Altered Function of Antigen-Presenting Cells in Type 1 Diabetes: A Challenge for Antigen-Specific Immunotherapy?

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Type 1 diabetes (T1D) arises from a failure to maintain tolerance to specific β-cell antigens. Antigen-specific immunotherapy (ASIT) aims to reestablish immune tolerance through the supply of pertinent antigens to specific cell types or environments that are suitable for eliciting tolerogenic responses. However, antigen-presenting cells (APCs) in T1D patients and in animal models of T1D are affected by a number of alterations, some due to genetic polymorphism. Combination of these alterations, impacting the number, phenotype, and function of APC subsets, may account for both the underlying tolerance deficiency and for the limited efficacy of ASITs so far. In this comprehensive review, we examine different aspects of APC function that are pertinent to tolerance induction and summarize how they are altered in the context of T1D. We attempt to reconcile 25 years of studies on this topic, highlighting genetic, phenotypic, and functional features that are common or distinct between humans and animal models. Finally, we discuss the implications of these defects and the challenges they might pose for the use of ASITs to treat T1D. Better understanding of these APC alterations will help us design more efficient ways to induce tolerance.

Type 1 diabetes (T1D) results from T-cell–mediated destruction of insulin-producing pancreatic β-cells, leading to hyperglycemia and associated complications (1). The etiology of T1D is not completely understood, but both genetic and environmental factors are known contributors in conjunction with a decline of central and peripheral tolerance mechanisms. T1D susceptibility genes substantially overlap with other polygenic autoimmune and autoinflammatory diseases (Supplementary Table 1), and T1D patients may develop other autoimmune diseases such as thyroiditis and celiac disease (2). Therefore, although a number of genetic traits may predispose to multiple autoimmune diseases, specific precipitating events may serve as a trigger and dictate which tissue(s) becomes targeted by autoreactive T cells. Whether it is deletion, anergy, or induction of regulatory T cells (Tregs), all mechanisms of tolerance require presentation of self-antigens to thymocytes or peripheral T cells by tolerogenic antigen-presenting cells (APCs), which are equipped to deliver, upon engagement of T cells, appropriate signals to prevent or shut down unwanted responses. The most studied are professional (hematopoietic) APCs, such as dendritic cells (DCs), macrophages (MΦs), and B cells. However, these APCs constitute double-edged swords in T1D because they may be inappropriately swayed toward immunogenic functions. Here, we comprehensively review APC features and functions that are relevant to tolerance (Fig. 1) and how they are altered or defective in humans and animals with T1D, including genetic associations that may influence these functions (Table 1 and Supplementary Tables 2 and 3). We then discuss the implications of such APC alterations on the efficacy of antigen-specific immunotherapies (ASITs), which aim to deliver particular self-antigens to the patient's endogenous populations of APCs for tolerance induction (Fig. 2 and Table 2). In the context of this review, we define APC as any cell that can present self-antigens and may potentially be the target of ASITs.

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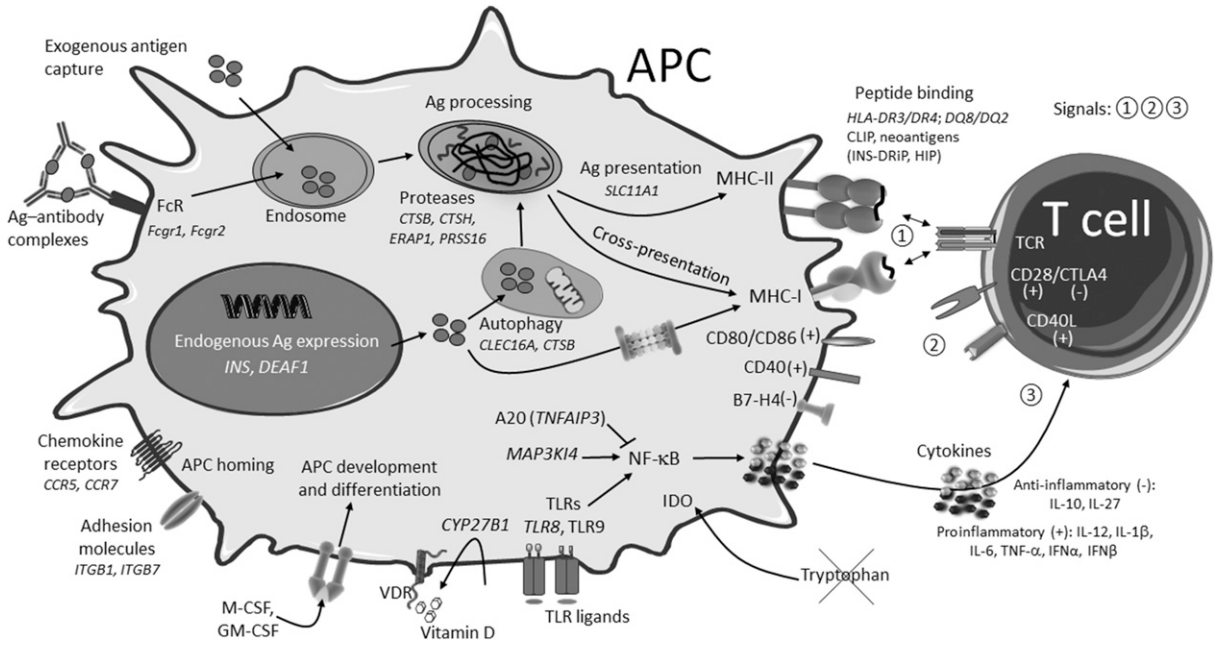

Figure 1—Summary of APC biological processes affected in T1D with examples. Processes shown are from multiple APCs (DCs, MΦs, B cells, mTECs, stromal cells) and may not all be found in a given type of APC. (+) and (-) denote immunogenic and tolerogenic signals, respectively. Ag, antigen; DRiP, defective ribosomal product; FcR, Fc receptors; HIP, hybrid insulin peptide; VDR, vitamin D receptor.

APC DEVELOPMENT AND FREQUENCIES

The relative proportion of different types of APCs may influence the frequency of their interactions with self-reactive T cells and the subsequent phenotype of these T cells. Peripheral blood DCs are differentially altered in their proportions depending on the age and disease stage (new-onset or long-term T1D vs. control group) of subjects (Supplementary Table 4*A*). The youngest patients appear to have a deficit in both myeloid DCs (mDCs) and plasmacytoid DCs (pDCs), although this difference is not apparent when evaluating a broader age range. The term mDC is now obsolete, but in most studies listed (Supplementary Table 4), it refers to $CD11c^+CD123^-$ DCs, and in a few studies, specifically to migratory $CD1c^+$ DCs. Furthermore, fewer monocyte-derived DCs (moDCs) were obtained from T1D or at-risk patients compared with control subjects (Supplementary Table 4*B*). Polymorphism in several human susceptibility genes may impact the development and number of APCs, including *SH2B3* for DCs, *IKZF1* for mDCs and pDCs, and *GAB3* and *ZFP36L1* for monocytes and MΦs (Supplementary Table 2).

