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The Circle between the Bedside and the Bench: Toll-Like Receptors in Models of Viral Induced Diabetes

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Abstract

Animal models provide many strategies to unravel the complex interplay of genetic, immunological, and environmental factors involved in the pathogenesis of type 1A (autoimmune) diabetes. Diabetes can be studied at multiple levels and new technological advancements provide insights into the functioning of organelle and cellular structures. The role of innate immunity in the response to environmental pathogens has provided possible biochemical and molecular mechanisms which can explain certain clinical diabetes events. These investigations may uncover new therapies and strategies to prevent type 1A diabetes.

Keywords

Toll-Like Receptors; Viruses; Type 1A diabetes

Introduction

Diabetes is not a single disease, but a group of disorders characterized by hyperglycemia. Type 1 diabetes (T1D), also known as insulin-dependent diabetes mellitus (IDDM) or juvenile diabetes, is characterized by an absolute insulin deficiency¹. Type 1A diabetes occurs as a result of an autoimmune process that leads to the destruction of pancreatic beta cells². A complex network of genetic and environmental factors exists that interact with the immune system to trigger beta cell destruction in T1D¹. However, due to the many impediments that exist in conducting T1D research in human subjects, many of our insights into the pathogenesis of the disease have been obtained through the use of animal models that exhibit type 1-like autoimmune disorders³. Using animal models, observations made in a clinical setting can be analyzed in depth in bench research in ways not possible in humans (Fig. 1). In turn, scientific discoveries at the laboratory bench have led to key therapeutic approaches that have been implemented in the clinic to treat T1D and its symptoms (Fig. 1). In this article, we focus on the interplay of clinical observations made at the bedside and insights obtained in experimental animal models at the laboratory bench which have helped to uncover the multifaceted interactions of genetic and environmental factors that lead to T1D.

‘Bedside’ - The Interplay of Genetics and Environmental Perturbations in Human T1D

1. Genetic predisposition in the pathogenesis of human T1D

It is well known that genetic susceptibility, especially with regard to certain major histocompatibility complex (MHC) haplotypes, is involved in the development of T1D. Certain HLA class I haplotypes have been linked to T1D⁴, but expression of the disease has been found to correlate more consistently with HLA class II haplotypes¹. Although the exact function of MHC gene products and their role in the pathogenesis of T1D are not completely understood, the association of these permissive haplotypes with T1D strongly suggests that the expression of diabetes requires a susceptible genetic background⁵. However, T1D concordance is only 30–50% among identical twins⁵. Thus, although there is a strong genetic predisposition for T1D disease development, the lack of 100% disease concordance among identical twins indicates that other factors, such as environmental influences, must act to initiate expression of the disease.

2. Viral infection and human T1D

Viral infections have been strongly implicated as the main environmental factor contributing to the pathogenesis of T1D. Viral infections such as mumps, rubella, enteroviruses, cytomegalovirus, rotavirus, and parvovirus have all been associated with human T1D⁶⁻⁷. Although the mechanisms by which viruses contribute to the onset of T1D remain elusive, there are multiple proposed mechanisms, including direct beta cell destruction, inflammation, or molecular mimicry by which viral infection could lead to autoimmunity, beta cell destruction, and eventual human T1D⁸.

The first mechanism proposes that direct infection of beta cells by viruses results in lysis of the beta cell and release of self-antigens. These released self-antigens are picked up by antigen presenting cells (APCs) that in turn activate self-reactive lymphocytes that mediate beta cell destruction, leading to the expression of hyperglycemia⁶⁻⁹. Second, it has been proposed that viral infection of APCs may cause an increased expression of cytokines that activate self-reactive lymphocytes, or directly mediate beta cell cytolysis⁹. A third mechanism known as ‘molecular mimicry’ proposes that viral antigens with homology to self-epitopes cross react, leading to the activation of self-reactive lymphocytes that mediate beta cell destruction¹⁰. Finally, a new mechanism recently proposed based on animal models hypothesizes that viral infections may cause a transient lymphopenia that disturbs the equilibrium between self-reactive lymphocytes and regulatory T lymphocytes, tipping the immune balance towards an autoimmune environment¹¹.

