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REVIEW

Complement activation in obesity, insulin resistance, and type 2 diabetes mellitus

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Abstract

Amplified inflammatory reaction has been observed to be involved in cardiometabolic diseases such as obesity, insulin resistance, diabetes, dyslipidemia, and atherosclerosis. The complement system was originally viewed as a supportive first line of defense against microbial invaders, and research over the past decade has come to appreciate that the functions of the complement system extend beyond the defense and elimination of microbes, involving in such diverse processes as clearance of the immune complexes, complementing T and B cell immune functions, tissue regeneration, and metabolism. The focus of this review is to summarize the role of the activation of complement system and the initiation and progression of metabolic disorders including obesity, insulin resistance and diabetes mellitus. In addition, we briefly describe the interaction of the activation of the complement system with diabetic complications such as diabetic retinopathy, nephropathy and neuropathy, highlighting that targeting complement system therapeutics could be one of possible routes to slow down those aforementioned diabetic complications.

Key words: Inflammation; Complement activation; Metabolic disorders; Obesity; Insulin resistance; Type 2 diabetic mellitus

Core tip: Inflammatory reaction is involved in cardiometabolic diseases such as obesity, insulin resistance, and diabetes. The complement system, a key component of innate immunity, was viewed as the first line of defense against microbial. Recent research has

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come to appreciate that the complement system is involved in such diverse processes as clearance of the immune complexes, complementing immune functions, tissue regeneration, and metabolism. The review is to update the role of the activation of complement system and the progression of metabolic disorders. We provided a paradigm that targeting complement therapeutics could be one of possible routes to slow down diabetic complications.

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INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is rapidly increasing globally and affects approximately one third of the worldwide population. The etiology of T2DM revolves around obesity and insulin resistance. Metabolic disorders like obesity[1-4], insulin resistance and diabetes mellitus[5-8] all possess inflammatory components. Although we are still in the early stage of fully understanding the relationship between inflammation and metabolic diseases, a wealth of data points to the fundamental role of inflammation in the initiation, development and progression of those metabolic disorders[9]. Activation of pattern recognition receptors (PRRs) that include NOD-like receptors (NLRs) and Toll-like receptors (TLRs) can initiate the immune responses by sensing both endogenous damage-associated molecular patterns (DAMPs) and exogenous pathogen-associated molecular patterns (PAMPs). Obesity-associated PAMPs and DAMPs have been revealed to trigger the NF-κB pro-inflammation pathway, and are associated with NLRP3 inflammasome activation in adipose tissue[10,11]. Several studies have revealed that metabolic inflammatory signaling can transcriptionally and post-transcriptionally interfere with insulin action and modulate metabolic pathways that promote insulin resistance[12,13]. The complement system is a central component of innate immunity and contributes substantially to homeostasis by eliminating infectious microbes, cellular debris, complementing immunological and inflammatory processes, and sending "danger" signals. The complement system was originally regarded as a supportive first line of defense against microbial invaders; however, research over the past decade has come to realize that the functions of the complement system extend far beyond the defense and elimination of microbes, partaking in such diverse processes including the clearance of immune complexes, mobilization of hematopoietic stem-progenitor cells, tissue regeneration, synapse maturation, angiogenesis, and lipid metabolism[14].

In this review paper we will look into the roles of several key components of the complement system in metabolic disorders and provide an updated overview of the complement system in the pathophysiology and development of obesity, insulin resistance, diabetic mellitus, and diabetes-related complications. In this way, we aim to elucidate the function of the complement system in the pathogenesis of metabolic disorders as well as highlight the complement system as a target for future potential therapeutics.

COMPLEMENT SYSTEM

The complement system, a crucial part of the human innate immune system, comprises of more than 50 soluble protein receptors and regulators in the plasma and cell membrane that act in a highly coordinated way to kill microbes, send danger signals, and expedite the elimination of apoptotic cells without damaging the healthy host cells. The complement system can be activated through PRRs that have evolved to recognize PAMPs and DAMPs, which include specific antibodies, mannan-binding lectin (MBL), ficolins, C-reactive proteins (CRP), C1q, and natural IgM. These PRRs activate three major complement pathways: The classical pathway (CP), the mannose-binding lectin pathway (LP), and the alternative pathway (AP). CP activation is initiated by the association of C1q with immune complexes (IgG or IgM) to form a complex with serine proteases C1r and C1s. The complex cleaves C4, then associates with the cleavage product C4b forming a new complex, which cleaves C2 (into C2a

and C2b) to form the C3 convertase C4bC2b. The LP activation starts by the association of MBL/ficolins/collectin 11 to the mannose residues on microbial surfaces. MBL-associated serine proteases (MASP)-1, MASP-2, and MASP-3 are activated by the binding of MBL to microbial surfaces, which function like C1r and C1s, and cleave C4 and C2 to form the same C3 convertase as that of CP. The AP is distinctive as it is activated without PRRs. Instead, a "tick over" mechanism starts with the spontaneous hydrolysis of C3 into $C3_{(H2O)}$, which acts similar to C3b and binds factor B. The AP C3 convertase (C3bBb) is generated through a chain of reactions involving factor D and properdin[15]. C3 convertase cleaves C3 into C3a and C3b. C3b then binds to the C3 convertase to form C5 convertases (C4bC2bC3b or C3bBbC3b), which cleaves C5 into C5a and C5b. C5b binds C6, C7, C8, and C9 molecules to form the membrane attack complex (MAC), the lytic machinery of the complement system (Figure 1). The complement system is finely tuned by positive and negative regulators, which can avoid a potentially misdirected or excessive activation of the system. However, due to dysfunction of the complement system, this defense system can be inappropriately triggered to attack host cells, which contributes to a broad range of immune, inflammatory, and age-related diseases. For example, when the dysfunction of the complement system occurs in the regulatory proteins, Factor H, the powerful cell-killing property of the complement system can be turned against the host self, causing diseases like atypical Hemolytic uremic syndrome, and paroxysmal nocturnal hemoglobinuria[14,16-18]. Unrestrained or undue complement activation is now recognized as one of key pathogenic drivers in a wide variety of immune-mediated and inflammatory diseases, including hematological and ageing-related ocular pathologies, cancer, autoimmunity, oral dysbiotic diseases, neuroinflammatory, neurodegenerative, and metabolic disorders[17].

