Chemokines as Drug Targets in Type 1 Diabetes

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Abstract: Acute or chronic inflammation is thought to play a major role in the etiology and/or pathogenesis of autoimmune disease. Often viral infections are the initial cause for a local inflammatory reaction resulting in tissue infiltration by activated leukocytes. The activation and trafficking of these leukocytes to the site of inflammation is conducted by chemoattractant cytokines, termed chemokines. Depending on the genetic background and the history of previous infections, such infiltrating leukocytes can potentially include autogressive lymphocytes with specificity to tissue antigens. The number of specific precursor lymphocytes, strength of activation and degree of counteracting immunoregulatory measures determine whether such an autoimmune incident ultimately results in autoimmune disease. Thus, by blocking the initial inflammatory insult one could in theory prevent the excessive attraction of autogressive lymphocytes to the inflammation site and the subsequent formation of a pattern that leads to autoimmune disease. This review focuses on blocking of chemokines in animal models of type 1 diabetes and discusses the possible applications of such treatments in human autoimmune disease.

INTRODUCTION

It is generally believed that autoimmune diseases occur as a result of genetic predisposition and environmental triggering. Acute and/or chronic inflammation caused by acute and/or persistent pathogen infection is one of the triggering factors suspected to play a major role in the etiology of human autoimmune diseases in susceptible individuals. Thus, targeted blocking of critical inflammatory mediators might prevent the subsequent development of autoimmunity. Chemokines are among the first inflammatory mediators expressed after pathogen infection. These chemoattractant cytokines are predominantly released locally by resident cells (endothelial cells, resident macrophages, stromal cells) upon activation by pro-inflammatory cytokines, such as γ-Interferon (IFNγ), (Interleukin 1β) IL-1β or tumor necrosis factor α (TNFα). The pattern of chemokine secretion determines the composition of immune competent cells recruited to the site of infection (for review see [1-3]).

CHEMOKINES AND TYPE 1 DIABETES

In the past few years, several chemokines that might have an influence on the pathogenicity of type 1 diabetes (T1D) have been identified in animal models (see below). Unfortunately, only a handful of studies have been conducted in humans and most of them were limited to the analysis of chemokine levels in the peripheral blood. Most prominently, it was found that patients with T1D display an elevated level of CXC chemokine ligand 10 (CXCL10), a chemokine that is predominantly attracting T-cells of the more aggressive Th1-type [4, 5]. In contrast, in a recent study with a group of susceptible individuals who did not progress to clinically overt T1D, Hanifi-Moghaddam et al. could not detect elevated serum levels of CXCL10 and other classical Th1-associated cytokines and chemokines, such as IFNγ, Interleukin 12 (IL-12) and CC chemokine ligand 5 (CCL5) [6]. They found however, in this cohort of islet-autoantibody-positive first-degree relatives of T1D patients significantly increased serum levels of CCL3 and CCL4 [6]. In another study, Lohmann et al. found increased levels CCL2, CCL3, CCL4, or CCL5 in the blood of newly diagnosed T1D patients [7]. Interestingly, the authors revealed a decreased presence of PBMCs expressing the CC chemokine receptor CCR5 (receptor for several chemokine ligands, such as CCL3, CCL4, and CCL5) and the CXC chemokine receptor 3 (receptor for CXCL9, CXCL10, and CXCL11) [7]. These data indicate a relocation of chemokine receptor bearing PBMCs into the inflamed pancreas. Interestingly, CCL2 was found to be produced by human pancreatic islets upon stimulation with IL-1β or TNFα [8, 9].

