The Journal of Immunology

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J Immunol 2008; 181:6109-6116; ;
doi: 10.4049/jimmunol.181.9.6109
http://www.jimmunol.org/content/181/9/6109

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Expression of CD86 on Human Islet Endothelial Cells Facilitates T Cell Adhesion and Migration[1]

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Pancreatic islet endothelial cells (ECs) form the barrier across which autoreactive T cells transmigrate during the development of islet inflammation in type 1 diabetes. Little is known about the immune phenotype of islet ECs that might shape their molecular interaction with autoreactive T cells before and during the development of islet inflammation. In this study we examined the expression and functional significance of costimulatory molecules by human islet ECs. Freshly isolated human islet ECs constitutively expressed CD86 (B7-2) and ICOS ligand but not CD80 (B7-1) or CD40 costimulatory molecules. The functional activity of islet EC-expressed CD86 was examined by coculture of resting islet ECs with CD4 T cells stimulated by CD3 ligation alone. Marked T cell proliferation in the coculture was completely abrogated by mAb blockade of CD86, confirming that costimulatory properties are conferred on ECs by CD86 expression. In view of its location on the vasculature, we hypothesized a role for CD86 in T cell adhesion/transmigration. In keeping with this, adhesion/transmigration of activated (CD3 ligated) memory ($CD45R0^+$) CD4 T cells across islet ECs was completely inhibited in the presence of CD86 blocking mAb. Identical results were obtained for T cell adhesion using either CTLA-4 blocking mAb or CTLA-4Ig (abatacept), indicating CTLA-4 as the T cell ligand for these CD86-mediated effects. These data suggest a novel role for CD86 expression on the microvasculature, whereby ligation of CTLA-4 on CD4 T cells by CD86 on islet ECs is key to the adhesion of recently activated T cells. ***The Journal of Immunology,*** **2008, 181: 6109–6116.**

Transendothelial migration and recruitment of autoreactive T cells into the pancreatic islets is a critical event during the development of chronic insulitis in type 1 diabetes (T1D)[3] (1). Activation of the islet endothelium by proinflammatory cytokines is a characteristic pathologic event in the development of T1D (2–4) and may either initiate or enhance subsequent leukocyte infiltration of the islets. Upon activation, endothelial cells (ECs) up-regulate the expression of cell adhesion molecules, which enable and facilitate T cell-EC interactions. Indeed, immunohistological analyses of human pancreata from newly diagnosed T1D patients have demonstrated hyperexpression of cell adhesion molecules on the islet microvasculature (4–6). A further sequel to EC activation that could play an important role in the development of insulitis is up-regulation of MHC class I and induction of MHC class II molecule expression. Immunohistological studies of inflamed islets have demonstrated that islet ECs surrounding the β cells express high levels of MHC class I and induced expression of MHC class II molecules (5–7). Although the functional importance of EC MHC expression in terms of its contribution to inflammation is not known with certainty, a considerable body of evidence suggests that inflamed ECs are capable of the processing and presentation of cognate Ag to T cells (8–10), which has potent effects on their migration both in vitro (11, 12) and in vivo (13–15).

Our own studies have added weight to this concept in relation to the development of T1D, showing that human vascular ECs process and present disease-related epitopes of the islet autoantigen glutamic acid decarboxylase-65 (GAD-65) to CD4 T cell clones in vitro, resulting in markedly enhanced transmigration (12, 16). Taken together, these findings support the hypothesis that the delivery of signal 1 (i.e., TCR ligation by peptide-MHC complexes) by inflamed ECs facilitates transmigration in vivo, enabling selective recruitment of T cells with relevant Ag specificities into the tissues. However, it could be argued that delivery of signal 1 alone by ECs, without signal 2 (i.e., costimulation) would be deleterious to cell function, because it can result in anergy (17). It is, therefore, an important matter of debate whether ECs possess the necessary repertoire of costimulatory molecules for adequate T cell activation, and what EC functionality this engenders.

In the present study we show that human islet microvascular ECs express CD86 and ICOS ligand (ICOS-L) costimulatory molecules constitutively. The expressed CD86 is functional and enables adhesion and migration of recently activated memory CD4 T cells. This novel role for costimulation may be important in the directed migration of recently activated lymph node migrant T cells, and its blockade may represent an additional mode of action in vivo of therapies that interfere with costimulation, such as CTLA-4Ig (abatacept).

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Received for publication February 11, 2008. Accepted for publication August 20, 2008.

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[1] This work was supported by grants from Diabetes U.K. and the Juvenile Diabetes Research Foundation (postdoctoral fellowship 3-2007-643 to B.L.-O.). The authors acknowledge financial support from the Department of Health via the National Institute for Health Research comprehensive Biomedical Research Centre award to Guy's and St Thomas' National Health Service Foundation Trust in partnership with King's College London.

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[3] Abbreviations used in this paper: T1D, type 1 diabetes; EC, endothelial cell; ICOS-L, ICOS ligand; MEC, microvascular endothelial cell.

Materials and Methods

Isolation and culture of human islet endothelial cells

Human islet endothelial cells were isolated from the human islets (8–10 × 10^4) of three cadaveric organ donors that were surplus to clinical transplant requirements using a modification of Ricordi's technique (18). Islet isolations were conducted in the Cell Isolation Unit at King's College Hospital, London, U.K. after obtaining informed consent and were approved by the local Ethical Review Committee. To obtain human islet endothelial cell lines, ~100,000 freshly obtained human islets that were 85–90% pure as assessed by dithizone staining were incubated at 37°C in RPMI 1640 (Invitrogen) supplemented with collagenase type I-A (1 mg/ml; Sigma Aldrich) and shaken every 15 min for 2 h as described (19). To remove larger debris, the digest was passed through a 70-μm filter and the remaining cell suspension was resuspended and washed at 300 × *g* for 10 min in RPMI 1640/2.5% FCS (Invitrogen). To obtain a single cell suspension, the resultant cell pellet was resuspended in 0.25% trypsin/1 mmol/L EDTA (Invitrogen) for 10 min at 37°C, washed, and resuspended in 80 μl of PBS/0.5% FCS supplemented with 20 μl of anti-CD105 immunomagnetic beads (Dynal). After 15 min at 4°C with an end-over-end rotation, cells were washed, resuspended in appropriate buffer, and positively selected using an MP3 magnetic cell particle separator (Dynal) according to the manufacturer's instructions. Isolated islet ECs were cultured in complete microvascular EC medium supplemented with EC growth factor (TCS CellWorks), 20% FCS, and antibiotics (Invitrogen) and grown to confluence. The purity and endothelial origin of the cultures were confirmed by the expression of EC surface markers CD105 (Serotec), CD31 (Serotec), and CD146 (Chemicon International) by flow cytometry. For all functional experiments, islet ECs were used at the earliest passages at which sufficient cells were available, and never beyond passage 5.