Complement system and inflammation

Acute and chronic inflammation is a recognized hallmark of many diseases. It has been understood now that the complement system acts as a sensor of pathogens and a contributor in various inflammatory diseases as well, especially those disorders with complement imbalances[17,18]. Insufficient control or excessive activation of the complement system can cause a malicious cycle between the complement system, inflammatory cells and tissue injury[16,17]. New findings have revealed an interesting cross-talk between the complement system and the inflammatory network[14,16,19-21]. During certain diseases and injuries, the complement and coagulation act in a coordinated fashion. Some enzymes of the coagulation system directly cleave and activate complement component C3 and C5; the products by such activation can affect coagulation. It was demonstrated that C5a, C5 activation fragment could stimulate tissue factor[22-24]. It has been recently recognized that the complement system and TLR, both described as "first line of defense" systems, are cooperated. The cross-talk between complement components CR3, C5aR, CD46, CD55, and gC1qR and TLR, CD14, and MyD88 of TLR system has been revealed to be important for antimicrobial defense but also contributes to inflammatory diseases[25-27]. It has been well described that the complement system essentially contributes to autoimmune and other diseases by the maturation and stimulation of B cells *via* CD21[28]. By directly acting on T cells or antigen presenting cells (APC), the complement system can modulate different phases of T cell immunity. Locally generated C3a and C5a stimulate a shift toward Th1 immunity by enhancing T cell proliferation and cytokine release on APC. In addition, complement regulator protein, CD46 was recognized as a key regulatory player in the IL-2-dependent conversion of Th1 cells[29,30]. C5aR and CD46 signaling were revealed to be associated with the function of γδT cells[31,32]. Conversely, the interaction of T cells and APC induces the enhanced secretion of C3, C5, Factor B, and Factor D with the down-regulation of CD55, which causes complement activation on immune cells. In addition, stimulation of C5aR promotes the expression of activated FcγR, by binding to autoantibodies to induce the local secretion of C5, hence activating the C5a/C5aR signaling pathway, revealing a positive feedback loop in cooperation of complement with FcγR to remove immune complexes[33]. Recent study discovered that high-galactose immune complexes associated with inhibitory FcγRIIB and lectin receptor dectin-1 could induce a signaling pathway that counteracts C5aR signaling pathway[34]. Studies have also shown that C1q is involved in age-related inflammation and tissue injury *via* upregulating the Wnt pathway that enhances age-related fibrotic muscle changes in mice skeletal muscle[35]. Lastly, the complement system has been demonstrated to regulate the functions of NKT and NK cells[36], myeloid-derived suppressor cells[37] and mast cells[38]. All these discoveries highlight the crucial functions of the complement system in the inflammatory network and indicate that dysregulation of the complement system may result in substantial clinical consequences by shifting the functions of T, B, and APC immune cells.

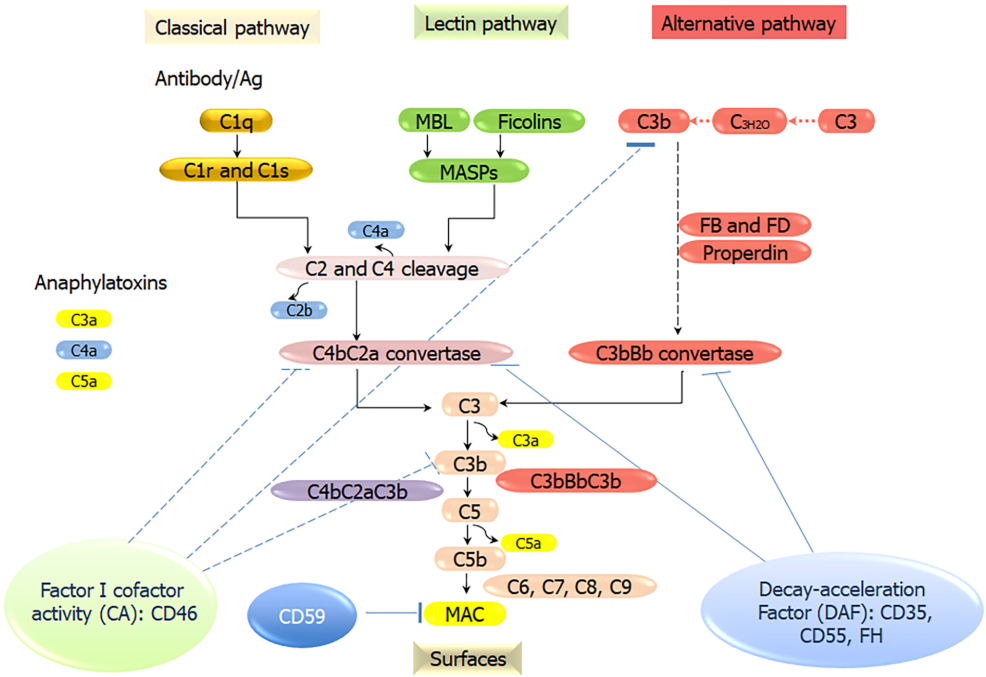

Figure 1 Overview of the activation and regulation of the complement pathways. Three different recognition and activation pathways, including classical pathway (CP), lectin pathway (LP), and alternative pathway (AP). The CP is triggered by the formation of antigen-antibody immune complexes that bind to the C1 complex (C1q, C1r2, C1s2). The LP is activated by the binding of mannan-binding lectin (MBL), ficolins, or collectin 11 to carbohydrates/mannan/other pathogen-associated molecular patterns. MBL-associated serine proteases (MASP)-1, MASP-2, and MASP-3 are activated. Serine protease C1s/C1r (CP) or MASPs (LP) sequentially cleave C4 and C2 to form C3 convertase C4bC2b. The AP C3 convertase (C3bBb) is generated through a chain of reactions involving factor B, factor D and properdin. C3b then binds with the C3 convertase to form C5 convertases (C4bC2bC3b or C3bBbC3b), which cleaves C5 into C5a and C5b. C5b binds C6, C7, C8, and C9 molecules to form the membrane attack complex (MAC), the lytic machinery of the complement system. MBL: Mannan-binding lectin; MASP: Mannan-binding lectin-associated serine proteases; FB: Factor B; FD: Factor D; MAC: Membrane attack complex.

COMPLEMENT ACTIVATION IN METABOLIC DISORDERS

Complement activation in obesity

Many complement components can influence the biology of adipose tissue. T2MD arises from the setting of inflammation and is especially targeted in obesity-related adipose tissue, particularly in white adipose tissue (WAT), which is also the site of numerous substances involved in pro-inflammatory pathways[39]. WAT secretes active cytokines such as TNFα, CRP, interleukins, plasminogen activator/inhibitor, fibrinogen, monocyte chemoattractant protein-1 and the anti-inflammatory factor adiponectin. Obesity induces adipocytes to go through hypertrophy and hyperplasia and gets invaded by macrophages and other immune cells[40]. This leads to a shift leaning towards the production of more pro-inflammatory than anti-inflammatory adipokines that results in chronic, low-grade inflammation[41]. Adipocytes are a primary source of human factor D, which plays an important role in the activation of alternative complement pathway. Adipsin, the mouse homolog of factor D, is essential for the differentiation of pre-adipocytes, revealing that the function of the complement system is far beyond the defense against microbial intruders. Further studies demonstrated that components from alternative complement pathways, C3, Factor B, properdin, Factor H, and Factor I are expressed in adipose tissue, emphasizing the hypothesis that local complement activation can substantially affect adipose tissue biology. In an aged population, complement C3 has been described as a strong marker of insulin resistance[42]. New studies have shown that adipose tissue can activate the alternative complement pathway in T2DM, which exhibits characteristic of the low-grade inflammation[43,44]. Increased C3 levels are associated with inflammation *via* cross-talk with TLR4 action of enhancing the production of pro-inflammatory cytokines *via* C3aR and C5aR signaling pathways[45,46]. Additionally, significantly increased levels of other complement components C3 and Factor D were found significantly in obese individuals as well[43,47,48]. The expression of complement in adipocyte can be used as a proxy measure of adipose tissue insulin resistance[49]. C3adesArg, identical to serum-derived acylation stimulating factor (ASP) has an important role in adipose tissue biology[50]. C3adesArg enhances adipocyte triglyceride synthesis, and increases plasma triglyceride levels, which are expected to be part of the causes in increasing insulin resistance and leading to the development