In contrast to studies in humans, more indications towards the role of chemokines during T1D have been derived from animal models. However as in humans, the helper T-cell 1 (Th1)-type chemokine CXCL10 seems to play a central role in the pathogenesis of T1D in both spontaneous (nonobese-diabetic (NOD) mouse, BB rats) as well as inducible models (RIP-LCMV mouse (see below), Insulin-promoter controlled influenza virus hemagglutinin (Ins-HA) mouse). In the NOD mouse, it was demonstrated that Th1-type T-cells, which produced CCL5, CCL7, CCL12 and CXCL10 migrate much quicker into the pancreas of the mouse). In the NOD mouse, it was demonstrated that Th1-type T-cells, which produced CCL5, CCL7, CCL12 and CXCL10 migrate much quicker into the pancreas of the insulin-deficient NOD.scid (NOD x severe combined immunodeficient) mice and only Th1-type but not helper T-cell 2 (Th2)-type cells induced diabetes [10]. In a recent study, Bouma et al. analyzed pancreas lysates and found a massive increase in CXCL10 levels around week 10 of age, which then decreased again back to baseline levels at week 20 of age [11]. In contrast, pancreatic expression of CCL5, which was found to be upregulated around week 10 as well, remained high until at least week 20 of age [11]. These increased CCL5 levels most likely reflect the degree of infiltration of pancreatic islets by autogressive T-cells, which are very well capable of producing CCL5. Similar data have been reported in recent publication by Gysemans et al., who...
demonstrated expression of CXCL10 as well as CCL2 and CCL20 at week 8 of age in female NOD mice [12]. Interestingly in this study, in vivo treatment of mice with 1.25-Dihydroxyvitamin D3 diminished the release of these chemokines in pancreatic islets and abrogated disease [12]. In another exciting study, Cameron et al. found that a high ratio of CCL3 to CCL4 expression correlated with destructive insulitis and progression to diabetes in NOD vs. NOR (nonobese diabetes-resistant) mice [13]. Furthermore, when NOD mice were treated with IL-4, which prevents T1D in NOD mice by polarizing intra-islet T-cell response towards a ‘regulatory’ Th2-type response, a dramatically diminished ratio of CCL3:CCL4 and a decreased expression of islet CCR5 was observed [13]. These data indicate that the pattern of pancreatic chemokine expression not only determines the population of infiltrating leukocyte, but subsequently also influences the outcome of disease in the NOD mouse. In another animal model for spontaneous T1D, the BB rat, it was found in a microarray study with pancreatic RNA that CCL11, a chemokine, which predominantly attracts eosinophils, was most prominently upregulated during a critical phase of disease development [14]. However, although analysis of chemokine expression in sera of T1D patients or in the pancreas of animals which develop spontaneous T1D gives some indication about the mechanisms involved in the ongoing destruction of the insulin-producing β-cells, only limited information can be obtained about the factors involved in the initiation of disease. The problem here lays in the fact that human individuals as well as individual mice, which develop spontaneous disease, are at a very heterogeneous stage of disease pathogenesis at the time of diagnosis or experimental analysis, respectively. However, there is a possibility to coordinate/accelerate the pathogenetic processes in the NOD mouse by injection of cyclophosphamide at around 6–8 weeks of age, which allows analysis of early events in a synchronized population of mice. By such an approach Matos et al. conducted microarray studies and found a prominent expression of CXCL1, CXCL5, CXCL9, CCL2, CCL5 and CCL7 [15].

However, the only reliable way to minimize the problem of inter-individual heterogeneity the autoimmune immune response and the pathogenesis of disease is the use of an entirely inducible animal model for T1D, in which the starting point of disease pathogenesis is clearly defined and the initiating antigens are very well characterized. The RIP-LCMV mouse model, which uses a viral protein as a target antigen in the β-cells, is probably the model system that was most often used in the past decade and I will discuss the data we obtained with this system in more detail below. Briefly, infection of RIP-LCMV mice (see below) with LCMV (lymphocytic choriomeningitis virus) causes a massive and unique expression of CXCL10 in the pancreas [16]. It was further found that CXCL10 was mainly produced by the β-cells themselves [17]. Since CXCL10 predominantly attracts those activated T-cells, which are of the more aggressive Th1-phenotype [18, 19], and more than 95% of LCVM protein (target antigen)-specific autoaggressive CD8 T-cells express CXCR3 (the CXCL10 receptor) [16], we hypothesized that CXCL10 might imprint a pattern of inflammation for the subsequent development of autoimmune disease. We used the RIP-LCMV model for T1D to test this hypothesis in more detail. In the following I will focus on our experiences with chemokine blockade in this virus-induced animal model and will discuss possible applications in therapy of human T1D.