Analysis of the immune phenotype of islet endothelial cells

For the analysis of the surface expression of immune phenotypic markers, confluent EC monolayers were detached from wells with Accutase (TCS CellWorks), washed in PBS/2% FCS, and stained for 30 min at 4°C with fluorochrome-conjugated mouse anti-human CD40, CD54, CD62E, and CD86 (all from Serotec); mouse anti-human CD80, CD106, HLA-DR, and HLA-ABC (all BD Biosciences and BD PharMingen); and mouse anti-human ICOS-L (Lab Vision) or appropriate isotype control mAbs.

For the analysis of ICAM-1 and VCAM-1 expression using specific mAbs (Serotec), islet ECs were stimulated with IFN-γ (500 IU/ml) and TNF-α (25 ng/ml) (both from Strathmann Biotec) for 24 h. The expression of E-selectin was examined following a 4-h stimulation of islet ECs with a combination of TNF-α (25 ng/ml), LPS (100 ng/ml) (Sigma-Aldrich), and IL-1β (10 ng/ml) (Strathmann Biotec). For the up-regulation of MHC class I, islet ECs were incubated with IFN-γ (500 IU/ml) for 24–48 h; for the up-regulation of MHC class II molecules, islet ECs were stimulated with IFN-γ (500 IU/ml) for 72 h. Costimulatory molecule expression (CD80, CD86, and CD40) on islet ECs was analyzed following IFN-γ (500 IU/ml) and/or TNF-α (25 ng/ml) stimulation for 24–72 h; for ICOS-L up-regulation, islet ECs were stimulated with TNF-α (25 ng/ml) for 24 h.

Purification of CD4 T cells and CD45R0$^+$ CD4 T cells by negative selection

CD4 T cells, CD45R0$^+$ CD4 T cells, and CD45RA$^+$ CD4 T cells were purified from PBMCs using the MACS magnetic cell sorting system (Miltenyi Biotec) with the appropriate CD4 T cell isolation kits (human memory and naive CD4 T cell subset column kit and human CD4 isolation kit I and II). Human CD4 T cells were cultured in RPMI 1640 medium (Invitrogen) supplemented with 2 mmol/L L-glutamine, 10% AB, and antibiotics. The purity of the selected cell subsets was analyzed by flow cytometry and was routinely >98%.

Functional analysis of the ability of human islet ECs to costimulate CD4 T cell proliferation

Resting islet ECs (3 × 10^4/well in 100 μl of medium) were seeded on attachment factor-coated (TCS CellWorks) 96-well flat-bottom tissue culture plates and grown to confluence. Purified human CD4 T cells were labeled with 2 μmol/L CFSE (Molecular Probes), washed twice in culture medium, and stimulated with 1 μg/ml soluble anti-CD3 mAb (clone UCHT1) with or without 1 μg/ml anti-CD28 mAb (clone CD28.2) (both BD Biosciences) for 30 min at 37°C. Subsequently, 2 × 10^5 CD4 T cells/well were added to the EC monolayers in 100 μl of medium. Before adding the CD4 T cells, resting islet ECs were preincubated with 15 μg/ml anti-CD86 (BD Pharmingen) and/or 15 μg/ml anti-ICOS-L (Lab Vision) blocking mAbs or an isotype-matched control for 2 h at 37°C, then washed with warm medium thrice before adding CD4 T cells. At the same time, CD4 T cells were incubated alone and stimulated with 1 μg/ml soluble anti-CD3 mAb with or without 1 μg/ml anti-CD28 mAb for 30 min at 37°C and cultured for 5 days. Following a 5-day culture, CD4 T cells were harvested from wells using warm medium and repeated micropipetting, washed, stained with mAbs of interest, and analyzed by flow cytometry for reduction in CFSE staining intensity.

Analysis of the role of CD86 in T cell transmigration

Islet ECs (3 × 10^4/well in 100 μl of medium) were seeded onto 24-well transwell clear polyester membranes (Corning) and grown to confluence as determined by successive electrical resistance measurements using electrical voltage ohm meter (World Precision Instruments) as described (12). Purified CD45R0$^+$ CD4 T cells were used unstimulated or prestimulated with 1 μg/ml anti-CD3 mAb (30 min at 37°C). Islet ECs were preincubated with anti-CD86 blocking mAb (15 μg/ml) or an isotype-matched control Ab, and unbound Ab was removed by washing the wells with warm medium. CD45R0$^+$ CD4 T cells at 5 × 10^5/well were then seeded onto resting islet ECs and migration was analyzed over 7 h. Naive unstimulated CD4$^+$CD45RA$^+$ cells (5 × 10^5/well) were used for comparison. Migrated cells in the lower chamber were collected and viable cells were counted (by trypan blue exclusion in a hemocytometer) at specific time points in triplicate by two independent observers. Percentages of migrated cells were calculated thus: (number of migrated cells/total number of input cells) × 100%.

