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Interference with islet-specific homing of autoreactive T cells: an emerging therapeutic strategy for type 1 diabetes

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Pathogenesis of type 1 diabetes involves the activation of autoimmune T cells, consequent homing of activated lymphocytes to the pancreatic islets and ensuing destruction of insulin-producing β cells. Interaction between activated lymphocytes and endothelial cells in the islets is the hallmark of the homing process. Initial adhesion, firm adhesion and diapedesis of lymphocytes are the three crucial steps involved in the homing process. Cell-surface receptors including integrins, selectins and hyaluronate receptor CD44 mediate the initial steps of homing. Diapedesis relies on a series of proteolytic events mediated by matrix metalloproteinases. Here, molecular mechanisms governing transendothelial migration of the diabetogenic effector cells are discussed and resulting pharmacological strategies are considered.

Islet-directed homing of autoreactive T cells: a key step in progression toward T1D

Type 1 diabetes (T1D) is a major debilitating human disease with an onset early in childhood. T1D progresses through the activation of autoreactive T cells, followed by homing of activated T cells into the pancreatic islets, and manifests itself through the elimination of β cells by cytotoxic T cells. Both $CD4^+$ and $CD8^+$ T-lymphocytes are involved in diabetogenesis [1,2]. Nonobese diabetic (NOD) mice lacking $CD4^+$ or $CD8^+$ cells do not develop diabetes [1,3]. The identification of insulin as an auto-antigen in the development of T1D and specifically insulin B chain-derived peptide InsBa.a.$^{15–23}$ as a major autoreactive determinant [4] enabled researchers to study various functions of $CD8^+$ T cells reactive to this peptide [4–6]. InsBa.a.$^{15–23}$-reactive $CD8^+$ (IS-$CD8^+$) T cells seem to have an important role during the early stages of progression toward T1D. At the initiation of insulitis, they dominate peri-islet infiltrates [4] and facilitate islet-bound traffic of other cell types [7].

Successful therapies of T1D will require the repair of immunological tolerance breakdown and the restoration of insulin-producing β-cell mass [8]. Current literature suggests that compensatory endogenous β-cell regenerative processes occur during at least initial stages of T1D [9–11]; however, the *de novo* developing β cells are probably continually affected by transmigrating autoimmune T cells. It is hypothesized that diminishing the rate of T-cell transmigration into pancreatic islets might facilitate restoration of functional β-cell mass and result in an associated increase of insulin production [12]. Thus, interfering with the homing process might translate into innovative and effective therapies for T1D.

General mechanism of T cells homing into target tissues

T cells follow certain rules when homing to the secondary lymphoid organs, to specialized barrier compartments and to sites of inflammation. Widely accepted mechanisms of T-cell homing [13] describe interactions between T cells and endothelial cells (ECs), which are mediated by several classes of adhesion molecules [14–16]. The homing process *per se* can be divided into three key steps: initial adhesion (tethering and rolling), firm adhesion (activation-dependent arrest of rolling) and diapedesis (Fig. 1).

Initial adhesion of T cells is mediated by the L-selectin, ligands for tissue-specific P- and E-selectins and integrins. These surface T-cell molecules interact with the respective EC ligands, enabling rolling of T cells on endothelial surface [16–18]. Activation of T-cell integrins, which increases integrins' affinity for the

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1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2010.05.013

