs TNF-α treatment.

**Fig. (6).** TNF-α Induced TK-1 Adhesion to PMEC. TNF-α significantly increased TK-1-pancreas endothelial cell adhesion. This increased adhesion was significantly inhibited by pre-treatment with MECA-367 (mouse MAdCAM-1) Ab (10mg/ml), but not by the addition of an isotype matched pre-immune IgG (not significant). Each value represents the mean ± s.e.; each group (n=6). *P < 0.05 vs TNF-α treatment.

**Fig. (7).** Expression of MAdCAM-1 in experimental pancreatitis. (A,B) phase photos of normal and cerulein-induced AP; (C,D) show MAdCAM-1 immunostaining for control and cerulein-induced AP respectively (200X magnification).
In our present study, we created a pancreatic endothelial cell line using endothelial selective medium containing D-valine with endothelial specific growth factors. It was reported that in D-valine supplemented media with endothelial specific growth factors, cultures are selected for endothelium and depleted of fibroblasts and other contaminating cell types [33, 34]. The mouse strain (H-2Kb-tsA58; Immortomouse) has been used extensively to generate several immortalized mouse cell lines for cell-based studies [35]. This mouse provides a source of nearly any selectable cell population, and avoids most of the limitations encountered when using immortalized agents including the sites of integration of genes into host genome and duplication of genes [25].

This cell model expresses a temperature sensitive T antigen and allows the elimination of T-antigen, its growth promoting activity and other phenotypic effects when the culture temperature is raised to 39°C. Immortomouse large T-antigen is under the control of IFN-γ. IFN-γ can alter several endothelial characteristics, like adhesion molecule expression, growth and survival. Therefore, because of this possible problem, we usually maintained our cells at the permissive temperature (33°C) and then cultured cells for 24 Hours at 37°C before performing experiments (which inactivates most of the temperature sensitive SV40 T-antigen). In addition to temperature treatment, we also removed IFN-γ from the culture medium for approximately 24 hours before initiating experiments [51, 52].

Our PMEC formed cobblestone monolayers at confluence (Fig. 1A). These cells were all immunostained by anti-VCAM-1 antibody (Fig. 1C) and showed positive metabolic uptake of Dil-AC-LDL (Fig. 1B). These results confirmed the identity of these cells as microvessel endothelial cells derived from the pancreas.

Late sepsis-related deaths resulting from infectious pancreatic necrosis are reduced by antibiotic treatment. Some early deaths may still occur because of severe remote multi-organ injury [38]. In AP, immune cells are the main triggers of the acute systemic inflammatory response that determines disease severity. However, the pathways through which the deleterious effects of pancreatic inflammation provoke distant organ injury are unclear. Activated pancreatic macrophages may release inflammatory cytokines (e.g., IL-1, IL-6 and TNF-α) as a response to local tissue injury [39]. These cytokines would be anticipated to aggravate local pancreatitis, and systemically may increase capillary permeability, recruit leukocytes and stimulate their extravasation [40].

Another important activity of macrophages is the presentation of antigens to T lymphocytes. IL-1β also promotes the release of IL-2 from T lymphocytes to increase lymphocyte expansion and activation [39]. Therefore local inflammatory responses could produce significant and remote effects throughout the body. We showed that MadCAM-1 was expressed on PMEC in response to TNF-α but not induced in response to IL-1β or IFN-γ (Figs. 2-4). We therefore assumed that TNF-α might be the key cytokine leading to these forms of diffuse pancreatic injury.

Acute pancreatitis is an inflammatory disease, and its severity varies widely, based on several factors which determine the clinical ‘grade’ of pancreatitis. It is generally believed that one of the earliest events in development of AP is a premature intra-acinar release of digestive zymogens and metalloproteinases which when activated damages acinar cells and help trigger forms of remote tissue injury. This concept is supported by several studies suggesting that severity in pancreatitis reflects levels of acinar cell injury [41]. This injury includes inflammatory cell recruitment and activation, the release of inflammatory cytokines, oxidants and proteases. Leukocyte-endothelial cell adhesion, the earliest step in inflammation, precedes injury in several forms of chronic inflammation e.g. gastrointestinal inflammation [42]. However, although adhesion molecules must likely play a role in pancreatitis, there is no evidence supporting pancreatic endothelial expression of adhesion molecules, and how this might impact the course of pancreatitis as is reported in other organs. Frossard et al. showed that pancreatitis and pancreatitis-associated lung injury were remarkably decreased in mice deficient in CD40L compared with that in wild type [43] suggesting CD40L-CD40L interactions. In another study, this group showed that ICAM-1 deficient mice show reduced pancreatitis severity and associated lung injury [7]. Importantly, neutrophil depletion also reduced pancreatitis severity and remote lung injury, events independent of ICAM-1 [7]. Demols et al. showed that T lymphocytes, particularly CD4+ T-cells, play a central role in cerulein pancreatitis. These results indicate that both neutrophils and lymphocytes contribute to pancreatitis [44].

