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Innate Immunity in Latent Autoimmune Diabetes in Adults (LADA)

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Abstract

Latent autoimmune diabetes in adults (LADA) is an autoimmune disease that shares some genetic, immunological, and clinical features with both type 1 diabetes and type 2 diabetes. Immune cells including $CD4^+$ T cells, $CD8^+$ T cells, B cells, macrophages, and dendritic cells (DCs) have been detected in the pancreas of patients with LADA and a rat model of LADA. Therefore, similar to type 1 diabetes, the pathogenesis of LADA may be caused by interactions between islet β-cells and innate and adaptive immune cells. However, the role of the immunity in the initiation and progression of LADA remains largely unknown. In this review, we have summarized the potential roles of innate immunity and immune-modulators in LADA development. Furthermore, we have examined the evidence and discussed potential innate immunological reasons for the slower development of LADA compared with type 1 diabetes. More in-depth mechanistic studies are needed to fully elucidate the roles of innate immune-associated genes, molecules, and cells in their contributions to LADA pathogenesis. Undertaking these studies will greatly enhance the development of new strategies and optimization of current strategies for the diagnosis and treatment of the disease.

Key words: Latent autoimmune diabetes in adults; Innate immunity; Autoimmune; Diabetes mellitus; Innate immune cells.

1. Introduction

Latent autoimmune diabetes in adults (LADA), also known as type 1.5 diabetes or slow-onset diabetes in adults, is an autoimmune disease that shares some genetic, immunological, and clinical features with both type 1 diabetes and type 2 diabetes(1-5). The disease was first described in 1983(6) and was defined as LADA in 1993(7). Although LADA accounts for approximately 1.5%-14.2% of the type 2 diabetes population(8-14), no unified criteria or management guidelines have existed for LADA. Recently, a panel of international experts defined a strategy for LADA management, which will be greatly helpful to the diagnosis and treatment of the disease in clinical practice(15).

GADA level is considered as the most valuable discriminatory parameter to predict the development of β-cell function in LADA(16). Compared with low level of glutamic acid decarboxylase antibody (GADA), high level of GADA in LADA is associated with faster insulin progression, indicating that the presence of high level of GADA was a significant predictor of insulin requirement in LADA(17). Our group also found that patients with high level of GADA showed a worse baseline and accelerated decline of β-cell function(18), while the metabolic phenotypes and β-cell function in LADA patients with a low level of GADA were similar to that in type 2 diabetes patients(19). It was reported that the presence of N-terminally truncated GAD65 autoantibody in individuals with adult-onset diabetes is associated with the clinical phenotype and can predict insulin therapy compared with the presence of full-length GAD65 autoantibody(20). IA-2A presence, in addition to GADA, is also highly predictive of future need for insulin therapy(21). Among seven IA-2 constructs, IA-2(256-760) fragment was identified as a marker with the highest sensitivity for detection of humoral IA-2 immunoreactivity in LADA patients, which is also able to identify IA-2 immunoreactivity among $GADA^-$ patients with type 2 diabetes(22).

It is believed that the initiation and progression of autoimmune diabetes, such as type 1 diabetes, involves complicated cross-talk between β-cells and immune cells from both innate and adaptive immune systems(23). In type 1 diabetes, $CD8^+$ T cells are the most abundant infiltrating cells in humans, with $CD4^+$ T cells, macrophages, and B cells simultaneously

detected in the inflammatory pancreatic islets(24). In animal models of human type 1 diabetes, dendritic cells (DCs) and neutrophils were also found to infiltrate the islets in the early stage of the disease development(25). Like type 1 diabetes, insulitis also seems to be the hallmark of the immune-mediated β-cell damage in LADA. The occurrence of insulitis in LADA was initially confirmed by pancreatic scintigraphy in the pancreata of LADA patients(26), and it was verified in a LADA animal model (a spontaneous rat model of LADA) recently(27). Compared with insulitis seen in type 1 diabetes, insulitis in LADA encompasses a changed ratio of tumor necrosis factor-α (TNF-α)/interleukin-1β (IL-1β) expression, an increased expression of IL-10, and an increased number of macrophages which predominate in the immune infiltration over $CD8^+$ T cells(27) (Figure 1). Apart from immune cell infiltration in islets, LADA patients displayed an altered frequency of B cell subsets, which is closely associated with altered β-cell function(28). Therefore, immunity may play a crucial role in the pathogenesis of LADA. In this review, we summarize the current studies investigating the role of innate immunity in LADA.