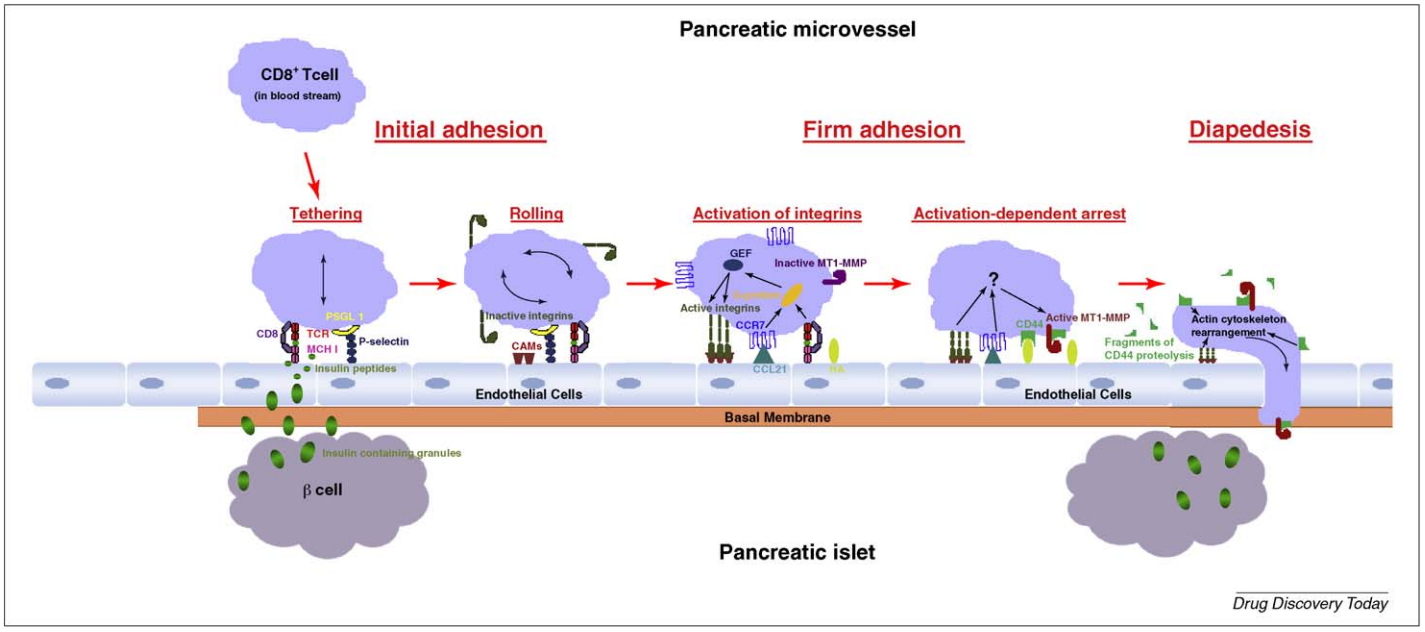

FIGURE 1

Molecular interactions mediating intra-islet homing of autoreactive insulin-specific CD8$^+$ T cells. Red arrows depict progression of T cell through all of the traffic stages indicated in red underlined text on top of the scheme; blue arrows indicate the direction of T-cell locomotion at each individual traffic stage. Black arrows illustrate intracellular signaling cascades. CAMs, cell-adhesion molecules; GEF, guanine-nucleotide exchange factor; HA, hyaluronan; MHC I, major histocompatibility complex class I; MT1-MMP, membrane-tethered matrix metalloproteinase 1; PSGL-1, P-selectin glycoprotein ligand-1; TCR, T-cell receptor.

corresponding EC-adhesion molecules (CAMs) [19–21], promotes the transition of slow rolling into the firm adhesion of T cells to endothelium [14,22]. Chemokines, expressed in a tissue-specific manner, mediate integrin activation by triggering cognate G-protein-coupled receptors on T cells [15,20,21,23]. Thus, tissue-specific adhesion molecules and chemokines contribute to the specificity of T-cell trafficking. T-cell diapedesis includes a series of lymphocyte cytoskeletal rearrangements mediated by chemokines; in addition, diapedesis is potentiated by a series of proteolytic events mediated by various proteases, including matrix metalloproteinases (MMPs) [24–26]. Proteolysis modifies adhesive strengths of lymphocyte–endothelial interactions and thereby enables extravasating T cells to successfully penetrate the basement membrane and the interstitial extracellular matrix (ECM) barriers.

In conclusion, a multitude of lymphocyte–EC receptor–ligand interactions are implicated in the homing process. Its specific use varies widely, depending on T-cell subset, homing compartment, site of T-cell priming and other, yet to be identified, factors. Dissecting molecular mechanisms that, when modulated, will interfere with homing of diabetogenic T cells is a scientifically challenging but rewarding task to perform.

Mechanism of islet homing: pancreatic traffic of insulin-specific CD8$^+$ T cells

Initial tethering and rolling

It is generally believed that antigen-experienced effector T cells travel more efficiently to inflamed tissues because they upregulate adhesion molecules and chemoattractant receptors for inflammation-induced ligands [13,27]. Neither the tissue-specific CAMs, upregulated by inflammation, nor the inflammatory chemokines did provide for the specificity of IS-CD8$^+$ T-cell traffic, however, because these cells efficiently homed to completely uninflamed islets of NOD-*scid*, NOD-*Rag1*$^{-/-}$, DBA/2 and BALB/C mice and did not home to inflamed salivatory and lacrimal glands of NOD mice [5]. Thus, it seems that not inflammatory but tissue-specific signals direct diabetogenic T-cell traffic and affect T-cell motility.