MadCAM-1 is expressed on high endothelial venules and mucosal vessels, where it guides lymphocyte traffic into Peyers patches and the intestine [45]. MadCAM-1 performs critical roles in several immune and inflammatory diseases like inflammatory bowel disease (IBD) through its ability to bind and retain β7-integrin expressing lymphocytes within the gut, where they appear to drive inflammation in IBD [16]. In this study, we showed that MadCAM-1 was expressed on the pancreatic endothelium in cerulein-induced pancreatitis (Fig. 7). The expression of MadCAM-1 was increased in mouse pancreatic endothelial cells in vitro after TNF-α stimulation (Figs. 2-5).

MadCAM-1 expression in the pancreas is somewhat unanticipated. MadCAM-1 has been previously thought to be restricted to specialized microvascular endothelial cells lining venules (high endothelial venules) and in some cases inflamed cerebral endothelium [28]. Our results indicate that MadCAM-1 expression on the pancreatic blood vessels might recruit lymphocytes to both aggravate local and remote multi-organ injury during AP.

Oshima et al. reported that TNF-α induced the expression of MadCAM-1 on lymph node derived endothelium (SVEC4-10) and bEnd.3, a brain capillary endothelial cell line, two models for evaluating mechanisms of human IBD and cerebral inflammation [28]. Prior results suggest that TNF-α-induced MadCAM-1 expression in the pancreas might also be controlled through tyrosine kinase, PKC, and perhaps PKG, MAPKs, and NF-κB, as has been reported for other endothelial adhesion molecules in colitis and IBD [17]. It is known that TNF-α receptors mobilize NF-κB and activate several levels of protein kinases, resulting in the release and proteosomal degradation of IκB from the inert NF-κB - IκB complex [23, 46]. The release of IκB permits the translocation of the activated p50/p65 NF-κB complex to the nucleus [47]. Read et al. reported that MG-132, a proteosome of the inhibitor, prevented IκBα degradation induced by
TNF-α and nuclear accumulation of NF-κB, efficiently inhibiting NF-κB function [48]. In our study, the expression of MAdCAM-1 was completely inhibited by MG-132, indicating that NF-κB helps to control MAdCAM-1 expression in PMEC. Recent studies show that NF-κB antagonist, (NF-κB antisense phosphorothioate oligonucleotide) and a 26S proteasome inhibitor successfully attenuate inflammation in experimental colitis in vivo [49, 50]. Therefore antagonists to these signaling cascades might also provide important therapeutic strategy to limit forms of MAdCAM-1-dependent inflammation. Some prior reports have suggested that NF-κB activation is critically involved in the cerulein induced model of pancreatitis. For example, Steinele et al. have suggested that NF-κB activation represents an important and direct effect of cerulein on acinar cells, rather than an indirect effect mediated by cytokines derived from activated mononuclear cells [51]. We assume that activation of NF-κB is essential in the responses to cytokines in our model, with some responses not related to cytokines as well.

We also demonstrated that MAdCAM-1 transcription and translation are regulated through the PKC, PARP, p38MAPKs, TK, and NF-κB signal cascades. MEK-1 and PKG inhibitors did not influence MAdCAM-1 expression. The signal transduction pathways in TNF-α-induced MAdCAM-1 expression are likely to differ somewhat among cell types in their sensitivity to PKC/MEK-1 inhibition. Interestingly, in previous reports, ERK1 inhibition did not block the upregulation of ICAM-1 in IL-1β-treated cardiac myocytes and fibroblasts [52]. It has also been reported that p38 MAPK may be required for NF-κB-dependent gene expression, and that inhibition of the p38 MAPK pathway prevented IL-1β-induced ICAM and VCAM translation [49, 53]. Here we have found that the signals from PKC, PARP, p38MAPKs and TK might be upstream of NF-κB in the case of TNF-α-induced pancreatic endothelial MAdCAM-1 expression.

When organ systems are exposed to intense inflammatory stimuli (e.g., cytokines), the expression of several cell adhesion molecules (CAMs), (P-selectin, ICAM-1 and VCAM-1), is often increased on the surface of the endothelium, and mediates a coordinated and sequential activation of leukocyte rolling, firm adhesion, and transendothelial diapedesis. It is known that ICAM-1 plays a significant role in leukocyte infiltration of the pancreas and that anti-ICAM-1 antibody therapy decreases acinar cell damage in experimental pancreatitis [54, 55]. Also, Nerin et al. showed the therapeutic effectiveness of chimeric TNF antibody, infliximab [56]. In our study, TNF-stimulated PMEC bind TK-1 lymphocytes directly and is decreased by anti-MAdCAM-1 antibody. Our observations are consistent with lymphocyte recruitment to the pancreas in inflammation (e.g. acute pancreatitis) as being MAdCAM-1/a4β7-integrin dependent suggesting that the MAdCAM-1/a4β7 pair bond may initiate pancreatitis. Future in vivo and in vitro may determine if interference with this binding (possibly using humanized antibodies against a4β7 e.g. Antegren/Natalizumab) might be useful prophylactic/therapeutic modalities for the treatment of pancreatitis.

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