An association between diabetes and virus infection was first made in 1864 in a patient who developed the disease following mumps infection. It is now known that the ssRNA enveloped mumps virus is capable of infecting islet and pancreatic cells *in vitro* and *in vivo*, respectively, and mediating direct beta cell cytolysis⁹⁻¹²⁻¹³. Similarly, rubella virus was first associated with human T1D in 1969. Epidemiological evidence suggests that if rubella virus infection is associated with the development of T1D, it is restricted to infected patients that are genetically susceptible to the development of T1D⁶⁻⁷. Since vaccinations to mumps and rubella have been developed and in use since the 1980s, additional epidemiological evidence indicates that preventing these infections by vaccinations did not decrease T1D incidence, thus suggesting that these infections may not act as major perturbations that lead to T1D⁶.

Enteroviruses are a class of nonenveloped RNA viruses that include coxsackie A and B viruses and polioviruses. Evidence for enterovirus infection contributing to T1D

pathogenesis has been obtained in studies where patients who recently developed T1D had an increase in enteroviral RNA in their sera as compared to healthy individuals^{6,13,14}. Additionally, cytomegalovirus (CMV) infection was linked to the development of T1D in 1979. In that report, a child infected with CMV developed T1D shortly after infection. Evidence from additional epidemiological studies shows a correlation between CMV infection and islet autoantibodies. However, analogous to mumps and rubella viral infection, enterovirus and cytomegalovirus infection may only contribute to increased development of T1D in certain subsets of genetically susceptible children⁶.

Like the dsDNA CMV, the nonenveloped dsDNA rotavirus has been linked to the development of T1D. Studies have associated rotavirus infection with the production of autoantibodies against islet antigens in genetically susceptible children^{6,7}. Also, infection by a ssDNA parvovirus has been shown to be associated with the development of T1D, but this is only by association. In animal models, there is strong evidence for parvovirus infection inducing diabetes in genetically susceptible rats^{6,15}. Thus, the link between T1D and rotavirus or parvovirus infection in humans remains correlative, but is strongly supported by animal models of type 1-like diabetes, suggesting a fruitful pathway for further research to validate the link between T1D and virus infection.

3. Why does a role for viral infections in human T1D remain elusive?

Although many viral infections have been associated with human diabetes, as annotated above, a direct causative role for viruses in T1D has not been established. One possible explanation to the lack of direct fulfillment of Koch's postulates in virus initiation of T1D may be that the link between infections and autoimmunity is multifactorial⁸. Several infections might act together, or in an appropriate temporal sequence, to trigger clinical autoimmunity. Coupled with this, the particular virus involved might be hard to detect systemically or in the target organ following initiation of the autoimmune response. Moreover, the precipitating virus infection may occur days or months before the clinical symptoms of diabetes are evident, making a direct relationship hard to establish. In addition, viral infections may not be sufficient to induce disease in the absence of other inflammatory factors, a model proposed in the “fertile field hypothesis”¹⁶. This model suggests that the presence of inflammatory factors caused by activation of an immune response is necessary for the induction of an autoimmune disease such as T1D, suggesting a role for activation of the immune system in disease pathogenesis¹⁶.

4. Adaptive and innate immunological responses in human T1D

There is strong evidence linking T1D pathogenesis to activation of an immune response following an environmental perturbation in genetically susceptible individuals. The human immune system is divided into two arms, an innate response and an adaptive response. Innate immunity is the primordial response that is the first arm of the immune response leading to inflammation and the production of cytokines involved with clearance of the infectious pathogen¹⁷. Innate immunity is mediated by cells of the myeloid and lymphoid system such as dendritic cells (DCs) and NK cells, respectively, which are important in creating the appropriate microenvironmental conditions for the induction of adaptive immunity.

The adaptive immune response is the induction of antigen-specific immune cells tailored to eliminate invading pathogens. This involves both B lymphocytes and T lymphocytes. Autoantibodies produced by B cells of adaptive immunity have been suggested to be indicative of the progression of T1D. Autoantibodies against insulin, glutamic acid decarboxylase (GAD), IA-2 antigens, and zinc transporter ZnT8 have been detected in pre-diabetic and diabetic patients with T1D, although a mechanism for a role of autoantibodies

in disease pathogenesis and the resultant autoimmune attack of pancreatic beta cells has not been identified¹. On the cellular adaptive immune arm, certain subsets of T lymphocytes can be autoreactive and invade islets of the pancreas leading to insulinitis and beta cell destruction. Further evidence for a role of adaptive immunity in T1D pathogenesis has been provided by data in identical twins. Pancreas and kidney transplantation from a discordant identical twin into a sibling with T1D leads to survival of the kidney, but rejection of the islets in the pancreas¹. These data suggest that the autoimmunity that destroyed the islets of the diabetic twin remains capable of destroying islets in the transplanted pancreas, a process referred to as autoimmune recurrence. Results of this study document that cells of the adaptive immune response are still capable of destroying beta cells long after T1D development¹. Collectively, studies such as these indicate a role for both the innate and adaptive immune system in T1D pathogenesis.