Analysis of the role of CD86 and CTLA-4 in T cell adhesion

Resting islet ECs (3 × 10^4/well in 100 μl of medium) were seeded onto attachment factor-coated, 96-well, flat-bottom tissue culture plates and allowed to grow to confluence (typically 24 h). When confluent, cells were incubated for 2 h at 37°C with anti-CD86 blocking mAb (15 μg/ml), abatacept (1–10 μg/ml; a fusion protein composed of the extracellular domain of CTLA-4 and the hinge domains of IgG that inhibits T cell costimulation; provided by Bristol-Meyers Squibb), or appropriate control Ab (isotype-matched to anti-CD86). Purified human IgG (Sigma-Aldrich) was included as control in experiments where abatacept was used. Unbound Ab was removed by washing the wells with warm medium. Purified CD45R0$^+$ CD4 T cells were used unstimulated or prestimulated with 1 μg/ml anti-CD3 mAb (30 min at 37°C).

In addition, where indicated a 1-h preincubation with 0.5 μg/ml anti-CTLA-4 blocking mAb (Chimerigen) was conducted. Subsequently, CD45R0$^+$ CD4 T cells (5 × 10^5/well in 100-μl medium) were seeded onto islet ECs and allowed to adhere for 30 min at 37°C. Nonadherent cells were removed by washing each well three times with warm medium and counted (trypan blue exclusion) in triplicate by two independent observers. Cell adherence to ECs was calculated thus: (number of nonadherent cells/total number of input cells) × 100%.

Analysis of LFA-1 clustering

LFA-1 clustering experiments were performed as described previously (20). In brief, freshly isolated CD4$^+$ CD45R0$^+$ memory T cells unstimulated or prestimulated with soluble anti-CD3 mAb (1 μg/ml for 30 min at 37°C) were seeded onto poly-L-lysine-coated (Sigma-Aldrich) Lab-Tec chamber slides (Nalge Nunc) or onto confluent resting islet ECs. After 30 min of incubation at 37°C, nonadherent cells were removed by washing the wells with warm PBS. Samples were then fixed in 3% paraformaldehyde for 10 min at room temperature, washed in PBS, and blocked with PBS/3% BSA and FcR blocking reagent (Miltenyi Biotec) for 1 h at room temperature. Next, cells were stained with the primary anti-LFA-1 mAb (BD Pharmingen) for 1 h at room temperature, washed, and incubated with the secondary Alexa Fluor 555 goat anti-mouse IgG (H + L) (Molecular Probes) for 1 h at room temperature. The samples were then washed, mounted onto coverslips, and analyzed using a laser scanning confocal microscope (model TCS SP2; Leica). Cells were visualized with a ×63 oil immersion objective and LFA-1 clustering was defined by the occurrence of a distinct polarized cap as described (20). At least five fields containing ≥300 cells were counted for each sample by two observers blinded to the conditions used.

Statistical analysis

Intergroup comparisons of T cell adhesion and migration across ECs as well as LFA-1 clustering under different experimental conditions were made using Student's *t* tests, and *p* values <0.05 were considered statistically significant. Statistical analyses were performed using GraphPad Prism 4 software.