2. Genetic studies in LADA patients identify polymorphisms in genes associated with innate immunity

Until now, most genetic studies of LADA have focused on genes relevant to type 1 diabetes (*HLA-DQB1*, *INS*, *PTPN22*, and *CTLA4*) and those associated with type 2 diabetes (*FTO*, *PPARG*, *TCF7L2*, and *SLC30A8*)(29, 30). Recently, Cousminer *et al*. performed the first Genome-Wide Association Study (GWAS) of patients diagnosed with LADA(3). The pathway analysis with DEPICT (an integrative tool that employs predicted gene functions to systematically prioritize the most likely causal genes at associated loci) revealed physiological abnormalities in natural killer (NK) cells, T lymphocytes, mTOR regulatory network, and cell cycle, supporting a strong immune role in the pathogenesis of LADA(3). In this study, it was found that *SH2B3* was also closely associated with the susceptibility to LADA. Moreover, a novel signal at the *PFKFB3* locus that can distinguish LADA from type 1 diabetes and type 2 diabetes was identified, but it needs further validation(3). Apart from the genes mentioned above, other genes that are closely associated with innate immune regulation have also been linked with disease susceptibility to LADA, including major histocompatibility complex class

I (MHC-I) chain-related gene (*MIC-A*), killer cell immunoglobulin-like receptors (*KIRs*), *TNF-α*, and *IL-10*.

2.1. *MIC-A*

Several studies investigated the effects of MHC class region, especially the HLA class II genes *HLA-DRB1* and *HLA-DQB1* in LADA. It was found in the population of European ancestry that *HLA-DRB1*0301*(*DR3*), *HLA-DRB1*0401*(*DR4*), *HLA-DQB1*0201*(*DQ2*) and *HLA-DQB1*0302* (*DQ3*) conferred susceptibility to LADA(31). In Chinses population, *HLA-DRB1-DQA1-DQB1* loci was found to have a close association with LADA susceptibility(32). Additionally, Mishra *et al.* investigated the relation of the MHC class I alleles with LADA, finding that class I genes (*HLA-A* and *HLA-B*) are not associated with LADA(33). *MIC-A* is a polymorphic gene located in the HLA region mainly expressed by monocytes, keratinocytes, and endothelial cells(34). Raache *et al.* found that patients with LADA from an Algerian population, like juvenile type 1 diabetes patients, had an increased frequency of valine at position 129 of *MIC-A* compared to healthy control subjects. This single amino acid change may affect the activation of NK and $CD8^+$ T lymphocytes, and thus, alter the risk for autoimmune diabetes(35). Moreover, the combination of the *MIC-A-129* Val/Val genotype with *DR3-DQ2* or *DR4-DQ8* was found to confer an increased risk for both adult-onset type 1 diabetes and LADA(35). In an Italian population, it was demonstrated that the presence of either *MIC-A5.1* or *MIC-A5* discriminated LADA patients from type 1 diabetes patients(36). *MIC-A5* was found to confer a genetic risk for type 1 diabetes, while *MIC-A5.1* was significantly increased among LADA patients. This was also demonstrated in subjects from Latvia and eastern Indian populations(37, 38); however, Carina *et al*. found that in a Swedish population, *MIC-A5.1* was increased in patients with LADA and type 1 diabetes, while heterozygosity for *MIC-A5/5.1* was the important genotype related to significant risk for LADA(39). Taken together, these studies indicate that *MIC-A5.1* may play an important role in the pathogenesis of slow-onset autoimmunity in LADA through altered activation of NK cells and T cells(36-38). However, Mishra and colleagues' recent report, which studied populations from UK, Germany and USA, showed that the independent effects of MHC class I observed in type 1 diabetes were not observed in LADA after conditioning on the leading

MHC class II associations(33). As these studies are from different populations and the sample size of some was relatively small, therefore, more studies with larger sample size among different populations, are warranted to confirm the role of these genes and the function of the proteins encoded by these genes in the pathogenesis of LADA.