Studies in NOD mice have made it possible to analyze APC frequency, phenotype, and function beyond the peripheral blood. NOD mice have consistently yielded fewer splenic DCs, particularly splenic $CD8\alpha^+$ DCs (Supplementary Table 5*A*). Similarly, the yield of bone marrow-derived DCs (BM-DCs) generated in vitro was reduced in NOD mice in the great majority of studies (Supplementary Table 5*B*). Defects affecting in vivo DC subsets, as well as pDC precursors, could be corrected by treatment with FLT3L (Supplementary Table 6). However, it is unclear if insufficient levels of FLT3L or other growth factors are responsible for the reduced number of certain DC subsets. Candidate genes for other pertinent growth factors in NOD susceptibility regions include *Csf1* (Idd18.3, encoding macrophage colony-stimulating factor [M-CSF]) and *Csf2* (Idd4.3, encoding granulocyte-macrophage colony-stimulating factor [GM-CSF]) (Supplementary Table 3). Polymorphism on these genes (and perhaps others) or altered responsiveness to these cytokines may affect the development and differentiation of myeloid APC progenitors or the function of myeloid cells (Supplementary Table 6). On the one hand, MΦs from NOD mice and monocytes from T1D patients and high-risk subjects express higher basal levels of GM-CSF, leading to persistent STAT5 stimulation. This increased GM-CSF production is somewhat surprising given the generally reported lower frequency of DCs and the therapeutic benefit of GM-CSF in T1D (3). On the other hand, responsiveness to M-CSF is reduced in NOD mice (Supplementary Table 6) and may be influenced in humans by the susceptibility genes *GAB3* and *PTPN2*, which encode signaling components downstream of the M-CSF receptor (Supplementary Table 2).

Merocytic DCs, a lesser-known population of $CD11c^+CD8\alpha^-CD11b^{-/low}$ DCs that can break the tolerance to apoptotic cell–derived antigens, are increased in NOD mice compared with other strains (4). Pancreatic islets contain several populations of DCs and MΦs (5–7). The majority of islet DCs are $CD11b^+CX3CR1^+$ DCs that are potentially proinflammatory and increase over time, whereas $CD103^+$ DCs represent a minority that typically migrate to pancreatic lymph nodes (PLNs) to present

Table 1—Summary of APC functions affected in T1D

APC functions and pathways			T1D (human)	T1D (rodents)
APC development (Supplementary Tables 4–6)		Cell number and yield	**Fewer blood DCs (young subjects); reduced moDC yield in vitro**	**Fewer splenic DCs in vivo; reduced BM-DC yield in vitro**
		APC generation and expansion	*IKZF1** DC subsets?; *SH2B3** DC numbers? *GAB3*/ZFP36L1** monocyte and MΦ generation? ***GAB3*/PTPN2*** **response to M-CSF**	***Csf1*^{M}/*Csf2*^{M}**; **responsiveness to M-CSF** ↓ in MΦs ***Ptpn2*^{M}**?
Antigen presentation	Antigen expression (Supplementary Table 7)	β-Cell autoantigens	***INS**; *IA-2/IGRP* (splicing)	*Iapp*^{M}; ***Ins***^{R}
		PTA regulation	***DEAF1*** (splicing)	***Deaf1*** (splicing)
	MHC (Supplementary Tables 8–10)	MHC-II haplotype	*HLA-DR3/4;* ***DQ8/2***	***I-A^{g7}*** (^{M}); *RT1^{u}* (^{R})
		MHC-II expression	HLA-DR ↑ ***SLC11A1****	MHC-II ↓ ***Slc11a1***^{M} (↑ Nramp)
		MHC-I	*HLA-B*; *HLA-A*	β2-microglobulin$^{M/R}$
	Antigen capture	Phagocytosis	***FCGR2A****	***Fcgr2***^{M} in MΦs; impaired clearance of apoptotic cells
	Antigen processing and loading (Supplementary Table 8)	Autophagy	*CLEC16A**; *CTSB**	
		Proteolysis	*CTSB**; ***CTSH***; *ERAP1**; *PRSS16**	***Ctsh***^{M}?
		Peptide binding	**CLIP**; neoantigens (DRiP, **HIP**)	**CLIP**, **HIP**
		Cross-presentation	***RAC2***?; *MAP3K14**?	Impaired in CD8α^{+} DCs; ***RAC2***^{R}?
APC activation and function		Maturation (Supplementary Table 11)	**NF-κB pathway hyperactivity** (monocytes, moDCs); *MAP3K14** *TNFAIP3** A20 ↓	**NF-κB pathway dysregulation and hyperactivity**; ***Nfkb1***^{R}
		Costimulation (Supplementary Tables 9 and 10)	No consensus on costimulatory molecule expression; *CD226**	No consensus on costimulatory molecule expression
		Cytokines (Supplementary Tables 12 and 13)	***IL10***; ***C1QTNF6***?; ***SLC11A1***?	***Il10***^{M}?; *CD101*^{M}? ***C1qtnf6***^{R}?
			IL-10↓ in B cells ***IL27****	***Slc11a1***^{M}? ***Il27***^{R}
			IL-12 ↓ or ≡ in DCs; *TYK2**?; *STAT4**? ***SLC11A1***?	IL-12 ↓ in DCs; ↑ in MΦs; no consensus in BM-DCs; ***Slc11a1***^{M}?
			IL-1β ↑ or ≡ in monocytes	***Il1a/Il1b***$^{M/R}$
			IFN-α/IFN-β ↑; *IFIH1** *TLR8**; ***TAGAP***; ***PTPN22***	**IFN-α/IFN-β** ↑ ***Tagap***^{M}?; ***Ptpn22***^{M}
			GM-CSF ↑ (monocytes)	**GM-CSF** ↑ (MΦs)
		Tolerogenic function (Supplementary Table 14)	Defective Treg induction by lamina propria DCs; *NRP1**?; **B7-H4** ↓; galectin-1 ↓; *CYP27B1**	Defective tolerance induction by CD8α^{+} DCs; **B7-H4** ↓; IDO ↓ in DCs and fibroblasts
APC adhesion and homing (Supplementary Table 15)		Cell adhesion	*ITGB1**; *ITGB7**; ICAM-1 ↓ (monocytes)	Fibronectin adhesion ↑; SLAM ↓
		Chemotaxis	**CCR2** ↓; ***CCR5***; ***CCR7***; ***CXCL12***; ***GPR183***; ***SKAP2***; *CD69**	**CCR2** ↓; **CCR5** ↓; ***CCR7***^{M}? **CXCL12** ↑; ***Skap2***^{R}

Genes associated with the disease are denoted by * for humans, ^{M} for NOD mice, or ^{R} for diabetes-prone BB rats; they are described in more details in Supplementary Tables 1, 2 (humans), and 3 (rodents). For more details about the genes or functions in each category, refer to the indicated supplementary tables. All functions listed under rodents are for NOD mice. Commonalities between patients and rodent models are in boldface type. A question mark indicates that the role of the gene in a particular function is speculative or that the gene association is not refined. When the same gene is linked to T1D in both humans and animal models, it is nonetheless possible that the function of that gene is affected differently between the two species. ↑, increased; ↓, decreased; ≡, unchanged. DRiP, defective ribosomal product; HIP, hybrid insulin peptide.

