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ANTIGEN PRESENTATION EVENTS IN AUTOIMMUNE DIABETES

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Introduction

This review considers the presentation of β-cell-derived antigens during the initial stages of autoimmune diabetes, focusing on the islet of Langerhans as well as on the lymph nodes (LN) that drain the pancreas. The islets contain distinct antigen presenting cells (APC). Recent evidence points to these APC as central cells in early diabetogenesis.

Islets normally have APC

The presence of APC within islets was documented in the late 1970's in the context of allogeneic transplantation studies performed by the groups of Paul Lacy and Kevin Lafferty (1, 2). They identified in donor-isolated islets “passenger leukocytes” expressing Major Histocompatibility Complex Class II (MHC II) molecules with ultrastructural characteristics resembling splenic dendritic cells (DC) (3, 4). The passenger leukocyte theory had been proposed by George Snell (5) and championed by several transplantation biologists, among them Kevin Lafferty (6). It stated that leukocytes carried in the transplant were a major stimulus for the allogeneic reaction (6, 7). Indeed, depletion of the “passenger leukocytes” of the islets delayed their rejection (1, 8). The initial findings were followed by the identification of the islet leukocytes in animals (9-13) and in humans (11, 14-17). The first report that directly identified the phenotype of the passenger leukocyte came from Lacy's group: they demonstrated that the MHC II+ cells within the islets stained for a DC marker (10). HLA-DR+ cells with characteristic of APC have been identified in human islets in limited evaluations (11, 14-17).

Features of the mouse islet APC

Observations have been made on the features, turnover time and function of islet APC (18-24). Islets from several non-diabetic mouse strains, including the diabetic-prone NOD mouse on the Rag-1$^{-/-}$ background, showed ~10 APC per islet (19, 20). There was a broad distribution of APC, and a relationship between the size of islet and content of APC. Small islets contained the fewest APC while the bigger islets (mega-islets), contained the largest number, (Figure 1a). No T cells or NK cells were found in islets of normal non-diabetic mouse strains.

As previously described in earlier studies (3, 9, 10, 12), the islet APC contained features of DC; we will refer to them as such here, although their phenotype overlaps with those of

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other APC. From our published data (19), all CD45+ cells in islets were identified as MHC II+ and CD11c+ cells. Islet endocrine cells and the endothelial cells did not express MHC II.

Most islet DC were positive for CD11b, CD11c and F4/80. Islet DC were also positive for ICAM-1, integrin α4, and CX3CR1 (21). Most were negative or weakly reactive for CD4, DEC205, Ly-6C, CD8α, CD101, 33D1, B7-H3, B7-H4, ICOS-L, PD-L1, PD-L2, CD40, CD40L, B220, 440c (plasmacytoid DC), and langerin). B7-1 expression was evident, but there was weak expression of B7-2, suggesting an immature state. The chemokine receptor profile showed about half of the islet DC to be positive for CCR5, CCR6, CXCR3 and CXCR4 (19).

From our unpublished data and reports by Merad's and Tang's group, (20, 21), two APC subsets are found within islets. The major population (~85% of the CD11c+) is the one detailed above in isolated islets, expressing CD11b, F4/80, CX3CR1 and SIRPα. The remaining ~15% expressed CD103 but lacked CD11b, F4/80 and CX3CR1.

Islet DC were relatively stable in numbers, decreased by 30%, three to seven days after whole body irradiation (19). Inflammation in the islets promoted either by administering low doses of streptozotocin (STZ) or by infiltrating diabetogenic T cells increased the number of DC in islets (19, 20, 24). The incoming DC during islet inflammation were phenotypically different from the resident DC expressing high levels of B7–2 (19, 20), as well as CD40, CD11b and ICAM-1 (24).

Localization of DC within the islet

Most DC were in tight association with the blood vessels. Electron microscopy analysis showed a stretched DC always next to blood vessels and often showing dendrites in close apposition to a thin basement membrane below the endothelium (19). Islet capillaries were positive for endoglin, ICAM-1, and PECAM-1, and negative for VCAM-1 (19).

Imaging studies disclosed a highly dynamic dendrite activity *in situ,* with extensions and retractions of long dendrites between the blood vessels and β-cells (19). Most DC had a point of attachment to the blood vessels with very little displacement. About one-third of the DC extensions penetrated the lumen of the vessel (a “periscope” function) (23). The periscoping of DC dendrites has been documented in the intestine (25-27), airways epithelium (28), and aorta (29). About 10% of the islet DC were highly motile, with less dynamic dendrite activity, and in some cases escaped from the islets (19).