of metabolic syndromes[51]. Reduced glucose tolerance and delayed postprandial triglyceride and non-esterified fatty acids clearance were reported in both C3- and Factor B-deficient mice as compared with wild-type mice on low-fat and high-fat diets[52]. Moreover, increased plasma levels of C3adesArg are also observed in obese individuals and patients suffering from type II diabetes[14,53]. Furthermore, receptors for C3a (C3aR) and C5a (C5aR1 and C5aR2) have been documented to be expressed in adipocytes[49]. Thus, adipose, in addition to producing complement components, is a potential target of complement action as well. Upon high-fat diet feeding, C3aR expression is increased in WAT. Both macrophage and adipose tissue within WAT express significant amount of C3aR. $C3aR^{-/-}$ mice on high fat diet transiently resist diet-induced obesity during 8-wk period compared with wild type mice. Improvement in glucose tolerance and insulin resistance are revealed in $C3aR^{-/-}$ mice. Furthermore, macrophage from $C3^{-/-}$ mice were polarized to the M1 phenotype, which demonstrated a considerable decrease in pro-inflammatory functions. Obesity-associated PAMPs and DAMPs have also been shown to activate nucleotide-binding domain and leucine-rich repeat-containing (NLR) protein NLRP3 inflammasome in adipose tissue[54]. In summary, the effects of complement components ASP, Factor B, C3a, C5a, and of their receptors in adipose tissue play essential roles in adipose tissue biology as well as the development of metabolic disorder.

Complement activation in insulin resistance

Insulin resistance is linked with elevated circulating complement factor C3 level, which involves the terminal and alternative complement pathways. Accumulating evidence revealed that anaphylatoxin C3a interaction with its receptor C3aR plays a role in metabolic disorders such as diabetes, obesity, and atherosclerosis. Animal studies revealed that improper complement stimulation exacerbates high-fat-diet-induced insulin resistance. C3 is particularly studied in insulin resistance due to its cleavage product, C3adesArg, also known as ASP, which has insulin-like properties by stimulating the uptake of glucose and the synthesis of triglycerides in adipose tissue[55]. The development of insulin resistance can be further explained by understanding ASP resistance in adipose tissue. Obesity-induced adipose tissue activates the alternative complement pathway, thereby creating C3adesArg, among other factors. This leads to a pro-inflammatory state due to an influx of pro-inflammatory biomarkers leading to ASP resistance, which contributes to the development of insulin resistance[55]. Another explanation could be that activated macrophages from adipose tissue, which can produce pro-inflammatory cytokines, like IL-6 and TNF-α, which can inhibit the insulin receptor functions and thereby induce insulin resistance[56]. The increased levels of C3 production from adipocytes also leads to increased macrophage infiltration into adipose tissue, thereby exacerbating insulin resistance[57]. Additionally, it was found that C1q in the CP could cause adipocyte apoptosis, which in turn leads to the infiltration of macrophages into adipose tissue and causes insulin resistance[58,59]. Clearly the components from complement system, especially complement C3, play crucial roles in causing insulin resistance. Thus, inhibition of the C1 and/or C3 complement activation and/or its regulators could be potential therapeutics for insulin resistance.

Complement activation in diabetic mellitus

The role of complement activation in diabetes mellitus has not been fully investigated. Some components of the complement system are thought to be involved in both Type I and II diabetes mellitus. Nevertheless, accumulating experimental and clinical evidence demonstrates a connection between the activation of the complement system and the pathogenesis of diabetic mellitus[60-63]. Pro-inflammatory cytokines IL-6 and IL-1 enhance complement C3 production from the liver[64,65]. The C3 gene is also observed highly expressed in adipose tissue[66]. Pro-inflammatory cytokines generated in adipose tissue, an organ with endocrine functions, have been associated with insulin resistance and impaired glucose uptake[67-69]. Systemic low-grade inflammation with the actions cytokines might explain the connection between C3 and the incidence of diabetes. Another possible link between C3 and diabetes could be due to C3adesArg or ASP, the proteolytic fragment of C3, which is a paracrine metabolic factor that can stimulate glucose uptake and lipid storage in adipose tissue[70,71]. ASP insufficiency was linked to the reduction of body weight in mice[72], and the correlation of high ASP levels and insulin resistance was observed in nondiabetic subjects[43,73]. However, C3-knockout mice have shown only modest alterations in insulin function and glucose metabolism, suggesting the activation of the C3-ASP system might function differentially in human and mice[74]. Complement C3 also provides a key link between inflammation and thrombosis in diabetes. It was reported that complement C3 interacts with fibrin leading to prolonged fibrinolysis in patients with T1DM and T2DM[75,76]. Another study has found that levels of C4 and C3 are both increased in

patients with diabetes[77]. Complement C4 has been less studied in metabolic disorders. Nevertheless, accumulated data suggested that C4 plays a role in T1DM[78-80]. TLR4 can interact with free fatty acids or free acid carrier (fetuin-A) to trigger the NF-κB pro-inflammation pathway in cells, which promotes adipose tissue inflammation and insulin resistance in diet-induced obesity[81,82]. Recent study demonstrated that C3a can increase insulin secretion[83]. To confirm this result, the study used complement factor D knockout mice which responded poorly to glucose tolerance due to a defect in insulin production[84]. In the same population, the authors of the study restored serum factor D levels, which increased plasma insulin levels, then additionally reversed this *via* treatment with a C3aR antagonist. Such findings allow for the conclusion that factor D is involved in the alternative complement pathway activation and C3a signaling, ultimately allowing for insulin secretion[84]. CD59 is a known complement inhibitor, but recent studies have shown a specialized function involving the secretion of insulin in β cells[84,85]. Specifically, CD59 seems to play a role in maintaining lipid raft stability in β cells to maintain basal insulin release rates. In addition, CD59 facilitates recycling of exocytotic core proteins to allow for the secretion of insulin granules[85]. Collectively, the components in the complement system, such as, C3, C4, factor D, and CD59 were shown to be involved in the pathogenesis of diabetic mellitus. Regulation of the activation of complement system might slow the pathological process of diabetes mellitus.