2.2. *KIRs*

KIRs are surface receptors expressed on NK cells that play crucial roles in the innate immune system by mediating the early killing of virally infected cells and tumors(40, 41). MHC molecule recognition by inhibitory KIRs usually leads to the suppression of the cytotoxic activity of NK cells, while the recognition by activatory KIRs can lead to the activation of cytotoxic activity of the cells(42, 43). It was reported that the activating of *KIR-HLA* genes was increased in young children diagnosed with type 1 diabetes in the first 5 years of life, indicating that type 1 diabetes patients may have altered NK cell responses(44). In LADA, *KIR* genes confer susceptibility to, or protection from the disease, and their roles in modulating disease susceptibility or protection are closely associated with the ethnicity of the subjects(45). In a Latvian population, *KIRs* 2DL1, 2DS2, and 2DS4 were all associated with susceptibility to LADA, while *KIR* 2DS5 was associated with protection. However, in an Asian Indian population, the *KIRs* 2DL5 and 3DL1 were associated with the susceptibility to LADA, and 2DS1 and 2DS3 were associated with protection(45).

2.3. *TNF-α* and *IL-10*

Cytokine gene polymorphisms may also contribute to the pathogenesis of LADA. *TNF* genes have a strong linkage disequilibrium with *HLA* class I/II genes and other *MHC* genes associated with immune-regulation(46). TNF-α, encoded within the *TNF* locus, is released by activated innate immune cells such as monocytes, macrophages, NK cells and adaptive immune cells (T and B cells)(46). Although *TNF-α*-308A/G single nucleotide polymorphisms (SNPs) do not contribute to the development of LADA(47), it was found that homozygosity for *TNF-α*-308A/A (*TNFα2/2*) is a risk factor for both LADA and type 1 diabetes(48). The genetic risk associated with *TNFα2/2* is likely to be due to linkage disequilibrium with *HLA-DR3-DQ2* and *DR4-DQ8*(48). IL-10, an anti-inflammatory cytokine, is a major suppressor of the immune system capable of regulating T cell proliferation and downregulating cytokine production from

activated innate (monocytes/ macrophages and DCs) and adaptive (T and B) immune cells(49). Tsiavou *et al.* found that the frequency of 1082*A *IL-10* alleles was increased in LADA patients, compared with individuals with type 2 diabetes, who had 1082*G alleles more frequently(47). This suggests that the A/G mutation at position -1082 of the *IL-10* promoter gene region may be one of the factors participating in the pathogenesis of LADA(47).

3. Innate immune cells in LADA

3.1. Monocytes/Macrophages

Monocytes constitute about 5-10% of circulating leukocytes in human peripheral blood and can be divided into two subsets - classical $CD14^{hi}CD16^{-}$ monocytes and non-classical $CD14^{+}CD16^{+}$ monocytes, the latter of which are more mature and resemble tissue macrophages(50). Macrophage can infiltrate the islets, involving in the initiation of the insulitis, β-cell destruction, and the development of type 1 diabetes(51-53). It was reported that resident macrophages in pancreatic islets can also initiate type 1 diabetes(54) and contribute to the inflammation affecting β-cell function in obesity(55). Several studies have shown that individuals with type 1 diabetes had increased monocytic activity compared with control individuals(56-59). Investigation of $CD14^{+}$ monocytes revealed altered gene expression between patients with LADA, juvenile-onset type 1 diabetes, adult-onset type 1 diabetes, type 2 diabetes, and healthy control subjects(60). Interestingly, $CD14^{+}$ monocytes from LADA and adult-onset type 1 diabetes patients showed similar immune characteristics distinct from juvenile-onset type 1 diabetes(60). No significant alterations were found in the proportions of $CD16^{+}$ monocytes in LADA patients when compared with healthy controls or patients with type 1 diabetes or type 2 diabetes(61) (Table 1); however, Pavlina *et al*. found that patients who had autoimmune diabetes, including patients with LADA and type 1 diabetes, had lower counts of $CD14^{hi}CD16^{-}$ monocytes and $CD14^{lo}CD16^{+}$ monocytes than healthy control subjects(62). Additionally, $CD68^{+}$ macrophages, together with $CD8^{+}$ T cells, were found to infiltrate the pancreatic islets in LADA patients(63). Recently, Jörns and colleagues have confirmed the pancreatic infiltration of macrophages and $CD8^{+}$ T cells in LADA by investigating the pancreata from LADA patients and a rat model of LADA(27). Here, the authors found that there was an increased ratio of macrophages to $CD8^{+}$ T cells in the pancreata of LADA when compared with macrophage/$CD8^{+}$ T cell from the pancreata of type 1 diabetes.

