Innate Immunity, Toll-Like Receptors, and Diabetes

Marcia F. McInerney[*,1], Lindsey A. Alexander[1], Michael P. Morran[2] and Massimo Pietropaolo[2]

[1]*College of Pharmacy, Department of Medicinal and Biological Chemistry, Center for Diabetes and Endocrine Research, University of Toledo, Toledo, Ohio, USA*

[2]*Laboratory of Immunogenetics, The Brehm Center for Type 1 Diabetes and Analysis, Division of Metabolism, Endocrinology & Diabetes, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA*

Abstract: Innate immunity is the first line of defense in guarding the host against foreign pathogenic invaders. Cells of the innate immune system express pattern recognition receptors, such as toll-like receptors (TLRs), that recognize common molecular patterns of bacteria and viruses. However, along with the recognition of foreign pathogens, TLRs have also been shown to respond to endogenous substrates including; RNA released from dead or dying cells, oxidized molecules such as low density lipoproteins, and free fatty acids. When TLRs are triggered, signal transduction occurs resulting in increased expression and production of various cytokines and costimulatory molecules. TLR activation due to the recognition of self endogenous antigen has been implicated in perpetuating multiple autoimmune responses by skewing immune responses toward strong proinflammation. TLR activation can prime an adaptive immune response, potentially possessing the ability to signal the generation of autoantibodies and autoreactive lymphocytes. Therefore, TLRs, their associated signaling molecules, and their triggered cytokines, are prime candidates for future research in type 1 diabetes (T1D) and autoimmunity. Recently, inflammation has been shown to be a component of type 2 diabetes (T2D). In T2D, signaling through TLRs, in association with free fatty acids, is correlated with insulin resistance. Furthermore, TLRs and their associated signaling molecules are increased in the adipose tissue of T2D patients where inflammatory cells can accumulate. This review focuses on innate immunity and TLR involvement in both T1D and T2D. Therapeutic measures to manipulate TLR signaling, the expression of signaling components, and inflammatory cytokines may help to alleviate the disease process.

Keywords: Autoimmunity, Type 1 diabetes, Type 2 diabetes, Toll-like receptors, innate immunity.

TYPE 1 DIABETES

Type 1 diabetes (T1D) is an autoimmune disease wherein the immune system specifically targets the destruction of pancreatic insulin producing β-cells. Once insulin production is lost, the body is unable to maintain normal glucose homeostasis and glucose levels rise in the blood and urine. Thus, individuals suffering from T1D must replace insulin by injection, multiple times daily, to prevent hyperglycemia and maintain normal glucose levels.

T1D is a complex polygenic disease [1] however, only four T1D susceptibility loci have been identified with convincing and reproducible statistical support: the HLA class II genes [2], the insulin gene [3, 4], the CTLA4 locus [5, 6] and the PTPN22 [7, 8]. A recent addition to this list is the interferon-induced helicase (IFIH1) region [9]. The DR genotype associated with the highest risk for T1D is the DR3/4-DQ8, where DQ8 is the heterozygous genotype DQA1*0301, DQB1*0302 [10]. Early HLA genotyping would help to identify individuals at high risk for T1D prior to clear signs of islet autoimmunity such as islet autoantibodies.

Animal model research has illustrated that the onset of T1D occurs in a two step process characterized initially by asymptomatic inflammation or insulitis, followed by autoimmune destruction of the β-cell mass and concomitant loss of β-cell function. Cells of both the innate and adaptive immune system are found within the insulitis lesion. Macrophages (MΦ) are the first cell type to arrive followed by T-lymphocytes, B-lymphocytes, and natural killer (NK) cells [11-17]. The autoimmune destruction of the pancreatic β-cells takes place in a cell-mediated organ specific manner that requires both CD^+4 and CD^+8 T-cells [12, 18, 19], as well as MΦs [16-19].

In the islet microenvironment, activated MΦs are able to secrete nitrogen and oxygen free radicals, as well as various cytokines including: interleukin-1β (IL-1β), interleukin-12 (IL-12), interleukin-8 (IL-8), and tumor necrosis factor-α (TNFα) [20]. The nitrogen and oxygen free radicals can directly penetrate cells causing intracellular damage to DNA, lipids, and proteins. The cytokines TNFα and IL-1β, produced by MΦ, along with interferon-γ (IFNγ), produced by neighboring T-cells, can have a direct toxic effect on β-cells [21]. Furthermore, IL-12 from activated MΦs can enhance the ability of the adaptive immune system to generate autoreactive β-cell specific cytotoxic T-cells in the NOD mouse. Alternately, loss of MΦ function leads to prevention of T1D in animal models [22-24]. These results indicate that activated MΦs can directly damage β–cells as well as lead to indirect β–cell damage by activating other cell types.

Once the innate immune system has been activated within the pancreas an adaptive immune response is subsequently triggered, generating autoreactive T-cells, B-cells, and antibodies. As pancreatic material is destroyed and diabetes progresses, the number of autoantigens targeted by T-

*Address correspondence to this author at the Department of Medicinal and Biological Chemistry, Center for Diabetes and Endocrine Research, College of Pharmacy, University of Toledo, 2801 W. Bancroft St, Toledo, OH 43606, USA; E-mail: Marcia.mcinerney@utoledo.edu

1573-3955/09 $55.00+.00

cells as well as autoantibodies can increase [25]. This phenomena is called epitope spreading and is observed in autoreactive T-cells that recognize islet self-antigens (insulin, glutamic acid decarboxylase (GAD), 65 kDa isoform, ICA512/IA-2 and I-A2β) and the autoreactive B-cells which generate the corresponding autoantibodies [25]. Therefore, both the innate and adaptive immune system and the interactions between them are involved in the development of diabetes. This review will also focus on the receptors and signaling molecules, utilized by the innate immune system, to examine their role in autoimmune and inflammatory diseases such as T1D.

TYPE 2 DIABETES

Type 2 diabetes (T2D) is a disease of insulin resistance, meaning that normally insulin-sensitive tissues such as liver, skeletal muscle, adipose, and the pancreas no longer respond to increasing amounts of insulin in the blood, which normally stimulates cellular uptake of glucose. Research has shown that an accumulation of MΦs, in hypertrophic adipose of obese animals and humans, plays an important role in the development and/or worsening of insulin resistance and T2D. Two mechanisms of MΦ infiltration into white adipose tissue (WAT) have been suggested [26, 27]. Paracrine and endocrine signaling of cytokines and chemokines can draw blood monocytes towards adipose, stimulated by mechanical modifications such as adipocyte hypertrophy and hyperplasia, which can activate local endothelial cells to favor monocyte attachment and transmigration leading to MΦ infiltration. Alternately, local hypoxia may trigger chemotaxis of MΦs, as it is known that MΦs play a role in angiogenesis [26]. Once in the hypertrophied adipose tissue, MΦs phagocytize dead and dying cells. Several studies provided evidence suggesting that the infiltrated MΦs are derived from bone marrow [28] and are not differentiated pre-adipocytes which, in an inflammatory environment, do have some macrophage-like functions and similarities in gene expression profiles [26, 27].

Excised adipose tissue from obese animals can be enzymatically separated into two fractions, 1) mature adipocytes and 2) the stromal vascular cells (SVCs) comprised of MΦs, dendritic cells (DCs), and other lymphocytes (possible with inflammation), pre-adipocytes, fibroblasts, and endothelial cells. MΦs and DCs are known for their extensive inflammatory cytokine secretion capabilities, as well as for their roles as professional antigen presenting cells (APC). These inflammatory cytokines can have both autocrine and paracrine functions that affect local cell types such as adipocytes, pre-adipocytes, and endothelial cells, along with endocrine systemic effects, which can have negative effects on other organs and body systems. Notably, inflammatory cytokines are involved in the pathogenesis of atherosclerosis and non-alcoholic steatohepatitis (NASH) or fatty liver disease [29]. Both of these two adipose tissue fractions have been shown to secrete varying levels of TNFα and macrophage chemotactic protein-1 (MCP-1), also known as chemokine CCL2, while the SVCs are the main producers of inflammatory interleukins, such as IL-6, inducible nitric oxide synthase (iNOS), and C-reactive protein (CRP). Although adipocytokines, such as leptin, adiponectin, and resistin, additionally play a role in obesity and diabetes, these molecules will not be addressed in this review article.

Inflammatory cytokine levels often correlate with insulin resistance severity [26, 27, 30]. TNFα, in particular, is known to affect insulin sensitivity in insulin target tissues such as liver and skeletal muscle, by interfering with insulin signaling [31, 32]. Serum of type 2 diabetic patients have elevated levels of acute-phase proteins, such as TNFα and IL-6 [26, 27, 30, 33-35]. There is a positive correlation between body mass index (BMI) and CRP, an inflammatory indicator [27]. Correspondingly, weight loss reduces inflammatory markers and associated complications [26, 27]. A further consequence of inflammatory cytokines and a contributing factor to insulin resistance, is the release of free fatty acids (FFA) *via* lipolysis of adipocytes both locally and into circulation. Therefore, inflammation and the innate immune system appear to play a role in T2D, as well as being the common thread between T1D and T2D.