Complement in diabetic complications

The pathophysiology of T2DM occurs in the setting of baseline and/or excessive chronic inflammation, contributing to the progression of diabetes. Emerging evidence has shown that complement-mediated chronic inflammation is linked to diabetic microvascular complications. In T2DM patients, the end product of complement activation, sC5b-9 and the MASP-2 were elevated. Such an environment contributes to endothelial dysfunction and reactive oxidative species production that plays a major role in the pathophysiology of diabetic complications. High concentration of complement C3 is reported to be connected to the increased risk of diabetic retinopathy, nephropathy, and neuropathy[86]. A study by Fujita *et al*[87] revealed the possible connection between complement activation, inflammation, and the development of atherosclerosis in diabetic conditions. Obesity-induced adipocytes overproduce C3desArg (ASP) from C3 and factor B. In this case, C3 is converted to C3a and C3b in the process C3 "tick over" AP. Then C3a is converted into C3desArg (ASP), which plays a role in insulin resistance as well as chronic inflammation. C3b goes through the terminal pathway and generates the MAC, which contributes to the formation of atheroma. The continuous activation of the complement AP in T2DM with obesity and dyslipidemia significantly contributes to microvascular complications[87]. This study suggests that significantly increased plasma ASP/C3a levels in severe microangiopathy may cause to the acceleration of such complications. Another recent study showed that a high concentration of complement C3 was associated with an increased risk of diabetic complications, such as retinopathy, nephropathy and neuropathy[86,88]. The complement system and inflammation are closely interconnected. Studies have shown that key complement proteins (such as C3, C4, MBL and Factor D) are correlated with a known marker of inflammation, like CRP[60,61,89]. Adipocytokines released from obesity-activated adipocytes induce the production of pro-inflammatory cytokines, causing the dysfunction of vascular endothelial cells and organ damage. After analyzing the relationship between complement-mediated inflammation and diabetic microangiopathy in obese T2DM patients *vs* normal individuals, plasma levels of C3, C4, iC3b, C3desArg (ASP), Factor B, and Bb were found to be significantly increased in T2DM patients. Furthermore, the level of complement components and their activation intermediates in plasma was tested with respect to the level of complication of the disease. The results of study revealed that the early phase of the alternative complement pathway was excessively activated, suggesting complement-mediated inflammation may contribute to the acceleration of diabetic microangiopathy. A statistical study revealed a close correlation between C3desArg (ASP), highly sensitive CRP, and body mass index. In the macroalbuminuric and proliferative retinopathy patient groups, plasma C3desArg (ASP) was significantly elevated. Diabetic retinopathy complement activation occurs in choriocapillaries of those with diabetic retinopathy. It was found in the eyes of patients with diabetic retinopathy with intense positive staining for MAC deposits, C3d and C5b-9[90,91]. Additionally, there was a reduction of CD55 and CD59 which are inhibitors of the complement pathway in the vessels of those with diabetic retinopathy[92]. Such findings may point to the activation of the AP in diabetic retinopathy[91]. The enhanced expression of C3 mRNA and protein in the glomeruli was positively correlated with the disease status of diabetic kidneys[93]. The increased levels of the complement components (such as C1q, MBL, MASP-2, factor B, C3 and

C5b-9) were found in diabetic nephropathy in rat, suggesting that the complement system is involved in aggravating the pathophysiology of this disease[94]. Specifically, most of these factors were found in the renal tubules, suggesting that complement also plays a role in tubular injury, not just in glomerular damage in diabetic nephropathy[94]. MAC was increased in diabetic patients' blood vessels as well as their kidneys. MAC insertion into cell membranes and into the glomerular mesangium will activate several intracellular signaling pathways leading to the production of reactive oxygen species[62,95-97]. Several other downstream effects of the insertion of MAC include cell proliferation and thrombosis formation. MAC can upregulate fibroblast growth factor and platelet-derived growth factor in glomerular mesangium causing the proliferation of mesangial cell, upregulation of TGF-β, MCP-1 and activation of collagen IV. Moreover, pro-inflammatory molecules such as P-selectin, vascular cell molecule 1, and E-selectin leak out through the MAC complex[98]. These effects of MAC could further explain the pathogenesis of diabetic nephropathy. Normally, MAC is inhibited by membrane proteins including Decay-acceleration factor (DAF) and CD59. Thus the decreased level of DAF or CD59 could result in increased level of MAC in diabetics. In the hyperglycemic state, the glycation of CD59 would increase the activation of MAC and then increase the release of the downstream effects of MAC, which contributes to the development of atherosclerosis and diabetic nephropathy[62,96]. Complement activation has been reported to cause nerve damage in peripheral neuropathies[99]. A recent study demonstrated that a single missense mutation of the CD59 gene, which results in the deficiency of the complement regulatory protein CD59 on the cell surface, causes chronic peripheral neuropathy in children[100]. It is also reported that there were activated complement proteins and MAC neoantigen are found in sural nerve biopsies from individuals with diabetes[101]. Moreover, the co-localization of MAC and CD59 was observed in the same nerve biopsies analyzed in that study[95]. In summary, the activation of the complement system is clearly involved in the diabetic complications, including nephropathy, retinopathy and neuropathy.

PERSPECTIVES AND THERAPEUTICS

Recent studies have revealed the functional link between immune and metabolic systems, which are two central pillars of survival pathways evolved from common ancestors. Human complement system is a global mediator of our innate immune system. It is recently understood that the complement system not only contributes substantially to homeostasis by eliminating infectious microbes, and cellular debris, complementing immunological and inflammatory processes and immune surveillance, but also contributes to various immune, inflammatory-related diseases. Undoubtedly, complement system may not be the chief driving force in these conditions, but it may still be a vital factor that can tip the scales between the initiation and resolution of inflammatory processes. It has been receiving increasing attention that the complement system plays a major regulatory role in metabolism and metabolic disorders. Accumulating evidence suggests that metabolic disorders like diabetes, obesity, insulin resistance, and arthrosclerosis possess important inflammatory components. In Table 1, we highlight those functions of some key complement components in obesity, insulin resistance, and diabetes mellitus (Table 1). In the past few years there is increasing attention in the development of therapeutic modulators of the complement system[17]. Such therapy should modify the complement system to sufficiently and precisely control the aberrant levels while allowing for the beneficial players to continue contributing to immune surveillance[91]. Some drugs are in clinical use for diseases other than metabolic diseases or diabetic complications. Given the strong etiologic components of the complement system activation, those medications might be worth to be evaluated for their ability to slow or halt the progression of metabolic disorders or related complications. Future studies are warranted to test the efficacy of long-term inhibition of activation of the complement system in the context of progression of T2DM and its related complications.

Table 1 The roles of complement proteins in obesity, insulin resistance, diabetes

Complement protein	Obesity	Insulin resistance	Diabetes
C3	Increases adipose tissue inflammation[45,46]; Highly expressed in adipose tissue[70,71]	Marker of insulin resistance[42]	Proinflammatory cytokine causing systemic low-grade inflammation, insulin resistance and impaired glucose uptake[67-69]; Link between inflammation and thrombosis[75,76]; Higher concentrations associated with increased risk of diabetic complications[86]
C3a and C3aR	Increased levels of C3 in obese individuals[43,47,48]; Increased C3aR in white adipose tissue[49]	Improved glucose tolerance and insulin resistance in C3aR$^{-/-}$ mice[54,55,84]	Proinflammatory cytokine enhanced by cross-talk with TLR4 and C3[45,46]; C3a augments insulin secretion from mouse pancreatic beta cells[83]
C3adesArg (ASP)	Increased levels found in obese individuals[14,53]; Obesity induced adipose tissue creates this product leading to a pro-inflammatory state and ASP resistance[55]	Insulin-like properties by stimulating uptake of glucose and synthesis of triglycerides in adipose tissue[55]	Stimulating glucose uptake and lipid storage in adipose tissue[70,71]; Plays a role in chronic inflammation and contribute to microvascular complications[87]
C5a and C5aR	Expressed in adipocytes[49]; Play role in adipose tissue biology and development of metabolic disorders		Affect coagulation and contribute to inflammation[22-27]
Factor I and H	Increased in adipose tissue, activated by complement activation and affecting adipose tissue biology[41,42]		
sC5b-9 and MASP-2			Elevated in T2DM; Contributing to endothelial dysfunction and ROS production in diabetes pathophysiology[62,95-97]; MASP-2 contributes to complications of diabetes[94]
CD 59 and MAC			Decreased levels of CD 59 in diabetics[62]; Increased levels of MAC found in diabetic patients and contributes to pathophysiology of complications[62,95-97]

MAC: Membrane attack complex; ROS: Reactive oxygen species; MASP: Mannan-binding lectin-associated serine proteases; ASP: Acylation stimulating factor; T2DM: Type 2 diabetes mellitus; TLR: Toll-like receptors.