It was documented that macrophages can produce more IL-1β than other types of immune cells(64), and β-cell destruction may be induced by interleukin 1β (IL-1β) released from activated monocytes/macrophages(51, 65). Jörns *et al*. found that LADA pancreas displayed a predominance of IL-1β in the infiltrated immune cells compared with the predominance of TNF-α found in the immune cell in the pancreas of type 1 diabetes. Although it is unclear whether the source of increased IL-1β is from macrophages or not in LADA pancreas, it was considered that increased IL-1β can recruit more macrophages to the islets to remove the apoptotic β-cells, which might be a major reason why LADA develops slower than type 1 diabetes(27).

3.2. Dendritic cells

Studies from both humans and animal models have shown that dendritic cells (DCs) are major players in modulating the development of type 1 diabetes by promoting the islet autoantigen presentation to T-cells, augmenting Th1 responses, or mediating the tolerance of autoreactive $CD4^+$ T cells(66-70). DCs, together with macrophages, were reported to infiltrate the pancreatic islets and activate $CD4^+$ T cells via IL-12 secretion(71). To date, few studies have investigated the importance of DCs in LADA. In 2009, Kelly and colleagues compared the phenotype of DCs in LADA patients with that in subjects with type 1 diabetes and healthy control individuals, with DCs defined as HLA-DR^+ cells that were deficient in lineage-specific markers (CD3, CD14, CD16, CD19, and CD57). Unlike monocyte-derived DCs, which showed both phenotypic and functional changes in patients with type 1 diabetes(72), the total number of DCs and the proportions of myeloid DC ($CD11c^+$) and lymphoid DC ($CD11c^-$) subsets were similar among patients with LADA, type 1 diabetes and control subjects(73). In addition, Kailash and colleagues also studied DC subsets, including the $CD123^-CD11c^+$, $CD123^+CD11c^+$, and $CD123^+CD11c^-$ plasmacytoid dendritic cells (pDCs) subsets, in healthy controls and patients with LADA, type 1 diabetes, and type 2 diabetes(61). In this study, no significant differences were found in the proportions of pDCs between the four groups; however, patients with LADA had a lower proportion of $CD123^-CD11c^+$ DCs compared to patients with type 1 diabetes(61). As $CD123^-CD11c^+$ DCs may prime T cells to target the insulin-producing β-cells(61), the decreased numbers of $CD123^-CD11c^+$ DCs found in patients

with LADA may provide further evidence of why the immune response is reduced and progression of β-cell failure is slower in LADA patients than type 1 diabetes patients(61).

3.3. Natural killer cells

The role of natural killer (NK) cells in type 1 diabetes development is unclear, and the studies regarding NK cell infiltration in islets of patients are inconsistent, with some reporting NK cell infiltrates(74) and others reporting no NK cell infiltration(75). Recently, NK cells were detected within the pancreatic islet of individuals and rats with LADA, representing approximately 10% of the islet immune cell infiltrated(27). Additionally, Akesson *et al.* showed that Caucasian patients who have LADA diagnosed within 5 years had a significant decrease in $CD3^{-}CD56^{+}$ NK cell frequency in the peripheral blood compared to healthy individuals(76). In contrast, newly diagnosed Caucasian patients with LADA showed a higher proportion of $CD3^{-}CD56^{high}CD16^{+}$ NK cells than both healthy controls and patients with type 2 diabetes(61). In a Chinese population, it was found that patients diagnosed with LADA within one month had a higher frequency of $CD3^{-}CD56^{+}$ NK cells, activated $NKp46^{+}$ NK cells, and interferon (IFN)-γ^{+} NK cells, but lower $3DL1^{+}$ (a KIR) NK cells than healthy control individuals(77). Moreover, the percentages of circulating $NKp46^{+}CD3^{-}CD56^{+}$ NK cells were negatively correlated with the levels of fasting plasma C-peptide. These studies have identified differences in NK cells; however, this may result from the varying disease duration of the patients studied. It is possible that an increased frequency of NK cells may be associated with the early stages of the pathogenic process underlying LADA development(77). Except for the KIRs mentioned above, a variety of receptors including NKp30, NKp46, NKG2D, the leukocyte immunoglobulin-like receptors (LIRs), and the CD94/NKG2A heterodimers are also found on NK cells(40, 41). Interestingly, patients with LADA displayed increased expression of activating NKG2D(76) and NKp46(77), but decreased inhibitory 3DL1 expression(76), indicating that the altered activation of NK cells may contribute to the development of LADA. Unlike patients with LADA, the NK cells in both type 1 diabetes patients(78) and the non-obese diabetic (NOD) mouse model of type 1 diabetes(79) display reduced NKG2D expression. Therefore, the higher expression of NKG2D receptor may correlate with the slower progression of β-cell failure in LADA(76). Although a variety of studies have been performed to investigate

the frequency of NK cell subsets and their receptor expression in patients with LADA, the function of NK cells in patients with LADA and their cross-talk with other immune cells such as DCs and $CD4^+CD25^+$ regulatory T cells have not been studied and need to be elucidated in the future.