TOLL-LIKE RECEPTORS

Innate immunity is the body's first line of defense against pathogenic microbes and is comprised of multiple cell types including: MΦs, DCs, natural killer (NK) cells, neutrophils, eosinophils, and mast cells [36]. Each cell type is capable of mediating specific functions in an innate immune response, from the phagocytosis of infectious pathogens to the direct targeted lysis of infected host cells [37].

The toll-like receptor (TLR) family, which is comprised of 13 members (TLR1- TLR13), plays a key role in pathogen recognition by the innate immune system [38]. TLRs are involved in innate immune responses, but also play a role in shaping adaptive immune responses through primary recognition of infectious pathogens [39, 40]. Although beneficial in host defense, TLRs have also been implicated in a number of diseases including T1D [41-43], as well as being involved in secondary complications associated with both T1D and T2D [44, 45].

TLRs are pathogen recognition receptors (PRR) that recognize molecules commonly expressed by infectious pathogens, known as pathogen associated molecular patterns (PAMPs), that are not expressed by the mammalian host [46]. Unlike adaptive immune system receptors, PRRs are not clonal receptors and are unable to clonally expand [38-40, 47]. Structurally, TLRs are type I integral membrane glycoproteins consisting of leucine rich repeats in the extracellular domain and a TIR (Toll/ IL-1R) cytoplasmic domain [38, 40]. As PRRs, the TLRs recognize many PAMPs including microbial membrane derived molecules *via* TLR4 and TLR2, bacterial flagellin *via* TLR5, as well as DNA and RNA from bacteria and viruses *via* TLRs 3, 7, 8, and 9 [38, 40, 47-49]. TLRs 1, 2, 4, 5, and 6 are expressed on the cell surface, whereas TLRs 3, 7, 8, and 9 are found within intracellular compartments such as endosomes [47].

The small number of innate immune receptors faces a large and diverse set of pathogens. In order to compensate, TLRs often recognize more than a single type of molecule. For instance, TLR2 responds to lipoteichoic acid and peptidoglycan from gram positive bacteria and lipoproteins of gram negative bacteria. TLR4 binds not only lipopolysaccharide of gram negative bacteria, but also envelope proteins of

some viruses, (reviewed in [38]). Lipopolysaccharide (LPS) from various pathogens are heterogeneous and stimulate TLR4 with various efficiencies [50]. Furthermore, subtypes of LPS have complex interactions including inhibiting the responses of others [51]. TLRs regulate and cross-regulate their own expression and signaling. TLR4 and TLR2 develop tolerance and are downregulated by a repeated exposure to a strong activating antigen [52]. TLR3 is upregulated by its ligand dsRNA [53], but at the same time can induce endotoxin tolerance by downregulating TLR4 [54].

TLRs can also recognize endogenous antigens or self molecules expressed by the host [49], especially if the molecules are indicators of stress or disease [55]. Furthermore, some self molecules, directly modified by the disease process, can become neoantigens to the immune system. At sites of oxidative stress or inflammation, breakdown of extracellular matrix components [56] or oxidation [57] can occur, resulting in conversion of self molecules into agonists of TLR activation. For example, free fatty acids, oxidized low density lipoprotein (LDL), and non-esterified fatty acids are endogenous ligands for TLR2 and TLR4 in inflammatory disease states [58]. TLR4 recognizes heat shock protein (HSP60), an endogenous product of inflammation, while both TLR2 and TLR4 are required for recognition of self-protein HSP70 [38, 55]. mRNA originating from damaged tissue, necrotic cells, or endocytosed cells is an endogenous ligand for TLR3 [59]. In particular, endogenous RNA released in necrotic synovial fluid can activate synovial fibroblasts in human rheumatoid arthritis *via* TLR3 [60].

The demand on host defense to protect against a large number of pathogenic microbes has to be balanced by the need to simultaneously spare the host, despite inherent cross reactivity and complex signaling patterns. In all probability, these conflicting interests must be met by a precise combinatorial tuning of the recognition, and the response of, both PAMPs and self-antigens. Such a fine balance can easily be disturbed by pathophysiological conditions during disease.

TLR SIGNALING

Ligand binding to a specific TLR initiates signal transduction that leads to cellular activation. TLR signaling molecules are similar to those used in the interleukin-1 receptor (IL-1R) signaling cascade [47]. Although TLRs recognize a variety of ligands, most TLRs utilize the adaptor protein myeloid differentiation factor 88 (MyD88) to initiate internal cell signaling. However, once internalized, multiple activation pathways exist for TLRs [47]. Signal transduction results in an up-regulation of the gene expression and protein production of numerous inflammatory Th1 cytokines, chemokines, and costimulatory molecules. This enhances innate immune cell sensitivity by promoting antigen presentation and activation of the adaptive immune system [38, 61-64].

A simplified model of TLR signaling and activation of cytokines and co- stimulatory molecules is presented in Fig. (**1**). TLRs 1, 2, 4, 5, 6, 7, 8, 9, and 11 promote cell activation *via* the MyD88 dependent pathway. Signal transduction through MyD88 elicits the interleukin-1 receptor-associated kinase (IRAK) family. Signal transduction continues with IRAK activation of tumor necrosis factor receptor-associated factor-6 (TRAF6), which then stimulates several molecules into the formation of transforming growth factor-β-protein kinase 1 (TAK1) complex. The activated TAK1/TABs complex signals both the NF-κB essential modulator (NEMO)/IKKs complex and the mitogen activated protein kinases (MAPKs). The NEMO/IKK complex catalyzes IκB, thus freeing nuclear factor-κB (NF-κB) subunits to translocate into the nucleus. The TAK1/TABs complex can also activate the MAPK pathway, activating protein-1 (AP-1), which becomes phosphorylated and moves into the nucleus. Both NF-κB and AP-1 control inflammatory responses through the induction of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6, IL-8, IL-12 and IL-18 [64-67].

TLR3 follows a MyD88 independent pathway (reviewed in [68]). The TIR domain-containing adapter inducing IFNβ (TRIF) pathway recruits TLR3, which then interacts with the tank-binding kinase-1 (TBK-1)/IKKi complex. The TBK-1/IKKi kinases mediate phosphorylation of interferon regulatory factor-3 (IRF3), which then dimerizes and translocates to the nucleus. TRIF can, however, also interact with TRAF6 and the signal from TLR3 activation can flow through the pathways to NF-κB and AP-1. The activation of IRF3, NF-κB and MAPK pathways is required for the induction of type 1 interferons (IFN) particularly IFNβ (Fig. **1**).

Uniquely, TLR4 can signal through both the MyD88 dependent pathway and the TRIF-dependent pathway. Signaling of TLR4 through MyD88 involves early phase activation of NF-κB which leads to the production of pro-inflammatory cytokines. Signaling of TLR4 through TRIF leads to IRF3 activation and late phase NF-κB activation, both of which lead to the production of IFNβ and the expression of IFN-inducible genes. MyD88 signaling through TLR7 and TLR9 which recognize viral DNA and RNA, can lead to phosphorylation and dimerization of IRF-7 leading to IFNα/β production along with inflammatory cytokines through NF-κB and AP-1 [64, 66, 67]. The Fig. (**1**) illustration of TLR signaling is generalized and does not include the pathway of signaling to IRF7 through MyD88.

NON-TLR PRRs

Among PRRs, TLRs have been extensively researched since their initial discovery in *Drosophila* in the late 1990s [38, 40]. However, several other non-TLR PRRs exist and have recently received attention. Cytoplasmic PRRs include the family of Nod-like receptors (NLRs) [69] and RNA helicase receptors [47]. Other non-TLR PRR include Triggering Receptors Expressed on Myeloid Cells (TREMs) [70], and C-type Lectin Receptors (CLRs) [71]. NLRs [69], are capable of detecting cytosolic bacterial proteins and can induce inflammatory responses upon activation [47, 69]. NLRs and TLRs may work together to survey intracellular and extracellular pathogens, but the exact details and interactive specific signaling pathways are not yet fully known. The cytoplasmic RNA helicase family consisting of Retinoic-acid-inducible protein-1 (RIG-1) and melanoma differentiation associated antigen 5 (MDA5) recognize several RNA viruses, especially viral genomes uncoated in the cell following infection and newly synthesized dsRNA. RIG-1 and the TLR system exert antiviral responses in a cell type specific manner, since fibroblasts and conventional DCs that are RIG-$1^{-/-}$ are unable to produce type 1 IFN after viral infection, whereas plas-