Trophic function of the islet DC

Phagocytes may have a role in the development of the pancreas and islets (30). As first reported by Jeffrey W. Pollard's laboratory, the CSF-1 mutant mice “op/op mice” showed small islets and reduced β-cell mass, about half the size of wild type islets (31). The islets of such mice showed a conspicuous reduction in the number of DC, (Figure 1b and 1c) (19). Thus the development of islet DC requires expression and signaling by CSF-1 receptor. These findings indicate a trophic role of the APC in islet physiology, be it by maintaining vascularization and/or by fostering β-cells viability. The nature of this supportive role has not been determined.

Antigen presentation in the islet and in the pancreatic lymph nodes (PLN)

Presentation of β-cell antigens by either MHC class I (MHC I) or MHC II molecules was examined using two different approaches. In one islet cells were tested for their relevant peptide-MHC (pMHC) complex *ex vivo* using diabetogenic T cells as probes. The second

approach tested diabetogenic T cells interactions with APC in *vivo*. Diabetogenic T cells were injected and their localization and site of activation examined in islets and PLN.

Presentation by islet DC to CD4 T cells

Under normal conditions, β-cells have low levels of MHC I and do not express MHC II. Presentation of β-cell antigens to autoreactive CD4 T cells involves the transfer of antigenic material from the β-cells to the APC. This situation was evident in the first reports by Haskins group examining the response of diabetogenic CD4 T cells to islet antigens (32, 33). In culture, β-cells (even allogeneic) activated CD4 T cells but only in the presence of syngeneic APC (34). *In vivo* experiments also pointed to direct lack of presentation by β-cells to CD4 T cells (35).

Are APC from resting islets (i.e., in non-inflammatory conditions), presenting to T cells or is β-cell damage or death a requirement for charging the APC with β-cell-derived antigens? These important questions are relevant for our understanding of how autoimmune diabetes develops. The answer is that islet DC are normally presenting peptides derived from β-cell proteins in high amounts, in a constitutive process unrelated to β-cell death. Islet DC from resting islets of non-diabetic NOD mice presented to and activated diabetogenic CD4 T cells, indicating that they contained the β-cell-derived pMHC complex (18, 19). DC isolated from islets of NOD mice activated a number of CD4 T cells of unknown specificity (34), as well as the BDC-2.5 transgenic CD4 T cell (19): this T cell was isolated by the Haskins laboratory (32) and its T cell receptor (TCR) genes cloned by Mathis and Benoist (36). A number of insulin-reactive T cells also were activated when cultured with islet DC (22). The presentation took place equally well by islet DC isolated from pre-diabetic NOD as well as NOD.scid mice where no inflammation or apparent cell death was evident (19, 22).

Islet APC from mice expressing the small protein hen egg white lysozyme (HEL) under the insulin promoter (37) likewise stimulated HEL-reactive CD4 T cells (19, 38, 39). In our studies most of the islet DC were positive for the HEL-pMHC complex testing with a monoclonal antibody (mAb) specific to the dominant pMHC complex (19). As in the NOD mouse, there was no apparent cell death when examined for terminal deoxynucleotidyl transferase dUTP nick end-labeling positive (TUNEL+) β-cells. We estimated that at a given time the islet DC contained about 2% of the HEL expressed by the islets. Beta cell-derived HEL was about 20-fold more effective than soluble HEL in charging APC (38). In brief, the capacity of the islet DC to present self-proteins is high (Figure 2).

Cytological examinations, including electron microscopy, confirmed the presence of secretory granules or their products inside most islet DC (19). Importantly, a mAb to the immunodominant peptide derived from the β-chain of insulin, residues 9-23, showed that most APC contained insulin granules (22). Considering that each granule has about 10^6 insulin molecules, it well explains the very high presenting activity of the islet DC. Insulin is a dominant autoantigen in autoimmune diabetes (40, 41). In agreement, examination of islets from NOD mice in which the green fluorescent protein (GFP) was expressed under the insulin promoter (42) showed most of the DC bearing GFP+ granules (20).

The role of the PLN

A major consideration in presentation of β-cell antigens is the role of the pancreatic lymph nodes (PLN). The PLN drain the acinar component of pancreas except for the islets, which lack lymphatic circulation. PLN also drain segments of the intestine (43, 44), a relevant issue regarding the influence of the microbiome in diabetes (45). All indications at present are that the PLN are essential in the initial activation of diabetogenic T cells, prior to their islet migration. This issue has been mostly explored with CD8 T cells. However, the number

of T cells examined with specificities to different β-cell antigens has been limited and generalizations need to be made with caution.