3.4. Neutrophils

Although autoimmune type 1 diabetes is an organ-specific autoimmune disease mediated primarily by auto-reactive T cells, neutrophils are found to be involved in the early development of the disease by activating plasmacytoid DCs (pDC) to produce IFN-α together with B-1a cells (25, 80). Moreover, numerous studies in patients with type 1 diabetes have shown a decreased number of neutrophils in the circulation(81-84). In patients with LADA, Singh *et al*. found no alteration in the proportions of $CD15^{low}$ neutrophils in the patients with a mean disease duration of close to 5 years(61). We recently found higher numbers of neutrophils in the circulation of newly diagnosed LADA patients (diagnosed within one year) compared to patients with type 1 diabetes(85). However, patients with LADA in our study have lower circulating neutrophil counts compared to patients diagnosed with type 2 diabetes within one year(85). Interestingly, the neutrophil counts in patients with type 1 diabetes or LADA were negatively correlated with the levels of GAD, IA2, and ZnT8 autoantibody. This was particularly evident in those individuals who were positive for all three autoantibodies, which had the lowest neutrophil counts, indicating that neutrophils are associated with the severity of the underlying autoimmune process and β-cell damage in the development of autoimmune diabetes including LADA. Moreover, neutrophils from patients with type 1 diabetes or LADA had impaired migratory ability when compared to either healthy controls or individuals with type 2 diabetes(85). By performing RNA sequencing (RNA-seq) analysis of peripheral blood immune cells, Wang *et al*. found that patients with LADA exhibited 277 differentially expressed genes (DEGs) compared to healthy controls. Further gene ontology (GO) analysis of these DEGs indicated that many of the enriched GO terms were closely related to immune functions, including positive regulation of neutrophil chemotaxis(86). Therefore, increased expression of neutrophil chemo-attractants may also contribute to the augmented inflammation observed in LADA(86). Recently, our group did RNA-seq using isolated neutrophils from

LADA patients and healthy controls, finding that differentially expressed genes between LADA and healthy controls are mainly involved in leukocyte degranulation, myeloid cell differentiation, and immune response-regulating signaling, revealing an abnormality in neutrophil disposition at the transcriptional level in LADA(87).

4. Mechanisms of innate immune activation in LADA

4.1. Toll-like receptors

Toll-like receptors (TLRs) are important pattern recognition receptors, which are widely expressed by various immune cells including monocytes, macrophages, DCs, B cells, T cells, as well as non-immune cells(88). Until now, thirteen mouse TLRs (TLR1-13) and ten human TLRs (TLR1-10) have been identified. Several TLRs including TLR2, TLR3, TLR4, TLR5, TLR7, TLR8, and TLR9 have been demonstrated to play important roles in the development of type 1 diabetes(89-91). However, only TLR2 and TLR4 expression on monocytes have been investigated in LADA. TLR2 and TLR4 can be activated by recognition of lipoteichoic acid (LTA) from Gram-positive bacteria, and lipopolysaccharide (LPS) from Gram-negative bacteria, respectively(92, 93). Our previous study found that LADA patients from a Chinese population had higher expression of TLR4, but not TLR2, on the surface of freshly isolated $CD14^+$ peripheral monocytes when compared to patients with type 1 diabetes(94). However, monocytes from patients with LADA or type 1 diabetes exhibited increased reactivity towards LPS and LTA *in vitro*, suggesting that tolerance to LPS and LTA could be dysregulated in both type 1 diabetes and LADA. Therefore, the increased proinflammatory monocyte responses to microbial components are likely to contribute to the pathogenesis of autoimmune diabetes(94).

