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Cure of chronic viral infection and virus-induced type 1 diabetes by neutralizing antibodies

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Abstract

The use of neutralizing antibodies is one of the most successful methods to interfere with receptor–ligand interactions *in vivo*. In particular blockade of soluble inflammatory mediators or their corresponding cellular receptors was proven an effective way to regulate inflammation and/or prevent its negative consequences. However, one problem that comes along with an effective neutralization of inflammatory mediators is the general systemic immunomodulatory effect. It is, therefore, important to design a treatment regimen in a way to strike at the right place and at the right time in order to achieve maximal effects with minimal duration of immunosuppression or hyperactivation. In this review, we reflect on two examples of how short time administration of such neutralizing antibodies can block two distinct inflammatory consequences of viral infection. First, we review recent findings that blockade of IL-10/IL-10R interaction can resolve chronic viral infection and second, we reflect on how neutralization of the chemokine CXCL10 can abrogate virus-induced type 1 diabetes.

Keywords: *Autoimmune disease, chronic infection, CXCR3, dendritic cells, LCMV, molecular mimicry*

Introduction

Lymphocytic choriomeningitis virus (LCMV) infection of mice has proven to be one of the most informative experimental systems for investigating various aspects of virology and immunology. The various LCMV models offer several acute and persistent infection systems that are well suited to reflect immune kinetics as they might occur in a variety of human chronic infections. In addition, transgenic expression of LCMV proteins as model target antigens in the pancreas or the CNS is being used in animal models for type 1 diabetes (Ohashi et al. 1991; Oldstone et al. 1991) and multiple sclerosis (Evans et al. 1996), respectively.

LCMV belongs to the arena virus family and is a natural pathogen for both humans and mice (Zinkernagel and Doherty 1974). The viral genome is comprised of two single stranded RNA segments; both segments are antisense, each encoding two proteins. The larger (L) segment encodes the L protein (the viral polymerase) and the Z protein with an undefined function. The short (S) segment encodes the nucleoprotein (NP) and the glycoprotein (GP), which undergoes post-translational cleavage to generate the two mature glycoproteins, GP1 and GP2 (Borrow and Oldstone 1997). LCMV can cause acute or persistent infection *in vivo* depending on the strain, route of infection and dose. Whereas adult mice inoculated intravenously with LCMV strain Armstrong (LCMV-Arm) rapidly clear the infection and remain immune-competent with the establishment of a stable memory T cell pool (figure 1) (Marker and Volkert 1973; Moskophidis et al. 1987), inoculation with the LCMV variant Cl13, which differs from its parent (LCMV-Arm) virus at only one amino acid

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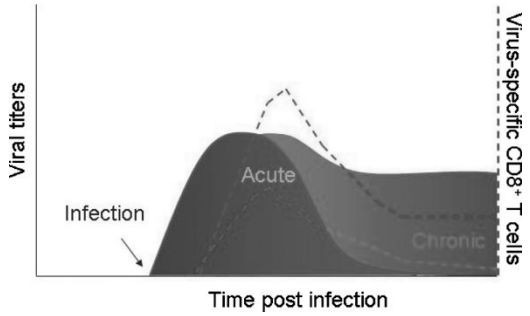

Figure 1. Mice infected with medium dose LCMV Armstrong develop an acute viral infection characterized by an initial expansion of anti-viral specific T $CD8^+$ cells (blue line) with the ability to efficiently eliminate virus infected target. During their expansion, viral antigen-specific $CD8^+$T cells acquire effector functions, including the ability to rapidly produce cytokines. Viral clearance is dependent on the presence of $CD8^+$ virus-specific cytotoxic T lymphocytes (CTLs) (Zinkernagel and Welsh 1976; Oldstone et al. 1986; Jamieson et al. 1987; Moskophidis et al. 1987); and the infection is cleared by day 10-12 from blood and spleen (Blue shaded area). After expansion of effectors and their contraction, a stable memory $CD8^+$T cell population is generated that can be maintained in the absence of antigen (blue line) (Lau et al. 1994; Murali-Krishna et al. 1999; Homann et al. 2001; Kaech et al. 2002). In contrast, infection with Cl13, results in a prolonged infection that persists (red shaded area). This chronic infection is associated with both the functional impairment, deletion of virus-specific CD8 T cells and due to lack of establishment of a memory T cells pool inability mount sufficient recall responses (red line).

positions in the virus GP is associated establishment of a chronic infection. This chronic infection is associated with both the functional impairment and deletion of virus-specific CD8 T cells and general immunosuppression (Moskophidis et al. 1993; Lin and Welsh 1998).