The importance of the PLN in the development of diabetes was shown in two experimental settings. First, surgical excision from NOD mice resulted in the absence of diabetes without apparent priming of T cells (46). Second, offspring of pregnant NOD mothers injected with lymphotoxin-β receptor fused to human Ig Fc lacked LN and did not develop diabetes (47). The level of autoreactivity was limited in these mice lacking PLN. In both situations, transfer of *activated* diabetogenic T cells resulted in diabetes.

Proliferation of diabetogenic CD4 T cells in the PLN was evident from experiments transferring labeled T cells. Transfer of un-activated BDC-2.5 CD4 T cells resulted in strong selective proliferation in the PLN (48). Direct examination showed the presence of DC containing antigen (19). In the PLN from insulin-promoter-HEL, APC presented to anti-HEL CD4 T cells (38, 39): 56% expressed the pMHC complex from the dominant peptide of HEL (19). In the study from Krummel, Tang and associates, nodes examined from insulin promoter-GFP mice showed DC containing GFP (49).

Diabetogenic CD8 T cells likewise proliferated strongly in the PLN. These observations were first made by Bill Heath's group examining the ovalbumin specific OT-1 T cells in mice displaying the protein under the insulin promoter (50-52). In NOD mice, two TCR transgenic CD8 T cells proliferated strongly in the PLN (53).

The process by which the PLN APC receives and presents β-cell antigens is not entirely known. Beta cell antigens released from β-cells, or some of the islet DC, or both, may move to the stroma to reach the lymphatic vessels that will drain their content into the PLN. Clearly, injections into the pancreas of cells or labeled proteins reach the PLN (54), although these are non-physiological conditions. It is not feasible to posit that the proteins or the APC leave the islet via blood, in which case there would not be PLN selectivity presentation.

We favor the hypothesis that the sensitization of the PLN is a constitutive process by way of the islet DCs, which at some point migrate out into the stroma to then enter the lymphatic network. This flow from islet antigen-presentation to the node could well be a normal biological process resulting from islet DC turnover or be influenced by biological changes of the islets (i.e., during inflammation). We consider the former to be the most feasible; however, inflammation may accelerate this process.

It is important to note that the APC network that surrounds the islets may not be presenting islet antigens (19). We examined the stromal APC in the insulin-promoter-HEL transgenic mice: while the islet DC were rich in HEL-pMHC complexes, the stromal DC were negative, and thus there was no apparent transport of β-cell HEL to the peri-islet areas. If this finding is applicable to all β-cell antigens, it has implications concerning the development of the peri-insulitic lesion prior to islet injury.

PLN from neonatal mice, up to about the third week of life, presented poorly to T cells (48, 53, 56, 57). Subsequently, the node became receptive to the activation of transferred T cells. There were no major differences in DC subsets of the PLN between young or old mice (48, 57). This was evaluated in B6 mice using the insulin promoter-ovalbumin system by Heath's group (57) and in the NOD mouse by Mathis and Benoist (48). The PLN from young mice developed antigen presenting capabilities following β-cell injury, either by transfer of activated CD8 T cells (57) or by administering STZ (the β-cell poison) (53). One interpretation is that sensitization of the node develops subsequent to reorganization or reconstruction of the islets which takes place a few weeks post-birth (57). A discrete wave of β-cell apoptosis takes place shortly post-birth in rats (58-61), mice (61, 62) and humans

(63). A relationship has been argued between this discrete wave and the acquisition of presenting capabilities by the nodes (57, 61, 64). This relationship has been questioned: manipulations that reduce or favor β-cell death have not affected the development of diabetes (65).

Presentation in vivo to CD4 T cells

An important issue to consider is the role of the islet DC in the migration and localization of either diabetogenic CD4 or CD8 T cells into islets bearing their cognate antigens. Specific T cells localized into islets where there was presentation of the pMHC complex. In all instances tested, the CD4 T cells required activation in order to directly enter islets.

Our laboratory examined the entry of diabetogenic CD4 T cells in B10.BR mice expressing HEL under the insulin promoter or in the NOD mouse (Table 1) (23, 24). T cells from TCR transgenic mice directed to the major segment of HEL presented by I-A^k molecules localized only to islets of mice expressing HEL under the insulin promoter. This specific localization was found to the same extent whether the mice were normal (untreated) or were lightly x-irradiated. For localization to take place, T cells had to be activated first by a short incubation with APC presenting the relevant peptide. Non-activated T cells did not localize to any extent. Neither did non-specifically activated CD4 T cells localize to islets of mice bearing HEL. Thus, the localization was a specific event that depended on the state of activation of the CD4 T cells and the expression of HEL by the islets. As noted before, most of the islet DC presented HEL peptides.