5. Clinical conundrums in human T1D development

Although a great deal of information on autoimmune diabetes has been garnered over the years, we will focus on three major groups of clinical observations that remain a conundrum to clinicians who treat individuals with T1D:

- a. Perhaps surprisingly, the great majority (~85%) of T1D patients have *no family history* of the disease! Thus, prior to diagnosis, there was no previous indication that the patient was genetically susceptible to T1D. Clearly, however, the patient must have inherited diabetes-susceptible MHC genes from parents/grandparents who themselves remained free of disease. Possible reasons for this discrepancy include a potential role of multiple genes that control T1D or a differential exposure to putative environmental ‘perturbants’ in individuals that are genetically susceptible to T1D.
- b. As discussed above, *identical twins show only 30–50% concordance* for T1D, again consistent with the premise that non-genetic environmental factors may play a role in disease initiation¹. Complicating this scenario, there may also be a genetic-environmental interaction. For instance, many human T1D susceptibility genes may be deleterious only in the context of environmental perturbation, *e.g.*, by amplifying the immune response and thereby enhancing disease progression¹⁸.
- c. Diabetes onset is *often preceded by exposure to a pathogen* or other deleterious environmental ‘perturbation’. Although the exact nature or identity of the ‘perturbant’ remains obscure, candidates for these contributory environmental factors include certain diets, toxins, vaccination, and infection¹. In regards to toxins, there is little convincing evidence to support a role in diabetes. With vaccination, there is strong evidence *against* an effect of vaccination in disease development. In terms of dietary factors, there is a plethora of data but no conclusive candidates have yet been identified. Therefore, the environmental factor with the strongest epidemiological data supporting an association with T1D thus far is viral infection.

Because human studies are limited by genetic variation and the inability to control the environment, there is a real need to perform controlled investigations with specific viral reagents that cause immune responses in genetically homogeneous animal models. In the next sections, we will focus on the use of animal models to understand the clinical observations associated with human T1D: 1) no previous family history, 2) 30 to 50% concordance in identical twins, and 3) previous exposure to pathogen(s).

‘Experimental Models’ - Animal Models of Induced Diabetes

1. Disadvantages of mouse models in studying induced autoimmune diabetes

The NOD (non-obese diabetic) mouse is undoubtedly the most well studied animal of *spontaneous* autoimmune diabetes, although other rodent models exist, such as the BioBreeding Diabetes Prone (BBDP) rat. By comparison, relatively few studies of environmentally induced diabetes have been reported. In addition, there has been no consensus on a ‘gold standard’ animal model for inducible diabetes. Transgenic mouse models have provided some information, but a concern is the inherent ‘artificial’ nature of these models. Non-transgenic mouse models of induced autoimmune diabetes are relatively few and in the NOD mouse the great majority of perturbants, including viral infection, reduce the frequency of diabetes or even prevent it entirely¹⁹.

2. Induced T1D in susceptible rat strains

Similar to the NOD mouse, autoimmune diabetes may appear spontaneously in certain rat strains (notably the BBDP rat) housed in viral-antibody-free (VAF) conditions²⁰. In contrast to the mouse, however, susceptibility to induced T1D in the rat is relatively common. Typically, diabetes may occur in response to immunological perturbations. These methods of induction include regulatory T cell depletion, toll-like receptor (TLR) ligation, and certain viral infections. Whether spontaneous or induced, all susceptible rat strains (with one exception) express the specific MHC class II allele, RT1 B/D^u. Diabetes susceptible rat strains include: BioBreeding (BBDP and BB Diabetes Resistant (DR)), LEW, LEW.1WR1, PVG and PVG-RT1^u, and several transgenic rats. These rat strains also have enhanced susceptibility to other autoimmune disorders, similar to what has been reported in humans with T1D²¹. The remaining sections will focus on various rat models of inducible T1D and how they may help us better understand the pathogenesis of diabetes in susceptible individuals which, in turn, may lead to therapies to prevent or treat human diabetes.