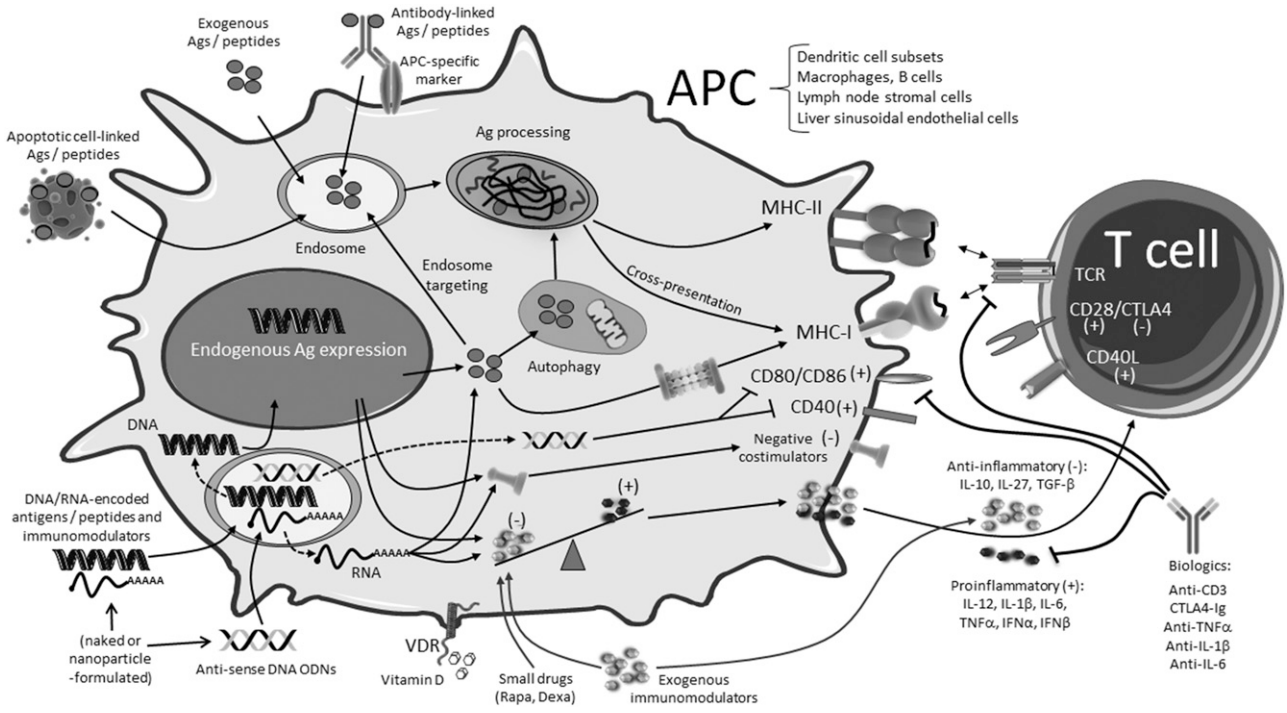

Figure 2—Summary of ASIT approaches and associated therapies for T1D. (+) and (-) denote immunogenic and tolerogenic signals, respectively. Not shown: Exogenous antigens/peptides may be formulated for codelivery with small drugs or other immunomodulators or conjugated with molecules other than the antibody for specific cell targeting. Ag, antigen; Dexa, dexamethasone; ODNs, oligodeoxynucleotides; Rapa, rapamycin; VDR, vitamin D receptor.

antigens (6) but are reduced in the islets of prediabetic NOD mice (8). Islet MΦs also take part in the initiation of disease (9). It is unclear if the frequency or phenotype of DCs and MΦs in NOD islets is abnormal prior to the inflammatory leukocytic infiltration since this process starts very early.

ANTIGENIC SIGNALS

Antigen Expression

In order to prevent autoimmunity, peripheral tissue antigens (PTAs) must be presented by tolerogenic APCs. Migratory DCs continuously acquire antigens in the tissue of origin to later present them to T cells in draining lymph nodes and, to some extent, in the thymus. Some antigens may also flow to local lymph nodes via lymphatics for uptake by local APCs. However, the predominant mechanism of tolerance varies per tissue. In mice, T cells that are specific to a pancreatic antigen and not deleted in the thymus tend to be ignorant and may become activated upon vaccination with this antigen and adjuvant (10). This is in contrast to ubiquitous antigens that primarily lead to deletion and to gut or lung antigens that induce Tregs (10). Shedding of self-antigens by the tissue does not apply to all PTAs, and many PTAs may also be ectopically expressed at very low levels by other cells, some of which have antigen-presenting and tolerogenic properties. Most importantly, medullary thymic epithelial cells (mTECs) express a variety of PTAs, owing to the activity of transcription regulators such as AIRE and FEZF2 (11,12), and play a crucial role in mediating deletion of autoreactive T cells and/or Treg induction while also serving as a local source of antigen for thymic DCs. Among β-cell antigens, insulin is expressed in mTECs under AIRE's control and also in a variety of other cells in the periphery, including subsets of DCs that also express AIRE (Supplementary Table 7). Lower expression of the *INS* gene in the thymus, due to risk variants affecting its promoter, may result in insulin-reactive T cells being less efficiently engaged for deletion or deviation toward Tregs. The *INS* gene is also associated with T1D in diabetes-prone BioBreeding (BB) rats (Supplementary Tables 1–3). Other β-cell antigens, including islet antigen 2 (IA-2), islet amyloid polypeptide (IAPP), and islet-specific glucose-6-phosphate related protein (IGRP), are also ectopically expressed in the thymus and peripheral lymphoid tissues (Supplementary Table 7), and IAPP is the only other β-cell autoantigen found in a susceptibility region (Idd6.2 of NOD mice) (Supplementary Table 3). The expression of some PTAs in lymph nodes is controlled by DEAF1, a transcriptional regulator that resembles AIRE. Both NOD mice and T1D patients have excessive splicing of *DEAF1* mRNA in PLNs, leading to loss of DEAF1 function, which correlates with reduced local expression of PTAs (13). Overall, defects in ectopic PTA expression could limit the availability of self-antigens in sites such as the thymus or lymph nodes for tolerance induction.

Table 2—Challenges ascribed to APC alterations and approaches to overcome them

Function affected	Challenge	Approaches
APC development	Some populations of tolerogenic DCs may be reduced in number	Current: APCs involved and their phenotype are not known Future: Identify alternative DC subsets that may be reprogrammed for tolerance; consider and harness nonhematopoietic cells as alternative APCs to increase antigen exposure; develop and evaluate artificial APCs
Antigen expression and distribution	Insufficient thymic expression; insufficient distribution of β-cell antigens beyond draining lymph nodes	Main rationale for ASIT: to improve availability of antigens to engage and tolerize autoreactive T cells Current: Typically one single antigen and one route, protection may be lost after treatment is discontinued Future: Delivering antigens to multiple sites may be beneficial; consider multiple β-cell antigens, especially those with limited exposure to the immune system; consider more sustained antigen exposure (long-term expression via DNA vaccines?)
Antigen capture	Defective acquisition of exogenous antigens	Not reported in patients; defects in NOD mice not an issue Current: Uptake may be limited for certain types of APCs Future: Use of micro- or nanoparticles may improve both capture and antigen load per cell; nucleic acid–encoded delivery may increase antigen load per cell
Antigen processing and presentation	Inability to generate certain neoepitopes from native antigens outside the islets	Current: Only native antigens Future: Neoepitopes to be considered for inclusion in ASIT (HIPs, mimotopes); ramp up identification of such neoepitopes
	Limited autophagy may limit Treg induction from endogenous antigens	Current: Antigens from DNA vaccines not presented to $CD4^+$ T cells unless released Future: Endosome targeting and/or active secretion of endogenously expressed nucleic acid–encoded antigens; improve targeting of exogenous antigens to DC subsets that are best at inducing Tregs
	Defective cross-presentation	Current: May result in poor engagement of $CD8^+$ T cells Future: Supplement classic (exogenous) antigen delivery with nucleic acid–based delivery
APC maturation	Excessive DC or MΦ maturation	Current: Only antigens administered Future: Combination therapy: block proinflammatory cytokines that act on APCs, limit exposure to TLR ligands (gut leakiness impacting PLNs?)
Costimulation	Imbalance between positive and negative costimulatory molecules	Current: Positive costimulatory molecules silenced with antisense oligonucleotide DNA in mice (not yet used in conjunction with antigens) Future: Combine with antigens, plus overexpress negative costimulatory molecules
Cytokines	Imbalance between proinflammatory and suppressive cytokines	Current: Overexpress suppressive and regulatory cytokines (in mice); neutralize proinflammatory cytokines produced by APCs Future: Combination therapy with antigens
Tolerogenic function	Defective stimulation or induction of Tregs, defective pathways (IDO, vitamin D) due to insufficient expression or responsiveness	Current: Vitamin D used in some trials Future: Boost the overall tolerogenic function of APCs and Treg induction with favorable dietary supplements (vitamins A and D, short-chain fatty acids) and locally delivered/targeted drugs (rapamycin, dexamethasone)
Homing	Same chemokines may recruit both proinflammatory and regulatory APCs (and T cells) to islets	Current: Mainly CXCL10 blockade in preclinical studies Future: Better understanding of the chemokine receptor profile of islet-infiltrating APCs and T cells in humans is needed; selective blockade of certain chemokines may be considered
	Defective homing of DCs to lymph nodes may also limit tolerance induction	Current: One pilot clinical study with intralymphatic injection Future: Novel antigen formulation to enhance delivery to lymph nodes may prove beneficial