Chronic-persisting protracted LCMV variant Clone 13

The LCMV variant Clone 13 (Cl13) was originally isolated from the spleen of a 2-month-old mouse infected at birth with LCMV-Arm (Matloubian et al. 1990). The molecular basis of persistence and suppression of the anti-LCMV cytotoxic T lymphocyte (CTL) response has been mapped to a single amino acid change in LCMV-GP; LCMV-Arm has a phenylalanine at amino acid position 260, whereas the variant Cl13 has a leucine at this position (Salvato et al. 1991; Dockter et al. 1996). Infection of mice with high doses of Cl13 leads to a persistent infection that is associated with a lack of viral antigen specific CD8 effectors (figure 1) (Moskophidis et al. 1993; Lin and Welsh 1998).

A peripheral membrane protein, alpha-dystroglycan (α-DG), was identified as the receptor for LCMV (Cao et al. 1998). Additionally, it was observed that Cl13 binds more strongly to α-DG as compared to the Armstrong strain (Smelt et al. 2001). The affinity of the viral binding to its receptor might be a crucial element in determining the outcome of infection with LCMV Armstrong (lower affinity to α-DG) versus Cl13 (high affinity to α-DG). It was shown that differences in binding affinities of LCMV strains to α-DG correlated with viral tropism and disease kinetics (Smelt et al. 2001). LCMV-Arm and Cl13 appear to exhibit differences in their tropism within the spleen, with Cl13 causing a higher level of infection of antigen presenting cells (APCs) in the white pulp, including periarterial interdigitating DCs. Cl13 could thereby render these cells targets for more effective destruction by the antiviral $CD8^+$ CTL response which is induced at an early time point following infection with Cl13 compared to LCMV-Arm. As a consequence this CD8-dependent destruction of dendritic cells (DCs) in the spleen of Cl13-infected mice leads to the ensuing immunosuppression (Borrow et al. 1995). *In vitro* studies showing a decline of DCs after infection with Cl13 but not LCMV-Arm support the notion that the loss was related to infection (Sevilla et al. 2004). Additionally, it was reported that DCs obtained from cultures infected with Cl13, but not LCMV-Arm were non-functional, as demonstrated by their ability to stimulate allogeneic T cells (Sevilla et al. 2004). Interestingly, upon closer look, this publication also shows that the loss of DCs was mostly restricted to the CD11c + CD8α population and affected CD11c + $CD8\alpha^{neg}$ DCs to a much lesser extent.

IL-10 as a modulator of APC function

Interleukin-10 (IL-10) is an immunosuppressive cytokine with implications for various immune and inflammatory diseases. It inhibits a broad spectrum of cellular immune responses, acting on APCs by, i.e. preventing DC maturation and thereby keeps the cells in an immature state and T cells by inhibiting pro-inflammatory cytokine production. Investigations have revealed that IL-10 play important roles in blocking cytokines production (Fiorentino et al. 1991) costimulatory molecules expression, there under MHC class II, CD80 and CD86 (Ding et al. 1993; Willems et al. 1994) as well as chemokine secretion (Jinquan et al. 1993; Kasama et al. 1994) and modification of chemokine receptor expression (Sozzani et al. 1998; Takayama et al. 2001, for review see Pestka et al. 2004). Apart from the reported potent immune-modulatory effects on APCs, IL-10 also affects T_H1- and T_H2-type immune responses, as evidenced by findings in asthma, transplantation and autoimmunity models (Moore et al. 2001). IL-10 regulates the proliferation and differentiation of T_H1-type T lymphocytes, which appear to control many effector immune responses (i.e. host defense, anti-tumor immunity, autoimmunity) *in vivo* (Fiorentino et al. 1991). In different cell types, the duration of stimulation affects IL-10 expression differentially. In T cells IL-10 expression occurs shortly after stimulation

and the levels of IL-10 increase with the duration of stimulation (Wolk et al. 2002). Elevated levels of IL-10 mRNA have been observed in immune-responsive vs. non-responsive metastatic melanoma lesions (Mocellin et al. 2001). Moreover, treatment with a combination of anti-IL-10 receptor (IL-10R) monoclonal antibody (mAb) and toll-like receptor 9 (TLR9) ligands has been shown to have potent therapeutic anti-tumor effects (Vicari et al. 2002; Vicari and Trinchieri 2004), pointing to the role of IL-10 in the development of cancer. Furthermore, IL-10 has also been shown to play a role in the establishment of certain chronic viral infections such as human immunodeficiency virus (HIV-1) (Granelli-Piperno et al. 2004), hepatitis C (HCV) and human cytomegalovirus (HCMV) infections (Rigopoulou et al. 2005). Interestingly, an increase in systemic IL-10 production has been demonstrated upon HCV infection (Accapezzato et al. 2004), and in some cases of infection with HIV (Akridge et al. 1994; Ameglio et al. 1994; Autran et al. 1995; Granelli-Piperno et al. 2004; Ji et al. 2005).