The ‘Bench’ – Laboratory Investigations of Induced Diabetes

1. ‘Spontaneous’ diabetes in a colony of BioBreeding Diabetes Resistant (BBDR) rats

In the 1980's, a fortuitous outbreak of autoimmune diabetes occurred in a colony of BBDR rats²². These animals had previously been impervious to the condition over many generations and, as such, could now serve as a tool to investigate human T1D in genetically susceptible individuals that had no previous family history of diabetes (Clinical Observation #1). Successive studies on these animals revealed valuable information germane to human disease. First, investigations uncovered a ‘break’ in the VAF housing conditions pointing to the presence of virus infections in affected rats expressing the disease. This discovery suggested a role for an environmental pathogen in diabetes induction, as has been proposed for some patients with T1D (Clinical Observation #3). The pathogen associated with diabetes development in the BBDR rats was subsequently determined to be Kilham rat virus (KRV), a ssDNA parvovirus²³. Interestingly, as mentioned above, human infection with ssDNA parvovirus have been associated with T1D induction^{6,15}. Finally, this diabetes outbreak in BBDR rats revealed a previously unappreciated genetic susceptibility of these animals to disease that could be capitalized on, as will be seen in the studies that follow.

2. KRV infection induces 30–40% T1D in BBDR rats

Further investigations using KRV obtained from diabetic BBDR animals showed that purposeful infection of BBDR rats housed under otherwise VAF conditions led to a recapitulation of the disease, fulfilling Koch's postulates and confirming that KRV was the causative agent of autoimmune diabetes in these animals. Titering of KRV documented that as few as 10^3 virions can generate a small percentage (<5%) of BBDR rats to express

diabetes, but the expression of diabetes in 100% of KRV infected BBDR rats has not been achieved, even using KRV doses as high as 10^8 virions. Over multiple trials, it was determined that KRV infection can induce diabetes in ~30 to 40% of BBDR rats. Intriguingly, this is consistent with the concordance rate for diabetes reported clinically with identical twins (Clinical Observation #2).

3. Diabetes induction following treatment with TLR agonists

To investigate further the role of viruses in diabetes in BBDR rats, we used polyinosinic-polycytidylic acid (poly I:C), a synthetic dsRNA that can act as a “viral mimetic”. BBDR/Wor rats treated with low doses (5 µg/g) of poly I:C develop diabetes at a rate of ~20%. At higher doses (10 µg/g), poly I:C treatment results in ~100% of BBDR rats developing diabetes within 2–3 weeks after the initiation of treatment. This poly I:C induced susceptibility to autoimmune diabetes is not unique to the BBDR rat, as several other rat strains tested also develop the disease following injection of high doses of poly I:C. Although many of these other strains have been shown to develop diabetes following treatment with virus and/or TLR agonists, we will focus on the BBDR rat as the most well studied of the diabetes-inducible strains of rats²¹⁻²⁴.

4. TLR agonists synergize with KRV infection to induce diabetes in BBDR rats

During efforts to increase the incidence of diabetes in KRV infected BBDR rats, several combination treatments were performed. The most potent protocol to induce diabetes in the BBDR rat was found to be treatment with the TLR3 agonist, poly I:C, followed by infection with KRV. This combination treatment induced diabetes in close to 100% of rats over a rapid time course (14–18 days). Likewise, poly I:C was not the only TLR agonist that could synergize with KRV to induce autoimmune diabetes. In BBDR rats infected with KRV, for instance, diabetes could be induced at rates of 60% and 100% following pre-treatment with heat killed *E. coli* and *S. aureus*, respectively²⁵. Heat killed *E. coli* and *S. aureus* are reported to act via TLR4 and TLR2, respectively, and neither treatment alone can induce diabetes in BBDR rats. Collectively, these data indicate that two environmental ‘hits’ may be required for optimal induction of diabetes and suggest a plausible scenario whereby genetically identical twins may have dissimilar diabetes outcomes.