We distinguish between current approaches that have already been evaluated clinically and possible future approaches to improve the current ones and to better address challenges from APC alterations. HIPs, hybrid insulin peptides.

Antigen Capture and Phagocytosis

A reduced ability to capture self-antigen for presentation may hinder tolerance induction. Defective clearance and persistence of immune complexes or apoptotic cells may also cause local inflammation and aberrant reactivity against self-antigens. The *Fcgr2* allele in NOD mice was associated with lower expression on MΦs (14), whereas the human gene *FCGR2A* is linked to T1D, though not as significantly as celiac disease (15). Furthermore, NOD MΦs are inefficient in the phagocytosis of apoptotic cells (16–18), which was linked to Idd5. This may contribute to the earliest stages of the disease pathogenesis by defective clearance: first, of dying β-cells, resulting in accumulation of DNA and antigens, and second, of immune complexes between natural antibodies and apoptotic components that can stimulate the production of IFN-α and other inflammatory cytokines by pDCs (19). Whether defects in clearance and antigen capture are implicated in human T1D has not yet been established, though they are typically found in systemic lupus erythematosus, a disease with a substantial overlap of susceptibility genes with T1D (Supplementary Table 1).

Antigen Processing

Endogenously expressed or exogenously acquired antigens must be processed into peptides within APCs for presentation to T cells. Defects that affect antigen processing can influence the abundance and diversity of generated peptides and their availability for presentation. Presentation of endogenously expressed antigens on MHC-II molecules can be achieved via autophagy, as seen in mTECs (20). The susceptibility genes *CLEC16A* and *CTSB* regulate autophagy, including in mTECs where they influence the thymic selection of autoreactive T cells (Supplementary Table 8). The processing of proteins into peptides involves the activity of various proteases that influence the repertoire of self-antigens presented for tolerance induction. Genetic variations in *CTSB* and *CTSH*, which encode cathepsins B and H, two lysosomal cysteine proteases expressed in DCs and MΦs, are associated with T1D (Supplementary Table 8). The risk allele of *CTSH* has also been linked to lower insulin expression and production in the islets (21), but whether it also affects the ectopic expression of insulin elsewhere is unknown. Two other protease-encoding genes are also associated with T1D/autoimmunity in humans: *ERAP1*, which controls the trimming of peptides for MHC-I loading, thereby influencing recognition by $CD8^+$ T cells, and *PRSS16*, which has been found to impact the deletion of several diabetogenic $CD4^+$ T cells in the thymus (Supplementary Table 8). Overall, impaired mechanisms of antigen processing can lead to qualitative and quantitative changes in the generation of epitopes derived from self-antigens that are presented to T cells.

Antigen Presentation

Different antigenic peptides are subject to presentation to T cells based on their ability to fit on MHC molecules. The MHC region confers the greatest genetic susceptibility to T1D, with particular MHC-II haplotypes playing an essential role. In humans, combinations of HLA-DR3/DR4 and DQ8/DQ2 predispose to disease to different degrees, a lot more than other haplotypes (22). Rodent models have a unique MHC-II haplotype ($I\text{-}A^{g7}$ in NOD mice and $RT1^u$ in BB rats) that is required for spontaneous disease development. The mouse $I\text{-}A^{g7}$ MHC molecule structurally resembles HLA-DQ8 with a nonnegatively charged amino acid at position 57 of the β-chain that allows more promiscuous peptide binding (23,24). Poor HLA-DM editing leads to increased levels of CLIP peptide on MHC-II in both NOD mice and T1D patients (Supplementary Table 8). These molecules may also preferentially bind neoepitopes, such as insulin's defective ribosomal product on HLA-DQ8/DQ2 or hybrid insulin peptides on $I\text{-}A^{g7}$ and HLA-DQ8/DQ2 (Supplementary Table 8).

Although various haplotypes are responsible for qualitative differences in their ability to bind key peptides, quantitative differences have also been reported on the amount of MHC molecules expressed on the surface of APCs. There is overwhelming evidence that both splenic DCs and BM-DCs from NOD mice express abnormally low levels of MHC-II relative to many different strains of mice, with or without stimulation (Supplementary Table 9). BM-DCs from diabetes-prone BB rats also express lower levels of MHC-II than those from control strains (25). In contrast, a few studies reported unchanged or increased HLA-DR expression on DCs from T1D patients (Supplementary Table 10), but these findings are mainly from the same group. MHC-II expression and antigen presentation can also be affected by polymorphism in the *SLC11A1* gene in humans and NOD mice (Supplementary Tables 1 and 2).

Diabetogenic $CD8^+$ T cells have also been identified both in humans and rodent models. T1D predisposition by MHC-I haplotypes within HLA-A and HLA-B loci has been observed in humans (26), whereas in rodents, it is the gene for the β2-microglobulin component of MHC-I that has been found in susceptibility regions in NOD mice and BB rats that can influence disease in NOD mice (Supplementary Table 3).

Cross-presentation refers to the ability of limited subsets of APCs, primarily Batf3-dependent DCs ($CD8\alpha^+$ resident DCs and $CD103^+$ migratory DCs), to process and present exogenously acquired antigens on MHC-I molecules to $CD8^+$ T cells. In NOD mice, splenic $CD8\alpha^+$ DCs show a reduced capacity to cross-present MHC-I–restricted antigens to diabetogenic $CD8^+$ T cells (27). This defect may limit the presentation of certain islet cell-derived epitopes to induce tolerance. Cross-presentation of certain β-cell antigens by B cells also exacerbates T1D (28). In humans, cross-presentation may be affected by polymorphism in the *RAC2* and *MAP3K14* genes (Supplementary Table 2).

TOLEROGENIC VERSUS IMMUNOGENIC SIGNALS

The type of immune response initiated by DCs ultimately depends on the context in which antigens were acquired, which influences the balance of immunogenic

and tolerogenic functions of APCs. Once autoreactive T cells are engaged by specific antigens (signal 1), their response will be dictated by the sum of signals that are delivered to T cells in the form of contact-dependent costimulation (signal 2) and cytokine-mediated immunomodulation (signal 3) (Fig. 1).