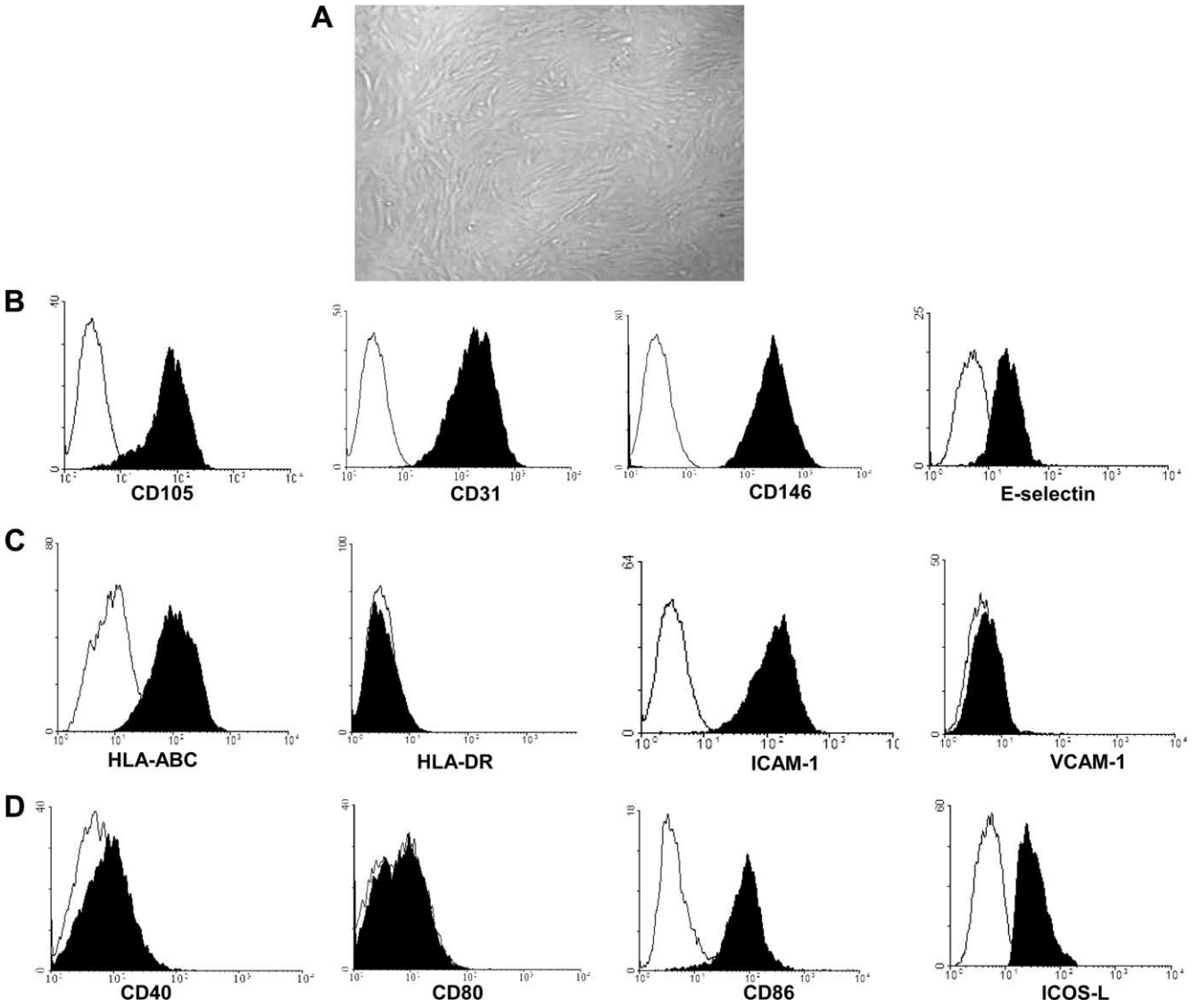

FIGURE 1. Phenotypic characterization of primary cultures of human islet ECs. *A* and *B*, The morphology of islet ECs at confluence observed by light microscopy (phase contrast) (*A*) and the expression of endothelial surface phenotypic markers on cultured human islet ECs including CD105, CD31, CD146 and E-selectin (*B*). *C* and *D*, The expression of MHC class I (HLA-ABC), MHC class II (HLA-DR), ICAM-1, and VCAM-1 adhesion molecules on resting islet ECs (*C*) and the basal expression of costimulatory molecules including CD40, CD80, CD86, and ICOS-L on resting islet ECs (*D*). Empty histograms represent staining with isotype-matched control mAbs. Study data are representative of at least three separate flow cytometric analyses conducted on islet ECs from all three islet donors.

Results

Phenotypic characterization of human islet ECs

Having generated primary cultures of islet ECs (from three donors), islet ECs were grown to confluence and their purity and endothelial origin were confirmed by the expression of endothelial surface phenotypic markers including CD105, CD31, and CD146 (Fig. 1*B*). The purity of the EC cultures was routinely >95% for at least two of three EC surface markers and >90% for all three EC markers. In addition, resting islet ECs expressed E-selectin, an adhesion molecule uniquely expressed by microvascular endothelium at low levels (Fig. 1*B*), which was further up-regulated following 4 h of stimulation with TNF-α (data not shown).

Expression of MHC and adhesion molecules on resting islet ECs

As shown in Fig. 1*C*, resting islet ECs express MHC class I (HLA-A, -B, and -C) but not MHC class II (HLA-DR). MHC class I expression was optimally up-regulated following 24 h of stimulation with IFN-γ while HLA-DR was optimally induced following IFN-γ stimulation for 72 h (data not shown). Similar results were obtained for islet ECs derived from three different donors. Resting islet ECs express ICAM-1 constitutively but not VCAM-1. Cytokine (IFN-γ and TNF-α) treatment for 24 h further enhanced ICAM-1 expression and induced the expression of VCAM-1 (data not shown). Islet ECs from all three donors behaved in similar fashion.

Expression of costimulatory molecules on resting islet ECs

T cell activation is dependent upon signals delivered through the Ag-specific TCR and costimulatory molecules. We therefore examined the expression of CD40, CD80, CD86, and ICOS-L costimulatory molecules on resting islet ECs (Fig. 1*D*). Cultured islet ECs as well as islet ECs freshly isolated from islets before subculture did not express CD40 or CD80 costimulatory molecules as determined by flow cytometry. Interestingly, islet ECs expressed

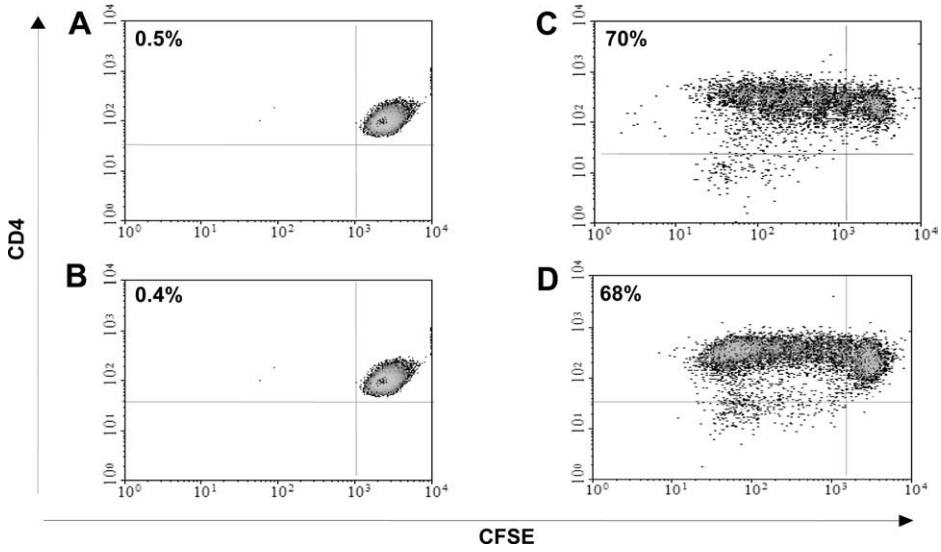

FIGURE 2. Functional analysis of ability of islet ECs to costimulate CD4 T cell activation. *A*, Coculture of resting islet ECs with CFSE-labeled, nonstimulated CD4 T cells did not result in T cell proliferation as assessed by flow cytometry. *B*, Likewise, CD4 T cells cultured alone and prestimulated with only anti-CD3 mAb did not proliferate. *C* and *D*, Coculture of resting islet ECs with anti-CD3-stimulated CD4 T cells resulted in proliferation of 70% of the cultured T cells (*C*), which was comparable to the proliferation of 68% achieved by CD4 T cells alone prestimulated with anti-CD3 and anti-CD28 mAbs (*D*). Study data are representative of at least three separate flow cytometric analyses conducted using islet ECs from two islet donors and CD4 T cells from three different donors.

CD86 and ICOS-L costimulatory molecules constitutively (Fig. 1*D*). Cytokine (IFN-γ and TNF-α)-stimulated islet ECs further up-regulated the expression of ICOS-L, but there was no change in the basal expression of CD86, CD80, and CD40 costimulatory molecules (data not shown).