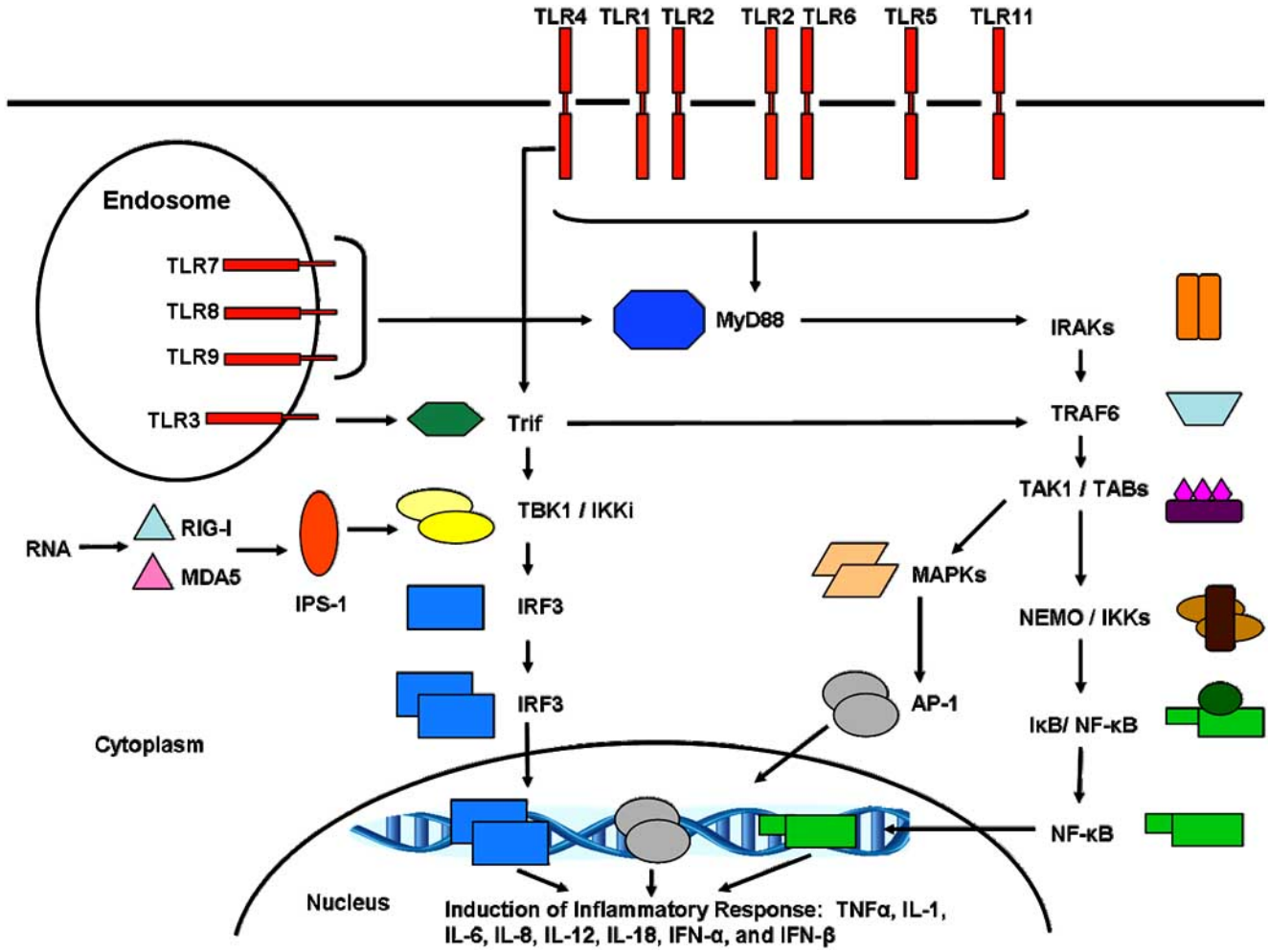

Fig. (1). TLR mediated cell activation and signaling, general model. TLRs (TLR-1, 2, 4, 5, 6, 7, 8, 9 and 11) promote cell activation *via* the MyD88-dependent pathway. MyD88 signals IRAKs, which lead to TRAF6 recruitment. TRAF6 mediates activation of the TAK1/TABs complex. The TAK1 complex signals both the NEMO/IKK complex, which results in IκB catalysis leading to the activation of the NF-κB, and the MAPKs, which phosphorylate AP-1, leading to AP-1 activation. TLR3 signaling utilizes the TRIF dependent pathway. TRIF activation mediates signaling through the TBK1/IKKi complex. The TBK/IKKi complex mediates the phosphorylation of IRF3. Phosphorylated IRF3 dimerizes and is free to translocate into the nucleus. TLR4 can signal through MyD88 dependent and independent pathways. Free activated IRF3, AP-1, and NF-κB can translocate into the nucleus where they influence expression of various inflammatory cytokines and co-stimulatory molecules.

macytoid DCs use TLR3. RIG-1 and MDA5 contain two caspase-recruitment domains (CARDS) and recognize dsRNA with the DExD/H box helicase domain. RIG-1 recruits a CARD-containing adaptor molecule called IPS-1. IPS-1 relays the signal to the kinases TBK1 and IKKi, with the result of IRF3 and IRF-7 phosphorylation and type 1 IFN production. The RIG-1 pathway bypasses the TLR3 pathway by utilizing IPS-1 instead of TRIF, see Fig. (**1**) [47, 72, 73].

TREMs act as immune modulators that can regulate the differentiation and function of MΦ and DCs as well as actively participating in amplifying the effects of inflammation [70]. When TREMs are defective, promotion of activation or amplification of inflammation leading to unnecessary innate and adaptive immune system responses could potentially occur. CLRs are an important control mechanism for innate immunity, since CLRs binding to PAMPs results in first activation and then inhibition of innate immune responses to pathogens or self [71]. Therefore, a malfunction of CLR signaling may promote inflammation due to a possible inability to properly signal the end of an innate immune response. Although not clearly determined as yet, the possibilities for synergy and cross talk between the innate TLR and non-TLR PRRs should enhance the potential to perpetuate and induce an adaptive immune response. Malfunctions in any of these innate systems or receptors, especially those responsible for amplifying or regulating an inflammatory response, could potentially trigger adverse and possibly detrimental immune responses leading to pathogenesis and disease.

PRR AND DISEASE

Activation of the innate immune system acts as a prerequisite for the induction of an adaptive immune response [39, 56, 57]. As discussed previously, TLRs can recognize and bind both non-self and self antigens [38, 40, 47, 49, 55-57]. Binding of a TLR is associated with production of pro-

inflammatory cytokines and mediators [38, 62, 64, 67]. This is the normal response expected to a non-self microbial infection. However, PRRs capable of interacting with self antigens may have the ability to break central tolerance and prime the adaptive immune system through cytokine and inflammatory signals to become auto-reactive toward self-antigens, thus ultimately controlling the progression to autoimmunity [74, 75]. Furthermore, the production of an uncontrolled inflammatory response could lead to inflammatory disease.

Besides their beneficial role in host defense, PRRs including TLRs have been implicated in a growing number of autoimmune and inflammatory diseases. Upregulated TLR4 expression is associated with pathogenicity in inflammatory bowel disease [76]. In Crohn's Disease, innate immune responses to bacterial flagella *via* TLR5-flagellin binding may actually promote an adjuvant-like effect on the adaptive immune system, thus prompting uncontrolled inflammatory responses resulting in autoimmunity [77]. Furthermore, polymorphisms in the PRR Nod2, leading to either strong activation or negative attenuation of NF-κB signaling, could be an underlying factor in the pathogenesis of Crohn's disease [78]. In atherosclerosis, an inflammatory disease and a secondary complication of diabetes, TLR4 function affects the initiation and progression of disease, (reviewed in [79]). TLR4 is expressed on MΦs in lipid-rich atherosclerotic plaques and TLR4 expression can be upregulated by oxidized low-density lipoprotein [80, 81]. Patients with hyporesponsiveness to LPS, due to a TLR4 polymorphism, are protected from carotid artery atherosclerosis [82-85]. Furthermore, it has been shown that single nucleotide polymorphisms (Asp299Gly and Thr399Ile) of TLR4 are associated with reduced incidence of diabetic neuropathy in T2D [45].

TLRs committed to nucleic acid recognition have also been implicated in autoimmune disease. RNA released from necrotic synovial fluid can activate synovial fibroblasts in human rheumatoid arthritis *via* TLR3 [60]. Nonspecific stimulation of TLR3 with Lymphocytic Choriomeningitis virus (LCMV) can precipitate autoimmune hepatitis. LCMV stimulates production of type 1 IFNs and TNFα from APC, resulting in chemokine production in liver tissue causing chemotaxis of antigen specific $CD8^+$ T cells ultimately leading to whole organ liver destruction [86]. Antibodies to RNA and DNA–containing autoantigens are characteristic of the autoimmune disease systemic lupus erythematosus (SLE). TLR7 in B cells is required to generate autoantibodies to RNA antigens *in vivo*, while TLR9 is required for efficient production of antibodies to relevant DNA autoantigens in SLE [87]. The interaction of TLRs 3, 7, 8 or 9 with self-DNA, RNA, or nucleotide complexes has been proposed as an initiating trigger for the development of autoimmunity, termed the toll hypothesis [74].

TLRs AND T1D

Cell surface expression, as well as mRNA for TLR2 and TLR4, is significantly increased in T1D patients compared to non-diabetics. Signaling molecules MyD88, TRIF, phosphorylated IL-1 receptor-associated kinase, and NF-κB were upregulated and increased production of cytokine products IL-1β and TNFα was observed [88]. Previously, a link between T1D and elevated levels of TNFα, by human mononuclear cells, was reported [89] in Danish males. Furthermore, recently diagnosed T1D patients display significantly increased levels of TNFα, IL-2, IFNγ, and other proinflammatory cytokines in their peripheral blood [90]. Therefore, TLR2 and TLR4 expression is increased in human T1D and may contribute to a proinflammatory state.