4.2. NOD-like receptors

Nucleotide oligomerization domain (NOD)-like receptors (NLRs) are another important class of pattern recognition receptors, which can detect microbial components in the cytosol(95). NLRs are also expressed in many cell types including both immune and non-immune cells e.g. epithelial cells. There are 23 NLR family members in humans and at least 34 NLR genes in mice(96). Both Nod1 and Nod2 are important NLRs, which upon recognition of their microbial ligand, can activate NF-κB and MAPKs to induce an immune response(95, 97). NLRP1, NLRP3, NLRC4, and NLRP6 proteins can form multi-protein complexes called

inflammasomes, which, when activated, play an essential role in caspase-1 activation and the subsequent release of proinflammatory cytokines such as IL-1β and IL-18(95). Importantly, NOD2 can modulate type 1 diabetes susceptibility by altering the microbial composition, which in turn affects the phenotype and functions of T cells and B cells in the NOD mouse model(98). In addition, in the NOD mouse, NLRP3 also alters susceptibility to type 1 diabetes by limiting the recruitment and infiltration of autoreactive $CD4^+$ T cells into the pancreas(99). However, the role of NLRs and inflammasome proteins in the pathogenesis of LADA has not yet been studied and should be investigated in the future.

4.3. Gut microbiota

TLRs, NLRs, and inflammasomes can be activated by microbial components and thus the composition of the microbiota may also alter the signaling of these molecules. Accumulating evidence has shown that gut microbiota play critical roles in the development, education, and function of innate and adaptive immune cells(100, 101). Furthermore, as an environmental factor, the gut microbiota, through interactions with the immune system, have been shown to modulate susceptibility to type 1 diabetes and the altered gut microbiota have been documented to contribute to the increased incidence of type 1 diabetes(102, 103). In 2008, it was firstly demonstrated that gut microbiota modulates type 1 diabetes development in NOD mice via the innate immune system(104). Since then, much research has been focused on the elucidation of the interaction between the host and the gut microbiome in health and disease. Our recent observations have identified that gut microbiota might contribute to the development of type 1 diabetes via modulation of neutrophil homeostasis (Huang *et al.* unpublished). Additionally, increasing evidence has shown that the composition of gut microbiota is altered during the development of type 1 diabetes, exhibiting a less diverse and less stable gut microbiome compared to healthy controls(105, 106). Furthermore, in individuals with type 1 diabetes, there are alterations in the ratio of *Firmicutes* to *Bacteroidetes* compared to healthy controls(105, 107, 108). Increasing evidence demonstrated that the changes in the microbiome might cause islet cell autoimmunity. Studies from human beings showed that the fecal microbiota of individuals with multiple islet autoantibodies displayed distinct bacterial diversity compared with the control subjects(105, 108, 109). Recently, it was found that the intestinal activation of

islet-reactive T cells requires the presence of gut microbiota and is abolished when mice are depleted of endogenous commensal microbiota by antibiotic treatment(110). However, to our knowledge, nothing is known about the gut microbiota composition in patients with LADA and the contribution of the gut microbiota to modulating LADA pathogenesis. This area of investigation would make an interesting future direction for LADA studies.

4.4. Cytokines

Cytokines are important regulators of both innate and adaptive immunity and contribute to the immune-mediated β-cell destruction observed in the development of type 1 diabetes(111, 112). Recently, Jorns *et al*. detected *TNF-α* and *IL-1β* gene expression in the pancreata of patients and rats with LADA, finding that there was a shift in the proinflammatory cytokine gene expression from *TNF-α* to *IL-1β* in the pancreata from LADA compared to that from type 1 diabetes (27). Additionally, other cytokines, such as IFN-γ, IL-6, and IL-15, may also contribute to the pathogenesis of LADA. Wang *et al*. detected that newly diagnosed LADA patients had higher percentages of inducible IFN-γ^+ NK cells and NKp46$^+$ cells than control subjects(77). As NK cells were recently identified in the pancreatic infiltrate of patients and rats with LADA(27), it is possible that IFN-γ secreted from NK cells may alter the insulin-producing β-cells and promote the development of LADA(77). Schloot *et al.* investigated the differences in systemic immune mediators between patients with type 2 diabetes and adult-onset autoimmune type 1 diabetes who had high GADA concentrations or low GADA concentrations (including classic adult-onset type 1 diabetes and LADA). In this study, they found no significant differences in the cytokines, including IL-10, TNF-α, C-C motif chemokine ligand (CCL)-2, CCL3, CCL4, soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) between adult-onset autoimmune type 1 diabetes and patients with type 2 diabetes, but it was observed that type 1 diabetes patients with high GADA level had lower concentrations of IL-6 and soluble E-Selectin compared with patients with type 2 diabetes(113). However, several studies demonstrated that the circulating IL-6 concentration is significantly higher in both patients with LADA and their healthy first-degree relatives compared to healthy control subjects, supporting the pro-inflammatory role for this cytokine in mediating susceptibility to LADA(114-116). For

IL-15, it was found that patients with LADA or type 1 diabetes had a higher level of IL-15 than both control subjects and their first-degree relatives, with relatives having positive autoantibodies showed a higher level of circulating IL-15 than those without autoantibodies(114). Moreover, the concentration of IL-15 is positively correlated with homeostatic model assessment-insulin resistance (HOMA-IR) in LADA and type 1 diabetes, and negatively correlated with estimated glucose disposal rate (eGDR) in LADA, suggesting that IL-15 is potentially a biomarker of the ongoing autoimmune process in humans, particularly in those with LADA(114).