Human islet ECs can provide costimulatory signals necessary for CD4 T cell proliferation

The ability of CD86 expressed on islet ECs to costimulate activation of CD4 T cells following TCR ligation by anti-CD3 mAb was studied using primary cultures of islet ECs isolated from two different donors and CD4 T cells purified from three different donors. Proliferation of CD4 T cells was selected as the most robust measure of costimulatory capacity. Because our aim was to understand how basal expression of CD86 can affect T cell activation and to minimize possible interference by allogeneic responses, no cytokines were used to activate the ECs (resting islet ECs do not express MHC class II molecules; see Fig. 1*C*). Proliferation was used as the measure of T cell activation. CD4 T cells, with or without prior stimulation with anti-CD3 mAb, were cocultured with resting islet ECs. In the absence of signal 1 provision in the form of anti-CD3 mAb, CD4 T cells cocultured with resting islet ECs did not proliferate (Fig. 2). Similarly, CD4 T cells failed to proliferate when cultured alone and prestimulated with anti-CD3 mAb but without provision of costimulation in the form of soluble anti-CD28 mAb. In contrast, CD4 T cells prestimulated with anti-CD3 mAb and cocultured with islet ECs proliferated with similar efficiency to that of CD4 T cells cultured alone after prestimulation with both anti-CD3 and anti-CD28 mAbs. These results show that islet ECs provide costimulation comparable to that of soluble anti-CD28 mAb.

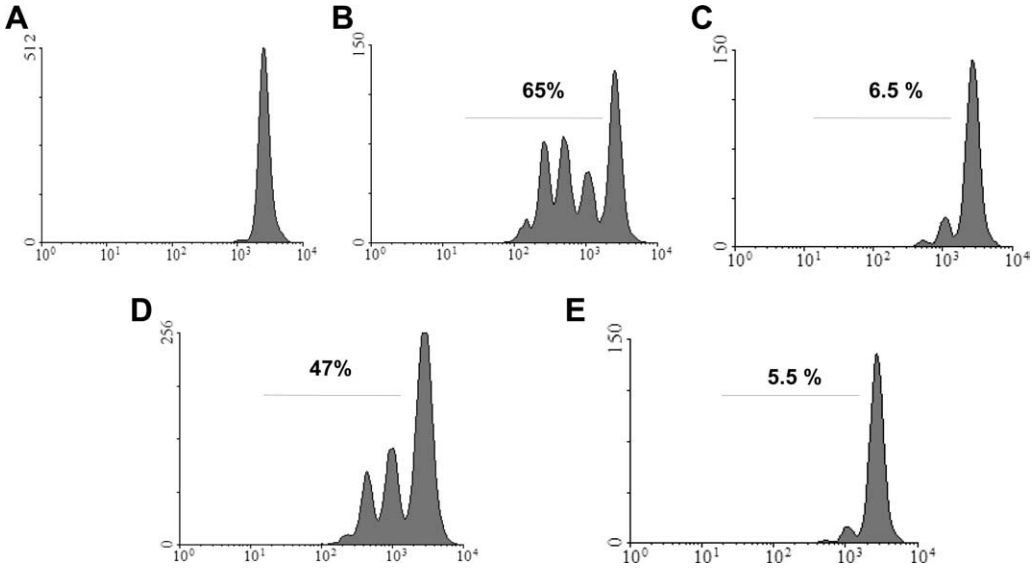

FIGURE 3. Relative contribution of CD86 and ICOS-L to islet EC-mediated T cell proliferation. *A*, Coculture of resting islet ECs with CFSE-labeled CD4 T cells without anti-CD3 mAb did not result in T cell proliferation as assessed by flow cytometry. *B*, Coculture of resting islet ECs with anti-CD3-prestimulated CD4 T cells resulted in proliferation of 65% of the cultured T cells. An identical result was obtained in the presence of the isotype control mAb. *C*, Preincubation of islet ECs with anti-CD86 mAb before coculture inhibited T cell proliferation. *D*, Addition of anti-ICOS-L mAb had a minor effect on the overall number of proliferating CD4 T cells but did reduce the number of cells undergoing more than one division. *E*, When used together, the blocking effect of anti-CD86 mAb and anti-ICOS-L mAbs was comparable to that achieved in the presence of anti-CD86 mAb alone. The functional T cell proliferation and blocking assays were repeated in three separate experiments using ECs from two different islet EC donors and CD4 T cells from three different T cell donors. The finding that blocking of CD86 on islet ECs inhibits CD4 T cell proliferation was reproduced in all experiments.