Multiple experiments utilizing transfer of diabetogenic lymphocytes confirmed precise homing specificity of transferred cells, which targeted only pancreatic islets, but not other tissues, whether inflamed or uninflamed. This fact, taken together with the well-documented specificity of diabetogenic T cells for the islet-derived antigens, led to the suggestion that pancreatic homing can be driven by the T cells receiving antigenic stimuli from endothelium of pancreatic microvessels. Indeed, homing of IS-CD8$^+$ cells depended on endothelial MHC class I expression and the ability of ECs to cross-present pancreatic antigen because adherence of IS-CD8$^+$ cells to EC monolayers was in direct correlation with availability of MHC class I–insulin peptide complexes *in vitro* [5]. In agreement, upon transfer of diabetogenic T cells into MHC class I deficient NOD.β*2m*$^{-/-}$ intra-islet trafficking of transferred cells was undetectable in the short term [5], and the formation of insulitis in recipient mice was extremely delayed in the long term [28]. Transgenic overexpression of β*2m* molecules in the β cells of *NOD*.β*2m*$^{-/-}$Tg*RIP*-β*2m* animals probably provided enough soluble β2m to restore MHC class I expression in all subsets of islet cells, including ECs, and resulted in return of speedy insulitis and subsequent diabetes after the transfer of diabetogenic T cells [28]. Cross-presentation of insulin-derived peptides is a unique property of islet ECs which routinely transport extremely high concentrations of locally secreted insulin and, thus, are likely to endogenously process it to peptides, load these peptides onto their MHC class I molecules and cross-present resultant MHC–peptide complexes to diabetogenic IS-CD8$^+$ T cells, facilitating T

cells islet-specific homing [5] (Fig. 1). Similarly, it was reported that the levels of MHC class I molecules on ECs loaded *in vitro* with the cognate peptides had a profound effect on the activation, adhesion and transmigration of pathogenic islet-reactive $CD8^+$ T cells [29]. In addition, antigen cross-presentation by endothelium has been recorded in transplantation models [30–32]. Hence, EC antigen cross-presentation is probably a general phenomenon in organ-specific autoimmunity crucially involved in the regulation of homing of autoreactive T cells. This notion is further supported by the observation that the cognate recognition of endothelium *in vivo* enhanced the tissue-specific traffic of HY antigen-reactive $CD8^+$ T cells [33].

The general consensus is that initial adhesion of T cells to ECs depends on selectins and their ligands: L-selectin (CD62L), PSGL-1 (CD162) and E-selectin ligands binding to their endothelial receptors, L-selectin ligands – GlyCAM, PNAd, CD34 and MAdCAM; P-selectin; and E-selectin, respectively. Selectin-mediated adhesion bonds have high 'on' and 'off' rates and last a few seconds or less, thus ideally contributing to short initial tethering of T-lymphocyte on the endothelial surface [34]. To date, no literature data connect PSGL-1 and E-selectin ligands, expressed on surface of some subsets of activated islet-specific T cells, with either their islet-specific homing or development of diabetogenic islet infiltrates. Monoclonal antibody, raised against mouse PSGL-1 (CD162), attenuated incidence of TID, most probably by direct induction of apoptotic death of activated T cells [35]. L-selectin, however, was implicated in the development of diabetes, despite being nondetectable in most diabetogenic $CD8^+$ T-cell species [5,7]. By contrast, $CD4^+$ T cells expressing high levels of L-selectin seem to home to the islets where they exhibit regulatory function. $CD4^+CD62L^+$ T cells, but not $CD4^+CD25^+$ T cells, have been reported to inhibit diabetes transferred into NOD-*scid* recipients by transgenic NOD.Tg*BDC2.5-scid* cells [36]. L-selectin, however, is dispensable for the spontaneous T1D development because NOD mice with disrupted L-selectin gene develop disease similarly to their nonmodified littermates [37]. This study is consistent with the observation that subcutaneous antigen priming produced islet-reactive T cells, which used neither VCAM-1 nor MAdCAM interactions to home to pancreatic islets [38]. Moreover, antibody neutralization of L-selectin or P-selectin ligand, alone or in combination, had no effect on either islet homing of these T cells or development of diabetes [38]. Thus, none of the molecules generally implicated in the regulation of initial T-cell adhesion can provide for the pancreas-specific homing of diabetogenic T cells.

It seems that not binding of selectins to their ligands, but rather TCR–MHC adhesive interactions, serve as a basis for initial tethering and rolling of islet-bound IS-$CD8^+$ T cells (Fig. 1). Recent studies demonstrated that the TCR coreceptor CD8 has a crucial role in antigen-specific binding of murine and human TCRs [39–41]. In some cases, CD8 acts as an adhesion molecule, stabilizing the TCR–MHC interaction that is sufficient to maintain firm T-cell adhesion under shear conditions [42].