Increased TLR expression, upregulated NF-kB, and overexpression of TLR4-induced proinflammatory cytokines TNFα, IL-12, and IL-6, in correlation with a significant decrease in regulatory cytokine IL-10, was observed at the onset of diabetes in the NOD mouse [91]. Similar TLR expression levels and TLR-induced proinflammatory cytokines have been observed in T2D mouse models reliant on elevated glucose levels and MAPK [92]. These observations may be reflective of a hyperglycemic state since a study by Kaizer *et al.* [93] showed similar increased expression levels of genes, especially IL-1β and c-myc in peripheral blood mononuclear cells obtained from both T1D and T2D patients. Furthermore, diabetic patients in a hyperglycemic crisis have elevated plasma levels of IL-1β, TNFα, IL-6 and IL-8, all pro-inflammatory cytokines [94]. However, hyperglycemia cannot be the full explanation for dysregulated cytokines since non-diabetic healthy family members of patients with T1D exhibit an overproduction of a number of cytokines that are implicated in diabetogenesis and further, the abnormalities did not correlate with islet cell autoantibodies or HLA-DR phenotype [95].

The importance of particular TLRs has been studied by utilizing TLR knock out (KO) mice on an NOD background and examining diabetes rates. Kim *et al.* [96] have proposed that TLR2 contributes to the initiation of autoimmune diabetes by sensing apoptotic beta cells undergoing secondary necrosis. Furthermore, the development of diabetes was markedly reduced in $TLR2^{-/-}$ KO mice, whereas $TLR4^{-/-}$ KO mice were unaffected. The authors propose that a TLR2 dependent activation of antigen presenting cells allows priming of diabetogenic T cells leading to autoimmunity [96]. Recent work by Wen *et al.* [97] supports the concept that TLR4 is not involved in diabetes development. In contrast however, using two independently derived TLR2 KO NOD mouse lines, the individual loss of TLR2 did not have any effect on the spontaneous rate of diabetes development [97]. Interestingly, in a Korean study, a single nucleotide polymorphism (SNP) in TLR2, known as TLR2-Ht4, correlated with T1D in that having more than one copy of TLR2-Ht4 was associated with strong protection against developing T1D [41]. Based on these studies TLR4 may not be involved in T1D development, while the exact role of TLR2 is questionable.

Knocking out expression of MyD88, the adapter protein responsible for most TLR signal transduction, protected NOD mice housed in specific pathogen free (SPF) conditions from the development of diabetes [97]. However, when the MyD88 KO NOD mice were re-derived as germ free, autoimmune diabetes was rampant [97]. It was determined that intestinal bacteria normally present in the gut, but lacking in the germ-free state, actually protects NOD mice from diabetes development. In fact, antibiotic treatment of the MyD88 KO NOD mice lowered resistance to diabetes development in the SPF conditions. Early work showed that treatment of NOD mice with complete Freund's adjuvant (CFA), contain-

ing mycobacteria, prevented diabetes [98, 99]. Furthermore, live attenuated *Salmonella typhimurium*, a gut pathogen, is able to halt diabetes in a wide age range of NOD mice by generating immunomodulatory DCs [100]. A very interesting point in the Wen study [97] is that the intestinal microbiota were able to protect in a MyD88 independent manner. This should also rule out other TLRs that signal through MyD88, such as TLR5. However, intracellular pattern recognition receptors should be examined for involvement in protection, such as NLRs that can recognize intracellular invasion by bacteria or the intracellular presence of bacterial products. Stimulation of Nod1 or Nod2 can lead to an inflammatory response through NF-κB (reviewed in [69]). These results also support the hygiene hypothesis [101] wherein introduction of hygienic measures reduces the amount of exposure to microbial agents and concomitantly lowers risk of infection. However, such environmentally clean conditions favor diabetes development in genetically susceptible animal models [102, 103]. Inversely, early exposure to microbial antigens modulates macrophage function [104] resulting in downregulation of cellular autoaggression against islet beta cells [105]. TLR stimulation may be involved in this phenomenon since T1D is prevented in NOD mice by treatment with LPS or TLR4 agonists [106] and by the TLR9 agonist, CpG [107].

Potential viral triggers of T1D include Adenovirus, Coxsackie B virus, Cytomegalovirus, Hepatitis C virus, Mumps virus, and the Rubella virus [108-111]. TLR3 is known to recognize viral intermediate dsRNA and its mimic poly(I)poly(C) [38, 40, 67], and therefore, has received attention as a possible link in the development of T1D and autoimmunity. TLR3 activation leads to the production of type 1 IFNs, which are key cytokines that induce an antiviral state in target cells, as well as serve to activate APCs and effect adaptive immunity by stimulating CD8 memory T cells (reviewed in [68, 112]). Bone marrow-derived MΦ at the onset of diabetes in the NOD mouse express significantly higher basal levels of TLR3 than that of pre-diabetic NOD mice and control strains [91] and stimulation with poly(I)poly(C), elicited a nine-fold increase in production of type 1 IFNs in diabetic animals compared to non-diabetic controls [91]. Furthermore, TLR-3 expression in islets may be partially responsible for diabetes development in rat insulin promoter-B7.1 transgenic BALB/c mice when diabetes is precipitated with poly(I)poly(C) and insulin B9-23 peptide [42].

A recent report indicated that loss of TLR3 expression has no effect on the development of diabetes in the NOD mouse [97]. However, following viral infection, dsRNA intermediates or dsDNA, in the cytosol, can trigger signal transduction leading to type 1 interferons by binding intracellular pattern recognition receptors, RIG-1 or MDA5, thus bypassing TLR3 [68]. In fact, type 1 IFN gene induction is still observed in cells from $TLR3^{-/-}$ mice after stimulation with poly(I):poly(C) (reviewed in [68]). Viral induction of type 1 IFN has been implicated in T1D development, although it has not been proven [113]. However, IFNα therapy can lead to the production of islet autoantibodies and IFNα is detected by histological analysis in the pancreata of patients with T1D, (reviewed in [113]). Autoimmune diabetes can be induced when IFNα is expressed as a transgene in islets of mice not genetically susceptible to the disease [114]. Furthermore, treatment of β-cells with poly(I):poly(C), in combination with IFN-α, -β or -γ significantly increases apoptosis [115]. Most importantly, a genome-wide association study of nonsynonymous SNPs identified the interferon-induced helicase (IFIH1) region as a susceptibility locus for T1D in humans. IFIH1 is also known as MDA5 [9]. Therefore, the involvement of RIG-1 and/or MDA5 in the development of T1D still needs to be assessed.

Involvement of nucleotide sensing TLRs in diabetes development has been studied in the Biobreeding diabetes-resistant rat (BBDR). Treatment with TLR3 ligand poly(I):poly(C), and the elimination of $ART2^{+}$ regulatory T cells, results in a 100% diabetes rate [116]. Treatment with diabetogenic Kilham rat virus (KRV) results in approximately a 30% incidence of diabetes. The KRV acts as a TLR9 ligand and upregulates proinflammatory genes. KRV can synergize with other agonists of various TLRs or whole heat killed bacteria (*Escherichia coli* or *Staphylococcus aureus*) to induce a 50-100% diabetes rate depending on the co-TLR agonist used. Therefore, in the BBDR rat model, the TLR9-signaling pathway is involved KRV-induced autoimmune diabetes [117, 118]. An individual role for TLR9 in spontaneous type 1 diabetes development in the NOD mouse has yet to be determined.

In summary, the TLR system is in place to protect the host against unwanted pathogenic microbes. However, TLRs triggered by self-ligands or inflammatory molecules, such as type 1 IFNs, could promote an unwarranted immune response which in turn, could non-specifically activate T cells through cytokine or inflammatory signals. Loss of down-regulation or chronic stimulation could exacerbate the problem and lead to cellular and, ultimately, tissue or organ destruction.

TLRs AND T2D

Several studies in the 1990s suggested a relationship between obesity and inflammation [31, 119-122]. These studies showed that type 2 diabetic patients and/or animal models had increased serum levels of acute-phase reactants and that TNFα was increased in adipose tissue in obese rodents. Importantly, when TNFα action was blocked, insulin sensitivity improved [31]. The link between inflammatory cytokines of the innate immune system, such as TNFα, IL-1β, MCP-1, and IL-6, and obesity is now firmly established [26, 27, 30, 35]. Therefore, obesity and type 2 diabetes are considered to be low-grade inflammatory diseases (reviewed in [26, 27, 29, 30, 33, 35, 123, 124]).