Current immunotherapeutic approaches in persistent infections

As the hallmark of chronic infection is an impaired virus specific effector cell response, numerous efforts have been undertaken to increase anti-LCMV immunity. Conventional immunotherapy attempting to augment anti-viral immunity directly in persistent infected individuals have failed to affect the outcome so far, but lowering the viral antigenic load (by interferons or anti-viral drugs) has clear beneficial effects. Additionally, specific immunization strategies have been combined with direct anti-viral drug treatments, for example protease inhibitors and highly active antiretroviral therapy (HAART) in HIV (Palella et al. 1998) and interferon administration, and ribavirin in hepatitis infections (Torriani et al. 2004; Kamar et al. 2005). In all the situations, where anti-viral drugs were employed, viral loads were significantly reduced and, for HIV, long-term deleterious consequences of the persistent infection were decreased. Interestingly, the outcome of ribavirin administration in chronic HCV was not quite as promising, although viral titers were lowered, since liver fibrosis was enhanced, possibly as a rather direct effect of the drug (Torriani et al. 2004; Kamar et al. 2005). However, complete elimination of the pathogen has remained elusive, despite the fact that anti-viral immunity was significantly increased in many instances.

Much attention has been paid to the role if IL-10 as a potent anti-inflammatory immuno-suppressive cytokine with important potential clinical applications. So far it remains unclear, whether IL-10 affects the outcome of infection, amount of immunopathology and complications and could be the actual cause for persistence. One major concern in clinically manipulating the levels of IL-10 is its critical role in the immune homeostasis. Long-term application of IL-10 could cause immunodeficiency, whereas continuous use of anti-IL-10 may lead to hyper immune reactivation. However, blocking the cytokine receptor itself for a short period of time is a relative novel approach to control the signaling effects of the cytokine. To gain further insight into the function of IL-10 in the establishment and maintenance of persistent viral infections, we have investigated how blockade of the IL-10/IL-10 receptor signaling pathway affects LCMV chronic infection in its natural host, the mouse.

Neutralization of IL-10 resolves chronic viral infection

LCMV Cl13-infection results in a prolonged period of elevated IL-10 production, which is predominantly produced by CD4 + T cells already early upon infection (Ejrnaes et al. 2006). This interestingly coincides with the loss of the systemic CTL response against the virus. Treatment with a neutralizing IL-10R antibody that was previously shown to block IL-10/IL-10R interaction *in vitro* (BD Biosciences) results in accelerated viral clearance (Ejrnaes et al. 2006). This is associated with a numeric increase of total spleen cells in comparison to non-treated mice indicating that development of lymphopenia is reversed upon anti-IL10R mAb treatment (Ejrnaes et al. 2006). Furthermore, this rapid resolution of viral infection is associated with diminished levels of endogenous IL-10 and enhanced anti-viral $CD8^+$ memory T cell responses (Ejrnaes et al. 2006). Importantly, overall clinical appearance is improved through such an intervention as reflected in an increase in bodyweight, healthy shiny coat, and increase in physical activity (Ejrnaes et al. 2006). Interestingly, this protection from chronic infection was achieved with only a few injections of neutralizing anti-IL-10R mAb immediately after virus infection. Thus, the duration of treatment could be minimized in order to avoid long-term systemic immunosuppressive effects.

Previous reports suggest that different DC subsets vary in their ability to prime effector T cells (Liu 2001), and in particular, evidence suggests that DCs can be converted to APCs, which skew the immune response towards T_H2-domination, when treated with anti-inflammatory cytokines such as IL-10 (Buelens et al. 1995; Liu 2001). In this context we found that activation of LCMV-specific T cells in chronically infected mice is preferentially achieved by $CD8\alpha^-$ DCs, which induced IL-10 production by virus-specific $CD4^+$ T cells (Ejrnaes et al. 2006). Subclasses of DCs have been shown to have the potential to differentially skew cytokine production towards