Localization started within a few minutes after injection peaking by about 24 hrs. We could not localize T cells in the DC network surrounding peri-islet areas. Examination of live islets by two-photon microscopy showed that about half of the CD4 T cells that crossed into the islet were in close contact with the DC. A visible immunological synapse was evident in many of the T cell-DC clusters (23, 24). The conclusion was that T cells entered directly from blood into islets crossing the intra-islet vessels. As noted before (23), islet DC protruded dendrites into the lumen, which could be the initial site of interaction with the circulating T cells. In support of this interpretation, injection of 0.5u beads coated with anti-MHC II mAb, led to specific localization in islet vessels always next to a DC (24). Islet blood circulation is slow and intermittent (66) which could foster the contact of the two cell types. An important consideration is whether T cells enter islets normally, at random, to then rapidly exit unless there is interaction with their cognate antigen. Although we could not eliminate this possibility, attempts to localize T cells in normal islets through this putative migratory stage failed. Even under circumstances where non-specifically activated T cells were abundant in blood, no localization was evident (23).

Similar findings were made in the NOD mice. The BDC-2.5 TCR transgenic CD4 T cells localized to the islets of NOD mice following a brief activation. BDC-2.5 T cells taken directly from the transgenic mice showed some localization (about 20% of the cells had activation markers). BDC-2.5 T cells did not localize to islets of NOD mice lacking MHC II or to NOD.$H2^b$ mice. Thus localization depended in the correct presentation of the I-A^{g7} pMHC (23).

The presence of cloned T cells in islets was examined by the Vignali laboratory, using “retrogenic” technology in which stem cells were transfected with retrovirus containing TCR genes from diabetogenic T cells (67). Some T cells directed to β-cell antigens were identified weeks later and correlated with the development of diabetes.

In our experiments, the entry of diabetogenic CD4 T cells was partially inhibited by administering blocking mAb to MHC II, prior to the transfer of the T cells. Partial inhibition

was also found by blocking ICAM-1 or by transferring T cells into NOD.ICAM-1$^{-/-}$ mice (23). We concluded that the two molecules cooperated in the entry of T cells. Of interest, treatment of diabetogenic CD4 T cells with pertussis toxin (PTx) had no influence in islet localization, indicating that signaling via G protein coupled receptors was not involved (23). The results suggest that diabetogenic T cells enter islets by contacting the islet DC, perhaps by their dendrites exposed inside the blood vessels.

Diabetogenic T cell entry caused rapid and profound changes in the islets (24, 55). Within a few hours post-entry, islet vessels expressed VCAM-1 and increased their expression of ICAM-1. Of note, β-cells also reacted by expressing ICAM-1 one or two days after the localization of T cells (23). Following the entry of diabetogenic CD4 T cells, islets developed profound amplification gene changes: transcriptome analysis disclosed a rapid induction of interferon genes within a short time after T cell entry (23). The strongest transcriptional changes were identified in the non-leukocyte-component of the islets (β-cells and endothelial cells).

Importantly, following localization of diabetogenic CD4 T cells, islets were receptive to the entry of non-specific T cells. Their entry mechanisms were far different from the diabetogenic CD4 T cells: entry was inhibited by blocking VCAM-1 which was not the situation with diabetogenic CD4 T cells. Importantly, PTx treatment inhibited the localization of the non-specific CD4 T cells (23) (Table 1 and Figure 3).

These findings using transfer of T cells need to be placed in the context of the normal diabetic process in which T cells start localizing early but diabetes does not take place until much later. Clearly a number of control events take place subsequent to T cell entry which modulate the effector reaction, an issue beyond the scope of this review. Another important issue is the status of the islet DC during the diabetogenic process. Regardless of the DC content of β-cell-pMHC complexes, the presenting capabilities via costimulatory molecules or cytokine expression are modulated and influence interactions with T cells. Such changes are to be expected (24).

Presentation in vivo to CD8 T cells

Most findings agree that cross presentation of β-cell antigens to unactivated diabetogenic CD8 T cells takes place in the PLN (51-53, 56). Direct entry of unactivated CD8 T cells into islets has not been identified. [A recent claim to this effect needs confirmation and further evaluation (68).]

