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Autoimmune and inflammatory diseases

Mechanisms of autoimmune heart disease

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Dilated cardiomyopathy is a common cause of heart failure, and often results from heart-specific autoimmunity following viral myocarditis. Clinical observations and animal models greatly advanced our knowledge on the pathogenesis of myocarditis. The experimental autoimmune myocarditis model (EAM) reflects the chronic inflammatory process that results from Coxsackie B3 infection in genetically predisposed rats and mice. Animal studies suggest that cardiac self-antigen released after tissue damage and non-specific activation of the innate immune system induces heart-specific autoimmunity. Furthermore, experiments with gene-targeted mice and neutralizing antibodies allowed the identification of cytokines that are critically involved in the development of autoimmune myocarditis. Understanding the underlying mechanisms is crucial for the development of novel therapeutic approaches including cytokine and/or chemokine targeting, tolerisation and vaccination.

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Myocarditis represents a formidable challenge to clinicians because it has an erratic course of development. It is now thought that inflammatory mechanisms play a crucial role in the pathogenesis of heart disease, including dilated cardiomyopathy – the most common cause of heart failure in young patients. The authors of this review are recognized as leaders in the field and have made major contributions to the area. In this article they review the molecular and cellular mechanisms underlying autoimmune myocarditis, with a focus on the current view of autoimmune mechanisms in the pathogenesis of post-viral inflammatory heart disease.

Introduction

Dilated cardiomyopathy is the most common cause of heart failure in young patients and often follows viral infections with enteroviruses such as coxsackievirus B3 (CVB3), and to a lesser extent adenovirus. Other infectious causes include bacteria and parasites such as trypanozoma cruzi. Non-infectious myocarditis denotes heart disease without an infectious etiology. Various histopathological patterns of non-infectious myocarditis have been described and are often associated with autoimmune diseases and/or systemic disorders [1].

Patients with myocarditis show varying development of disease. Many patients manifest minor symptoms, or are entirely asymptomatic, whereas other patients follow an acute or fulminant disease course with heart failure and/or severe arrhythmias. Finally, another group of patients develop slow progressive inflammatory cardiomyopathy, as suggested by the detection of not only cardiotropic viruses such as enteroviruses [2], adenoviruses [3], cytomegalovirus (CMV), Ebstein–Barr virus (EBV), but also parvovirus B19, and hepatitis C virus in heart muscle biopsies of many patients.

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Mechanisms of post-inflammatory heart disease

Evidence for autoimmunity in human cardiomyopathy

In human, clinical studies provide indirect evidence that autoimmunity plays a crucial role in disease development. Autoimmune features include abnormal expression of human leukocyte antigen (HLA) class II on endothelial cells, a weak but significant association with HLA–DR4, and the detection of autoantibodies in approximately 30% of patients with myocarditis and dilated cardiomyopathy [4,5]. The observation that myocarditis is induced in severe combined immunodeficiency (SCID) mice after the transfer of mononuclear blood cells from cardiomyopathy patients, but not from patients with ischemic heart disease, directly supports a role for autoimmune mechanisms in mediating cardiac damage [6].

The idea that autoimmune mechanisms play an important role in the pathogenesis of post-viral cardiomyopathy suggests a beneficial effect of immunosuppression in affected patients. Despite the fact that a large clinical trial failed to demonstrate that immunosuppression improved survival and/or cardiac ejection in biopsy-proven myocarditis [7], several recently published studies suggest that autoimmune features are relevant to treat certain patients subgroups [8,9]. A randomized placebo-controlled study in patients with dilated cardiomyopathy associated with endomyocardial HLA upregulation demonstrated positive effects of immunosuppression [8]. Another study reported that immunosuppressive treatment benefited only patients with myocarditis associated with circulating autoantibodies, but with no evidence of cardiotropic infection in their myocardium [9].

Thus, increasing evidence suggests that autoimmune mechanisms play an important role in the pathogenesis of post-viral heart disease.

In vivo *models mirror the pathogenesis of viral and autoimmune myocarditis*

To develop specific treatment strategies research should (i) define the mechanistic steps leading to disease, and (ii) focus on those aspects that are accessible to therapeutic interventions. In the context of myocarditis and post-viral cardiomyopathy, our knowledge is derived primarily from animal experiments (Fig. 1).

Infection of several mouse and rat strains with coxsackievirus B3 (CVB3) results in a biphasic myocarditis characterized by (i) an early acute stage five to eight days after inoculation, followed by (ii) a chronic stage of autoimmune-mediated low-grade inflammation. This ultimately results in end-stage heart failure [10]. This biphasic disease pattern is genetically determined and only observed in Lewis rats and mice expressing $H2^a$, $H2^f$, $H2^k$ and $H2^d$ haplotypes [10]. Both viral infection and ongoing cardiac inflammation might impair cardiac function due to viral persistence [11] and/or the development of heart-specific autoimmunity [10].