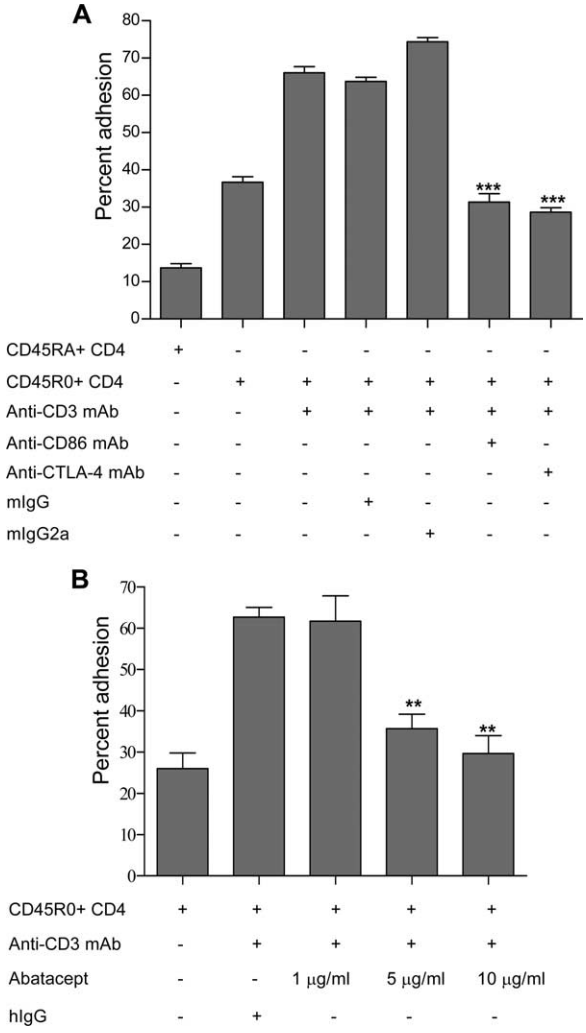

FIGURE 4. Role of islet EC-associated CD86 in CD45R0$^+$ CD4 T cell adhesion. *A*, Percentage of adhered CD45R0$^+$ CD4T cells on resting islet ECs measured following a 30-min period of coculture in the presence or absence of anti-CD86 and anti-CTLA-4 blocking mAbs and isotype-matched control mAbs. Blocking of CD86 function on islet ECs using either reagent significantly inhibited the adhesion of anti-CD3 prestimulated CD45R0$^+$ CD4 T cells (***, $p < 0.0001$, comparing adhesion of CD45R0$^+$ CD4 T cells in the presence of CD86/CTLA-4 blockade vs relevant isotype control mAb). mIgG/mIgG2a, mouse IgG/IgG2a. *B*, Blocking of islet EC expressed CD86 using CTLA-4 Ig (abatacept) resulted in significant inhibition of anti-CD3 prestimulated CD45R0$^+$ CD4 T cell adhesion to islet ECs (**, $p < 0.001$, comparing adhesion of CD45R0$^+$ CD4 T cells in the presence of CTLA-4 Ig vs relevant control human IgG mAb). Data are representative of three separate experiments with similar results. hIgG, human IgG.

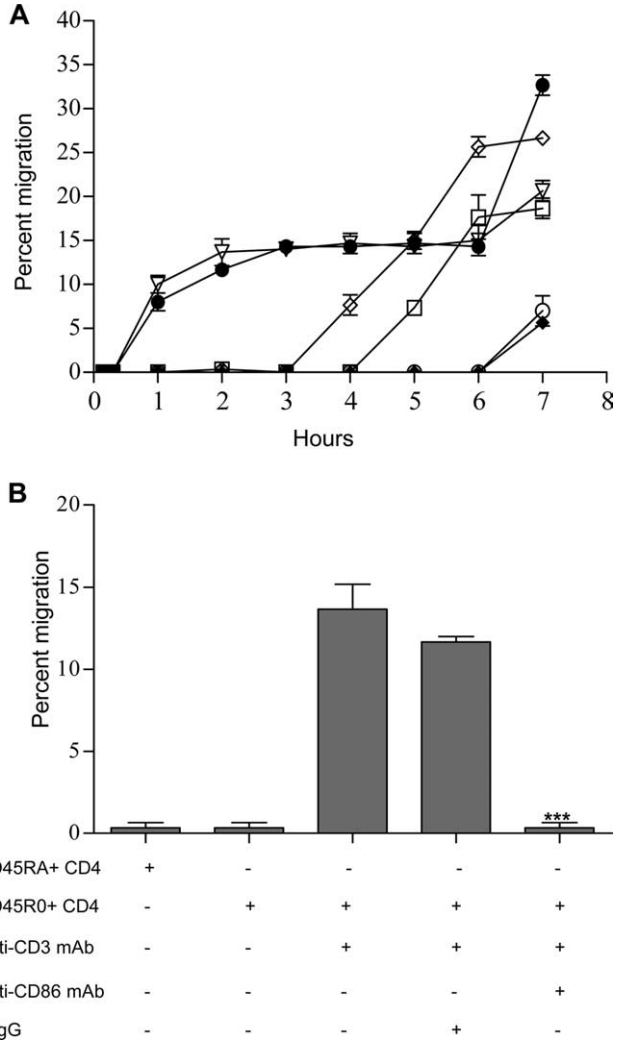

FIGURE 5. Role of islet EC-associated CD86 in CD45R0$^+$ CD4 T cell migration. *A*, Percentage of CD45R0$^+$ CD4 T cells migrating across resting islet ECs over 7 h in the presence or absence of anti-CD86 blocking mAb and isotype control mAb. Resting CD45R0$^+$ CD4 T cells (□) or CD45R0$^+$ CD4 T cells prestimulated with anti-CD3 mAb (▽) and anti-CD3 plus anti-CD28 mAb (◇) were allowed to adhere and migrate across resting islet ECs in the presence (○) or absence (▽) of anti-CD86 blocking mAb and isotype control mAb (●). A filled diamond (◆) represents naive CD45RA$^+$ T cells. *B*, Blocking of CD86 expression on islet ECs significantly inhibited the migration of anti-CD3-prestimulated CD45R0$^+$ CD4 T cells as measured at 2 h under conditions shown in *A* (***, $p < 0.001$, comparing migration of CD45R0$^+$ CD4 T cells in the presence of CD86 blockade vs relevant isotype control mAb). Study data are representative of at least three separate experiments. mIgG, murine IgG.

Relative contribution of CD86 and ICOS-L to islet EC- induced CD4 T cell activation

Having demonstrated that islet ECs can provide costimulation necessary for CD4 T cell activation, we addressed the question of which islet EC-associated costimulatory molecules are involved. Blocking of CD86 on islet ECs using specific mAb resulted in 90% reduction in CD4 T cell proliferation and number of cell divisions when compared with the isotype control mAb (Fig. 3). There remained the possibility that CD4 T cell costimulation in these cultures could be provided in *trans* (21). However, in our experiments islet ECs were washed extensively to remove unbound anti-CD86 blocking mAb, reducing the likelihood that the observed effect could be due to blockade of CD86 on activated CD4 T cells. The most conservative estimate is that a small proportion (6.5% proliferation; Fig. 3*C*) of the observed proliferation is attributable to *trans*-costimulation. Addition of anti-ICOS-L mAb to the EC/CD4 T cell coculture resulted in 50% reduction in the number of cell divisions that proliferating T cells underwent, but it had a relatively modest effect on the overall percentage of proliferating CD4 T cells. These findings were reproduced using primary islet EC cultures established from two different islet donors and CD4 T cells derived from three different donors.