5. Mechanisms of T1D pathogenesis during induced diabetes

a) No role found for molecular mimicry—As mentioned above, infection of BBDR rats typically results in diabetes onset in ~30 to 40% of the animals over a two to three week period. One hypothesis that may explain the mechanism of KRV-induced T1D is molecular mimicry. However, in studies that directly tested this hypothesis in BBDR rats by infection with viral vectors expressing KRV proteins, no diabetes occurred. Furthermore, even though adaptive and innate immune responses were generated, including expansion of CD8⁺ T cells in infected animals, no virus-specific CD8⁺ T cell responses were found. Together, these data fail to support a role for molecular mimicry in KRV induced diabetes.

b) Imbalance in regulatory T cell populations—Many investigations have focused on the role of the adaptive immune response during induced diabetes. Thus, another hypothesis that may explain how KRV induces diabetes in BBDR rats is by altering the balance between regulatory T cells (T_{reg}) and autoreactive effector T (T_{eff}) cells. This seems plausible as KRV and other parvoviruses, such as H-1, are known to infect lymphocytes, but do not directly infect pancreatic islets. Although both parvoviruses induced adaptive and innate immune responses when injected intraperitoneally into BBDR rats, only KRV caused a decrease in the population of splenic T_{reg} cells²⁶. Moreover, KRV but not the highly similar H-1 parvovirus, induced diabetes in BBDR rats. Taken together, these data are

consistent with a role for an imbalance in T_{reg} to T_{eff} cells in the pathogenesis of virus-induced autoimmune diabetes.

c) Activation of innate immunity—Pre-treatment of BBDR rats with low doses of poly I:C, a TLR3 agonist and known innate immune activator, acts synergistically with KRV to induce diabetes in up to 100% of treated rats. These studies suggest that activation of the innate immune system plays a key role in the induction of diabetes in this model. This finding was not limited to TLR3 agonists, as several TLR ligands also synergized with KRV infection to increase the incidence of diabetes²⁵. KRV infection itself also activates the innate immune response, as measured by increases in IL-12p40, IFN γ -inducible protein-10, and IFN γ mRNA, particularly in the pancreatic lymph nodes. However, the innate immune response to H-1 virus infection (which fails to induce diabetes) was much lower in magnitude. These data strongly suggest that, similar to clinical human observations, activation of the innate immune system is critical to the initiation of environmentally induced diabetes.

d) TLR9 signaling in KRV induced diabetes—The above studies suggest that KRV activates the innate immune system as evidenced by the production of proinflammatory cytokines by DCs and macrophages *in vitro*. A similar pattern of cytokine response was seen when the cells were treated with CpG ssDNA, a synthetic TLR9 agonist²⁷. As KRV is a ssDNA virus, we hypothesized that KRV may also act through the TLR9 receptor. Support for this hypothesis was engendered by studies with TLR9-deficient mice. DCs from wild type, but not TLR9-knockout mice, produced high levels of IL-12p40 in response to CpG or genomic DNA isolated from KRV virus. Additional support was provided by studies with the TLR9 inhibitors, inhibitory CpG (iCpG) and chloroquine. Inhibitory CpG is an oligonucleotide containing an inhibitory CpG motif and chloroquine is thought to act via endosomal acidification, thereby preventing signaling of TLRs localized to the endosome (Fig. 2). BBDR spleen cells incubated in the presence of either CpG or KRV DNA showed significant reductions in the secretion of IL-12p40 when in the presence of iCpG or chloroquine. More importantly, treatment of BBDR rats with chloroquine was shown to reduce the incidence of KRV induced diabetes. Taken together, these studies identify a role for TLR9 signaling in virus induced diabetes in the BBDR rat. Furthermore, these studies suggest potential therapeutic targets for intervention in human diabetes.

6. Recent discoveries at the ‘bench’

The above sections highlight some of the recent discoveries made in the BBDR rat model of type 1-like diabetes that pertain to virus induced diabetes. Progress in understanding diabetes pathogenesis, of course, has been made on many fronts, using different animal models and/or *in vitro* systems. A major question in diabetes pathogenesis relates to the role of the pancreatic beta cell. Does the beta cell play an active or passive role in its own demise? In particular, recent research has focused on endoplasmic reticulum (ER) stress in the beta cell. It is well known that the beta cell is subject to ER stress as a secondary consequence of the synthesis and secretion of high levels of insulin. We have postulated that ER stress may result in beta cell death that *precedes* immune cell infiltration in the islets and may actually *contribute* to immune infiltration. Alternatively, ER stress may result in misfolding of beta cell proteins, *e.g.*, insulin, in such a way as to generate ‘neo-autoantigens’¹¹. In either case, ER stress and beta cell death may instigate the initial autoimmune assault that ultimately proceeds to absolute insulin deficiency and diabetes. The answers to these interesting questions await further clinical and laboratory studies.