**THE RIP-LCMV MODEL FOR TYPE 1 DIABETES**

One possible mechanism of how pathogens can initiate autoimmunity is molecular mimicry. This hypothesis involves breaking existing self-tolerance due to an inherent structural similarity of pathogen molecules to self-components [20-25]. The concept of molecular mimicry has been successfully integrated into animal models for various human autoimmune diseases such as T1D, multiple sclerosis, or systemic lupus erythematosus (for review see [23]). Among these models, the RIP-LCMV mouse model for T1D was established in 1991 in the laboratories of Michael Oldstone [26] and Rolf Zinkernagel [27]. RIP-LCMV transgenic mice express the glycoprotein (GP) or nucleoprotein (NP) of LCMV under the control of the rat insulin promoter (RIP) selectively in the insulin-producing β-cells of the islets of Langerhans in the pancreas. The transgenically expressed viral protein is considered a component of ‘self’ and thus RIP-LCMV mice are tolerant and do neither generate an LCMV-specific immune response nor develop insulitis or T1D. However, upon infection with LCMV itself this self-tolerance is broken, auto-aggressive LCMV-specific T-cells are generated and expanded, the β-cells are being destroyed and ultimately clinical overt diabetes results.

Similar to the proposed mechanisms involved in human T1D, the onset of diabetes in RIP-LCMV mice depends on autoreactive CD4 and CD8 T-cells and correlates with the numbers of autoaggressive lymphocytes generated. In accordance, the incidence of disease varied between the individual transgenic lines ranging from 2 weeks (RIP-LCMV-GP lines) to 1-6 months (RIP-LCMV-NP lines). Further studies revealed the mechanism involved in the rapid compared to the slow onset diabetes: LCMV-GP transgenic mice expressed the GP exclusively in the β-cells of the islets and manifested rapid-onset T1D (10-14 days after viral challenge) [28]. In these mice the high systemic numbers of auto-aggressive CD8 T-cells were sufficient to induce diabetes and did not require ‘help’ from CD4 cells. In contrast, LCMV-NP transgenic mice expressed the NP-transgene in both the β-cells and in the thymus and thus anti-self (viral) cytotoxic T lymphocytes (CTL) were of lower affinity and CD4 T-cell help was essential to generate anti-self (viral) CD8 lymphocyte-mediated T1D, which took much longer to occur following LCMV infection [28]. Importantly in context with this review, mouse models in which transgene-encoded ‘target-antigens’ are expressed in the pancreatic β-cells, such as the RIP-LCMV and the INS-HA [29, 30] mouse, have demonstrated that the presence of auto-aggressive T-cells alone is not enough to cause disease. For example, crossing RIP-LCMV mice with an IFNγR deficient mouse line resulted in a drastically reduced diabetes incidence [31]. Furthermore, blockade of TNFα at an early stage after LCMV-infection of RIP-LCMV mice abrogated T1D [32]. These data indicate that unspecific ‘bystander factors’, such as cytokines and chemokines generated during the acute inflammation after LCMV infection, are important to drive the...
auto-aggressive response (β-cell destruction) in ‘antigen-specific’ models for T1D.

Hence, the RIP-LCMV model has become a very useful tool to study the etiology and the mechanisms of human autoimmune diabetes and to evaluate possible treatments, such as blockade of specific inflammatory factors, as discussed below [16, 32], oral tolerance induction [33] or DNA-vaccination [34]. The strengths of the RIP-LCMV models are (i) a clearly defined initiation point (LCMV-infection), which allows studying the precise kinetics of immunopathogenesis, (ii) an autoimmune-mediated disease that is restricted to the islets of Langerhans and (iii) the presence of extensively characterized target antigens (GP, NP). Importantly, the immune response against these target antigens can be easily visualized using flow cytometry by stimulation of splenocytes with LCMV-peptides and subsequent analysis of intracellular cytokine expression (ICCS) or direct detection of CD8 T-cells with MHC class I-LCMV-peptide tetramers [35]. In addition, we recently demonstrated tracking of LCMV-specific CD8 T-cells by in situ MHC class I-peptide staining of quick-frozen tissue sections [36].