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MINIREVIEWS

Current understandings of the pathogenesis of type 1 diabetes: Genetics to environment

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease that usually strikes early in life, but can affect individuals at almost any age. It is caused by autoreactive T cells that destroy insulin-producing beta cells in the pancreas. Epidemiological studies estimate a prevalence of 1 in 300 children in the United States with an increasing incidence of 2%-5% annually worldwide. The daily responsibility, clinical management, and vigilance required to maintain blood sugar levels within normal range and avoid acute complications (hypoglycemic episodes and diabetic ketoacidosis) and long term micro- and macro-vascular complications significantly affects quality of life and public health care costs. Given the expansive impact of T1D, research work has accelerated and T1D has been intensively investigated with the focus to better understand, manage and cure this condition. Many advances have been made in the past decades in this regard, but key questions remain as to why certain people develop T1D, but not others, with the glaring example of discordant disease incidence among monozygotic twins. In this review, we discuss the field's current understanding of its pathophysiology and the role of genetics and environment on the development of T1D. We examine the potential implications of these findings with an emphasis on T1D inheritance patterns, twin studies, and disease prevention. Through a better understanding of this process, interventions can be developed to prevent or halt it at early stages.

Key words: Type 1 diabetes genetics; Type 1 diabetes epigenetics; Role of genetics in type 1 diabetes; Diabetes prevention; Type 1 diabetes environment; Type 1 diabetes twin

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Core tip: Type 1 diabetes (T1D) is one of the most common childhood chronic conditions with its incidence increasing annually. Its arduous management requires vigilance to avoid complications that impact quality of life and health care costs. A better understanding of how T1D develops can help find a way to prevent it from developing at all. In this review, we discuss the current understanding of the complex relationship between the roles of genetics and the environment in the development of T1D.

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INTRODUCTION

Type 1 diabetes (T1D) is one of the most common childhood chronic conditions. Though its peak incidence is between the ages of 10 and 14, its clinical presentation can occur at almost any age from early infancy to as late as the 9th decade of life[1-3]. See Figure 1[3], for the relative incidence at different age groups. Data from large epidemiologic studies worldwide have indicated that the incidence of T1D has been increasing by about 2%-5% worldwide annually and that the prevalence of T1D has increased to approximately 1 in 300 in the United States by 18 years of age[1]. It affects 2.28 in 1000 children as compared to the 1.24 of 1000 youths (< 20 years old) affected by cancer and 120 of 1000 youths suffering from asthma[1,4]. Additionally, T1D can significantly affect the quality of life of patients and families because they are required to vigilantly monitor their blood sugars with needled lancets or other monitoring devices and administer insulin with syringes, pens, or insulin pumps several times a day. Moreover, there is a significant public health impact with an estimated total cost of $132 billion in the United States in 2002[4,5].

The high number of people affected by T1D and its health burden has spurned a robust growth of clinical research aimed at improving the quality of life of those affected and basic research looking at ways to better understand the pathogenesis of this disease in the quest for a cure. Though significant advances have been made, there is still much we do not understand about what triggers T1D and how to effectively control this disease, and thusly much research work is still needed. This review article will focus on our current understanding of the development of T1D from an immunologic, genetic, and environmental standpoint. We will highlight promising and interesting studies that seem closest to uncovering why and how T1D develops.

HIGHLIGHTS OF PATHOGENESIS OF T1D

Diabetes mellitus (DM) is a condition characterized by a state of relatively insufficient or complete lack of insulin production. There are various types of DM that include: T1D, T2D, gestational, post-pancreatectomy, Mature Onset Diabetes of the Youth, neonatal diabetes, and medication-induced diabetes. T1D "contributes up to about 10% of the estimated 422 million diabetes cases worldwide"[6].

T1D results from the destruction of the insulin-producing cells in the pancreas called beta cells by the adaptive immune system. This process is promoted by an incompletely understood interaction between a person's genetics and their environment. Genetic factors (*i.e.*, individuals with an overexpression of human leukocyte antigen or HLA class molecules DR4, DQ8, and DQ2 increasing their susceptibility is present in approximately 90% of T1D patients) and one or more environmental factors lead to the recognition of beta cell components as autoantigens that the immune system erroneously recognizes as foreign leading to an autoimmune attack[1,7,8]. Identified autoantigens include insulin B chain peptide (11-23) and other

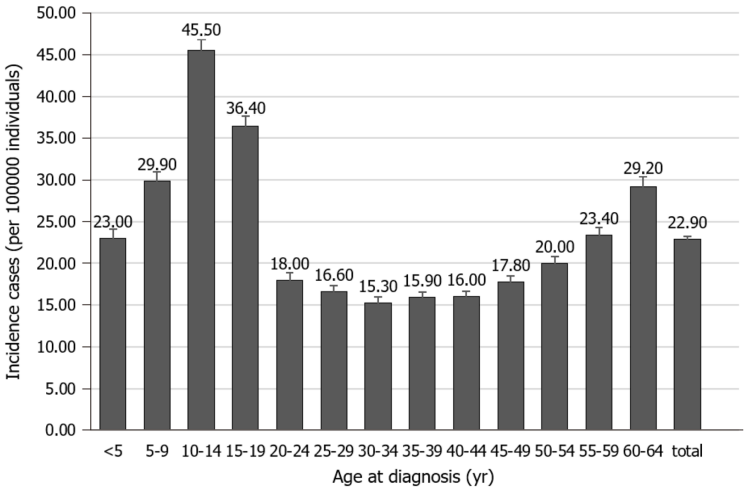

Figure 1 Incidence of type 1 diabetes in the United States by age.

components of beta cell secretory granules such as glutamic acid decarboxylase 65, protein phosphatase-like IA-2, and transmembrane Zn transporter. The presence of one known autoantibody confers a moderate risk of developing T1D while the presence of each additional autoantibody increases the risk exponentially[7].

These autoantigens are presented by HLA molecules major histocompatibility complexes (MHC) I and II on antigen presenting cell (APCs) to diabetogenic autoreactive T cells. Autoreactive CD4 T cells stimulate APCs, including B cells that produce high-affinity autoantibodies against beta cells. Autoreactive CD4 T Cells also help diabetogenic CD8+ T cells to acquire cytolytic activity and attack beta cells through the release of cytokines (including TNF-α and IFN-γ, Fas/FASL and perforin/granzyme). Released cytokines also stimulate macrophages and other innate immune cells to further damage beta cells yielding a positive feedback loop with the production of more toxic cytokines to propagate further beta cell destruction[7,8].

There has been much debate regarding the identity of the nidus that initiates the autoimmune destruction of beta cells. Other groups are currently studying potential players in the immune system such as FasL B cells and dual expresser (DE) cells that may play a role in T1D pathogenesis. Using Flow Cytometry, high-throughput sequencing, and transcriptional analysis utilizing RNA-seq, interesting subsets of CD5+ B Cells that include IL-10 producing B-reg cells, FasL-expressing B cells and DE cells that express both B cell receptor (surface immunoglobulin, Ig) and T cell receptor have been discovered[9]. Findings suggest that the CDR3 region of the heavy chain of surface Ig on the DE cell expresses an extremely potent diabetogenic T-cell autoantigen that is able to stimulate other T cells and may contribute to the development of T1D and similarly potent x-autoantigens with different CDR3 sequences could be involved in pathogenicity of other autoimmune diseases[10]. Another study also suggests that T1D could be caused by a paucity of anti-inflammatory IL-10-producing Breg cells by apoptosis mediated by FasL expressing B cells leading to the unchecked proliferation and expansion of diabetogenic T cells[11]. This is supported by the elevated levels of FasL-expressing CD5+ B cells in the splenocytes and lymph nodes of T1D patients compared to controls and the prevented development of T1D in non-obese diabetic (NOD) knockout mice or mice treated with FasL-neutralizing monoclonal antibody[11]. Further studies are being performed to determine the significance of blocking FasL in humans and targeting DE cells as a form of immunotherapy.