T_H1- or T_H2-profiles (Mosmann and Coffman 1989). Notably, it has been suggested that $CD8\alpha^-$ DCs induce T_H2-profiles whereas $CD8\alpha^+$ DCs preferentially stimulate IFN-γ production and therefore induce T_H1 profiles (Maldonado-Lopez et al. 1999), a result we could confirm when analyzing the ability of $CD8\alpha^+$ and $CD8\alpha^-$ DCs to polarize naïve LCMV-reactive $CD4^+$T cells (Ejrnaes et al. 2006). The generalized state of lymphopenia induced by Cl13 infection might be mediated by APCs inducing IL-10 production. However, the mechanisms by which IL-10 enables Cl13 to persist are unknown. IL-10 could either down-regulate pro-inflammatory responses in a general manner or, more specifically, inhibit the induction or expansion of anti-viral CTLs. In fact, IL-10 may directly decrease the viability of $CD8\alpha^+$ DCs as has been previously suggested (Maldonado-Lopez et al. 2001; Re and Strominger 2004). In the state of chronic Cl13 infection, prominent $CD8\alpha^-$ DCs with reduced ability to prime T_H1/T_C1 effectors "by default" become the modulators of the T cell response and thus derail anti-LCMV immunity through the production of IL-10 (figure 2). While the precise mode of action of IL-10 is unknown, LCMV-specific $CD4^+$T-cells activated in this context may in turn acquire the ability to produce IL-10 and provide inappropriate or insufficient anti-viral help to other cell types, in particular $CD8^+$T cells, thus leading to persistent infection (figure 2). Additionally, it is possible that the remaining $CD8\alpha^-$ DCs, which appear ill-equipped to propagate anti-viral effectors, will continue to support IL-10 production. The resulting high concentration of IL-10 in the milieu may thus lead to further modulation of DC function and in this way become a self-fulfilling phenomenon (figure 2). As a consequence, only disruption of IL-10 signaling will have the ability to break this vicious circle and enable the recovery of appropriate anti-viral immunity by the infected host. Since blockade of IL-10 signaling likely directly acts on DCs, a central switch in immunity could be implanted in this way directly at the core, where most immune responses are orchestrated.

Virus-induced type 1 diabetes

Another possible consequence of virus infection and its accompanying inflammatory cascades is the initiation or acceleration of autoimmune disease. One scenario that might be involved in the etiology of autoimmune disease is that virus infection could break self-tolerance due to an inherent structural similarity to self-components (Oldstone 1989; Cantor 2000; Miller et al. 2001; Olson et al. 2001; Christen et al. 2004b; Christen and Herrath 2004). This concept of molecular mimicry has been integrated into animal models for various human autoimmune diseases such as type 1 diabetes (T1D), multiple sclerosis (MS), or systemic lupus erythematosus (SLE) (Christen and von Herrath 2004a). The RIP-LCMV mouse model for type 1 diabetes that was established in 1991 in the laboratories of Michael Oldstone (Oldstone et al. 1991) and Rolf Zinkernagel (Ohashi et al. 1991). These mice express the GP or NP of LCMV-Arm in the insulin-producing β-cells of the islets of Langerhans in the pancreas. Such β-cells are the target of the auto-aggressive immune system in T1D. Since the transgenically expressed viral protein is considered a component of "self", RIP-LCMV mice are tolerant and do not mount an immune response to the LCMV protein. However, infection with LCMV-Arm itself breaks this self-tolerance, auto-aggressive

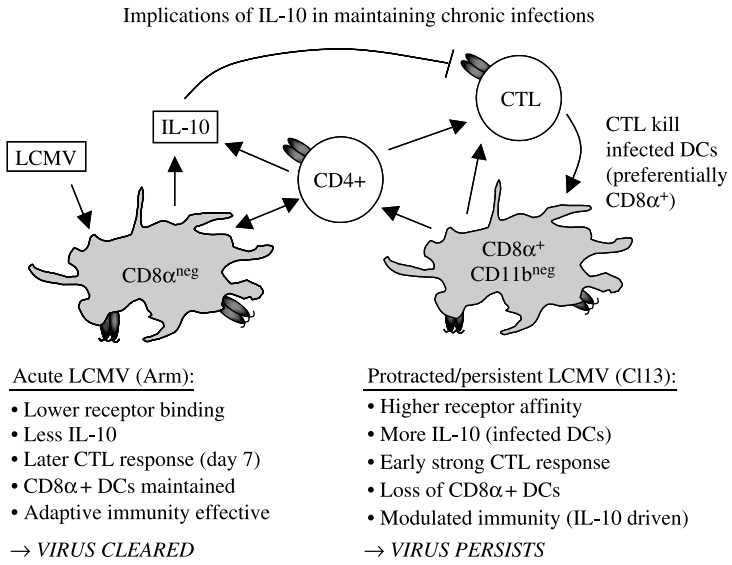

Figure 2. LCMV Cl13 persistence is the result of an early CTL-mediated loss of $CD8\alpha^+$ DCs, which indirectly supports immune responses high in IL-10 production that might be predominantly driven by the remaining $CD8\alpha^{neg}$ $CD11c^+$ DCs.