APC Maturation and Regulatory Function

Mature DCs express higher levels of MHC and costimulation molecules, which are required to stimulate effector cells. Many studies have compared levels of costimulatory molecules on APC populations between NOD mice and control strains (Supplementary Table 9) or between T1D patients and control subjects (Supplementary Table 10), reporting variable and conflicting results, possibly due to a large variety of tested conditions. As an important regulator of APC maturation, the nuclear factor-κB (NF-κB) pathway was found to be altered in T1D in various ways (Supplementary Table 11). Several genes associated with T1D in humans may impact NF-κB activation, including NIK-encoding *MAP3K14* and A20-encoding *TNFAIP3* (Supplementary Table 2), the latter being an important negative regulator of DC immunogenicity associated with multiple autoimmune diseases (Supplementary Table 1). Other genes associated with DC maturation include *ERBB3* and *CD226* in humans (Supplementary Table 2) and genes within the Idd10/17/18 region in NOD mice (Supplementary Table 3).

Loss of tolerogenic function has been reported in multiple APC subsets from NOD mice. First, $CD8\alpha^+$ DCs ($CD40^{hi}$) are defective at inducing $CD4^+$ T-cell tolerance after antigen delivery targeted to these DCs, but after CD40 blockade, they improve in their ability to mediate T-cell deletion and reduce Th1 stimulation (29). A deficiency of DCs to mediate T-cell deletion in PLNs was previously mapped to Idd3 (including *Il2* and *Acadl*, underexpressed in stimulated NOD DCs) and Idd5 (*Slc11a1*, overexpressed in stimulated NOD DCs and MΦs) (Supplementary Table 2) (30). Second, both pancreatic $CD8\alpha^+CD103^+Langerin^+$ and $CD11c^+CD8\alpha^-$ DC populations also appear less tolerogenic than those from control strains based on phenotype and gene expression (8,31). Irradiated splenocytes from NOD mice also induced less suppressive Tregs than their B6 counterparts (32). Similarly in humans, both APCs from peripheral blood mononuclear cells and DCs from the lamina propria of T1D patients were defective in the induction of $Foxp3^+$ Tregs (33,34). T1D patients with suboptimal glycemic control produce moDCs that have reduced tolerogenic potential (35), indicating a negative influence of hyperglycemia. This loss of tolerogenic function is not understood but may be contributed by imbalance between positive and negative costimulatory signals or, as we will see later, between proinflammatory and immunoregulatory cytokines. For instance, NOD mice exhibit a gradual loss of the negative costimulatory molecule B7-H4 on the surface of APCs (compared with other strains) due to proteolytic cleavage, which correlates with increased levels of circulating soluble B7-H4; about half of T1D patients have high levels of soluble B7-H4 as well (36).

Cytokines and TLR Signaling

Full DC maturation commonly results in production of proinflammatory cytokines that support T-cell differentiation into effector T-cell subsets (IL-12 for Th1, IL-1β and/or IL-6 for Th17), whereas semimature DCs may express relatively high levels of costimulatory molecules without secreting proinflammatory cytokines and regulatory DCs may correspond to a terminally differentiated state with switch toward suppressive cytokines to regulate the elicited T-cell response. Cytokine imbalance may contribute to inappropriate T-cell responses to self-antigens (Supplementary Tables 12 and 13). Furthermore, aberrant Toll-like receptor (TLR) response to viruses, bacteria, and other microbes may lead to excessive secretion of proinflammatory cytokines.

IL-10 and IL-27 are two major anti-inflammatory cytokines produced by APCs and whose genes are associated with T1D in humans, though the markers associated with the human genes are in noncoding regions and it is not clear how the expression and/or function of these cytokines is affected by these polymorphisms. The same genes are found in susceptibility regions in NOD mice (*Il10*) and BB rats (*Il27*) (Supplementary Table 3). Other susceptibility genes can influence the production of IL-10 by APCs, including *Cd101* in NOD mice (Supplementary Table 3) and *C1QTNF6* in humans (Supplementary Table 2) and BB rats (Supplementary Table 3). Studies performed in NOD mice did not provide a consensus on whether IL-10 production is defective, although both $CD8\alpha^+$ and $CD8\alpha^-$ DCs in the pancreas seem to express less IL-10 (Supplementary Table 12*A*). IL-10 production does not appear to be defective in DCs and monocytes from T1D patients, and if anything, it may be increased in some cases (Supplementary Table 13). B cells can also serve as APCs and as a source of regulatory cytokines. T1D patients have altered frequency of different B-cell subsets, in particular they have fewer $CD19^+CD5^+CD1d^{hi}$ cells, which are thought to be IL-10–producing regulatory B cells (B10 cells), and B cells from T1D patients are defective in IL-10 production in response to TLR ligands (Supplementary Table 13).

IL-12 is secreted by DCs and MΦs to promote the differentiation of Th1 cells, the main type of pathogenic $CD4^+$ T cells in T1D. Although studies in NOD mice consistently point to a defective ability of splenic $CD8\alpha^+$ DCs to produce IL-12, this defect is overcompensated by increased expression of IL-12 by MΦs (Supplementary Table 12*A*), attributed to abnormal NF-κB signaling (Supplementary Table 11) and linked to the Idd4 region (Supplementary Table 3). Thus, assessing IL-12 production in only one type of APC may not provide a full picture of the milieu to which T cells are exposed. Studies using BM-DCs from NOD mice yielded variable results, though most studies reported increased production of IL-12, linked to excessive NF-κB

activity and to the Idd10/17/18 regions (Supplementary Tables 3, 11, and 12). In T1D patients, APCs express normal amounts of IL-12, although DCs may produce less IL-12 under certain conditions (Supplementary Table 13). Dysregulated IL-12 production may be influenced by variants of the *TYK2* and *STAT4* genes, whereas *SLC11A1*-encoded NRAMP1 may also affect the IL-10/IL-12 balance (Supplementary Table 2).

Proinflammatory cytokines TNF-α, IL-1, and IL-6 are usually undesirable in autoimmune diseases and commonly targeted by neutralizing biologics. When assessed in NOD mice, the production of these cytokines has been variable and inconsistent depending on the analyzed APCs, the control strains used for comparison, and the type of stimulation (Supplementary Table 12*B*). The *Il1a* and *Il1b* genes are associated with T1D in both NOD mice and BB rats (Supplementary Table 3). In T1D patients, there is also no clear consensus on how the expression of these cytokines is altered in DCs and monocytes (Supplementary Table 13).

Type I interferons are typically overexpressed when APCs from NOD mice or T1D patients are stimulated with resiquimod, CpG, or flu virus (Supplementary Tables 12*B* and 13). As cytokine expression by APCs is generally assessed after stimulation with TLR ligands, such as peptidoglycan (TLR2), polyinosinic:polycytidylic acid (TLR3), lipopolysaccharide (TLR4), resiquimod (TLR7/8), and CpG (TLR9), the observed alterations of cytokine expression also point toward abnormal TLR expression (37) or TLR signaling (38–40). The risk variant of the human *IFIH1* gene is associated with a greater production of type I interferons and responsiveness to self-RNA ligands (Supplementary Table 2). Other risk alleles associated with T1D and TLR signaling include *TLR8*, *PTPN22*, and *TAGAP* (Supplementary Table 2). Certain infections and changes in the microbiome can alter the function of APCs through TLR stimulation, at least in certain tissue compartments. The role of TLRs in T1D has been more extensively reviewed elsewhere (41).