In summary, the dynamic nature of the initial tethering and rolling process, along with the complexity of the signals involved in its regulation, has hindered progress in identifying potential drug targets and candidate molecules capable of interfering with the initial step of adhesion of diabetogenic T cells to the endothelium. One attractive approach that could be tested in animal models of T1D involves the blocking of insulin-reactive TCRs on autoreactive T cells using soluble MHC–insulin–peptide fusion complexes. Soluble MHC–peptide treatment has the potential to prevent intra-islet transmigration but can also trigger multiple changes in T-cell activation, drive insulin-reactive T cells into an anergic state or induce apoptosis of diabetogenic T cells. Thus, this approach might translate into meaningful therapeutic solutions in due time.

Activation of integrins

Circulating T cells maintain their integrins mostly in an inactive state and must undergo *in situ* modulation to develop high avidity of integrins for their endothelial ligands to establish firm shear-resistant adhesion on endothelium of target organs [43]. This process is known as activation of integrins; it occurs within a fraction of a second of initial tethering and rolling [44] and manifests as the regulated increase of integrins' adhesiveness in the absence of marked changes in integrins' cell-surface expression levels. Activation of integrins on the surface of islet-bound diabetogenic T cells is driven by two independent, but co-operating, mechanisms: TCR engagement is the specific component, and chemokine receptor signaling is the nonspecific component of the pathway.

TCR-mediated integrin activation

This process requires presentation of pancreatic antigens, such as insulin, by ECs. TCR ligation induces activation of interleukin-2-inducible T-cell kinase, which potentiates formation of the linker for activation of T cells (LAT)–VAV1–SLP76 signaling complex, which, in turn, is linked to the activation of phospholipase C-γ1 (PLC-γ1) [45–48]. In T cells, PLC-γ1 acts through activation of several guanine-nucleotide exchange factors (GEFs) and leads to activation of small GTPase – RAP1, which, in turn, binds its ligand RAPL [49,50]. The RAP1-RAPL pair seems to be the centerpiece in the crossroads of inside-out signaling because it is activated by both TCR ligation and chemokine receptor triggering. After TCR ligation, activated RAP1 associates itself with RAPL, which then forms complex with LFA-1 and quickly initiates LFA-1 conformational changes and membrane translocation. Dominant-negative RAPL inhibits TCR-ligation-induced upregulation of LFA-1 avidity [51]. RAPL-deficient T cells also show defective LFA-1-mediated adhesion after stimulation through the TCR [52]. Moreover, TCR activation results in direct phosphorylation of GEF2 at Ser960 by PKC-θ, which translates into RAP1 activation and drastic increase of LFA-1 adhesiveness to ICAM-1 [53]. In agreement, IS-$CD8^+$ cells upregulated integrin avidity upon stimulation of their TCR [5].

Chemokine receptor-mediated integrin activation

Stimulation of T cells with chemokines induces patch-like clustering of LFA-1 and micro clustering of VLA-4, increasing T cells' adhesion to ICAM-1 molecules [20] and providing for transition from tethering into T cells' firm adhesion under shear flow [27]. Chemokines act through G-protein-coupled receptors on the surface of T cells. They activate several signaling pathways including the phosphatidylinositol 3-kinase, PLC, RAS- and RHO- (RAS homolog) family small GTPases, as well as mitogen-activated protein kinase signaling cascades, which result in the activation of the same RAP1/RAPL pair (as does TCR signaling) [52]. Activated

RAP1 binds RAPL and forms a complex with intracellular LFA-1. This results in LFA-1 clustering (which modulates LFA-1 valency at the leading edge) and potentiates establishment of the firm adhesion of T cell [52].

In summary, we can infer that the complex of small GTPase RAP1 and its intracellular ligand RAPL leads to integrin activation on T cells [54]. RAP1 is activated by a variety of external stimuli, including chemokines CCL21 and CXCL12 [55] and TCR signaling [56,57]. Inhibition of RAP1 by signal-induced proliferation-associated protein 1 – a RAP1-specific GTPase-activating protein that promotes GTP hydrolysis – abrogates chemokine-induced adhesion that is mediated by both LFA-1 and VLA-4 [55]. Hence, RAP1 has an important role in inside-out signaling triggered by both chemokine receptors and TCR engagement.

Several chemokines are associated with the development of T1D. Increased levels of T_H1 cell-derived chemokines CCL3, CCL4 and CXCL10 were found in the serum of patients recently diagnosed with T1D [58–60]. In addition, a negative correlation between the blood levels of CCL3 and C-peptide was identified in cohorts of T1D patients [61]. Diabetogenic T_H1 cells in NOD mice expressed the CCR5 receptor and its ligand CCL3 [62,63]. Deletion of CCL3 in NOD mice ameliorated symptoms of insulitis and delayed autoimmune diabetes. These effects seemed to be rather monocyte- than T-cell-mediated, however, because CCL3 preferentially attracts macrophages and/or monocytes and CCL3 production in the islets is completely infiltrate-dependent [62].