LCMV-specific T cells are generated and expanded, the β-cells are being destroyed and ultimately clinical overt diabetes results (figure 3). In the context of this review article it is important to reiterate here that LCMV-Arm is one of the isolated LCMV strains that has no immunosuppressive properties and does not result in persistent infection. In the past, this model was used to investigate mechanisms of how autoimmune processes are involved in the pathogenesis of T1D and to evaluate possible treatments for human T1D in an animal model.

Just as proposed for human T1D, the onset of diabetes in RIP-LCMV mice depends on the action of both, autoreactive CD4 and CD8 T-cells and correlates with the numbers of auto-aggressive lymphocytes generated. In accordance, the incidence of disease varied between the individual transgenic lines ranging from 2 weeks (RIP-GP lines) to 1–6 months (RIP-NP lines). Further studies revealed the mechanism involved in the rapid compared to the slow onset diabetes: Transgenic lines expressing the LCMV-GP transgene exclusively in the β-cells of the islets manifested rapid-onset T1D (10–14 days after viral challenge) (von Herrath et al. 1994). In these lines 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, in lines expressing the LCMV-NP transgene in both the β-cells and in the thymus, T1D took longer to occur after subsequent LCMV challenge. Several lines of evidence indicated that in RIP-NP mice the anti-self (viral) CTL were of lower affinity and that CD4 T-cells were essential to generate anti-self (viral) CD8 lymphocyte-mediated T1D (von Herrath et al. 1994). In addition, mouse models in which transgene-encoded "target-antigens" are expressed in the