Other Tolerance-Regulating Mechanisms

Defects affecting other mechanisms of tolerance maintenance have also been reported. Monocytes from T1D patients produce less galectin-1, an immunosuppressive molecule, than control subjects (Supplementary Table 14). Indoleamine 2,3-dioxygenase (IDO) limits T-cell activation by depriving the milieu of the tryptophan necessary for cell growth and contributes to Treg development and to the protective role of pDCs in NOD mice (Supplementary Table 14). However, DCs and stromal cells (fibroblasts) from NOD mice are impaired in the induction of IDO in response to IFN-γ (Supplementary Table 14). Finally, vitamin D regulates the tolerogenic function of DCs and the maintenance of Tregs. Therefore, alterations in the production of or responsiveness to vitamin D may contribute to autoimmunity. The genes *CYP27B1* (encoding an enzyme converting vitamin D into its active form) and *VDR* (encoding the vitamin D receptor) are associated with T1D in humans and BB rats, respectively (Supplementary Tables 2, 3, and 14). The expression of *CYP27B1* is reduced in APCs of T1D patients (Supplementary Table 14).

CELL ADHESION AND CHEMOTAXIS

Adhesion molecules facilitate contact between cells and help with the proper transmission of regulatory signals to and from APCs. Integrin genes *ITGB1* and *ITGB7* are associated with human T1D (Supplementary Table 2), with ITGB7 playing an important role in T-cell (and likely DC) recruitment to islets and homing of $CD103^+$ migratory DCs (Supplementary Table 15). Both integrins are involved in the binding of DCs to fibronectin, and mature BM-DCs from NOD mice show increased adhesion to fibronectin in vitro (Supplementary Table 15). Other altered adhesion molecules include ICAM-1 (reduced in moDCs of T1D patients) and SLAM (reduced in mature mDCs of NOD mice), the latter resulting in an impaired induction of "regulatory" natural killer T cells (Supplementary Table 15). SIRPα is expressed on MΦs and DC subsets to block cell phagocytosis via the SIRPα–CD47 interaction. Although not regarded as an adhesion molecule, the protein encoded by the NOD variant of *Sirpa* binds more strongly to CD47, resulting in enhanced T-cell proliferation induced by DCs (Supplementary Tables 3 and 15). It is unclear if this effect results from signaling on either side of the SIRPα–CD47 axis or simply from a tighter contact between the two cells. Variations in the strength and duration of adhesion may impact the potency of T-cell stimulation (APC–T-cell interaction) and the ability of APCs to exit one tissue (attachment to extracellular matrix) or access another (e.g., transendothelial migration).

Chemokine receptors also play a crucial role in the trafficking of migratory APCs to lymph nodes and to sites of inflammation. Both CCR2 and CCR5 play distinct but important roles in the recruitment of leukocytes to inflamed islets, reflecting differential attraction of proinflammatory versus regulatory APCs (Supplementary Table 15). CXCL12 may also control homing of certain APCs to the pancreas (Supplementary Table 15). CCR7 is required for the homing of migratory DCs to draining lymph nodes. In humans, *CCR7* is associated with T1D, and in NOD mice, mature BM-DCs have a defect in migration toward CCL19, one of CCR7 ligands (Supplementary Table 15). Polymorphism in the *SKAP2* and *GPR183* genes may also alter MΦ and DC migration (Supplementary Table 2). Finally, *CD69* is also associated with human T1D, and in addition to regulating retention of T cells in tissues, it may also control the ability of DCs to leave peripheral tissues to lymph nodes (Supplementary Table 2). However, all the aforementioned chemokine receptors and other homing/retention molecules are expressed in many immune cells, so their specific role in positioning APCs in the context of T1D and tolerance is still not clearly established.

APC ALTERATIONS IN T1D: CAUSE OR CONSEQUENCE?

Some of the changes seen in APC development, phenotype, and function may contribute to disease, result from disease, or simply occur in parallel as a reflection of the genetic makeup of the individual. As mentioned above, many aspects of APC frequency and function are affected by susceptibility genes, and genetically controlled APC alterations are more likely to be causative as they predate the initiation of disease. However, it is plausible that some of these alterations manifest themselves only in the context of inflammation. In T1D patients, changes in APC frequency or function (e.g., excessive secretion of proinflammatory cytokines or overreaction to such cytokines or TLR ligands) are most often evaluated after disease onset. The heterogeneity of patients, discussed below, leads to data inconsistencies, whereby alterations in APC parameters vary with disease stage, age-group, and criteria to measure responsiveness and maturation of APCs (examples can be found in Supplementary Tables 4, 10, and 13). This complicates our understanding of the contribution of APC alterations to predisposing to or perpetuating disease. Persistent inflammation and uncontrolled hyperglycemia can also have adverse repercussions on APC functions in mice (42) and patients (35).

IMPLICATIONS FOR ASIT

The Hurdles of Translating Therapies From Mice to Patients

In this review, we have identified multiple layers of APC function that are defective or simply vary in NOD mice or T1D patients (Fig. 1). These alterations are influenced by genetic polymorphism, response to infections, changes in microbiota, and other environmental factors. Although there are appreciable similarities between animal models and T1D patients (Table 1), the latter constitute a heterogeneous population in terms of genetic risk factors. The NOD mouse model is very valuable to understand the role of certain human genes when the genes of both species are associated with T1D. However, there are also limitations to the animal models when testing therapeutic approaches. Despite the numerous alterations in APC function reported in NOD mice, it has been possible to achieve antigen-specific tolerance by delivering antigens in different forms (protein, peptide, DNA) and via different routes (oral, subcutaneous, intramuscular, etc.). However, the most promising of these approaches that were evaluated in clinical trials had no or little beneficial effect in secondary prevention and treatment (recent onset) settings. Ineffective induction of tolerance may be due to a number of reasons, including insufficient antigen coverage or inadequate choice of antigens, inability to generate certain epitopes from delivered protein antigens or to properly bind them on MHC (e.g., inadequate HLA haplotype), improper expression of positive and negative costimulatory molecules on APCs, inability to target critical subsets of APCs, or resistance of antigen-specific T cells to the tolerogenic effect of APCs and/or Tregs, for example. The approaches currently in clinics may fail at different levels (Table 2). For example, the current practice of administering a single antigen (or multiple peptides of the same antigen) by a single route may not achieve the in vivo antigen distribution and broad engagement of diabetogenic T cells that may be required for complete tolerance induction (further discussed below). Moreover, low and sustained antigen levels may be preferable over antigen levels that fluctuate between different administrations. Less conventional approaches, including nanoparticle-based delivery and apoptosis-associated uptake (43), may facilitate immunomodulation or targeting of atypical tolerogenic APCs (44) but have not yet been translated to T1D patients. Occasionally, a transient effect (delayed loss of C-peptide) has been observed in particular subsets of patients or with unconventional delivery strategies (Table 3). These few studies suggest that *1*) antigens selected based on high autoantibody reactivity may have a greater therapeutic impact, *2*) HLA haplotype may influence the efficiency of antigen presentation for T-cell deletion, and *3*) efficient delivery to lymph nodes may have a more profound effect. As we enter the new era of precision medicine to address patient heterogeneity, understanding patient-specific patterns and defects will enable us to apply ASIT more effectively with relevant antigens and appropriate delivery platforms.