CD86 on human islet ECs facilitates T cell adhesion and transendothelial migration

Firm adhesion to endothelial cells is a prerequisite for successful entry of activated T cells into inflamed tissues. In addition to its

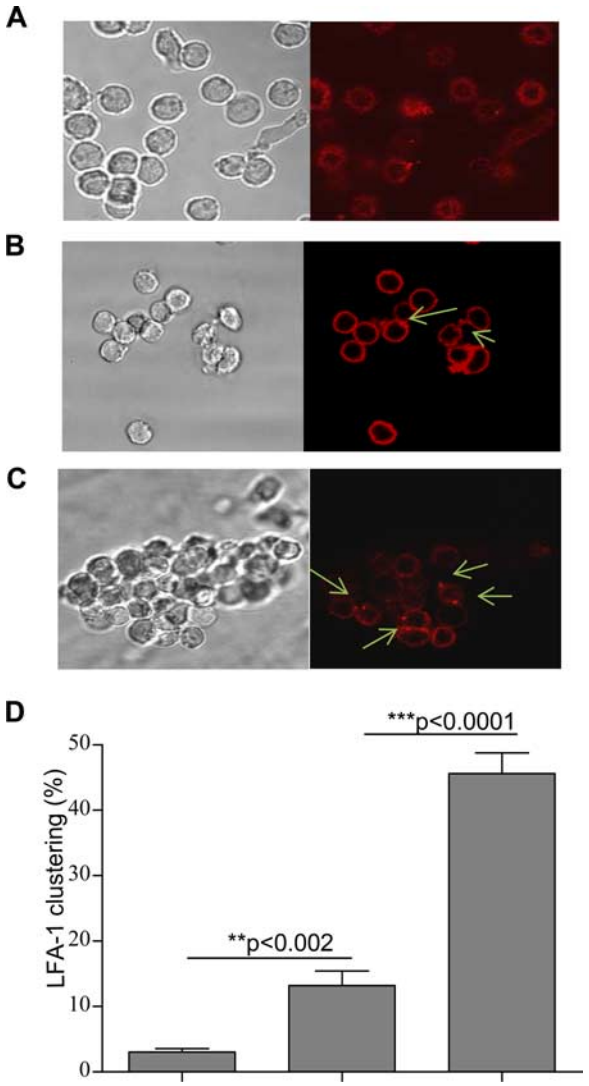

FIGURE 6. LFA-1 clustering is enhanced in CD45R0$^+$ CD4 T cells cocultured with islet ECs. *A*, Example of LFA-1 clustering observed on CD45R0$^+$ CD4 T cells cultured alone without anti-CD3 mAb stimulation, viewed as light and fluorescent photomicrographs. *B* and *C*, CD45R0$^+$ CD4 T cells stimulated with anti-CD3 mAb alone (*B*) or with islet ECs (*C*). Arrows indicate a polarized LFA-1 cap. *D*, Graph shows mean percentage (± SD) of T cells that have formed polarized LFA-1 caps under the different conditions. Percentage clustering shown on the *y*-axis denotes the percentage of counted T cells where one or more LFA-1 clusters were observed. At least five fields containing ≥300 cells were counted for each sample by two observers blinded to the conditions used. Results are representative of two independent experiments.

well-established role in costimulating T cell activation, CD86 has been shown to actively participate in the formation and stability of the immunological synapse through mobilization of LFA-1 to the T cell/APC contact area (22). We therefore set out to examine whether CD86 plays a role in T cell adhesion to and migration across islet ECs. For the adhesion and migration assays we used CD45R0$^+$ memory CD4 T cells known for their ability to migrate across resting endothelial cells in vitro and in vivo (23–25). CD45R0$^+$ CD4 T cells unstimulated or prestimulated with anti-CD3 mAb were allowed to adhere to and migrate across resting islet ECs for up to 7 h in the presence or absence of blocking Abs to CD86. As shown in Fig. 4, the adhesion of CD45R0$^+$ CD4 T cells measured over 30 min in the presence or absence of blocking mAbs to CD86 and CTLA-4 was significantly inhibited, and similar inhibition was seen in the presence of CTLA-4Ig (abatacept), the therapeutic agent designed to block CD86-dependent interactions (Fig. 4). These findings suggest a novel role for EC associated CD86 expression in T cell adhesion via CTLA-4.

These data indicate a dependency of CD4 T cells on the CD86/CTLA-4 axis for adhesion to islet ECs. Because adhesion is a prerequisite for transmigration, we considered it likely that blockade of this axis would also impact upon the ability of cells to transmigrate. This was confirmed in studies in which the addition of anti-CD86 blocking mAb to islet ECs resulted in significant inhibition of T cell migration (Fig. 5).

Increased LFA-1 clustering following T cell culture with islet ECs

One plausible way by which ligation of CTLA-4 by islet EC-associated CD86 could support T cell adhesion and migration is through the activation of LFA-1 on T cells. Indeed, a recent study by Schneider et al. described a powerful role for CTLA-4 in T cell adhesion. CTLA-4 ligation led to the activation of Rap1, which in turn increased LFA-1 clustering and thus T cell adhesion (20). We therefore examined LFA-1 clustering on CD45R0$^+$ CD4 T cells following coculture with islet ECs (Fig. 6). Culture of T cells with islet ECs significantly increased LFA-1 clustering compared with LFA-1 clustering on T cells cultured alone with only anti-CD3 as stimulus.

Discussion

In the present study we demonstrate for the first time that human islet ECs, when examined soon after organ retrieval and subsequently in primary cultures, constitutively express the costimulatory molecules CD86 and ICOS-L but not CD80 and CD40. Resting islet ECs were used to investigate whether this basal expression of CD86 is sufficient to costimulate anti-CD3 mAb-induced T cell activation. CD4 T cells prestimulated with anti-CD3 mAb proliferated during coculture with islet ECs but not when cultured alone, indicating that islet ECs provide robust costimulation. Anti-CD3-induced T cell proliferation was abrogated in the presence of anti-CD86 blocking mAb, suggesting that CD86 on islet ECs represents the major costimulatory signal capable of providing adequate costimulation for T cell activation. We speculated that their location on the inner lumen of the microvasculature reflects a role for costimulatory molecules in T cell adhesion and transmigration. This proposal was confirmed by our demonstration that costimulation-enhanced CD4 T cell adhesion is dependent upon ligation of CTLA-4. Taken together, these studies contribute to the weight of evidence that cognate interactions between ECs and T cells can promote T cell adhesion and transmigration. In the context of T cell-mediated autoimmune diseases such as T1D, this work also adds to the rationale for using costimulatory molecule blockade as a therapeutic modality (26). However, there are numerous axes in addition to CD86/CTLA-4 that might contribute to T cell adhesion and *trans*-endothelial migration, and further work will be required to elucidate the relative contributions of each.