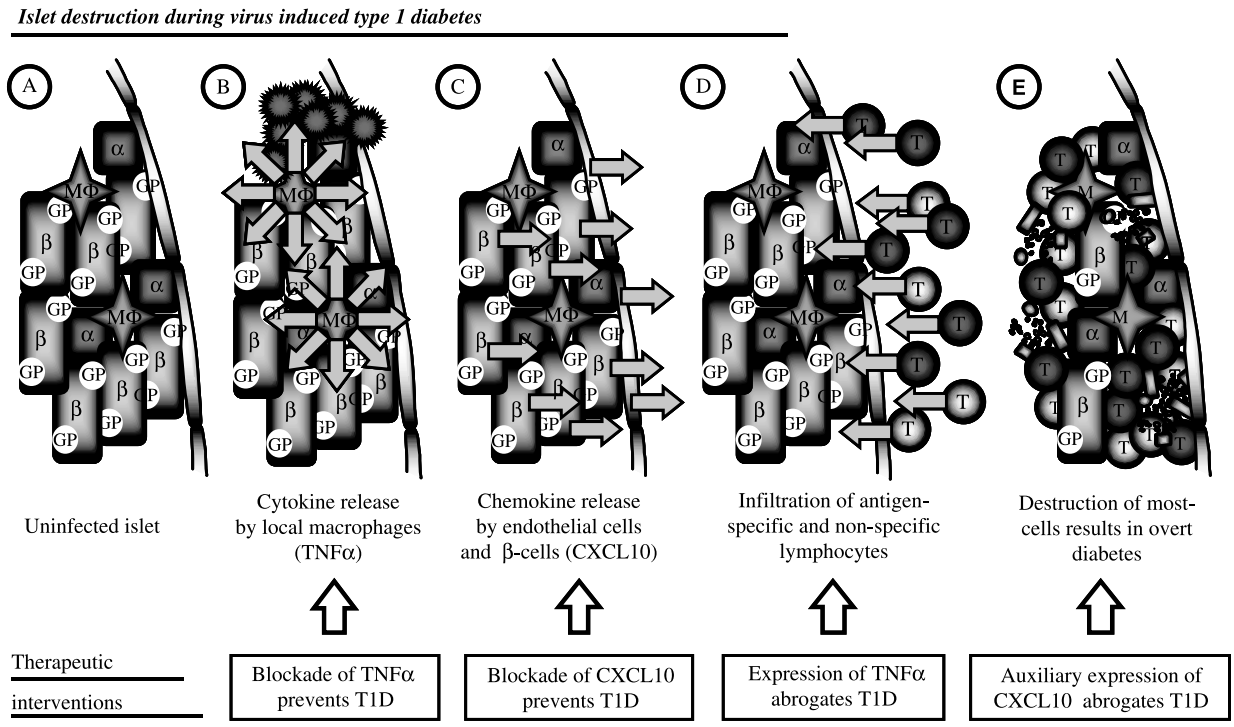

Figure 3. Therapeutic intervention during the ongoing destruction of islet cells in the pancreas. After LCMV infection active virus is found in the pancreas but not necessarily in the islets. In an initial stage local resident macrophages or dendritic cells are activated and release pro-inflammatory cytokines, such as TNFα. Blocking of TNFα at this early stage after infection can abrogate T1D (Christen et al. 2001 Christen and von Herrath 2004c) (panel B). Chemokines, most prominently CXCL10, are released by endothelial cells and β-cells (Christen et al. 2003, Frigerio et al. 2002) (panel C) which in turn attract islet-specific auto-aggressive T lymphocytes as well as non-specific bystander cells into the islets of Langerhans (Christen et al. 2003, Rhode et al. 2005) (panel D). At that time neutralization of CXCL10 prevents the subsequent development of T1D by interfering with the migration of auto-aggressive T lymphocytes to the pancreas (Christen et al. 2003). When not prevented from doing so, the first islet-specific auto-aggressive CD8 cells destroy some β-cells by perforin dependent cytolysis resulting in release of β-cell antigens (panel E). These antigens include transgenic viral proteins and additional non-viral components and are processed and presented to infiltrated CD8 and CD4 cells by APC (panel E). In the terminal stage of immunopathogenesis the majority of β-cells are being destroyed by auto-aggressive CD8 cells in a IFNγ dependent manner (Seewaldt et al. 2000). It is only in this final stage where clinically overt diabetes is apparent and can be assessed by blood glucose measurements. The destructive process at this end stage can however be reversed in the presence of high local concentrations of TNFα (islet-specific TNFα expression in a transgenic system) which can induce a status of hyper-activation resulting in apoptosis of auto-aggressive CD8 (Christen et al. 2001, Christen and Von Herrath 2002) (panels D and E). Similarly, expression of CXCL10 at an auxiliary site, such as the PDLN, can cause a recruitment of auto-aggressive CD8 cells away from the islets and a subsequent apoptosis in the PDLN by hyper-activation (Rhode et al. 2005).

pancreatic β-cells, such as the RIP-LCMV and the INS-HA (Lo et al. 1992, 1993) mouse, have demonstrated that the presence of auto-aggressive T-cells alone is not enough to cause disease. For example, when RIP-LCMV mice were crossed with mice expressing an inactive mutated form of IFNγR the diabetes incidence was drastically reduced (Seewaldt et al. 2000). These results 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 (Christen et al. 2001, 2003), oral tolerance induction (Homann et al. 1999) or DNA-vaccination (Coon et al. 1999). Besides having a clearly defined initiation point (LCMV-infection), the advantage of the RIP-LCMV system over other established models for T1D, such as the NOD mouse, is the presence of extensively characterized target antigens (GP, NP). The immune response against these target antigens can be visualized using flow cytometry by stimulation of splenocytes with LCMV-peptides or direct staining of CD8 T-cells with MHC class (-peptide tetramers (Murali-Krishna et al. 1998). In addition, we recently demonstrated tracking of LCMV-specific CD8 T-cells by *in situ* MHC class-peptide staining of quick-frozen tissue sections (McGavern et al. 2002).

CXCL10—A sentinel chemokine during inflammation

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. In general, ligands of the CXCR3 chemokine receptor including CXCL10 (interferon-induced protein of 10 kDa, IP-10), CXCL9 (monokine induced by gamma-interferon, Mig) and CXCL11 (interferon-inducible T-cell α chemoattractant, I-TAC) have been suggested to play an essential role during inflammation and autoimmunity. CXCR3 chemokine ligands are mainly expressed by keratinocytes, macrophages, fibroblasts and endothelial cells upon stimulation with IFNγ or TNFα (Luster et al. 1985; Luster and Ravetch 1987) but are also generated by activated T-cell hybridomas, normal T-cells, and thymocytes (Gattass et al. 1994). CXCL10 was suggested to function as a "sentinel molecule" in host defense against viruses (Liu et al. 2000) and is generated very early after infection with a wide variety of viruses such as HIV, adenovirus, LCMV, Theiler's virus and mouse hepatitis virus (Asensio and Campbell 1997; Lane et al. 1998; Charles et al. 1999; Hoffman et al. 1999; Kolb et al. 1999). Further, CXCR3 chemokine ligands are have been implicated in the host defense against foreign pathogens by promoting local inflammation. Blockade of CXCL10 disturbed the control of parasite propagation after *Toxoplasma gondii* infection by abrogating T-cell migration into tissues and impairing antigen-specific T-cell function resulting in amplified tissue parasite burden and an increased mortality (Khan et al. 2000). Further, transgenic (tg) CXCL10 expression in keratinocytes resulted in delayed wound healing and disorganized neo-vascularization due to a more intense inflammatory phase (Luster et al. 1998).