Primary cultures of murine and human pancreatic islets expressed and secreted CCL2 [64]. High basal CCL2 production by human islets correlates with a poor clinical outcome after islet transplantation in patients with T1D [65]. During the course of diabetes, macrophages are probably the first cells to infiltrate islets of NOD mice and BB rats; depletion or inactivation of macrophages prevented development of TID [66]. CCL2 and CCL5 attract macrophages and other monocytes; their expression in the islets of NOD mice parallels disease progression and contributes to the formation of insulitis [62,64,65,67]. Transgenic expression of CCL2 in the islets succumbed T1D-resistant mice to develop spontaneous diabetes. Development of diabetes in these mice correlated with the accumulation of large numbers of monocytes in the islets, however, and did not depend on T and B cells [68]. This accumulation was reversed by CCR2 deficiency [68] and is in agreement with studies in *CCR2*$^{-/-}$ NOD mice, which exhibited delayed inflammatory cell recruitment [69]. By contrast, deletion of CCR5 led to accelerated diabetes, which was associated with aggressive insulitis and was accompanied by altered leukocyte migration into islets of NOD.*CCR5*$^{-/-}$ mice. Nevertheless, diabetogenic cells from NOD.*CCR2*$^{-/-}$ and NOD.*CCR5*$^{-/-}$ mice showed similar potency to adoptively transfer diabetes into NOD.*scid* recipients, suggesting that deletion of *CCR2* or *CCR5* on diabetogenic T cells did not affect their migratory properties [69]. Depending on the stage of the autoimmune process, interference with chemokine receptors might lead to unexpected consequences; monoclonal anti-CCR5 antibody treatment, used late in the autoimmune process, accelerated diabetes onset in NOD mice [70].

Both newly diagnosed and longstanding T1D patients were reported to have reduced amounts of CCR4$^+$ cells in peripheral blood [71]. In the NOD mice, expression of CCR4 ligand CCL22 on pancreatic islets induced intensive recruitment of CCR4$^+$ cells and accelerated disease, whereas the use of CCL22-neutralizing antibodies attenuated onset of T1D after adoptive transfer of diabetogenic cells. This effect, however, was attributed to the block of homing of CD4$^+$ T cells not to the islets but to pancreatic lymph nodes, where CCL22 is primarily expressed on dendritic cells [72].

Unlike other chemokine receptors, CXCR4 molecule is expressed widely and is involved in basal trafficking of naive lymphocytes [73]. In humans, polymorphisms in *CXCL12* gene, which codes the natural ligand for CXCR4, are linked to susceptibility to TID [74,75]. Transgenic overexpression of CXCL12 in the islets of C57BL/6.Tg*RIP-CXCL* mice provided resistance to streptozocin-induced β-cell apoptosis and diabetes and promoted β-cell survival via activation of the antiapoptotic Akt kinase [76]. CXCR4 RNA levels increase early in the islets of NOD mice [77]. Aboumrad and colleagues showed that in NOD mice, CXCR4$^+$ T cells specific for islet antigens displayed mostly regulatory function and, thus, completely abolished capacity of diabetogenic T cells to transfer diabetes. Administration of specific CXCR4 inhibitor AMD3100 accelerated adoptive transfer of diabetes, reduced numbers of CXCR4$^+$ T regulatory cells in the islets and increased numbers of autoreactive T cells and severity of insulitis [78]. The most recent study by Leng *et al.* [79], however, reported an opposite effect of CXCR4 inhibition, showing that continuous AMD3100 treatment of prediabetic NOD animals decreased accumulation of T cells in the bone marrow, significantly increased numbers of T regulatory cells in the periphery, dramatically reduced insulitis and protected animals from development of spontaneous diabetes. Consistent with this observation, injection of antibodies neutralizing CXCL12 was effective in inhibiting diabetes and insulitis without affecting autoimmune sialoadenitis in NOD mice [80]. These seemingly conflicting results can, at least in part, be explained by different adhesion properties of subsets of CXCR4$^+$ T cells with regulatory and cytotoxic phenotypes because CXCL12 was able to block adhesion of certain populations of diabetogenic T cells to the islet EC monolayers *in vitro* in a dose-dependent manner, whereas inhibition of T cells' CXCR4 with neutralizing antibodies reversed these SDF-1 chemorepulsive effects [81]. Thus, the CXCL12–CXCR4 axis indeed has a considerable role during T1D pathogenesis, providing for islet homing of autoreactive T cells and promoting β-cell survival.