ROLE OF GENETICS IN T1D

T1D is a multifactorial disease that is affected by a number of genes. Even in the case of high-risk conferring haplotypes that are present in 90%-95% of T1D children, only 5% or fewer with the haplotype actually develop overt T1D in the general population[1]. This supports the fact that there must be other factors, genetic, epigenetic, and/or environmental that play a role in ultimately determining disease manifestation. According to genome wide association studies, over 60 genes can affect the risk of the developing T1D especially those that affect the HLA loci[12,13]. Up to 30%-50% of the genetic risk in T1D is related to HLA class II alleles. Of the HLA class

II genes, the mutations found on the HLA region of chromosome 6p21 have been shown to be a major susceptibility locus for T1D. There are also several non-MHC loci that have a lesser contribution to disease risk, such as IFIH1, IL2RA, PTPN22, and CTLA4[13-15]. See Table 1 for a list of prevalent T1D-associated genes associated with risk factors[16,17] and protective factors[18,19].

Interestingly, although the association of T1D with the HLA class II genes DQ and DR has been well validated, individuals who are heterozygous for HLA-DRB1*04 and HLA-DRB1* 03 type have been found to be at highest risk[14]. It has been suggested that almost 50% of children with the HLADR3/4-DQ8 heterozygous genotype will develop anti-islet autoimmunity before the age of 5[14,15]. Children with the DR3/4-DQ8 or DR4/DR4 homozygous genotype and a family history of T1D have more than a 1 in 5 risk to developing islet autoantibodies, whereas children with no family history and DR3/4-DQ8 or DR4/DR4 genotype have a 1 in 20 risk[15].

Therefore, it stands to reason that HLA typing could be a cost-effective method to screen for T1D susceptibility and identify high risk individuals at an early age if targeted immunotherapy is available to prevent disease onset. There are many ongoing international projects, including DIPP, TEDDY, TRIGR, and TrialNet that are monitoring high-risk individuals, their risk alleles and their progression to T1D to better elucidate the positive and negative predictive values of identifying the presence of high-risk alleles. Thus far, it is clear that the inheritance of T1D is complex and development of overt disease involves both a genetic predisposition and an environmental trigger(s) since the presence of high-risk genetic determinants does not yield a 100% positive predictive value.

The strong multifactorial genetic component of T1D is further supported by the effects of familial inheritance. Interestingly, the offspring of mothers with T1D have a reduced risk of developing T1D when compared to the offspring of fathers with T1D. Harjutsalo's group's study of at-risk (offspring of T1D patients) Finnish children found that the risk of developing T1D was 1.7 times higher if the father had T1D as compared to when the mother had T1D (incidence of 7.8% vs 5.3%, respectively)[20]. They also found that sons (more than daughters) of a parent with T1D had an increased risk of developing T1D. A younger age of the male parent (at diagnosis) was also found to be a significant predictor with fathers diagnosed at the age of ≤ 4 years being 2.66 times more likely to have children with T1D than fathers diagnosed at the age of 15-17 years[20].

The reason why mothers with T1D are less likely to pass on T1D to children than fathers with T1D remains unclear. Some hypothesize that it may be caused by selective loss of fetuses in mothers with T1D since the rate of miscarriage is higher (15%-30%) in T1D mothers than the general population usually as a result of hyperglycemia at conception and early pregnancy[21-23]. Others hypothesize that it may be due to upregulation of CD4+CD25+FOXP3+ Treg cells that have been found to be significantly elevated in the offspring of mothers with T1D in comparison to infants of healthy mothers[24]. In addition, studies have found that children born before maternal onset of T1D have a higher risk of T1D than infants born after maternal disease onset. Their studies also showed that in vitro insulin stimulation of CD4+CD25+ cells upregulated the expression of anti-inflammatory genes and FOXP3 + Treg cells suggesting that insulin treatment of T1D mothers during pregnancy increases the expansion of anti-inflammatory regulatory cells in their offspring thereby decreasing their risk of T1D development as compared to the offspring of fathers with T1D[24].

TWIN STUDIES AND EPIGENETIC MODIFICATION

Studies in twins have demonstrated the important role of gene and gene modification in the development of T1D. Dizygotic (non-identical) twins and siblings share about 50% of their genes, while monozygotic or identical twins share 100% of their genes meaning differences found between monozygotic twin pairs are likely the result of environmental factors or post-translational modification of histones, the activation of microRNAs, the methylation of DNA, or acquired postconceptional genetic discordance. Multiple studies indicate a significantly higher concordance rate for T1D in monozygotic twins than dizygotic twins (23%-61% *vs* 0-27% probandwise, respectively). Table 2 compares the concordance rates of a co-twin developing T1D when there is a proband twin diagnosed with T1D. In addition, it was noted that "more monozygotic twins were positive for > 1 autoantibodies that dizygotic twin siblings"[25]. A study followed siblings for 3 years after screening for autoantibodies to see how many would develop T1D and showed that the T1D rate increases with the increasing number of autoantibodies present in the co-twin when the proband twin had been diagnosed with T1D[26]. It also showed that a larger proportion of

Table 1 Genetic risk and protective factors of type 1 diabetes[1]

Risk factors[16,17]	Protective factors[18,19]
HLA DR3/DQ2	DRB1*15:01
HLA-DR4/DQ8	DQA1*01:02
HLA-A*02:01	DQB1*06:02
Increased PTPN22 activity	Rare variant of IFIH1
INS polymorphisms	
IL2RA variants	
Increased expression of common variant IFIH1	
1st degree relative with T1D	

[1]These genetic factors represent more prevalent risk factors associated with type 1 diabetes and is not all inclusive. PTPN22: Protein tyrosine phosphatase non-receptor type 22; INS: Insulin gene; IL2RA: Interluekin-2 receptor alpha; IFIH1: Interferon-induced with helicase C domain 1; T1D: Type 1 diabetes.

monozygotic twins are likely to have positive antibodies than dizygotic twins or full siblings[26], see Table 3. Interestingly, a North American study also found that the relative risk of developing TID increased if the proband was diagnosed at an earlier age. It noted that "if the proband is diagnosed before 15 years of age, the long-term risk to the co-twin is estimated at 44% (monozygotic) and 19% (dizygotic); it reaches 65% for the co-twin of a monozygotic proband diagnosed before 5 years of age with time after the proband's diagnosis"[27]. Additionally, the discordance time among the concordant could range from 1-36 years[25,28] with the mean time being 3.3 (+/- 0.6) years for monozygotic twins and 6.1 (+/- 1.5) years in dizygotic twins[27]. This suggests that while genetics play an important role in the ultimate development of T1D, there must be other factors that contribute since the concordance rate is not 100%.