Santamaria's group made a detailed analysis of a CD8 T cell to the islet-specific glucose-6-phospatase catalytic subunit protein (IGRP) (69, 70). IGRP contains a strong MHC I-epitope presented by K^d molecules (71), and a TCR transgenic mouse (the 8.3 CD8 T cell) was generated to it. Unactivated 8.3 T cells proliferated in the PLN and apparently did not enter islets until after a period of activation. In contrast, *activated* 8.3 T cells entered islets readily, agreeing with the findings made in CD4 diabetogenic T cells. One important issue is the cellular site of MHC I expression required for the localization of activated diabetogenic CD8 T cells. The response to IGRP was completely abolished by having the adenovirus E19 protein expressed in β-cells (69). Transfer of 8.3 T cells into these mice resulted in PLN proliferation but without localization in islets. NOD mice selectively lacking MHC I on the β-cells showed insulitis and low diabetes incidence. Although this experiment indicated that expression of MHC I in islets favored diabetes penetrance, it did not address CD8 T cell localization in such islets (72). Selective absence of MHC I on the APC lacked presentation in the PLN, indicating, a lack of cross-presentation and activation of the CD8 T cell (73).

Chervonsky's laboratory examined the migration of the CD8 T cell reactive to an insulin peptide, the IS-CD8 clone (74). T cells migrated specifically into islets bearing the appropriate MHC I (K^d) (75, 76). Their findings suggested that the initial localization of CD8 T cells was to the islet endothelium. Chemokine signaling was essential for specific CD8 T cell migration into the islet. Additional work showed the requirement of VCAM-1 expression in the islet vasculature for diabetogenic CD8 T cell migration to occur (55). The differences in localization between CD8 and CD4 T cells, point to distinct differences in local antigen presentation.

Concluding Remarks

Evidence is growing that the islet DC is a central cell in diabetogenesis. The islet DC is heavily charged with class II pMHC complexes due to constitutive uptake of β-cell granules. As a result of this uptake, the islet DC is instrumental in the initial sensitization, most likely by moving to the PLN. How this movement takes place and how generalizable it is for priming of all diabetogenic T cells needs further analysis. Islet DC are also the central cells in the localization of activated CD4 T cells. The extent to which changes in islet biology and of the β-cell alters these processes needs to be examined, but is a likely possibility. One issue that remains to be evaluated is the capacity of the islet DC to cross present MHC I-epitopes. Most of the islet DC have monocyte /macrophage features but a small percentage are CD103+; which of the two if any cross presents β-cell granules needs to be examined. Moreover the mechanism of localization of the diabetogenic CD8 T cells appears to be different from CD4 T cells, involving cells other than the islet DC.

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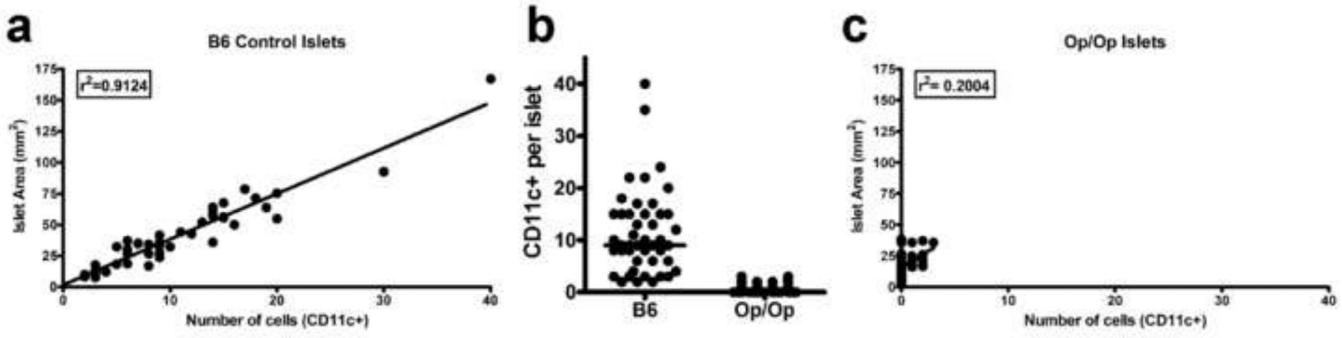

Figure 1. Islet size and DC content in normal and in CSF-1-/- mice (Op/Op)

(a) Distribution of islet area vs. CD11c+ cells in islets of 6 week old C57BL/6 mice. **(b)** Comparison of islet CD11c+ content in islets of C57BL/6 and Op/Op mice. **(c)** Distribution of islet area vs. CD11c+ cells in islets of 6 week old Op/Op mice. From reference 19.