CXCR3 is the only cellular receptor for CXCL9, CXCL10 and CXCL11 identified to date, and is predominantly found on activated Th1-type T-cells (Loetscher et al. 1996; Sallusto et al. 1998). Thus, CXCR3 chemokines direct the anti-viral defense towards the more aggressive type 1 T-cell-domination and act as "bystander effectors" that unspecifically activate T-cells (including autoreactive T-cells) and subsequently drive an auto-aggressive immune response that may result in autoimmune disease.

Unique role of CXCL10 in imprinting a pattern for autoimmune disease

Data from our lab suggest that among CXCR3 chemokines, CXCL10 plays a unique role in imprinting a pattern for the subsequent development of autoimmunity (Christen et al. 2003; Christen and von Herrath 2004b). As previously reported for infection of the CNS by various viruses (Asensio and Campbell 1997; Lane et al. 1998; Charles et al. 1999; Hoffman et al. 1999; Kolb et al. 1999), infection of mice with LCMV caused a very rapid and strong expression of CXCL10 in the pancreas (Christen et al. 2003). Interestingly, 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). Further, chemokines such as CCL3 (MIP-1α) or CCL5 (RANTES) were not increased until day 7 post-infection, a time where most pro-inflammatory cytokines including TNFα, and IFNγ are strongly expressed as well (Christen et al. 2003). Very recently, the important role of CXCL10 during virus-induced diabetes was underlined in RIP-CXCL10 transgenic mice, which showed a spontaneous infiltration of islets by a mixed leukocyte population (Rhode et al. 2005). 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 (Rhode et al. 2005). In addition, when crossed to the RIP-LCMV mouse line the RIP-CXCL10 mice had accelerated T1D after

infection with LCMV (Rhode et al. 2005). Thus, CXCL10 is a prime candidate for a neutralization attempt to rescue mice from autoimmune disease.

Neutralization of CXCL10 abrogates type 1 diabetes

We used a very characterized neutralizing monoclonal antibody to CXCL10 that was previously shown to block the biological activity of CXCL10 *Toxoplasma gondii* infection model (Khan et al. 2000). Briefly, neutralization of CXCL10 inhibited the influx of activated T-cells into tissue and a 1000-fold higher parasite burden resulting in a higher mortality of infected mice (Khan et al. 2000). In our T1D mouse model we found that indeed blockade of CXCL10 with this neutralizing antibody abrogated disease in $>60\%$ of all LCMV-infected mice (Christen et al. 2003). In contrast blockade of CXCL9 with a neutralizing anti-CXCL9 antibody neither reduced the incidence nor prolonged the onset of diabetes (Christen et al. 2003). Mechanistically, blockade of CXCL10 interfered with the expansion of LCMV-specific auto-aggressive CD8 T-cells and their migration to the pancreatic islets of Langerhans (Christen et al. 2003). Thus, neutralization of one critical inflammatory factor at the right time could prevent the subsequent development of autoimmune disease. It is important here 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 Ref. Christen et al. 2003).

The same monoclonal anti-CXCL10 antibody was used to neutralize CXCL10 in the CNS of mice with experimental autoimmune encephalomyelitis (EAE) (Fife et al. 2001). Similar to our experiences in the RIP-LCMV model, CXCL10 neutralization decreased the accumulation of antigen-specific lymphocytes to the site of autoimmune damage (Fife et al. 2001). A different approach to neutralize CXCL10 was recently published by the Narumi group who made use of the non-obese diabetic (NOD) mouse model for spontaneous T1D (Morimoto et al. 2004). They administrated young NOD mice with a DNA plasmid encoding CXCL10. This DNA vaccination approach resulted in the *in vivo* generation of neutralizing anti-CXCL10 antibodies and suppressed the development of diabetes (Morimoto et al. 2004). In contrast to the findings in the RIP-LCMV model, the treatment did neither reduce insulitis nor alter the islet-specific immune response. It rather enhanced the proliferation of the insulin-producing β-cells resulting in a higher β-cell mass (Morimoto et al. 2004). Another interpretation of these data would be that CXCL10 neutralization relieves the islets from "inflammatory stress". Thus, β-cells from DNA-vaccinated NOD mice might proliferate at a normal rate, whereas β-cells from untreated NOD mice show reduced proliferation rates and therefore insufficient insulin production. However, the experiments by Morimoto et al. demonstrate that neutralization of CXCL10 can be successful even in a spontaneous model such as the NOD mouse.