APC Heterogeneity and the Need for Better Profiling

The same APC population may have contrasting roles depending on the stage of disease. For example, pDCs are critical for the initiation of T1D (19,45) but also play a tolerogenic/regulatory role later on (46,47). Most data from patients and NOD mice suggest that this population is reduced in later stages of the disease (Supplementary Tables 4 and 5). The frequency and phenotype of certain human APCs may vary depending on the compared parameters, such as age range (children vs. adults) and disease status (autoantibody positive, new-onset T1D, long-term T1D, etc.) (Supplementary Tables 4, 10, and 13). Since these early studies on patient APCs, multiparametric technologies have come a long way (spectral flow cytometry or mass cytometry, high-throughput single cell sequencing) and would now allow for a very detailed profiling of different APCs before and after therapy. Revisiting blood APCs using these new technologies, for example, is warranted to stratify groups of patients and better correlate responsiveness to therapy (or lack thereof) with subgroups of patients. Although blood APCs are not perfect surrogates of lymphoid tissue APCs, they may nonetheless reveal genetically imprinted impairments that may be extrapolated and taken into account. This information, combined with genetic and serological data, as well as a similar multiparametric profiling of T-cell populations, will help us to better appreciate the heterogeneity of T1D patients and, as far as ASIT is concerned, to deliver pertinent antigens to appropriate APCs. Approaches to bolster the number of

Table 3—Partial successes in antigen-specific prevention and intervention in humans: lessons learned

Trial	Treatment	Results	Lessons learned	Ref.
Oral insulin (DPT-1)	Secondary prevention in high-risk individuals with autoantibodies (372 treated subjects)	No prevention, except in a subgroup of patients with highest insulin autoantibody levels where loss of C-peptide was delayed	Unlike in NOD mice, proinsulin may not be a driving antigen in all patients; selecting antigens based on strong evidence of autoreactivity may be required	75,76
Proinsulin DNA (BHT-3021)	Phase 1 study in T1D patients with 5 years of onset and with residual C-peptide, involving intramuscular delivery of proinsulin-encoding plasmid (80 T1D patients)	Significant delay in C-peptide loss up to 15 weeks after treatment with 1-mg dose; significant decrease of proinsulin-reactive $CD8^+$ T cells in treated HLA-$A3^+$ patients	Presentation of proinsulin-derived peptides (at least HLA-A3 restricted) may mediate peripheral deletion of some autoreactive $CD8^+$ T cells and delay $CD8^+$ T cell–mediated β-cell destruction	77
GAD65-Alum (DIAGNODE-1)	Ongoing pilot study involving intralymphatic delivery of GAD65-alum and oral vitamin D (6 new-onset patients, all with GAD65 autoantibodies)	Promising results of C-peptide preservation relative to historical studies with GAD65-alum or anti-CD3; these data remain very preliminary	Intralymphatic delivery may provide better exposure of antigens and leverage nonmigratory subsets of APCs	78

DIAGNODE-1, GAD-Alum (Diamyd) Administered into Lymph Nodes in Combination with Vitamin D in Type 1 Diabetes; DPT-1, Diabetes Prevention Trial–Type 1 Diabetes.

certain underrepresented APCs populations (e.g., pDCs) and/or to dampen APC hyperactivity (e.g., excessive NF-kB induction and related susceptibility genes) (Supplementary Table 11) may then be considered alongside ASIT.

Expanding the Panoply of Self-antigens for Tolerance Induction

The need to deliver exogenous antigens for ASIT is fueled by the insufficient or lack of expression/presentation of relevant β-cell antigens outside the islet-draining PLNs, which becomes an unsuitable environment for tolerance induction in T1D. The *INS* gene is a good example of a β-cell antigen that may not be sufficiently expressed outside the pancreas. Although insulin in its mature form is available systematically for uptake and processing, certain epitopes unique to (pre)proinsulin can only be produced from endogenously expressed protein. This provided a rationale to use proinsulin in ASIT, supported by promising results in inducing tolerance in mice. Similarly, GAD65, which is not expressed in mTECs (48), has been an autoantigen of choice for delivery to T1D patients based on preclinical efficacy. However, clinical studies have not used insulin and GAD65 in combination, nor have other β-cell antigens been used so far. When antigens are generated exclusively in the islets, for example neoepitopes under excessive stress (posttranslational modifications [49]) or as a result of high concentrations of peptides from different β-cell proteins (hybrid peptides [50]), tolerance would rely entirely on tolerogenic presentation of these antigens by DCs in draining lymph nodes. However, the recent evidence that islet-infiltrating pathogenic T cells recognize all these antigens (51–53) suggests that this mechanism of autoantigen presentation fails to achieve tolerance in T1D individuals and provides a rationale to include these new antigens in ASITs and deliver them to sites that are more conducive for tolerance. Modified epitopes (mimotopes) have been produced to stabilize binding to MHC-II molecules. Mimotopes of $InsB_{9–23}$ that bind better to HLA-DQ8 (or $I\text{-}A^{g7}$ in NOD mice) are able to better engage populations of diabetogenic T cells (54,55), and preclinical studies were indicative of a superior therapeutic benefit of mimotopes (56–58), though not consistently (59). More studies are needed to ascertain their safety and benefit in patients. The use of soluble peptides or multiepitopes polypeptides can circumvent antigen-processing defects and make it possible to include neoepitopes that are otherwise not present in native proteins (56,60). Reassuringly, the efficacy of therapies that rely on apoptotic cell uptake (61) does not appear to be hindered by the phagocytosis defects reported in NOD mice (16–18). Finally, the consistent and robust decrease of MHC-II expression on DCs from NOD mice (Supplementary Table 9) may possibly be a factor in their better responsiveness to ASIT. At an equivalent antigen dose, NOD DCs may engage T cells with lower avidity than their human counterparts, which may be beneficial for Treg induction. Indeed, it has been proposed that lower/subimmunogenic stimulation favors Tregs in NOD mice (56,57,62).

Targeting and Modulating Endogenous APCs

It may be challenging to control which APCs participate in the presentation of antigens after ASIT. Many delivery methods may involve any type of APC that is capable of taking up local or systemic antigen, whereas others target specific DC subsets (e.g., antigens conjugated to DEC205 or DCIR-targeting antibodies) or specific compartments (e.g., nasal or oral mucosa). However, some DC populations may be functionally deficient, and dysbiosis (microbiota

imbalance) may cause the mucosal interface to be more inflammatory in some patients. Changes in gut microbiome have been observed in T1D in patients (63,64). Interestingly, T1D-associated genes most overlap with gut-associated diseases (Crohn disease, ulcerative colitis, celiac disease) (Supplementary Table 1), and PLNs also drain regions of the gut, at least in mice (65). Thus, gut leakiness of bacterial products may also enhance the immunogenicity of APCs in PLNs (66). Lingering inflammation, whether it is in the pancreas, draining lymph nodes, or the gut, can alter the phenotype of DCs and MΦs in a way that hinders effective tolerance induction. Unlike patients, NOD mice are subject to minimal microbiome variation within a colony, as they need to be kept under specific pathogen-free conditions for them to develop T1D. This needs to be taken into account when transitioning ASITs based on oral tolerance from preclinical to clinical studies. Importantly, the delivery of immunomodulators in conjunction with antigens will likely be needed to rectify the signals provided by APCs to T cells (Fig. 2). Such immunomodulators include regulatory cytokines, negative costimulatory molecules, and metabolic modifiers. Biologics that neutralize proinflammatory cytokines (TNF-α, IL-1β, IL-6) or costimulatory molecules (CTLA4-Ig, antisense oligonucleotides against CD80, CD86, and CD40) have been assessed on their own but should be evaluated in combination with ASIT (Fig. 2). Alternatively, certain inhibitory ligands and/or immunoregulatory molecules may be overexpressed along with antigens when using nucleic acid–based strategies. New vehicles and routes for antigen delivery continue to be investigated to target alternative APCs and delivery sites to substitute or mitigate functionally altered APCs that may otherwise interfere with the tolerogenic process. Although protein antigens are primarily used by APCs that have efficient endocytosis and endosomal degradation (DCs and MΦs), nucleic acid–based delivery may be more efficient for other nonprofessional APCs that have reduced endosomal degradation. Examples of such APCs include lymph node stromal cells and a variety of other nonhematopoietic APCs that can present antigens without positive costimulation and that have been implicated in tolerance induction and maintenance (44,67). Endogenous and specific antigen expression in these cells has been achieved with vectors using tissue-specific promoters and miR-142 target sites, preventing expression in professional APCs (68,69). At present, the extent of nonprofessional APCs' contribution to tolerance induction under steady-state conditions is unclear. Further, whether their function is defective in T1D and whether they constitute worthy targets as alternative APCs in ASIT remain to be determined.