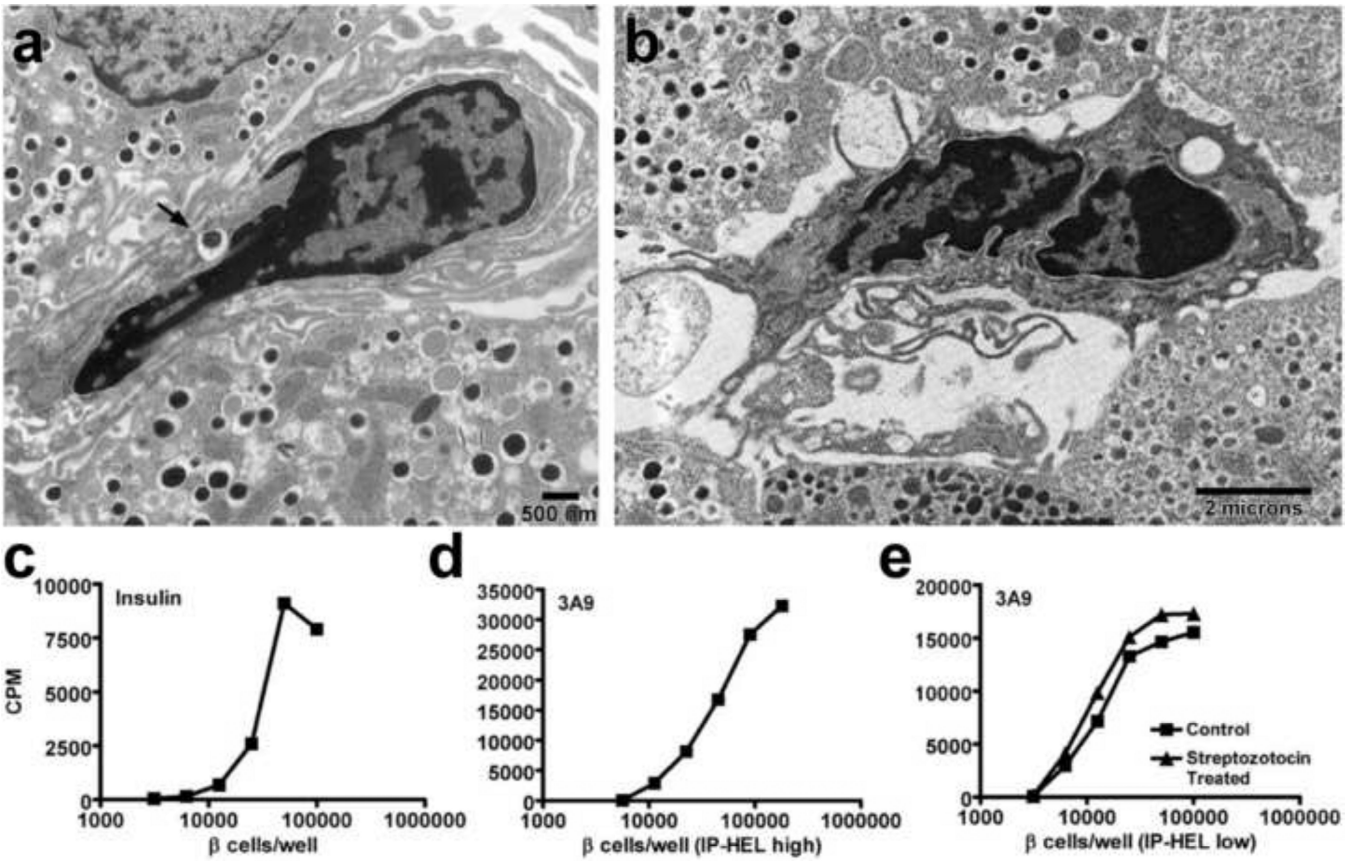

Figure 2. Islet DC present β-cell derived antigen to specific T cell hybridomas

(a and b) Electron microscopy analysis of NOD.Rag-1$^{-/-}$ islets showing islet DC with an insulin granule inside a vacuole (arrow) **(a)** and islet DC with dendrites extending to adjacent β-cells **(b)**. **(c to e)** Dispersed islet T cell assays showing: insulin-specific T cell hybridomas cultured with titrating amounts of NOD.Rag-1$^{-/-}$ dispersed islets (c), 3A9 T cell hybridomas cultured with dispersed islets from high producer IP-HEL mice (ILK3 strain) (d) or cultured with dispersed islets from low producer IP-HEL mice (117 strain) with or without low dose STZ treatment 4 days before islet isolation. Panel (a), (c), (d) and (e) published as Figure 3 and 6 from reference 19, with permission from PNAS. Panel (b) is from unpublished data.