Several lines of evidence suggest an important role for chemokine receptor CXCR3 and its ligands CXCL9, CXCL10 and CXCL11 in the pathogenesis of diabetes. Pancreatic β cells produce CXCL10 and CXCL9 chemokines, which specifically attract T-effector cells via the chemokine receptor CXCR3 [67]. A relevant role for these locally produced chemokines was observed in the mouse model of lymphocytic choriomeningitis virus (LCMV)-induced autoimmune diabetes. In these mice, complete absence of CXCR3 delayed onset of insulitis and diabetes, and blockade of CXCL10 – but not that of CXCL9 – prevented the development of T1D after LCMV infection [67]. Mechanistically, CXCL10 blockade impeded the expansion of peripheral Ag-specific T cells and obstructed their migration into the pancreas [82]. Conversely, LCMV-induced overexpression of CXCL10 accelerated the onset of T1D [82]. T1D-resistant B6 mice, engineered to express CXCL10 in the pancreatic islets (C57BL/6Tg.*RIP-CXCL10*), had limited insulitis and no spontaneous diabetes; however, when crossed

to C57BL/6Tg.*RIP-NP* mice, expressing LCMV nucleoprotein in the β cells, such animals had massively accelerated T1D after LCMV infection, characterized by a drastic increase in nucleoprotein-specific, autoaggressive $CD8^+$ T cells in the pancreas [83]. In DO11.10 TCR transgenic (RIP)-mOVA mouse model of T1D, subset of $CD4^+$ Tregs potently suppressed islet tissue infiltration via downregulation of CXCR3 on the surface of T_H1 effector cells, which failed to respond to pancreatic islet-derived CXCL10 and infiltrate the islets [84]. Similarly, T1D patients showed a reduced expression of CXCR3 on $CD4^+$ peripheral blood T cells at the time of diagnosis, but not 12 months later, suggesting that $CXCR3^+$ peripheral T cells are reduced in a narrow time window, possibly because of extravasation into the inflamed pancreas [71]. In addition, secretion of CXCL10 by autoaggressive IS-$CD8^+$ T-cell clones obtained from NOD mice determined their efficient intra-islet homing and high diabetogenic capacity [7]. Taken together, these findings suggest that the CXCL10–CXCR3 axis might present a broadly applicable target for the inhibition of T-cell recruitment into the pancreatic islets during T1D.

The other important set of chemokine–chemokine receptor molecules directly involved in the intra-islet migration of autoreactive T cell seems to be CCR7 and its ligands CCL21 and CCL19. CCL19 and CCL21 are expressed in immediate islet vicinity, in paraductal areas and their expression was increased in islet areas rich in inflammatory cells [85,86]. CCR7 regulates migration of T cells and other leucocytes into secondary lymphoid organs [87,88], where it is implicated in control of cytotoxic T-cell priming [89,90], and also plays a part in the induction and maintenance of central and peripheral tolerance [91,92]. A recent study has implicated that differences in surface expression of CCR7 affected migration pattern of transferred diabetogenic T cells [93]. Earlier, it was shown that CCL21 expression within the immediate islet vicinity is an absolute prerequisite for the intra-islet homing of diabetogenic IS-$CD8^+$ T cells [5]. In addition, diabetogenic $CD4^+$ T cells sensed and migrated toward the gradient of CCL21 [94]. In agreement, the higher percentages of $CD8^+CCR7^+$ lymphocytes and the lower percentages of $CD8^+CD45RO^+$ cells were detected in peripheral blood of patients with recent onset of T1D, compared with healthy children. This might reflect the selective recruitment of activated $CCR7^+CD8^+$ T cells into the pancreas [95]. Another recent study found that CCR7-deficient NOD mice (NOD.$CCR7^{-/-}$) never developed diabetes but showed severe inflammation in multiple tissues, including thyroid, lung, stomach, intestine, uterus and testis [96]. This suggests a complexity in the generation of multiple autoimmune phenotypes in NOD mice and indicates that CCR7 is a key molecule affecting their development.