Our results extend previous reports that costimulatory molecule expression on ECs can provide important supplementary signals for T cell responses in vitro, despite levels being less than those observed on professional APCs. As an example, Seino et al. reported that blocking CD86 expression on human microvascular endothelial cells (MECs) and human umbilical vein ECs reduces T cell alloresponsiveness, and blockade of CD86 and CD80 on human brain MECs potently inhibited anti-CD3-induced T cell proliferation (27). Proliferation of human T cells cocultured with porcine aortic ECs is also inhibited by anti-CD80 and anti-CD86 mAbs or CTLA-4 Ig (28). There is, however, conflicting evidence

in the literature regarding the expression of CD80 and CD86 molecules on ECs, with some reports showing their presence and others reporting their absence. Human brain MECs have been found to express CD80 and CD86 in vitro (29, 30), as have human intestinal MECs (31), human cardiac MECs (32), and human umbilical vein ECs (27). Seino et al. demonstrated constitutive CD86 expression on human microvascular endothelial cells but not expression of CD80 and, similar to our findings, the CD86 expression was not altered by cytokine treatment (27). In contrast, others did not detect CD80 or CD86 expression on passaged human ECs from several sources (21). These discrepancies may reflect different culture conditions and serial passages of cells, or inherent differences between ECs derived from different vascular beds. Furthermore, it is possible that conflicting results regarding the tissue-specific phenotype and cytokine responsiveness of ECs could arise from the use of different isolation techniques or different selection of EC subpopulations during subculture. Most importantly, depending on the tissue from which ECs are isolated, the cultured cells might be predominantly microvascular, macrovascular, or a combination of the two, which will have an effect on the cultured cells' phenotype. At present there is no reliable marker available that can be used to distinguish between microvascular and macrovascular endothelial cells.

Islet ECs also expressed ICOS-L constitutively, which was further up-regulated following TNF-α stimulation. Addition of anti-ICOS-L mAb to the islet EC/T cell coculture led to only a modest inhibition of T cell proliferation. This finding was not entirely surprising considering the current view that, unlike CD28-mediated costimulation that is deemed essential for IL-2 production and T cell proliferation, the engagement of ICOS by ICOS-L is more important for the enhancement of secondary effector T cell functions (33).

The finding of a novel role for EC-associated CD86 in T cell adhesion through binding of CTLA-4 is of particular interest, in that it suggests an alternative mechanism of action of this classical costimulatory molecule at the blood/tissue interface. Our LFA-1 clustering experiments lend support to the previously proposed mechanism of the role of CTLA-4 ligation in T cell adhesion (20). Schneider et al. described a powerful role for CTLA-4 in T cell adhesion. Engagement of CTLA-4 potently up-regulated LFA-1-mediated T cell adhesion via activation of Rap1, a molecule known for its ability to augment LFA-1 avidity and clustering (20). The active conformation of LFA-1 is necessary for interaction with and Ag presentation by APCs, and for the migration of T cells to sites of inflammation. It is possible that in vivo ligation of TCR by pMHC complexes on ECs, in addition to selectively recruiting T cells of relevant Ag specificities, would increase LFA-1 affinity, whereas ligation of CTLA-4 by CD86 molecules expressed on ECs would activate Rap 1 and thus increase LFA-1 clustering and avidity. This would allow firm adhesion to the endothelium and increased transendothelial and interstitial motility. Indeed, our observation that islet EC/T cell coculture significantly enhances LFA-1 clustering supports such a scenario.

The novel role of EC-associated CD86 in T cell adhesion and migration suggested by our findings may also offer an additional mechanism of action for interventions based on the use of CTLA-4 Ig (abatacept) for the control of chronic inflammation. Inclusion of abatacept in our adhesion/migration assays significantly inhibited T cell adhesion to islet ECs. CTLA-4 Ig was originally developed as an agent capable of inhibiting or preventing T cell activation by blocking the B7/CD28 pathway of T cell costimulation (34). In therapeutic terms it would thus be predicted to have much greater efficacy in the inhibition of priming (e.g., when used in the context of allotransplantation) than in controlling primed memory T cells responsible for chronic inflammation. Surprisingly, however, administration of CTLA-4 Ig to patients with psoriasis led to marked clinical improvement, and the drug has also been licensed for use in rheumatoid arthritis. Significantly, administration of abatacept to patients with psoriasis was associated with a marked decrease in lesional T cell infiltration (35), leading the authors to conclude that the vascular recruitment of effector T cells is susceptible to CD86 blockade.

We previously reported that human ECs are able to process and present disease-relevant epitopes from islet autoantigens and that this facilitates transmigration of autoreactive T cell clones. The present study identifies an additional route through which cognate interactions between tissue ECs and recently activated effector T cells have the effect of enhancing T cell transmigration. Taken together, these and other studies (14) provide evidence for a mechanism of peripheral tissue-specific T cell migration that is dependent upon local Ag presentation at the site of inflammation. The ability of microvascular ECs to process and present exogenous Ag and costimulate adhesion and transmigration may be highly relevant to autoimmune disease. Our findings in relation to the costimulatory capacity of islet ECs lend further support to the notion that islet ECs may play an important role during the initiation of islet inflammation and progression to T1D.

Acknowledgments

We are grateful to Bristol-Meyers-Squibb for provision of abatacept for use in this study.

Disclosures

The authors have no financial conflict of interest.

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