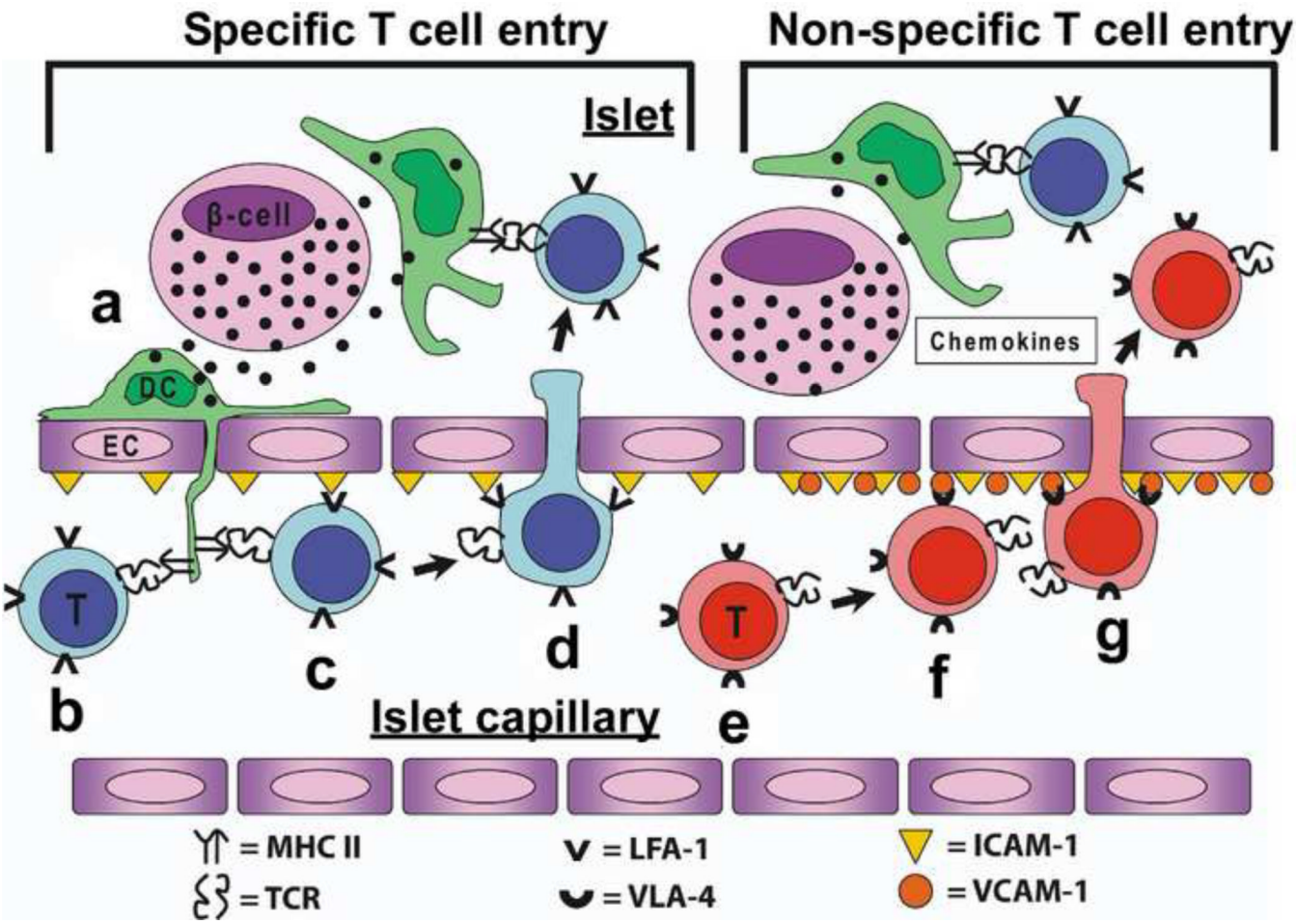

Figure 3. Proposed model of CD4 T cell entry into the islets of Langerhans ***Specific T cell entry*** **(a)** Secretory granules are taken up by the islet DC (shown in green), which are then processed and its peptides presented by MHC II. **(b)** Specific CD4 T cells (shown in blue) encounter their antigen presented by islet DC protruding through the fenestrated endothelium of the vessel. **(c)** MHC II recognition and the interaction of LFA-1/ICAM-1 favors the retention and adhesion to the endothelium of the specific T cell in the vessel. **(d)** The retention allows the entry of the T cell into the islet. Once inside the islet, T cell interacts with islet DC and become activated. These steps will trigger inflammatory signals and gene changes in the islet increasing ICAM-1, VCAM-1 and inflammatory chemokines. ***Non-specific T cell entry:*** **(e)** Non-specific T cells in circulation (shown in red) will encounter the inflammatory signals (chemokines and VCAM-1) that will allow its retention and adhesion to the islet endothelium. **(f and g)** Attachment to VCAM-1 and the inflammatory chemokine gradient will lead to non-specific T cell entry into the islet.