In summary, the specificity and complexity of the chemokine system – which implies release of specific chemokines in different tissue compartments at different time-points of T1D pathogenesis, on the one hand, and tightly regulated expression of chemokine receptors on different subsets of T cells, on the other hand – makes it extremely difficult to identify the unique role of individual chemokine–receptor pairs in islet-specific migration of diabetogenic T cells. However, a recent study utilizing overexpression of murine gammaherpesvirus-68 M3 gene in islets of NOD mice [97] opened a very promising avenue on interference with chemokine signaling-induced T-cell homing events during T1D pathogenesis. The M3 gene encodes a secreted 44-kDa protein with no sequence similarity to known chemokine receptors. It was shown to bind with high affinity a broad range of chemokines, including the ones upregulated in the NOD model of TID (i.e. CCL2, CCL3, CCL4, CCL5 and CCL21). The M3 gene product binding efficiently blocked chemokines from interactions with their receptors and inhibited chemokine-induced elevation of intracellular calcium levels [98,99]. Islet-specific expression of the pan-chemokine blocker M3 abrogated inflammatory cell infiltration of the islets and completely blocked the development of diabetes in NOD mice [97], suggesting that chemokines mediate afferent and efferent immunity in T1D. Thus, broad chemokine blockade might represent a viable strategy to interfere with islet homing of diabetogenic T cells.

Mechanistically, endothelial cross-presentation of islet antigens enhances the strength of T-cell adhesion and works in concert with chemokine receptors. These events account for the transition from initial tethering and rolling to the firm adhesion via integrin activation (Fig. 1).

Arrest of rolling or firm adhesion of T cells on endothelium

Firm adhesion of lymphocytes occurs mostly in high endothelial venules and requires *in situ* activation of at least one of the three main integrins: VLA-4, LPAM-1 and LFA-1. LFA-1($\alpha_L\beta_2$)–ICAM-1, VLA-4($\alpha_4\beta_1$)–VCAM-4 and LPAM-1($\alpha_4\beta_7$)–MadCAM-1 adhesive interactions are important for the development of diabetes [100–103]. Although Hanninen *et al.* [38] suggested that LFA-1 is dispensable for diabetes development, a report by Barlow *et al.* [104] highlighted the essential role of β_2 integrins during insulitis formation. In agreement, genetic absence of either of the LFA-1 chains β_2 and α_L completely prevented the development of insulitis and overt diabetes in NOD mice. Whereas β_2-chain deficiency completely abrogated T-cell–EC adhesion, lack of α_L molecules probably affected T-cell activation [105]. Moreover, prevention of autoimmune diabetes in NOD mice by PPAR-gamma agonists, thiazolidinediones, was reported to be associated with suppression of IL-1β-induced ICAM-1 expression, leading to a reduced vulnerability of pancreatic β cells during the effector stage of islet destruction [106]. Accordingly, lack of ICAM-1 expression prevented diabetes by abrogating insulitis in NOD.*ICAM-1*$^{-/-}$ mice [107].

Strategies to interfere with integrin-mediated adhesion during T-cell migration into target tissues in the course of autoimmune diseases have already been translated into the clinic. Natalizumab, a monoclonal antibody to the α_4 integrins that blocks binding of $\alpha_4\beta_1$ (VLA-4) to VCAM-1 on brain-infiltrating T cells and binding of $\alpha_4\beta_7$ (LPAM-1) to MadCAM-1 on gut-infiltrating T cells, has been used successfully for the treatment of multiple sclerosis and Crohn's disease, respectively [108,109].

Given the restricted expression and function of integrins in different subsets of diabetogenic T cells, 'bispecific' antibodies directed to target both suspected integrin and its binding partner might function in a cell-type-restricted way and be more selective than the 'monospecific' integrin-blocking antibodies or drug antagonists.

The other important player supporting firm adhesion of T cells to the vascular bed is the CD44 molecule. CD44, a transmembrane multifunctional cell adhesion protein, is the principal receptor for hyaluronan (HA) and can be considered crucial in support of firm adhesion of T cells to endothelium and further extravasation steps

[110]. CD44 has a crucial role in a variety of cellular behaviors including adhesion, migration, invasion and survival [111]. Through its cytoplasmic domain, CD44 interacts with the ezrin, radixin and moesin proteins; members of the Rho-family GTPases and their exchange factors; and adaptor molecules that link CD44 to the actin cytoskeleton and promote CD44-induced cytoskeletal rearrangements [112]. CD44 ligand, HA (a nonsulfated linear glycosaminoglycan) is abundantly present in the ECM and on the surface of ECs and, together with its fragments, is promigratory [112,113]. HA synthesis can be induced by proinflammatory stimuli such as TNFα, IL-1β, IL-15 and Lipopolysaccharide (LPS), mostly on ECs derived from microvascular, but not large, vessel sources, consistent with the dominant role of microcapillaries in leukocyte trafficking [114,115]. An increase in surface levels and substrate-binding affinity of CD44 is a well-known consequence of T-cell antigenic stimulation [116] and is considered to reflect activity of autoimmune disease [114,117]. The activated form of CD44 supported rolling and adhesive interactions on HA substrates both *in vitro* and *in vivo* [118–120]. CD44–HA interactions are required for the extravasation of a wide variety of pathogenic T cells because CD44-mediated lymphocyte migration has been prominently associated with human arthritis, collagen-induced murine arthritis and mouse model of multiple sclerosis [114,117,121–123]. Most importantly, targeting CD44 with neutralizing antibodies, as well as pretreatment of NOD mice with hyaluronidase, induced resistance to T1D in the adoptive transfer model and CD44 neutralization also protected NOD mice from the development of spontaneous T1D [124].