EARLY INFLAMMATORY EVENTS AFTER LCMV INFECTION IN THE PANCREAS

Here I will focus on the initial inflammatory reactions generated as a direct response to virus infection as the initiating event in LCMV-induced T1D. In the past years we acquired a whole body of information that helped us modeling a script of individual immunopathogenic events directing from LCMV-infection of the pancreas to establishment of overt diabetes (Fig. 1). Chemokines, most prominently CXCL10, were the first inflammatory mediators we could detect after LCMV-infection [16]. These chemokines can be generated by endothelial cells as well as by β-cells themselves [17]. This expression of CXCL10 is most likely induced by the presence of high local concentrations of TNFα released by virally activated resident macrophages. In a second step, the high chemokine concentrations attract LCMV-specific and non-specific leukocytes which extravasate into the islets of Langerhans. Some initial damage is done by LCMV-specific CD8 T-cells in a perforin-dependent manner to some β-cells. This initial β-cells destruction is not sufficient to cause overt diabetes, since the remaining β-cell population upregulate insulin-production to compensate for the smaller total number of β-cells. In the following additional β-cell antigens are presented by antigen-presenting cells (most likely in the pancreatic draining lymph node or alternatively directly in the islets themselves) and most of the β-cells are destroyed by expanded LCMV-specific CD8 T-cells as well as by other islet-specific T-cells resulting in clinically overt diabetes.

Interestingly and similar to previous reports for the infection of the CNS by various viruses [37-41], infection of wildtype and RIP-LCMV mice with LCMV caused a very
rapid and strong expression of CXCL10 in the pancreas [16]. Importantly, CXCL10 was the only chemokine whose expression was strongly induced as early as 1 day after infection. The other two CXCR3 chemokine ligands were either expressed later (CXCL9) or were only faintly upregulated (CXCL11). Other chemokines such as CCL3 or CCL5 were not increased until day 7 post-infection, a time where most pro-inflammatory cytokines including TNFα, and IFNγ are strongly expressed as well [16]. The immediate upregulation of CXCL10 was not restricted to LCMV-infection. We observed that all viruses that directly infect the pancreas, such as the LCMV strains Armstrong and Pasteur, encephalomyocarditis virus variant B (EMC-B) or Coxackie virus B4 (CVB4), caused a rapid and strong pancreatic CXCL10 expression, whereas viruses with a tropism for other organs (encephalomyocarditis virus variant D (EMC-D), Thieier’s murine encephalomyelitis virus) did not cause any CXCL10 expression in the pancreas [16].

In summary these data suggest that LCMV-induced CXCL10 expression was direct consequence of virus infection and is not linked to the RIP-LCMV model system as such. CXCL10 was suggested to function as a ‘sentinel molecule’ in host defense against viruses before [42] and was shown to be generated very early after infection with a wide variety of viruses such as HIV, adenovirus, LCMV, Thieier’s murine encephalomyelitis virus, and mouse hepatitis virus [37-41]. In general, the ligands of the CXCR3 chemokine receptor (CXCL9, CXCL10, and CXCL11) have been suggested to play an essential role during inflammation and autoimmunity. They are mainly expressed by keratinocytes, macrophages, fibroblasts, and endothelial cells upon stimulation with IFNγ or TNFα [43, 44] but are also generated by activated T-cell hybridomas, normal T-cells, thymocytes and diabetogenic T-cell clones [45, 46]. CXCR3 is predominantly found on activated Th1-type T-cells [18, 19]. Thus, CXCR3 chemokine ligands are thought to direct the anti-viral defense towards the more aggressive Th1-type immune response and act as ‘bystander effectors’ that un-specifically activate T-cells (including autoreactive T-cells). Subsequently they propel an auto-aggressive immune response in a way that may result in autoimmune disease. Indeed, CXCL10 seems to have a significant impact on the subsequent development of autoimmunity, since blocking of CXCL10 by neutralizing antibodies abrogated T1D [16].

NEUTRALIZATION OF CXCL10 ABROGATES TYPE 1 DIABETES

We used a very well characterized neutralizing monoclonal antibody to CXCL10 that was previously shown to block the biological activity of CXCL10 Toxoplasma gondii infection model [47] and was used to neutralize CXCL10 in the CNS of mice with experimental autoimmune encephalomyelitis (EAE) [48]. In our system, blockade of CXCL10 with this neutralizing antibody abrogated T1D in >60% of all LCMV-infected mice [16]. Mechanistically, CXCL10 neutralization interfered with the expansion of LCMV-specific auto-aggressive CD8+ T-cells and their migration to the islets of Langerhans [16]. It is important to note, that the treatment had to be administered at the precise time when CXCL10 was expressed at high density. Rescued mice received 5 intravenous injections of 100 µg neutralizing antibody at days 0, 1, 2, 4, and 6 post infection. Treatment of mice before LCMV-infection or at a later time (days 7-14) was not successful in preventing autoimmune disease (U.CH. unpublished observations and reference [16]).