Once cytokines are implicated in disease pathology, TLR involvement needs to be explored. Cell types involved in obesity, such as adipocytes and MΦs, are known to express TLR2 and TLR4 [58, 125-128]. Obese *db/db* mice have greater mRNA levels of TLR4 in epididymal fat pads, compared to *db/+* littermates [128]. Separation of whole, white adipose tissue into adipocyte and SVC fractions revealed that the increased TLR4 expression resided with the adipocytes [128]. TLR4 mRNA expression is also increased in diet induced obese WT, *ob/ob*, and *db/db* mice in comparison to controls [127]. Furthermore, the TLR4 is functional as determined by LPS stimulation and resultant IL-6 production

[127]. Creely *et al.* [125] showed that human adipocytes from patients across a range of BMI express TLR2 and TLR4 mRNA and protein, with higher expression in obese compared to lean subjects. In addition, *ex vivo* MΦ and DCs derived from bone-marrow stem cells were confirmed to display cell surface TLR2 and TLR4 on over 80% of the total cells [58].

Many studies are investigating the possibility of free fatty acids (FFA) as TLR ligands. *In vitro* experiments using NF-κB luciferase reporter-transfected 293T cells showed that a mixture of the most abundant nutritional fatty acids palmitate and oleate can activate TLR4 [127]. Positive results have been found using many of the medium chain (carbon length 12-18) saturated fatty acids for stimulating TLR4. Unsaturated FFA generally do not stimulate TLR and some are capable of blocking LPS or FFA induced inflammatory changes [127]. A mixture of saturated and unsaturated free fatty acids stimulate both TLR2 and TLR4 on DCs and MΦs however, bone marrow-derived dendritic cells seem to have higher inflammatory capabilities compared to bone marrow-derived macrophages by expression of cyclooxygenase 2 (COX-2), chemokine (C-C motif) receptor 2 (CCR2), IL-1, IL-6, and TNFα expression [58]. Therefore, palmitate and certain other saturated fatty acids can activate the TLR4 present on adipocytes, DCs, and MΦs in a dose-dependent manner.

Evidence from cell culture and animal tissue studies supports a role for signaling molecules NF-κB [125, 129], c-jun-N-Terminal Kinase (JNK), IκBα [127] and IKKβ [58, 130] during TLR4-mediated inflammation. Corresponding increases in TNFα and IL-6 mRNA are seen in response to FFA stimulation and NF-κB activation [127, 129]. TLR4 interference of any kind reduces FFA-induced signaling and inflammatory protein expression in mouse tissues, primary cells, and cell-lines.

Studies using TLR4 knock-out or C3H/HeJ mice with a mutant TLR4 receptor and deficient LPS responsiveness, have proven extremely useful in understanding the role of TLR4 in obesity-related inflammation [126, 127, 130, 131]. Importantly, $TLR4^{-/-}$ and TLR4 defective mice, fed a high fat diet, displayed increased feeding efficiency, increased metabolic rates, attenuated increases in serum and adipose tissue concentrations of TNFα, IL-6, and, in one case, serum FFA compared to control mice [126, 130, 131]. Surprisingly, there was no significant difference between the TLR4-defective mouse and control strains for MΦ infiltration in adipose after a 16 week high fat diet [126] suggesting that TLR4 mutation does not affect MΦ infiltration. Studies reported increases in adipose and skeletal muscle insulin sensitivity. Therefore, inflammatory processes decrease and insulin sensitivity improves in diet-induced obesity and cells treated with free fatty acids when TLR4 is either not present or not fully functional. Thus, TLR4 does appear to play a role in T2D, while involvement of TLR2 is still unconfirmed.

DIABETES AND INFECTIOUS DISEASE

Diabetes is often complicated by acute or chronic infection, and diminished responses to infection may underlie the complications of diabetes [132-134]. Macrophage and other innate immune system cells also play a key role in inflammatory and immune mediated disease complications [135]. Diabetics are at severe risk to develop periodontitis [136]. A high prevalence of *Helicobacter pylori* infection is correlated with dyspeptic symptoms in T2D patients [137]. An increased liver fibrosis stage and faster fibrosis progression rate is the consequence of the association between diabetes and hepatitis C virus infection (reviewed in [138]). In India, tuberculosis occurs at a rate 2-5 times higher in diabetics compared to the general population [139]. *Staphylococcus aureus* multiplies in the blood of diabetic mice with hyperglycemia due to diminished leukocytic respiratory burst [140]. Polymicrobial infections were significantly more severe in diabetic mice, exhibiting a 5 to 35 times increase in the number of colony forming units compared to control mice [141]. An accelerated form of atherosclerosis is associated with diabetes and cytomegalovirus infection [142]. Coxackievirus-B4 can trigger cytokine production through TLR4 in human pancreatic cells [143]. Furthermore, vascular inflammation, a common complication in both T1D and T2D, is the result of infectious load [144-146]. Diabetes complications affect many different organs systems and altered inflammatory responses and impaired responses to infection are observed. Therefore, diabetes has been classified as a secondary immune deficiency disorder [132-134].

Neither diminished nor exaggerated signaling through TLR would be beneficial to the host. The inability to resolve chronic infection by pathogenic organisms leads to chronic stimulation of macrophages. Products of macrophages, such as nitrogen and oxygen free radicals, TNFα and prostaglandin 2 (PGE_2), can damage soft tissue and active osteoclasts to resorb bone in periodontitis [136]. Monocytes from diabetic patients are capable of secreting proinflammatory cytokines (TNFα, IL-1β and PGE_2) in an exaggerated fashion, compared to controls, in response to *Porphyromonas gingivalis*, a know periodontal pathogen [147, 148]. Therefore, diabetics can respond to an equivalent oral bacterial burden with inappropriate inflammatory responses leading to severe periodontitis [147, 148].

A major question is whether all diabetics are prone to developing infections because there is a problem with the innate immune system and TLRs. Hyperglycemia in diabetes is associated with the synthesis of proinflammatory cytokines during infection [149]. High glucose induces multiple proinflammatory cytokines and chemokines in monocytes, cells that are involved in the secondary complications of diabetes [150]. Diabetic patients in a hyperglycemic crisis have elevated plasma levels of IL-1β, TNFα, IL-6, IL-8, reactive oxygen species (ROS), and cardiovascular risk factors [94]. Human peripheral blood cells activated by high glucose *via* oxidant stress express increased levels of TNFα and NF-κB [151]. Activated monocytes that adhere to endothelium play a key role in inflammatory and cardiovascular diseases. Alternately, the reduction of glucose levels below 200 mg/dl improves granulocyte adherence and granulocytosis, key elements of the innate immune system in the defense against infectious microorganisms. TLR4 sensitivity in the diabetic state is a factor that underlies the increased risk of certain vascular complications in diabetes [44]. In NOD mice, macrophages differentiated from stem cells under hyperglycemic conditions have increased expression of TLR2, TLR4,

and TNFα. Furthermore, stimulation of TLRs at the onset of diabetes leads to exaggerated inflammatory responses to bacterial and viral ligands [91]. The overproduction of proinflammatory molecules could lead to chronic inflammation and impaired, or inappropriate, responses to infectious pathogens in diabetes, due in part to the altered TLR responsiveness of diabetic macrophages.

The growing evidence for a role of the innate immune system in autoimmune diabetes, inflammatory diseases, and diabetic complications warrants a more detailed investigation of TLR expression and signaling in macrophages and dendritic cells. Modulation of the expression, responsiveness, and signaling of TLRs in the prediabetic state leading to diabetes, and in the diabetic state itself, will have an affect on the inflammatory environment and, consequently, islet transplantation, the ability to repair wounds, resolution of infection without tissue damage, and the progression to complications [152-155].

CONCLUSION

The immune system has developed to sustain the health of the host and protect against foreign invaders. However, in instances such as autoimmune and inflammatory diseases, a dysregulated immune response could contribute directly, or indirectly, to pathogenesis of disease. We have reviewed the purposed roles of TLR and other innate immune components in the pathogenesis of T1D and T2D. Presently, and in the near future, along with expanding the knowledge of basic TLR function and signaling, research should focus on manipulating TLR ligands and signaling molecules to treat or prevent disease. Certainly enough is known to begin the process of developing small molecule antagonists, targeting treatment to diseased organs such as the pancreas and the adipose tissue, and specific neutralization of inflammatory cytokines for clinical applications testing.

ABBREVIATIONS

TLR	=	Toll-like Receptor
MΦ	=	Macrophages
MHC	=	Major histocompatibility complexes
PAMPs	=	Pathogen Associated Molecular Patterns
PRRs	=	Pattern Recognition Receptors
MyD88	=	Myeloid differentiation factor 88

ACKNOWLEDGEMENTS

This review article on innate immunity is dedicated to the memory of Dr. Charles A. Janeway Jr, mentor. This work was supported by the United States Department of Agriculture/Cooperative State Research, Education and Extension Service grant 2008-38903-19049 (MFM), by the National Institute for Dental and Craniofacial Research R21DE0144 86 (MFM), and the National Institutes of Health grants R01 DK53456 and R01 DK56200 (MP).

REFERENCES

[1] Thompson G. DR antigens and susceptibility to insulin-dependent diabetes. Am J Hum Genet 1984; 36: 1309-17.

[2] Cucca F, Lampis R, Congia M, *et al.* A correlation between the relative predisposition of MHC class II alleles to type 1 diabetes and the structure of their proteins. Hum Mol Genet 2001; 10: 2025-37.