4.5. Chemokines

Chemokines, also called chemotactic cytokines, play essential roles in attracting leukocytes to tissues during inflammation(117). Over 40 chemokines have been identified, and are subdivided into families based on the relative position of their cysteine residues, including α-chemokines (cysteine-X amino acid-cysteine, or CXC) and β-chemokines (cysteine-cysteine, or CC)(118). Previous studies have shown that the serum concentrations of CCL2 and CCL4 did not differ between patients with type 1 diabetes or LADA(113, 119), whereas CCL3 levels were higher in patients with LADA and type 1 diabetes, compared to those with type 2 diabetes and controls(119). By performing RNA-seq analysis from PBMCs of patients with LADA and healthy control subjects, Ji *et al.* found that gene expression levels of chemokine ligands including *CXCL8*, *CCL2*, and *CCL23* were upregulated in patients with LADA(86). It is known that CXCL8 was originally identified as a potent neutrophil chemotactic factor(120), and both CCL2 and CCL23 are potent chemo-attractants for monocytes(121). Therefore, the increased expression of these chemo-attractants for neutrophils and monocytes may contribute to the augmented pancreatic inflammation in LADA(86).

5. Therapeutic approaches targeting innate immunity may be efficacious in LADA

The current approaches for the treatment of patients with LADA include insulin sensitizers, insulin, sulfonylureas, dipeptidyl peptidase-4 (DPP-4) inhibitors, sodium-glucose cotransporter 2 inhibitors (SGLT2i), glucagon-like peptide 1 receptor agonists, and GAD-alum(15). DPP-4, a serine peptidase also known as CD26, is a cell surface antigen (DPP-4/CD26) expressed on T and B lymphocytes, macrophages, NK cells and acinar, endothelial,

and epithelial cells(122). 1,25(OH)2D3, the active form of vitamin D, is an immunomodulator targeting various immune cells including monocytes, macrophages, DCs, as well as T cells, and B cells(123). Therefore, both DPP-4 inhibitors and vitamin D3 can have substantial effects on the immune system. Prospective studies from our groups(124-126) and post hoc analysis by Buzzetti *et al*.(127) have shown that DPP-4 inhibitors and/or vitamin D3 benefit patients with LADA by preserving pancreatic reserves, as indicated by higher C-peptide secretion, after treatment. The exploratory analysis by Johansen *et al.* also showed that linagliptin treatment may attenuate the rate of decline in C-peptide levels compared with glimepiride treatment in LADA patients, which may act through elevating the level of endogenous glucagon-like peptide 1 (GLP-1) and/or modulating peptides involved in cell signaling and autoimmunological pathways(128). Combination therapy with DPP-4 inhibitors and vitamin D3 are believed to induce immunoregulation and reduce the inflammatory response in autoimmune type 1 diabetes(129). Our previous study found that 1,25(OH)2D3-treated monocytes from patients with LADA showed significantly reduced IL-1β and TNF-α production compared to monocytes cultured with LTA or LPS(94). Similar results were also observed in monocytes from patients with type 1 diabetes and healthy controls(94). Although DPP-4 inhibitors and/or vitamin D3 can help to preserve pancreatic reserves in LADA patients, their roles in the regulation of immunity remain unclear; thus, more investigations are needed to elucidate the effects of vitamin D3 and/or DPP-4 inhibitors in immune regulation. Furthermore, understanding which patients should receive therapy and when the therapy should start, in order to elicit the greatest effect, will also need to be studied.

Conclusion

Innate immunity plays a crucial role in the pathogenesis of LADA; however, the specific contribution of the innate immune cells and immune modulators to disease development is largely unknown, and most studies related to LADA lack in-depth mechanistic investigation. The rat model of spontaneous LADA, which also develops insulitis offers a promising tool for the mechanistic investigation. This will greatly help us to better understand LADA pathogenesis with more details, and also offering a model for pre-clinical studies of LADA, which will provide new insights into the diagnosis and treatment of the disease.