Table 1

Early islet cell entry evaluation performed with adoptive transferred specific and non-specific CD4 T cells

CD4 T cell evaluated	Permutation	Recipient strain	Islet entry
Manipulation			
3A9*			
Non-activated	None	IP-HEL	No
Activated	None	IP-HEL	Yes
Activated	None	Non-IP-HEL	No
Activated	Anti-class II	IP-HEL	Reduced
Activated	Anti-ICAM-1	IP-HEL	Reduced
Activated	Anti-class II + Anti-ICAM-1	IP-HEL	Reduced
Activated	Anti-VCAM-1	IP-HEL	Yes
Activated	Anti-PECAM-1	IP-HEL	Yes
Activated + PTX	None	IP-HEL	Yes
Activated	Anti-IFN-γ	IP-HEL	Yes
B10.BR‡			
Non-activated	None	IP-HEL	No
Non-activated	None	Non-IP-HEL	No
Activated	None	IP-HEL	No
Activated	None	Non-IP-HEL	No
Activated	STZ	IP-HEL	No
Non-activated	Co-transfer with 3A9	IP-HEL	Reduced
Activated	Co-transfer specific	IP-HEL	Yes
Activated + PTX	Co-transfer specific	IP-HEL	No
Activated	Co-transfer specific + anti-VCAM-1	IP-HEL	No
Activated	Co-transfer specific + anti-IFN-γ	IP-HEL	Reduced
BDC2.5*			
Non-activated	None	NOD.Rag-1$^{-/-}$	Reduced
Activated	None	NOD.Rag-1$^{-/-}$	Yes
Activated	None	B6.g7	Yes

CD4 T cell evaluated		Permutation	Recipient strain	Islet entry
	Activated	None	BALB/c	No
	Activated	None	NOD.Class II$^{-/-}$	No
	Activated	Anti-Class II	NOD.Rag-1$^{-/-}$	Reduced
	Activated	None	NOD.ICAM-1$^{-/-}$	Reduced
	Activated	Anti-Class II	NOD.ICAM-1$^{-/-}$	Reduced
	Activated	Anti-CD44	NOD.Rag-1$^{-/-}$	Yes
	Activated + PTX	None	NOD.Rag-1$^{-/-}$	Yes
	Activated	None	NOD. IFN-γR$^{-/-}$	Reduced
B6.g7‡				
	Non-activated	None	NOD.Rag-1$^{-/-}$	No
	Non-activated	None	B6.g7	No
	Activated	None	NOD.Rag-1$^{-/-}$	No
	Activated	None	B6.g7	No
	Activated	Co-transfer specific	NOD.Rag-1$^{-/-}$	Yes
	Activated + PTX	Co-transfer specific	NOD.Rag-1$^{-/-}$	No
	Activated	Co-transfer specific + anti-VCAM-1	NOD.Rag-1$^{-/-}$	No
	Activated	Co-transfer specific	NOD. IFN-γR$^{-/-}$	No

This Table summarized findings of ours reported in references 23 and 24.

*__3A9__ is a CD4 T cell that recognizes the 48-62 peptide of HEL bound to I-A^{k} molecules. Cells were isolated from a T cell receptor transgenic mice and transferred directly (non-activated), or following a short period of activation with the peptide (activated). Recipients were mice that express HEL under the insulin promoter (IP-HEL) (37, 39). Results were identical in lightly irradiated and non irradiated mice. PTX refers to Pertussis toxin. STX refers to streptozotocin.

‡**B10.BR** refers to CD4 T cells from normal mice, incubated or not incubated with concanavalin A (ie activated and non-activated, respectively).

***BDC2.5** refers to the diabetogenic CD4 T cells isolated from T cell receptor transgenic mice (36). Cells were activated by incubation with a mimotope peptide.

‡**B6.g7** refers to CD4 T cells isolated from non-diabetic B6.g7 mice.