[3] Bell G, Horita S, Karam J. A polymorphic locus near the human insulin gene is associated with insulin-dependent diabetes mellitus. Diabetes 1984; 33: 176-83.

[4] Barratt BJ, Payne F, Lowe CE, *et al.* Remapping the Insulin Gene/IDDM2 Locus in Type 1 Diabetes. Diabetes 2004; 53: 1884-9.

[5] Nistico L, Buzzetti R, Pritchard L, *et al.* The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. Hum Mol Genet 1996; 5: 1075-80.

[6] Ueda H, Howson JMM, Esposito L, *et al.* Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003; 423: 506-11.

[7] Smyth D, Cooper JD, Collins JE, *et al.* Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. Diabetes 2004; 53: 3020-3.

[8] Bottini N, Musumeci L, Alonso A, *et al.* A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet 2004; 36: 337-8.

[9] Smyth D, Cooper J, Bailey R, *et al.* A genome-wide assoication study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region. Nat Genet 2006; 38: 617-9.

[10] Todd JA, Bell JI, McDevitt HO. HLA-DQbeta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 1987; 329: 599-604.

[11] Pietropaolo M, Barinas-Mitchell E, Kuller LH. The heterogeneity of diabetes: unraveling a dispute: is systemic inflammation related to islet autoimmunity? Diabetes 2007; 56: 1189-97.

[12] Delovitch TL, Singh B. The nonobese diabetic mouse as a model of autoimmune diabetes: immune dysregulation gets the NOD. Immunity 1997; 7: 727-38.

[13] Katz J, Benoist C, Mathis D. T helper cell subsets in insulin-dependent diabetes. Science 1995; 268: 1185-8.

[14] Rabinovitch A, Suarez-Pinzon W, Sorenson O. Interleukin-12 mRNA expression in islets correlates with beta-cell destruction in NOD mice. J Autoimmun 1996; 9: 645-51.

[15] Von Herrath M, Oldstone M. Interferon-gamma is essential for destruction of beta-cells and development of insulin-dependent diabetes. J Exp Med 1997; 185: 531-9.

[16] Lee K, Amano K, Yoon J. Evidence for initial involvement of macrophage in development of insulitis in NOD mice. Diabetes 1988; 37: 989-91.

[17] Jansen A, Homo-Delarche F, Hooijkaas H, *et al.* Immunohistochemical characterization of monocytes-macrophages and dendritic cells involved in the initiation of the insulitis and beta-cell destruction in NOD mice. Diabetes 1994; 43: 667-75.

[18] Wong F, Janeway CJ. The role of CD4 and CD8 T cells in type 1 diabetes in the NOD mouse. Res Immunol 1997; 148: 327-32.

[19] Yoon J, Jun H, Santamaria P. Cellular and molecular mechanisms for the initiation and progression of beta-cell destruction resulting from the collaboration between macrophages and T cells. Autoimmunity 1998; 127: 109-12.

[20] Beyan H, Buckley LR, Yousaf N, Londei M, Leslie RDG. A role for innate immunity in type 1 diabetes? Diabetes Metab Res Rev 2003; 19: 89-100.

[21] Rabinovitch A, Suarez-Pinzon W. Cytokines and their roles in pancreatic islet B cell destruction and insulin dependent diabetes mellitus. Biochem Pharmacol 1998; 55: 1139-49.

[22] Jun HS, Yoon CS, Zbytnuik L, van Rooijen N, Yoon JW. The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. J Exp Med 1999; 189: 347-58.

[23] Oschilewski U, Kiesel U, Kolb H. Administration of silica prevents diabetes in BB-rats. Diabetes 1985; 34: 197-9.

[24] Lee K, Pak C, Amano K, Yoon J. Prevention of lymphocytic thyroiditis and insulitis in diabetes prone BB rats by the depletion of macrophages. Diabetologia 1988; 31: 400-2.

[25] Atkinson M, Eisenbarth G. Type 1 Diabetes: new prespectives on disease pathogenesis and treatment. Lancet 2001; 358: 221-9.

[26] Cancello R, Clement K. Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue. Br J Obstet Gynaecol 2006; 113: 1141-7.

[27] Bastard JP, Maachi M, Lagathu C, *et al.* Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 2006; 17: 4-12.
[28] Weisberg SP, McCann D, Desai M, *et al*. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112: 1796-808.
[29] Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006; 116: 1793-801.
[30] Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 2004; 27: 813-23.
[31] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259: 87-91.
[32] Feinstein R, Kanety H, Papa MZ, Lunenfeld B, Karasik A. Tumor necrosis factor-alpha suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. J Biol Chem 1993; 268: 26055-8.
[33] Alexandraki K, Piperi C, Kalofoutis C, *et al.* Inflammatory process in type 2 diabetes: The role of cytokines. Ann N Y Acad Sci 2006; 1084: 89-117.
[34] Crook M. Type 2 diabetes mellitus: a disease of the innate immune system? An update. Diabet Med 2004; 21: 203-7.
[35] Fernandez-Real JM, Pickup JC. Innate immunity, insulin resistance and type 2 diabetes. Trends Enodcrin Met 2008; 19: 10-6.
[36] Janeway CJ, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002; 20: 197-216.
[37] Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature 2007; 449: 819-26.
[38] Takeda K, Kaisho T, Akira S. Toll-like Receptors. Annu Rev Immunol 2003; 21: 335-76.
[39] Dabbagh K, Lewis D. Toll-like receptors and T-helper-1/T-helper-2 responses. Curr Opin Infect Dis 2003; 16: 199-204.
[40] Pasare C, Medzhitov R. Toll-like receptors and acquired immunity. Semin Immunol 2004; 16: 23-6.
[41] Park Y, Park S, Yoo E, Kim D, Shin H. Association of the polymorphism for Toll-like receptor 2 with type 1 diabetes susceptibility. Ann N Y Acad Sci 2004; 1037: 170-4.
[42] Wen L, Peng J, Li Z, Wong FS. The effect of innate immunity on autoimmune diabetes and the expression of Toll-like receptors on pancreatic islets. J Immunol 2004; 172: 3173-80.
[43] Lang KS, Recher M, Junt T, *et al.* Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. Nat Med 2005; 11: 138-45.
[44] de Kleijn D, Pasterkamp G. Toll-like receptors in cardiovascular diseases. Cardiovasc Res 2003; 60: 58-67.
[45] Rudofsky G, Jr, Reismann P, Witte S, *et al.* Asp299Gly and Thr399Ile Genotypes of the TLR4 Gene Are Associated With a Reduced Prevalence of Diabetic Neuropathy in Patients With Type 2 Diabetes. Diabetes Care 2004; 27: 179-83.
[46] Medzhitov R, Janeway CA. Innate Immunity: The Virtues of a Nonclonal System of Recognition. Cell 1997; 91: 295-8.
[47] Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. Cell 2006; 124: 783-801.
[48] Burns E, Bachrach G, Shapira L, Nussbaum G. Cutting edge: TLR2 is required for the innate response to porphyromonas gingivalis: activation leads to bacterial persistence and TLR2 deficiency attenuates induced alveolar bone resorption. J Immunol 2006; 177: 8296-300.
[49] Johnson G, Brunn G, Platt J. Activation of mammalian Toll-like receptors by endogenous agonists. Crit Rev Immunol 2003; 23: 15-44.
[50] Hajjar AM, Ernst RK, Tsai JH, Wilson CB, Miller SI. Human Toll-like receptor 4 recognizes host-specific LPS modifications. Nat Immunol 2002; 3: 354-9.
[51] Dobrovolskaia MA, Medvedev AE, Thomas KE, *et al.* Induction of *in vitro* reprogramming by toll-like receptor (TLR)2 and TLR4 agonists in murine macrophages: effects of TLR "homotolerance" versus "heterotolerance" on NF-kappaB signaling pathway components. J Immunol 2003; 170: 508-19.
[52] Muthukuru M, Jotwani R, Cutler CW. Oral mucosal endotoxin tolerance induction in chronic periodontitis. Infect Immun 2005; 73: 687-94.
[53] Guillot L, Le Goffic R, Bloch S, *et al.* Involvement of toll-like receptor 3 in the immune response of lung epithelial cells to double-stranded RNA and Influenza A Virus. J Biol Chem 2005; 280: 5571-80.
[54] Jiang W, Sun R, Wei H, Tian Z. Toll-like receptor 3 ligand attenuates LPS-induced liver injury by down-regulation of toll-like receptor 4 expression on macrophages. Proc Natl Acad Sci USA 2005; 102: 17077-82.
[55] Ohashi K, Burkart V, Flohe S, Kolb H. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. J Immunol 2000; 164: 558-61.
[56] Termeer C, Benedix F, Sleeman J, *et al.* Oligosaccharides of Hyaluronan Activate Dendritic Cells *via* Toll-like Receptor 4. J Exp Med 2002; 195: 99-111.
[57] Buttari B, Profumo E, Mattei V, *et al.* Oxidized beta2-glycoprotein I induces human dendritic cell maturation and promotes a T helper type 1 response. Blood 2005; 106: 3880-7.
[58] Nguyen MTA, Favelyukis S, Nguyen AK, *et al.* A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids *via* Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem 2007; 282: 35279-92.
[59] Kariko K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA Is an Endogenous Ligand for Toll-like Receptor 3. J Biol Chem 2004; 279: 12542-50.
[60] Brentano F, Schorr O, Gay R, Gay S, Kyburz D. RNA released from necrotic synovial fluid cells activates rheumatoid arthritis synovial fibroblasts *via* toll-like receptor 3. Arthritis Rheum 2005; 52: 2656-65.
[61] Godfrey DI, Hammond KJL, Poulton LD, Smyth MJ, Baxter AG. NKT cells: facts, functions and fallacies. Immunol Today 2000; 21: 573-83.
[62] Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol 2001; 1: 135-45.
[63] Akira S. Toll receptor families: structure and function. Semin Immunol 2004; 16: 1-2.
[64] Kawai T, Akira S. TLR signaling. Cell Death Differ 2006; 13: 816-25.
[65] Hayden M, West A, Ghosh S. NF-kB and the immune response. Oncogene 2006; 25: 6758-80.
[66] Kawai T, Akira S. Signaling to NF-kappaB by Toll-like receptors. Trends Mol Med 2007; 13: 460-9.
[67] Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol 2004; 4: 499-511.
[68] Honda K, Taniguchi T. IRFs: Master regulators of signaling by Toll-like receptors and cytosolic pattern recognition receptors. Nat Rev Immunol 2006; 6: 644-58.
[69] Fritz JH, Ferrero RL, Philpott DJ, Girardin SE. Nod-like proteins in immunity, inflammation and disease. Nat Immunol 2006; 7: 1250-7.
[70] Klesney-Tait J, Turnbull IR, Colonna M. The TREM receptor family and signal integration. Nat Immunol 2006; 7: 1266-73.
[71] Robinson MJ, Sancho D, Slack EC, Leibund Gut-Landmann S, Sousa CRe. Myeloid C-type lectins in innate immunity. Nat Immunol 2006; 7: 1258-65.
[72] Kato H, Takeuchi O, Sato S, *et al.* Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 2006; 441: 101-5.
[73] Kato H, Sato S, Yoneyama M, *et al.* Cell type-specific involvement of RIG-1 in antiviral response. Immunity 2005; 23: 19-28.
[74] Martin DA, Elkon KB. Autoantibodies make a U-turn: the toll hypothesis for autoantibody specificity. J Exp Med 2005; 202: 1465-9.
[75] Meagher C, Sharif S, Hussain S, *et al.* Cytokines and chemokines in the pathogenesis of murine type 1 diabetes. Adv Exp Med Biol 2003; 520: 133-58.
[76] Cario E, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. Infect Immun 2000; 68: 7010-7.
[77] McSorley SJ, Ehst BD, Yu Y, Gewirtz AT. Bacterial flagellin is an effective adjuvant for CD4+ T cells *in vivo*. J Immunol 2002; 169: 3914-9.
[78] Abbott DW, Wilkins A, Asara JM, Cantley LC. The Crohn's disease protein, NOD2, requires RIP2 in order to induce ubiquitinylation of a novel site on NEMO. Curr Biol 2004; 14: 2217-27.
[79] Li H, Sun B. Toll-like receptor 4 in atherosclerosis. J Cell Mol Med 2007; 11: 88-95.
[80] Edfeldt K, Swedenborg J, Hansson GK, Yan Z. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. Circulation 2002; 105: 1158-61.