In summary, several potentially beneficial approaches that involve interfering with firm adhesion of autoreactive T cells can be suggested and tested in the corresponding experimental settings. They include 'bispecific' β_2 integrin–ICAM-1 monoclonal antibodies and CD44-neutralizing agents, as well as HA-modifying enzymes.

Diapedesis

The last step in the autoreactive T cell's journey from the bloodstream into the islets of langerhans is termed diapedesis, or transmigration. Mechanistically, it is the passage of T cells through the capillary walls and underlying basement membrane into the islet tissue. Besides well-documented integrin–CAM involvement, this process depends first on rearrangement of lymphocyte's cytoskeleton, coordinated by GTPases RAP1 and RhoA and their multiple downstream effectors [52,55], and second on a series of proteolytic events promoting leukocyte motility over and across endothelial barriers. Several proteases, and in particular MMPs, are upregulated during transendothelial migration. Localized proteolysis by migrating leukocytes can expose cryptic ECM ligands, modulate chemokine-binding specificity and release ECM-stored chemokines and inflammatory cytokines involved in leukocyte locomotion and retention [125].

T cells express several individual MMPs, including a membrane-tethered MT1-MMP enzyme [26,126–129]. MT1-MMP is distinguished from soluble MMPs by a C-terminal transmembrane domain and a cytoplasmic tail [130]. MT1-MMP is synthesized as a latent zymogen and requires proteolytic processing for activation. Once activated, MT1-MMP can be inhibited by its natural inhibitors, tissue inhibitors of metalloproteinases (TIMPs), with TIMP-2 being the most potent one [130]. Recent reports indicate that T-cell MT1-MMP becomes active after the firm adhesion of T cells and show that the proteolysis of CD44, integrins, transglutaminase, LRP, E-cadherin and related adhesion-signaling cellular receptors is the important function of MT1-MMP [131–134]. During cell migration, MT1-MMP forms a complex with CD44 via the hemopexin domain [111,135,136]; in this complex, CD44 is a direct substrate of MT1-MMP for shedding [137].

MT1-MMP is the major cell-surface-associated proteinase that contributes to the shedding of CD44 in the adherent autoimmune IS-CD8$^+$ T cells [128,129,138]. MT1-MMP becomes active only in adherent diabetogenic T cells [12,26,128,129]. Active MT1-MMP zymogen cleaves T-cell CD44, releasing its extracellular domain from IS-CD8$^+$ T-cell surfaces, thus rendering the CD44 cell receptor inactive [12,26,128,129]. MT1-MMP proteolysis of CD44 seems to control the severity of the diabetic disease and mediates the transition of T-cell adhesion on the endothelium to transendothelial migration, which results in T-cell homing into the pancreatic islets. TIMP-2, but not TIMP-1 (a poor inhibitor of MT1-MMP), is shown to efficiently block CD44 shedding in T cells [12,26,128,129]. Moreover, inhibition of MT1-MMP proteolysis of CD44 by hydroxamate inhibitor extended adhesion of IS-CD8$^+$ T cells to the vascular endothelium, impeded their intra-islet homing efficiency and significantly delayed onset of diabetes transferred by either IS-CD8$^+$ T cells or total diabetogenic splenocyte population [12,26,128,129]. Similarly, TIMP-2, but not TIMP-1, decreased T-cell transmigration and preserved insulin production in a T1D organ culture model [139].

We suggest that in addition to interference with islet homing of diabetogenic T cells, MT1-MMP inhibition is likely to block MT1-MMP-driven proteolysis of basement membrane laminin and rescue laminin-stimulated β_1 integrin signaling in β cells and, thus, might also contribute to β-cell survival.

In summary, most recent data convincingly demonstrated that the prolonged pharmacological inhibition of MT1-MMP-dependent CD44 shedding resulted in a therapeutic effect in spontaneously diabetic NOD mice. More specifically, it was shown that using the MMP inhibitor AG3340, T-cell intra-islet transmigration could be blocked, resulting in partial restoration of β-cell function, increased insulin production and alleviation of the severity of T1D in acutely diabetic NOD mice [12]. These results indicate that MMP inhibitors might provide a meaningful tool to explore the effects on intra-islet homing of autoimmune T cells in T1D patients.

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