To address this issue, some have looked to epigenetic factors such as DNA methylation, which is important in gene expression and transcriptional regulation. Rakyan's group performed an epigenomic-wide association study looking at DNA methylation profiles from T1D monozygotic discordant twin pairs and diabetes-associated antibodies from longitudinally sampled pre-and post-diagnosis T1D singletons to identify CpG sites with T1D-MVPs (T1D-associated methylation variable positions), genetic differences, and epigenetic variations. In this manner, they identified a number of genes including INS-IGF2, SH2B3, MEG3 and ORMDL3, that are known to be correlated with T1D and had differential CpG methylation when they compared T1D-affected to non-affected twins[29]. This demonstrates that in addition to inheritance and the presence of risk genes/alleles, epigenetic factors (such as DNA methylation or exposure to insulin in utero) that regulate gene expression or upregulated anti-inflammatory cells could determine whether T1D would develop.

ROLE OF THE ENVIRONMENT

Though many genetic factors have been implicated in the development of T1D, it's widely believed that environmental factors must be involved. Genetic susceptibility persists for a lifetime. Individuals can develop T1D at any time from age 1 to 100 years old suggesting that "T1D precipitates in genetically susceptible individuals, very likely as a result of an environmental trigger"[1,14]. This is also supported by the temporally discordant times of T1D development (if at all) in monozygotic twins. Several environmental factors have been suggested as potential risk and protective factors[30,31] affecting T1D incidence such as viruses, anthropometric development[32-34], ethnicity, maternal age and weight[35], microbiota[36-38], number of siblings[35], season, diet, and geographic location[18,31] among other factors. See Table 4 for a list of environmental factors likely contributing to T1D development.

Viruses

Of the external factors, viruses have long been suggested as a potential environmental trigger for the disease and has some of the strongest evidence as a potential factor. Though there has been strong evidence to suggest the association of viruses with T1D, evidence of a definitive causative relationship leading to the initiation or acceleration of islet autoimmunity is circumstantial[39-41]. Viral infection by enteroviruses such as coxsackieviruses (CVB) or echoviruses have been strongly implicated as potential

Table 2 Concordance rate of monozygotic and dizygotic twins in the indicated countries

Study population	Relation	No. of twin pairs	No. of concordant pairs	Probandwise concordance rate (%)
Australia[95]	Monozygotic	14	6	61
	Dizygotic	32	2	12
Finland[28]	Monozygotic	44	12	42.90
	Dizygotic	183	7	7.40
Japan[96]	Monozygotic	19	7	53.8[1]
	Dizygotic	13	1	14.3[1]
United States[25]	Monozygotic	53	12	36.9[1]
	Dizygotic	30	0	0
Denmark[97]	Monozygotic	26	10	53
	Dizygotic	69	4	11
Finland[98]	Monozygotic	26	3	23.10
	Dizygotic	83	2	4.80
North America[27]	Monozygotic	132	38	45
	Dizygotic	92	13	25
United Kingdom[99]	Monozygotic	49	15	25 (1 yr)[1]
				40 (5 yr)[1]
				50.7 (10 yr)[1]

[1]Rate not listed in original study, but calculated here based on the equation (2C/2C+D), where C is the number of concordant twin pairs and D is the number of discordant twin pairs.

disease triggers. Several epidemiological studies have linked the association of CVB infection with T1D onset. One study performed between 1964-1984, found that among children diagnosed with T1D, 67% of them had positive CVB IgM serology[42]. Similarly, among 14 children presenting with new-onset T1D, 64% of them were found to have serologic evidence of enteroviral infection, which were mainly CVB3 and CVB4[43]. CVB1 was found to increase the risk of developing T1D in studies looking at children who were at high risk or recently developed T1D[44,45].

The exact mechanism by which viral infections contribute to the destruction of islet cells is still unknown. However, various mechanisms have been suggested such as molecular mimicry, inflammation, endoplasmic reticulum (ER) stress, and bystander activation or suppression of T cells, which are detrimental to beta cell function and survival[30,31]. Some studies have reported cross-reactivity between islet cell autoantigens and certain viral proteins. However, there is no proof that this cross-reactivity leads to T1D autoimmunity[30]. Interestingly, at-risk children with insulin autoantibodies have a reduced ability to produce antibodies to VP1 (markers of viral infections), possibly facilitating enterovirus infection and persistence[46]. It was found that inflammation and ER stress induced by viruses can cause beta cell dysfunction and protein misfolding, which could lead to an abnormal presentation of autoantigens and thus to autoimmunity[47,48]. Viral infection can also stress the ER causing beta cells to release exosomes loaded with autoantigens and immuno-stimulatory chaperones, which are then taken-up by APCs[30,49]. These can then be presented to the adaptive immune system and cause beta cell destruction.

Such associations have also been found in experimental mouse models. One study was able to induce diabetes in mice using a coxsackie virus (CVB4E2) isolated from a patient with diabetic ketoacidosis[50,51]. Additionally, coxsackievirus infection has been found to escalate the progression of T1D in mice engrafted with human islets[52]. Transfer of CVB-specific antibodies from mothers to their offspring in NOD mouse models decreases the risk in the offspring for CVB infection and protects them from T1D development[53,54]. However, a conclusive link between viral infection and the onset of T1D in humans is still lacking. While mouse models have shown a potentially causative relationship between viruses and T1D development, there have mostly been epidemiological studies in humans and no conclusive trials to establish a causative link. However, if a viral link were confirmed, it would garner support for research to develop an enterovirus vaccine to help prevent T1D.

Seasonality

Interestingly, studies have revealed that migrants tend to develop the same level of risk of developing T1D as the population in the area of their new residence, despite

Table 3 Progression to type 1 diabetes in siblings within 3 years based on number of autoantibodies at screening

Relation	0 Autoantibodies		1 Autoantibody		≥ 2 Autoantibodies	
	No. of individuals	Progressed to T1D	No. of individuals	Progressed to T1D	No. of individuals	Progressed to T1D
Monozygotic Twins	89	1.50%	25	69%	29	69%
Dizygotic Twins	231	0%	22	13%	17	72%
Full Siblings	13944	0.50%	1456	12%	900	47%

T1D: Type 1 diabetes.

their origin being from a low incidence region of the world. This supports the theory of the environmental effects of T1D development, which could be influenced by factors such as seasonality or geographic location.

Numerous reasons have been proposed for the apparent link between seasons and the onset of T1D. Some of these theories include that there is a seasonal variation in the blood glucose and insulin levels possibly due to a typically reduced level of activity in young people in the cold winter months and seasonal viral infections. Several studies suggest a seasonality that conforms to a sinusoidal model that peaks in the winter and troughs in the summer[55,56]. Epidemiological studies following birth cohorts found that many people (79%) with high risk genotypes seroconverted during the fall/winter months[57]. A large international study found that 42 out of 105 centers exhibited this seasonal trend with 28 centers having peaks of diagnosis in the winter and 33 had troughs in the summer[58].

However, other studies contradicted this correlation, stating that their findings indicated that there was no seasonality in the month of onset, rather that there was significant seasonality in the month of birth (peaking in November to January)[59]. In addition, the seasonality pattern appears to be dependent on the geographical position, in regards to the northern/southern hemisphere dichotomy. The correlation of seasonality appeared to disappear after adjustment was made for latitude, where centers furthest away from the equator were more likely to exhibit significant seasonality[58]. However, most of the data from the study came from the northern hemisphere, and there was inadequate information from Asia and Africa. Therefore, this correlation was deemed inconclusive, as more data on the population living below the 30th parallel north was needed for the information to be accurate[58]. Though still inconclusive, this data likely indicates that there is an increased susceptibility to developing T1D during times when people are most vulnerable to viral infections. This is generally during cold (viral infection) seasons, which may vary based on geographic location and could explain why regions in colder climates or with temperate seasons have higher T1D incidence rates than regions with warmer climates and tropical seasons.