UNIQUE ROLE OF CXCL10 IN IMPRINTING A PATTERN FOR AUTOIMMUNE DISEASE

Among the inflammatory mediators generated upon LCMV-infection of the pancreas, CXCL10 proved to play a key role in imprinting a pattern for the subsequent development of autoimmune disease in the RIP-LCMV model. Recently, we could further confirm the important role of CXCL10 during virus-induced diabetes by investigating the kinetics of T1D in RIP-CXCL10 / RIP-LCMV double transgenic mice [49]. We generated transgenic mice which express CXCL10 under the control of the rat insulin promoter specifically in the β-cells. Such RIP-CXCL10 mice showed a spontaneous infiltration of islets by a mixed leukocyte population [49]. These mice were not diabetic but had a reduced capacity to response to a high glucose challenge possibly due to ‘inflammatory stress’ to the islets [49]. When crossed to the RIP-LCMV mouse lines the RIP-CXCL10 mice had accelerated T1D after infection with LCMV. Interestingly, islet-specific expression of CXCL10 in RIP-LCMV-NP mice, which in its single transgenic RIP-LCMV-NP state display a slow onset of disease, accelerated T1D to a degree, where its kinetic appeared very similar to the rapid-onset disease observed in RIP-LCMV-GP mice [49].

CXCL10 NEUTRALIZATION A POSSIBLE CURE FOR HUMAN T1D?

Based on these study one might come to the conclusion that CXCL10 is a prime candidate for therapy of human T1D. However, there is one major problem in translating the data obtained from the inducible RIP-LCMV mouse model to the human situation, namely the exact timing of CXCL10 neutralization. As mentioned before, the administration of neutralizing antibodies to CXCL10 had to be administered at the time of maximal CXCL10 expression, which was immediately after infection of mice with LCMV. Neutralization of CXCL10 at a later time had no influence on the course of disease, since CXCL10 is not persistently expressed during the ongoing autoimmune destruction of the β-cells. Therefore, when the theory that environmental triggering factors, such as viruses, are responsible for the initiation of T1D in susceptible individuals indeed holds true, we are still in the unfortunate position of not knowing when the detrimental triggering event is taking place. At the time of disease diagnosis (or even of islet-autoantibody detection) the triggering virus might be long gone and may not even leave any traces behind that would allow proving its existence. This circumstance has been as well hampering the attempt of firmly proving that viruses indeed are the initiating factors for human T1D and the ‘virus-hypothesis’ therefore is still based on indirect prove and epidemiological studies associating virus-infection with T1D (see [50, 51] for recent reviews on that topic).

There is however, a situation in which blockade of CXCL10 might be successful: Islet transplantation. Transplantation of islets has become one of the major hopes for patients suffering from T1D and several clinical trials are
currently being conducted. Most of these trials follow the ‘Edmonton Protocol’ according to which isolated islet transplants are being injected into the recipient’s hepatic portal vein [52]. Islet clusters subsequently settle down in the liver and start insulin production. Although the ‘Edmonton Protocol’ provides a steroid-free immuno-suppression regimen, a constant delivery of immunosuppressive drugs, such as sirolimus, tacrolimus, and daclizumab has to be maintained to prevent acute islet allograft rejection [52]. At the end islet transplantation is not a cure for the disease, it merely reestablishes a state of regulated insulin-production. From the viewpoint of the autoreactive immune system islet transplantation provides new ‘food’ for the auto-aggressive islet-specific T-cells. Only little is known about how autoaggressive CD8 T-cells are attracted to and infiltrate into islet allografts. However, CXCR3 chemokine ligands are likely candidates for mediating the recruitment of destructive cells to the transplanted islets because, first, CXCR3 chemokines are held responsible for initiating allograft rejection of various tissues including heart, lung, liver, cornea, kidney and the skin [53-58]. Second, islets are capable of producing CXCR3 chemokine ligands after virus infection [16, 17]. Therefore, blocking of CXCR3 chemokine ligands, such as CXCL10, which has been proven to be a driving force behind virus-induced T1D in the mouse, should be considered for administration during islet transplantation therapy. Thus, CXCL10 could indeed be a drug target for the treatment of human T1D, if not to directly reverse the ongoing destruction of insulin-producing β-cells, then at least to prevent or delay islet graft rejection.

REFERENCES


Received: 10 May, 2006 Accepted: 26 July, 2006