[81] Xu XH, Shah PK, Faure E, *et al.* Toll-Like Receptor-4 Is expressed by macrophages in murine and human lipid-rich atherosclerotic plaques and upregulated by oxidized LDL. Circulation 2001; 104: 3103-8.

[82] Kiechl S, Lorenz E, Reindl M, *et al.* Toll-like receptor 4 polymorphisms and atherogenesis. N Engl J Med 2002; 347: 185-92.

[83] Kiechl S, Wiedermann CJ, Willeit J. Toll-like receptor 4 and atherogenesis. Ann Med 2003; 35: 164 - 71.

[84] Ameziane N, Beillat T, Verpillat P, *et al.* Association of the toll-like receptor 4 gene Asp299Gly polymorphism with acute coronary events. Arterioscler Thromb Vasc Biol 2003; 23: e61-4.

[85] Arbour NC, Lorenz E, Schutte BC, *et al.* TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nat Genet 2000; 25: 187-91.

[86] Lang K, Georgiev P, Recher M, *et al.* Immunoprivileged status of the liver is controlled by Toll-like receptor 3. J Clin Invest 2006; 116: 2456-63.

[87] Christensen SR, Shupe J, Nickerson K, *et al.* Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity 2006; 25: 417-28.

[88] Devaraj S, Dasu MR, Rockwood J, *et al.* Increased toll-like receptor (TLR) 2 and TLR4 expression in monocytes from patients with type 1 diabetes: further evidence of a proinflammatory state. J Clin Endocrinol Metab 2008; 93: 578-83.

[89] Pociot F, Briant L, Jongeneel CV, *et al.* Association of tumor necrosis factor (TNF) and class II major histocompatibility complex alleles with the secretion of TNF-a and TNF-b by human mononuclear cells: a possible link to insulin-dependent diabetes mellitus. Eur J Immunol 1993; 23: 224-31.

[90] Hussain M, Peakman M, Gallati H, *et al.* Elevated serum levels of macrophage derived cytokines preceed and accompany the onset of IDDM. Diabetologia 1996; 39: 60-9.

[91] Mohammad MK, Morran M, Slotterbeck B, *et al.* Dysregulated Toll-like receptor expression and signaling in bone marrow-derived macrophages at the onset of diabetes in the non-obese diabetic mouse. Int Immunol 2006; 18: 1101-13.

[92] Sherry CL, O'Connor JC, Kramer JM, Freund GG. Augmented lipopolysaccharide-induced TNF-alpha production by peritoneal macrophages in type 2 diabetic mice is dependent on elevated glucose and requires p38 MAPK. J Immunol 2007; 178: 663-70.

[93] Kaizer EC, Glaser CL, Chaussabel D, *et al.* Gene expression in peripheral blood mononuclear cells from children with diabetes. J Clin Endocrinol Metab 2007; 92: 3705-11.

[94] Stentz FB, Umpierrez GE, Cuervo R, Kitabchi AE. Proinflammatory cytokines, markers of cardiovascular risks, oxidative stress, and lipid peroxidation in patients with hyperglycemic crises. Diabetes 2004; 53: 2079-86.

[95] Hussain M, Maher J, Warnock T, *et al.* Cytokine overproduction in healthy first degree relatives of patients with IDDM. Diabetologia 1998; 41: 343-9.

[96] Kim HS, Han MS, Chung KW, *et al.* Toll-like receptor 2 senses beta-cell death and contributes to the initiation of autoimmune diabetes. Immunity 2007; 27: 321-33.

[97] Wen L, Ley R, Volchkov P, *et al.* Innate immunity and intestinal microbiota in the development of Type 1 diabetes. Nature 2008; 455: 1109-13.

[98] McInerney M, Pek S, Thomas D. Prevention of insulitis and diabetes onset by treatment with complete Freund's adjuvant in NOD mice. Diabetes 1991; 40: 715-25.

[99] Sadelain M, Qin H, Lauzon J, Singh B. Prevention of type I diabetes in NOD mice by adjuvant immunotherapy. Diabetes 1990; 39: 583-9.

[100] Raine T, Zaccone P, Mastroeni P, Cooke A. *Salmonella typhimurium* infection in nonobese diabetic mice generates immunomodulatory dendritic cells able to prevent type 1 diabetes. J Immunol 2006; 177: 2224-33.

[101] Kolb H, Elliott R. Increasing incidence of IDDM a consequence of improved hygiene? Diabetologia 1994; 37: 729.

[102] Like A, Guberski D, Butler L. Influence of environmental viral agents on frequency and tempo of diabetes mellitus in BB/Wor rats. Diabetes 1991; 40: 259-62.

[103] Wilberz S, Partke HJ, Dagnaes-Hansen F, Herberg L. Persistent MHV (mouse hepatitis virus) infection reduces the incidence of diabetes mellitus in non-obese diabetic mice. Diabetologia 1991; 34: 2-5.

[104] Yagi H, Matsumoto M, Kishimoto Y, Making S, Harada M. Possible mechanism of the preventive effect of BCG against diabetes mellitus in NOD mouse : II. Suppression of pathogenesis by macrophage transfer from BCG-vaccinated mice. Cell Immunol 1991; 138: 142-9.

[105] Shehadeh NN, LaRosa F, Lafferty KJ. Altered cytokine activity in adjuvant inhibition of autoimmune diabetes. J Autoimmun 1993; 6: 291-300.

[106] Tian J, Zekzer D, Hanssen L, *et al.* Lipopolysaccharide-Activated B Cells Down-Regulate Th1 Immunity and Prevent Autoimmune Diabetes in Nonobese Diabetic Mice. J Immunol 2001; 167: 1081-9.