The Case of Exogenous APCs

As an alternative ASIT strategy, antigens may be provided to ex vivo generated DCs that have been conditioned to adopt robust and stable tolerogenic properties before being reintroduced into patients in a more personalized approach, which is reviewed elsewhere (70–72). Such ex vivo "engineered" exogenous APCs (typically DCs) may overcome deficiencies of endogenous APCs to induce tolerance. In this respect, an outstanding question remains as to whether exogenous DCs need to be provided with antigens in order to effectively engage diabetogenic T cells or whether they are able to acquire these antigens in situ. Although antigen provision has been used in DC therapy for rheumatoid arthritis and multiple sclerosis, this has not yet been tested clinically in T1D (72). When DCs with and without antigen provision were compared side by side in preclinical models, DCs with antigen were more often protective (Supplementary Table 16). Other studies have shown that tolerogenic DCs without antigens or with irrelevant antigens can nonetheless be protective. In the most recent study, provision of relevant (GAD65) or irrelevant (OVA) antigen surprisingly abrogated the therapeutic benefit of unpulsed tolerogenic DCs (73). Given the limited number of such studies and the inconsistency of conditions used (e.g., DC generation and treatment, dose, route, antigen(s), preclinical model, age of mice at treatment) (Supplementary Table 16), it is difficult to draw solid conclusions. The merits and caveats of antigen provision to exogenous APCs are summarized in Fig. 3. Although most of the islet-derived antigens are expected to be found in islet-draining PLNs, the majority of cells or antigens administered in ASIT likely end up in other sites. In mice, intraperitoneal (and to a lesser extent intravenous) delivery best achieves targeting to PLNs (65,74). One important caveat is the lack of CCR7 expression on immature DCs that limits homing to that site, and ex vivo treatments that enable upregulation of CCR7 (and inhibitory ligands such as PD-L1) without increasing costimulatory molecules will be essential. Although it is undeniable that DCs have therapeutic potential without antigen provision, it remains unclear whether this effect involves uptake and presentation of islet-derived antigens in situ or is basically antigen-independent and primarily immunomodulatory in nature. These important considerations should be addressed experimentally, particularly in the context of the delivery routes used in humans.

CONCLUSIONS

Overall, the simple provision of antigens in T1D patients for ASITs faces challenges related to the possible limited ability of endogenous APCs to properly induce tolerance with these antigens. A number of approaches may be implemented to overcome these limitations and improve their efficacy (Table 2). Defects affecting T cells, not covered in this review, will also need to be taken into consideration, particularly those that make T cells resistant to regulation by APCs or Tregs. Although successful outcomes have been achieved in NOD mice despite a considerable number of reported APC defects, the heterogeneity of T1D patients makes it unlikely that an off-the-shelf ASIT strategy that suits all patients will be available soon. Instead, combination therapies will be required to

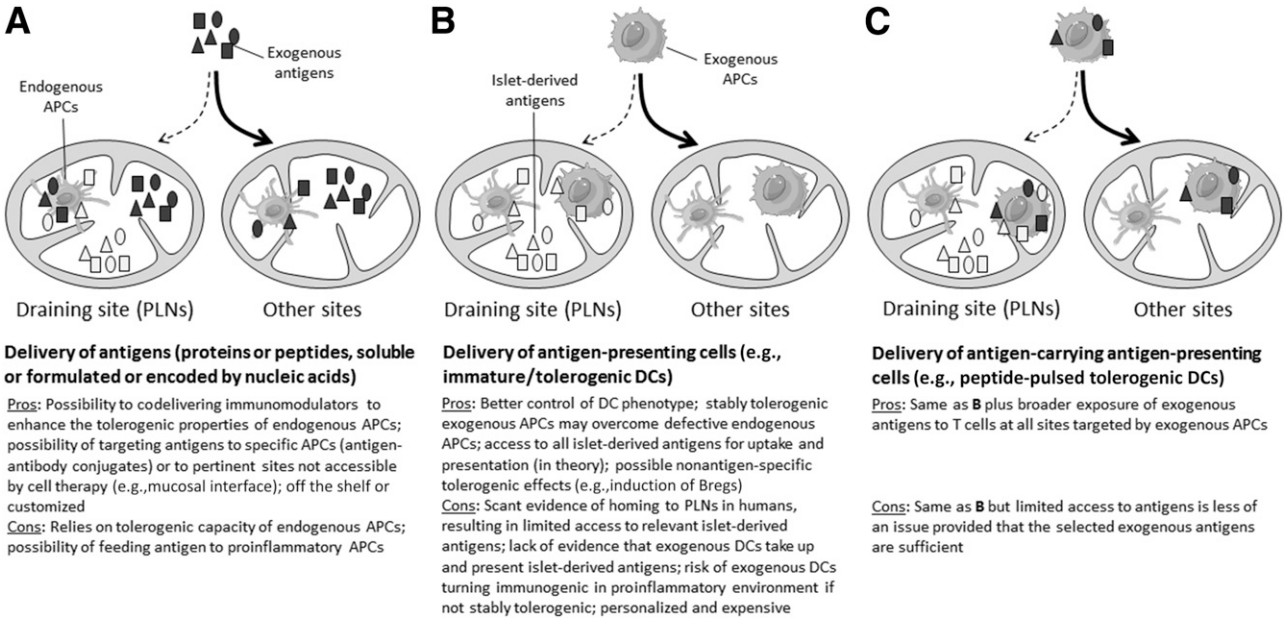

Figure 3—Different ASIT approaches relying on endogenous vs. exogenous APCs and on islet-derived vs. exogenously provided antigens. *A*: Delivery of exogenous antigens to endogenous APCs. Delivery of exogenous APCs without (*B*) or with (*C*) exogenous antigens. Bregs, regulatory B cells.

tackle multiple defects and to better control the way T cells are being stimulated. We will need to better delineate APC defects in patients and, in particular, how the function of a particular gene in APCs is affected by risk alleles. Eventually, once a sufficient number of contributing genes have been characterized, their polymorphism may be tested as part of an assay panel to better customize the type of intervention that patients may require. Identification of targeted epitopes in human, which has undergone a renaissance in the past few years, will continue to play a critical role in the effort to sort out patient-specific epitopes from those that are commonly shared. Genomic evaluation should include HLA typing to ensure that epitopes selected are tailored for the patient's HLA if not using the whole protein as antigen. ASIT is a safe approach to treat tissue-specific autoimmune diseases such as T1D. As we enter the era of precision medicine, ASIT can also be tweaked to address specific deficiencies, thereby making the treatment even safer and more effective.

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