It was previously demonstrated that CXCL10 is predominantly expressed by β-cells upon LCMV-infection of the pancreas (Frigerio et al. 2002; Rhode et al. 2005). However, recent observations in our group show that diabetogenic T-cell clones produce CXCL10 as well (Ejrnaes et al. 2005). In this study, CD8 T-cell clones specific for the islet antigens insulin, glutamic acid decarboxylase (GAD) and LCMV-GP were analyzed for expression of various chemokines and cytokines. Interestingly, there was a clear correlation between diabetogenicity and the expression of CXCL10 (Ejrnaes et al. 2005). Additional CXCL10-expression by auto-aggressive T-cells might lead to a perpetuated infiltration rate of the islets and subsequent acceleration of the destructive process.

Auxiliary CXCL10 expression can abrogate autoimmune disease

Whereas expression of CXCL10 at the site of autoimmune damage clearly accelerates the immunopathogenic process and exacerbates disease (Rhode et al. 2005), auxiliary expression of CXCL10 can, under certain circumstances, abrogate the ongoing autoimmune destruction. In the late 1980ies it was observed that infection of NOD mice with LCMV can be used as a therapeutic intervention to prevent T1D (Oldstone 1988, 1990). In the RIP-LCMV model T1D could be abrogated by a secondary infection with one particular strain of LCMV (strain Pasteur, LCMV-Past) but not with LCMV-Arm (strain Armstrong) that was used for the initial induction of disease (Christen et al. 2004a). The major difference between those two LCMV strains, that share all the immunologically relevant epitopes, is a striking discrepancy in viral growth in the pancreatic draining lymph node (PDLN). Secondary infection with LCMV-Past resulted in higher viral titers in the PDLN when compared to the pancreas and to infection with LCMV-Arm. Along with this augmented viral proliferation a differential expression of the inflammatory chemokine CXCL10 between PDLN and pancreas was observed (Christen et al. 2004a). Interestingly, a similar increase in PDLN-specific CXCL10 expression could be detected in NOD mice infected with LCMV-Past as well as LCMV-Arm (Christen et al. 2004a). In both LCMV-Past-infected

NOD and RIP-LCMV mice a significant decrease in the infiltration of islets and an increase in cellular apoptosis in the PDLN was detected (Christen et al. 2004a). Apparently, auto-aggressive cells migrated away from the islets and got stuck in the PDLN due to the high CXCL10 concentration. Once arrived at the highly inflamed PDLN the cells were hyper-activated and died by apoptosis. Thus, in such a scenario, the LCMV-infected, highly inflamed auxiliary site may the act as a filter for auto-aggressive lymphocytes (Christen and von Herrath 2005).

Summary

Neutralizing antibodies have been widely used to interfere with receptor–ligand interactions *in vivo*. Blockade of soluble inflammatory mediators and/or their cellular receptors is a highly effective way to down-regulate inflammation or prevent its negative consequences. In contrast to non-specific chemical immunomodulators or systemic treatment with cytokines, such as IFN-α, which act on a relatively broad range and thus can cause either unwanted severe side effects or general immunosuppression or -activation, neutralizing monoclonal antibodies act in a more specific way by preventing the binding of key mediators of inflammation to their cellular receptor. Nevertheless, long-term systemic administration of neutralizing antibodies inactivates the function of such mediators in their natural role in defense against harmful pathogens, such as viruses. It is, therefore, important to design a therapeutic regimen in way to reach maximal efficacy with a minimal side effects. This can only be achieved by a detailed knowledge of the mechanisms of action and the expression kinetics of such inflammatory mediators. In this review, we highlighted two recent findings from our lab that demonstrate that short term neutralization of inflammatory mediators can abrogate or reverse adverse effects of chronic inflammation. First, blockade of IL-10/IL-10R interaction can resolve chronic viral infection. Mechanistically, IL-10 neutralization restores the anti-viral immune response that was derailed by viral infection of a specific subgroup of APCs, namely the CD8α- DCs. Second, short-term neutralization of CXCL10 after in virus infection prevented the progression from inflammation to autoimmune disease in a mouse model for type 1 diabetes. In both examples, the precise timing and duration of administration of neutralizing antibodies was critical for success. Similarly, treatment of LCMV-infected RIP-LCMV mice with TNFR55-IgG1 fusion protein to neutralize TNFα was only successfully, when given early after infection (Christen et al. 2001). The finding that transgenic expression of TNFα early after infection enhanced the incidence of T1D, whereas late TNFα-expression reversed the auto-destructive process, suggested that TNFα has actually a dual role in T1D depending on the time of its expression (Christen et al. 2001). Thus, for applications of neutralizing antibodies as therapeutic agents one has to consider carefully on one hand the precise timing of treatment in order to achieved the desired protective effect and on the other hand the duration of the treatment to avoid permanent suppression or hyper-activation of the immune system.

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