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Figure 1. Immune cells and autoimmune diabetes. The initiation of type 1 diabetes development starts with self-antigens that are released after β-cell death and are presented to $CD4^+$ T and $CD8^+$ T cells by macrophages, conventional dendritic cells (cDC), and B cells(130). The β-cell specific autoreactive $CD4^+$ T cells usually destruct β-cells by producing inflammatory cytokine IFN-γ and activating macrophages as well as dendritic cells, while $CD8^+$ T cells damage β-cells through secreting perforin/granzyme(131, 132). In addition to presenting antigens, cDCs can promote the disease development by activating T cells through producing inflammatory cytokine interleukin-12 (IL-12)(133). Macrophages also release IL-12, IL-1β, and tumor necrosis factor-α (TNF-α) after activation(134, 135), all of which are inflammatory. Together with B-1a cells, neutrophils can initiate diabetogenic T cell response by activating plasmacytoid DCs (pDC) to produce interferon (IFN)-α(25). Natural killer (NK) cells, as important innate effector immune cells, destruct β-cell by recognizing NK cell ligand expressed on β-cell(136, 137). These pathogenic immune responses in type 1 diabetes development can be tempered by regulatory cells including IL-4 producing NKT cells(138) and regulatory T (Treg) cells(139). Additionally, cDC, pDCs and NK cells might play protective roles in disease development by expanding Treg cells(139), expressing indoleamine 2,3-dioxygenase (IDO)(140), or producing IFN-γ(141), depending on the microenvironment and insulitis stage. For latent autoimmune diabetes in adults (LADA), $CD8^+$ T cells and macrophages are the most abundant immune cell types found in the pancreas of patients and a rat model of LADA(27, 63). In addition to these cells, $CD4^+$ T cells, B cells, and natural killer cells also infiltrate the pancreas during the development of LADA. These infiltrating immune cells were found to secrete multiple cytokines including inflammatory IFN-γ, IL-1β, and TNF-α as well as anti-inflammatory IL-10. In comparison with type 1 diabetes, there is an increased absolute number of macrophages (which may promote the removal of apoptotic β-cells from the inflamed islets), an altered ratio of TNF-α/IL-1β expression, and an increased expression of anti-inflammatory IL-10 in the pancreas of LADA(27). These changes may account for the slower progression of islet autoimmunity in LADA, but more studies are needed to elucidate the pathogenesis of LADA.

Table 1. Overview of circulating innate immune cell studies in patients with LADA

Cell type	Defining markers	Country	Age (Years)	Disease duration	Sample size	Alteration	Ref
Monocytes	$CD16^+$	Sweden	65.7 ± 2.4	4.9 ± 0.3 months	13/14/16/16 (HC/LADA/T1D/T2D)	No change (vs HC, T1D, and T2D)	(61)
Dendritic cells	$CD11c^+$DCs	Canada	37.8 ± 16.8	1.5 ± 1.3 years	7/5/9 (HC/LADA/T1D)	No change (vs HC and T1D)	(73)
	$CD11c^-$DCs	Canada	37.8 ± 16.8	1.5 ± 1.3 years	7/5/9 (HC/LADA/T1D)	No change (vs HC and T1D)	(73)
	$CD123^+CD11c^-$ pDC	Sweden	65.7 ± 2.4	4.9 ± 0.3 months	13/14/16/16 (HC/LADA/T1D/T2D)	No change (vs HC,T1D and T2D)	(61)
	$CD123^-CD11c^+$ DCs	Sweden	65.7 ± 2.4	4.9 ± 0.3 months	13/14/16/16 (HC/LADA/T1D/T2D)	Decreased (vs T1D)	(61)
Natural killer cells	$CD3^-$ $CD16^+CD56^+$	Sweden	30 - 70	< 5 years	20/46 (HC/LADA)	Decreased (vs HC)	(76)
	$CD3^-$ $CD56^{high}CD16^+$	Sweden	65.7 ± 2.4	4.9 ± 0.3 months	13/14/16/16 (HC/LADA/T1D/T2D)	Increased (vs HC and T2D)	(61)
	$CD3^-CD56^+$	China	30 - 60	< 1 month	20/27 (HC/LADA)	Increased (vs HC)	(77)
	$NKp46^+$ NK cells	China	30 - 60	< 1 month	20/27 (HC/LADA)	Increased (vs HC)	(77)

LADA, latent autoimmune diabetes in adults; HC, healthy controls; T1D, type 1 diabetes; T2D, type 2 diabetes.