Back to the 'Bedside' – New Clinical Therapies

Many studies have focused on clinical intervention therapies for autoimmune T1D, both during the evolution of the disease and at disease onset. The therapeutic objective, before disease onset, is to inhibit the immune destruction of pancreatic beta cells and thereby delay or prevent clinical disease. When disease onset has already occurred, the goal is to halt the destruction of the remaining beta cells, which may allow residual beta cells to recover function, thereby decreasing the severity of clinical signs and slowing the progression of the disease. An important aspect of setting up clinical trials for potential therapeutics, of course, is to accurately identify individuals at risk for T1D.

Potential therapies investigated in large clinical trials have first been found to be effective in animal models of autoimmune diabetes, as well as in small cohorts of human volunteers. Based on this data, some of the earliest large-scale studies were undertaken to investigate the therapeutic value of the B vitamin, nicotinamide, as well as both oral and parenteral insulin. Unfortunately, even though the initial pilot studies showed promise, none of these treatments proved to be effective in large-scale human trials²⁸.

Additional studies are currently focused on newborns with genetic risk factors for T1D. One such study is investigating whether the frequency of T1D can be reduced by preventing exposure to cow milk protein during early life²⁴. A second pilot study is examining the effectiveness of the omega-3-fatty acid, docosahexaenoic acid (DHA), in delaying or preventing diabetes. Moreover, several agents are being tested in patients with new onset T1D, including immunomodulatory therapies such as the p277 peptide from heat shock protein 60, anti-CD3 and anti-IL2-receptor monoclonal antibodies, and a GAD vaccine²⁸. In each case, initial promising results must await longer-term and larger-scale studies to validate their effectiveness.

Overall, many of the earliest and most promising potential therapeutics to slow the progression of T1D have proven disappointing. Although there has been progress in implementing studies designed to arrest or delay disease progression, there remains a real need to develop new and effective clinical therapies. Careful elucidation of molecular mechanisms involved in the pathogenesis of induced diabetes in animal models should facilitate this need. For example, as chloroquine was shown to inhibit TLR9 signaling and reduce the incidence of diabetes in KRV infected BBDR rats, this agent would represent a promising potential therapeutic for human T1D (Fig. 2). In addition, evidence from David Baltimore's laboratory has demonstrated a role for specific microRNAs (miRNA-155) in regulation of the inflammatory response²⁹. Manipulation of this miRNA may provide yet another means to ameliorate or prevent T1D (Fig. 2). These and other cutting-edge data gathered and assimilated from a large body of clinical and laboratory research should provide additional innovative treatments for human diabetes.

Conclusion

In summary, autoimmune diabetes in humans results from an interplay of genetic, immunological, and environmental factors. In particular, three clinical observations of patients with T1D have puzzled clinicians: 1) most patients have no previous family history, 2) there is only 30 to 50% concordance in identical twins, and 3) many patients had a previous exposure to virus. Here we demonstrate how these clinical observations led to carefully controlled laboratory studies using animal models of type 1 diabetes. These studies, in turn, have identified specific molecules and pathways involved in the initiation of disease that may be targeted as rational therapies and brought back—full circle—to the clinics to prevent or slow the progression of autoimmune diabetes in human patients.

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Figure 1. The circle between the bedside and the bench

Clinical examination of patients at the bedside can be utilized to design experiments in animal models at the laboratory bench in order to further knowledge about T1D. In turn, information gained through scientific research at the bench can be used to develop therapeutics for treatment of T1D and its clinical symptoms. The study of diabetes in the “bedside to bench” model begins at the population level and continues to the molecular level of genomic research. In the future, answers to many challenging questions surrounding T1D pathogenesis may lie in the very atoms that comprise the molecules of the cell (adapted from Majno and Joris (2004)30).

Figure 2. A model of TLR signaling in dendritic cells during virus induced diabetes

Signaling via the endosomal localized TLR3 (poly I:C) or TLR9 (KRV) during diabetes induction (step 1) leads to downstream signaling events to the nucleus (step 2). These signals activate transcription factors, such as NF κ B and IRF, which transcribe type 1 IFNs and other important cytokines (step 3) that have direct effects on the adaptive immune system and possibly on beta cells. Research data suggests that chloroquine inhibits TLR9 signaling and decreases KRV induced diabetes, thus making it a potential therapeutic candidate in the clinic. Other possible therapeutic candidates may exist, such as miRNAs, which are produced following TR3 ligation and capable of regulating inflammatory responses.