[107] Quintana FJ, Rotem A, Carmi P, Cohen IR. Vaccination with empty plasmid DNA or CpG oligonucleotide inhibits diabetes in nonobese diabetic mice: modulation of spontaneous 60-kDa heat shock protein autoimmunity. J Immunol 2000; 165: 6148-55.

[108] Green J, Casabonne D, Newton R. Coxsackie B virus serology and Type 1 diabetes mellitus: a systematic review of published case-control studies. Diabet Med 2004; 21: 507-14.

[109] Barbu AR, Akusjarvi G, Welsh N. Adenoviral-mediated transduction of human pancreatic islets: importance of adenoviral genome for cell viability and association with a deficient antiviral response. Endocrinology 2005; 146: 2406-14.

[110] Chen LK, Chou YC, Tsai ST, Hwang SJ, Lee SD. Hepatitis C virus infection-related Type 1 diabetes mellitus. Diabet Med 2005; 22: 340-3.

[111] Varela-Calvino R, Peakman M. Enteroviruses and type 1 diabetes. Diabetes Metab Res Rev 2003; 19: 431-41.

[112] Theofilopoulos AN, Baccala R, Beutler B, Kono DH. Type I interferons in immunity and autoimmunity. Annu Rev Immunol 2005; 23: 307-35.

[113] Devendra D, Eisenbarth G. Interferon alpha- a potential link in the pahtogenesis of viral-induced type 1 diabetes and autoimmunity. Clin Immunol 2004; 111: 225-33.

[114] Alba A, Planas R, Clemente X, *et al.* Natural killer cells are required for accelerated type 1 diabetes driven by interferon-beta. Clin Exp Immunol 2008; 151: 467-75.

[115] Rasschaert J, Ladriere L, Urbain M, *et al.* Toll-like Receptor 3 and STAT-1 Contribute to Double-stranded RNA+ Interferon-gamma-induced Apoptosis in Primary Pancreatic beta-Cells. J Biol Chem 2005; 280: 33984-91.

[116] Ellerman K, Richards C, Guberski D, Shek W, Like A. Kilham rat triggers T-cell-dependent autoimmune diabetes in multiple strains of rat. Diabetes 1996; 45: 557-62.

[117] Zipris D, Lien E, Xie JX, *et al.* TLR activation synergizes with Kilham rat virus infection to induce diabetes in BBDR rats. J Immunol 2005; 174: 131-42.

[118] Zipris D, Lien E, Nair A, *et al.* TLR9-signaling pathways are involved in Kilham rat virus-induced autoimmune diabetes in the biobreeding diabetes-resistant rat. J Immunol 2007; 178: 693-701.

[119] Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997; 40: 1286-92.

[120] Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 1998; 41: 1241-8.

[121] Crook M, Tutt P, Simpson H, Pickup J. Serum sialic acid and acute phase proteins in type 1 and type 2 diabetes mellitus. Clin Chim Acta 1993; 219: 131-8.

[122] Spiegelman B, Choy L, Hotamisligil G, Graves R, Tontonoz P. Regulation of adipocyte gene expression in differentiation and syndromes of obesity/diabetes. J Biol Chem 1993; 268: 6823-6.

[123] Jellinger PS. Metabolic consequences of hyperglycemia and insulin resistance. Clin Cornerstone 2007; 8 (suppl 7): S30-42.

[124] Kolb H, Mandrup-Poulsen T. An immune origin of type 2 diabetes? Diabetologia 2005; 48: 1038-50.

[125] Creely SJ, McTernan PG, Kusminski CM, *et al.* Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol-Endoc M 2007; 292: E740-7.

[126] Suganami T, Mieda T, Itoh M, *et al.* Attenuation of obesity-induced adipose tissue inflammation in C3H/HeJ mice carrying a Toll-like receptor 4 mutation. Biochem Biophys Res Commun 2007; 354: 45-9.

[127] Shi H, Kokoeva MV, Inouye K, *et al.* TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116: 3015-25.
[128] Song MJ, Kim KH, Yoon JM, Kim JB. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. Biochem Biophys Res Commun 2006; 346: 739-45.
[129] Suganami T, Tanimoto-Koyama K, Nishida J, *et al.* Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. Arterioscler Thromb Vasc Biol 2007; 27: 84-91.
[130] Tsukumo DML, Carvalho-Filho MA, Carvalheira JBC, *et al.* Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes 2007; 56: 1986-98.
[131] Poggi M, Bastelica D, Gual P, *et al.* C3H/HeJ mice carrying a toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet. Diabetologia 2007; 50: 1267-76.
[132] Calvet H, Yoshikawa T. Infections in diabetes. Infect Dis Clin North Am 2001; 15: 407-21.
[133] Pozzilli P, Leslie R. Infections and Diabetes: Mechanisms and prospects for prevention. Diabet Med 1994; 11: 935-41.
[134] Moutschen M, Scheen A, Lefebvre P. Impaired immune responses in diabetes mellitus: analysis of factors and mechanisms involved. Relevance to increased susceptibility of diabetic patients to specific infections. Diabete Metab 1992; 18: 187-201.
[135] Brownlee M. Glycation products and the pathogenesis of diabetic complications. Diabetes Care 1992; 15: 1835-43.
[136] Campus G, Salem A, Uzzau S, Baldoni E, Tonolo G. Diabetes and Periodontal Disease: A Case-Control Study. J Periodontol 2005; 76: 418-25.
[137] Gulcelik N, Kaya E, Demirbas B, *et al.* Helicobacter pylori prevalence in diabetic patients and its relationship with dyspepsia and autonomic neuropathy. J Endocrinol Invest 2005; 28: 214-7.
[138] Ratziu V, Heurtier A, Bonyhay L, Poynard T, Giral P. Review article: An unexpected virus-host interaction- the hepatitis C virus-diabetes link. Aliment Pharmacol Ther 2005; 22: 56-60.
[139] Banerjee S, Banerjee M. Diabetes and tuberculosis interface. J Indian Med Assoc 2005; 103: 318-22.
[140] Rich J, Lee J. The pathogenesis of Staphyloccus aureus infection in the diabetic mouse. Diabetes 2005; 54: 2904-10.
[141] Mastropaolo MD, Evans NP, Byrnes MK, *et al.* Synergy in Polymicrobial Infections in a Mouse Model of Type 2 Diabetes. Infect Immun 2005; 73: 6055-63.
[142] Roberts B, Cech I. Association of type 2 diabetes mellitus and seroprevalence for cytomegalovirus. South Med J 2005; 98: 686-92.
[143] Triantafilou K, Triantafilou M. Coxsackievirus B4-induced cytokine production in pancreatic cells is mediated through toll-like receptor 4. J Virol 2004; 78: 11313-20.
[144] Rothenbacher D, Brenner H, Hoffmeister A, *et al.* Relationship between infectious burden, systemic inflammatory response, and risk of stable coronary artery disease: role of confounding and reference group. Atherosclerosis 2003; 170: 339-45.
[145] Espinola-Klein C, Rupprecht HJ, Blankenberg S, *et al.* Impact of infectious burden on progression of carotid atherosclerosis. Stroke 2002; 33: 2581-6.
[146] Espinola-Klein C, Rupprecht HJ, Blankenberg S, *et al.* Impact of infectious burden on extent and long-term prognosis of atherosclerosis. Circulation 2002; 105: 15-21.
[147] Salvi G, Collins J, Yalda B, *et al.* Monocytic TNF alpha secretion patterns in IDDM patients with periodontal diseases. J Clin Periodontol 1997; 24: 8-16.
[148] Salvi G, Beck J, Offenbacher S. PGE2, IL-1 beta, and TNF alpha responses in diabetics as modifiers of periodontal disease expression. Ann Periodontol 1998; 3: 40-50.
[149] Engebretson S, Hey-Hadavi J, Erhardt F, *et al.* Gingival crevicular fluid levels of interleukin-1beta and glycemic control in pateints with chronic periodontitis and type 2 diabetes. J Periodontol 2004; 75: 1203-8.
[150] Shanmugam N, Reddy M, Guha M, Natarajan R. High glucose-induced expression of proinflammatory cytokine and chemokine genes in monocytic cells. Diabetes 2003; 52: 1256-64.
[151] Guha M, Bai W, Nadler JL, Natarajan R. Molecular mechanisms of tumor necrosis factor alpha gene expression in monocytic cells *via* hyperglycemia-induced oxidant stress-dependent and -independent pathways. J Biol Chem 2000; 275: 17728-39.
[152] Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. Nat Immunol 2004; 5: 975-9.
[153] Hajishengallis G, Sharma A, Russell MW, Genco RJ. Interactions of oral pathogens with toll-like receptors: Possible role in atherosclerosis. Ann Periodontol 2002; 7: 72-8.
[154] Stoll LL, Denning GM, Weintraub NL. Potential role of endotoxin as a proinflammatory mediator of atherosclerosis. Arterioscler Thromb Vasc Biol 2004; 24: 2227-36.
[155] Gordon S. Do macrophage innate immune receptors enhance atherogensis? Dev Cell 2003; 5: 666-8.

Received: September 29, 2008 Revised: December 9, 2008 Accepted: December 22, 2008