Vitamin D

There is increasing evidence that demonstrates a strong association between vitamin D signaling and many biological processes involved in regulating immune responses. A deficiency in vitamin D is associated with increased risk of autoimmune diseases such as multiple sclerosis, systemic lupus erythematosus, and T1D[60]. The vitamin D receptor (VDR) is expressed on several immune cells (B cells, T cells and antigen-presenting cells) and affects "cell proliferation and differentiation and immunologic effects resulting in an ability to maintain tolerance and promote protective immunity"[61].

FokI (rs10735810) has been identified as one of four polymorphisms in the VDR gene. Its presence has been suggested to cause a dysfunctional structural change on the VDR protein[62]. Studies demonstrated that mononuclear cells from subjects with the dysfunctional VDR genotype had a lower ED50 of 1,25 (OH)2D when compared with the normal VDR genotype[63]. Studies also reported that monocytes and dendritic cells from subjects with the dysfunctional genotype had a higher expression of interleukin-12 (pro-inflammatory cytokine) and proliferated more significantly after stimulation than those with the normal genotype[64].

A causal relationship between FokI polymorphism and vitamin D status in T1D has not been reported, but several studies have suggested a correlation. Vitamin D deficiency has been found to be higher in children with T1D in comparison to controls[65-67]. A birth cohort following over 12000 live births found that children receiving regular Vitamin D supplementation had a reduced risk of developing T1D

Table 4 Environmental risk and protective factors of type 1 diabetes

Risk factor	Protective factors
Group B Coxsackieviruses[42,44,45]	Longer breastfeeding duration[78-80]
Early introduction of cow's milk duration[76,77]	Vitamin D intake supplements[68,69]
Early cereal introduction[72,87]	Polyunsaturated fatty acids[89,90]
High latitudes[18,31]	Intestinal microbiome[36-38]
Cold seasons[55-57]	Multiple living siblings[35]
Acceleated linear growth and weight gain[32-34]	
White ethnicity[35]	
Maternal age > 35 yr[35]	

with a RR of 0.22 (0.05-0.89), while children suspected of rickets (related to vitamin D deficiency) had a RR of 3.0 (1.0 -9.0) of developing T1D[68]. Another study involving patients with latent auto-immune diabetes found that c-peptide levels and beta cell function was better preserved in the group who received vitamin D supplements[69]. Some aspects regarding the relationship of islet autoimmunity and development of T1D remain controversial. Though still controversial, vitamin D appears to have a beneficial effect in preventing T1D, likely due to its role in modulating potentially pathogenic inflammatory processes.

Diet

Epidemiological studies have noted that a majority of children that develop T1D before the age of 10 years old, seroconvert within the 1st two years of life[57]. This suggests that early life exposures could play an important role in environmental factors leading to T1D such as nutrition and diet.

Dietary factors that have been explored include infant food exposures such breastfeeding, cow's milk formula, and cereal. Their role in the development of T1D is controversial with a number of studies having conflicting conclusions and insufficient evidence to prove a causal effect[70-74]. However, a majority of birth cohort studies investigating high-risk infants indicate that short durations of breastfeeding (earlier than 2-4 mo)[75] and early introduction of cow's milk (earlier than 4 mo)[76,77] may have a predisposing factor with an overall odds ratio of 1.43 (95%CI: 1.15-1.77) and 1.63 (95%CI: 1.22-2.17), respectively[78]. While a longer duration of exclusive breastfeeding (longer than 4 months) has a protective factor with an odds ratio of 0.17, 95%CI: 0.03-0.86[75]. The exact mechanism for these predispositions are still speculative. It is proposed that breastfeeding provides the benefit of additional immune protection from the passing of maternal antibodies that may help decrease the frequency of early enteroviral infections[79,80] and decreased intestinal permeability, which may protect from potentially T1D inciting exposures[81]. The predisposition caused by early exposure to cow proteins is thought to be mediated by inflammation of the intestinal mucosa[82], increased intestinal permeability[81] and a dysregulated immune response to the milk proteins[83-85]. Intertwined with this concept is the role of intestinal microbiome. Intestinal "microbes influence lipid and glucose metabolism, as well as immunity and systemic inflammation outside of the intestine"[86]. Available studies on this subject are currently inadequate to confirm a definitive connection, but there appears to be a trend of lower microbial diversity in people with T1D with a dominance of a microbiome less conducive to maintaining gut integrity[86,87].

Another study looking at exposure to cereal found that initial cereal exposure before 4 mo of age[72] and after 6 mo of age was associated with an increased risk of T1D or islet autoimmunity with a HR = 5.55 (95%CI: 1.92-16.03) and 12.53 (95%CI: 3.19-49.23), respectively[88].

Another dietary factor that has been investigated is the effect of polyunsaturated fatty acids diets, given its association in modulating the immune system and curtailing inflammatory responses[89,90]. An observational study in the United States found that omega-3 fatty acid intake was inversely associated with the risk of developing T1D or islet autoimmunity[91]. Another study noted linoleic acid had an inverse, marginal association with the development of T1D[92]. Conversely, other studies have found no correlation between fatty acids and islet autoimmunity. It is also interesting that some regions (*i.e.*, India or Africa) that have relatively low polyunsaturated fatty acids in their diet do not necessarily have high T1D incidence rates. This could be because these fatty acids do not play a major role as an environmental factor, increase intake confers a protective factor, but its lack does not yield a risk factor. It could also be possible that other factors such as geography offset

these possible risk factors.

CONCLUDING THOUGHTS

Some of the first cases ascribed to T1D were discovered by the ancient Indians around 1500 BC when patients were noted to have excessive urination that would attract ants due to its sweetness[93]. This condition had been recognized later as a disease and eventually given the name T1D. Early on, T1D was a death sentence because no one understood the pathogenesis or could manage it. Those affected would suffer from insatiable hunger and slowly waste away and die from acute complications. After the seminal discoveries by Joseph von Mering and Oskar Minkowski in 1889 of the pancreas' role in T1D and Frederick Banting and Charles Best ability to purify insulin (in the 1920s) from animal pancreas for the use in treatment of T1D patients, life with diabetes has become an issue of management and not certain death[94].

Since then, significant progress has been made in understanding T1D as a disease with a multifactorial etiology, including genetic factors, primarily affecting the adaptive immune system, and environmental triggers. However, our understanding of T1D pathogenesis remains incomplete. There are still many questions such as why particular genetic variants predispose to the disease, how epigenetics and environmental factors contribute to the disease onset (especially given the 40%-60% disconcordance rate in identical twins), and whether other unrecognized genetics and/or immunologic factors play a pivotal role in T1D development and progression (*i.e.*, DE cells or FasL)[10,11].

Answering these questions is urgently needed as patients still suffer from reduced quality of life given the large treatment burden, the risk of life-threatening hypoglycemia and potentially debilitating end organ complications. Current research focusing on these parameters to clarify the pathogenesis of this disease are expected to help in the development of novel treatments that could prevent disease onset or slow progression by identifying new targets for immunotherapy or vaccines to prevent its initiation. Overall, while the battle against T1D is still ongoing, research has provided us the tools to gain an upper hand and the knowledge to hopefully win the battle in the early stages or before it has begun.

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