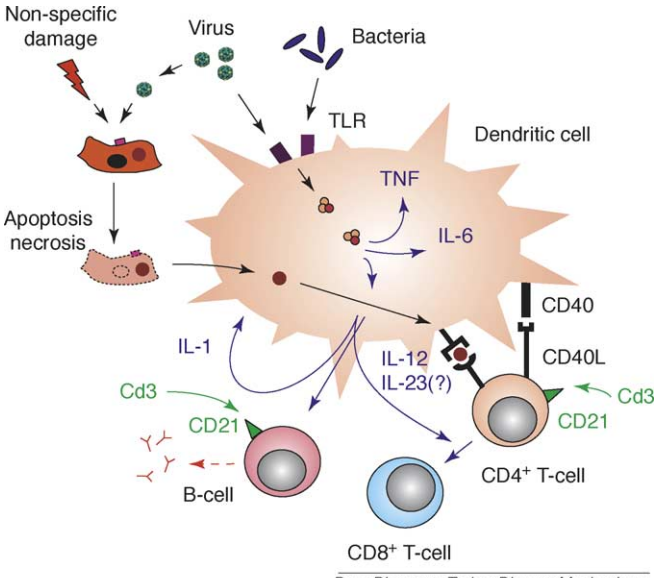

Figure 1. integrates our idea on the pathogenesis of autoimmune heart disease post-pathogen infection. Dendritic cells scavenge proteins from necrotic tissue and damaged cells and present self-antigens. They become activated by bacterial or viral products through Toll-like receptors resulting in the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, Interleukin (IL)-1, IL-6, and IL-12 (p40/p35), which play a crucial role in priming and/or differentiation of autoreactive $CD4^+$ T cells. Activated $CD4^+$ T cells upregulate CD40L and augment DC survival and IL-12 secretion by interaction with CD40. Furthermore, they help self-reactive B cells to produce IgG autoantibodies and they can sustain cytotoxic $CD8^+$ T cell memory. Activation of the complement cascade and production of complement components such as C3d and C3b as part of the acute phase response can promote the activation of both B and $CD4^+$ T cells.

If autoimmune mechanisms are important in developing chronic inflammatory heart disease, can heart-specific T- or B-cell responses be induced by immunization with self-proteins? In fact, immunization of susceptible mice [12] and rats [13,14] with α-myosin or α-myosin peptides [15] and potent adjuvants induces autoimmune myocarditis with a similar histological picture to that observed during the chronic phase of viral myocarditis [10,12]. In the experimental autoimmune myocarditis animal model, disease severity assessed by histology usually peaks three weeks after immunization [12], followed by ventricular dilation and heart failure several weeks later [16,17]. It is important to note that the histological picture varies among different mouse strains. In rats, distinct histological patterns suggest a morphology that closely resembles human giant cell myocarditis [13]. These experimental findings implicate autoimmunity in the development of post-viral cardiomyopathy.

Molecular mimicry and heart-specific autoimmunity

Immunodominant peptides derived from cardiac α-myosin have been characterized, that can mediate autoimmune myocarditis in BALB/c ($H2^d$) [18] and A/J ($H2^k$) mice [19]. The

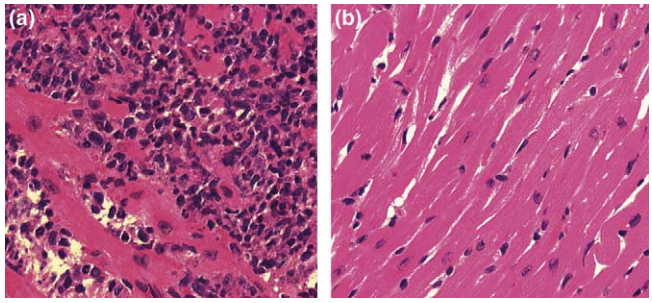

Figure 2. Autoimmune myocarditis develops after immunization of BALB/c ($H2^d$) mice with α-myosin heavy chain peptide MYHC-α loaded dendritic cells, activated with lipopolysaccharide (LPS) (b) but not after injection of activated dendritic cells loaded with irrelevant, non-cardiac peptide (a). Inflammatory infiltrates consist of macrophages, granulocytes, eosinophils and lymphocytes. (hematoxylin and eosin-staining; 400× original magnification).

dominant autoaggressive epitope located in the head-like structure of the molecule (MYHC-α, aminoacids 614-643) contains a motif xMAxxSTxx, which is also found in cardiotropic bacteria such as borrelia and treponema [18]. Furthermore, common amino-acid sequences between rat cardiac myosin and coxsackie B3 virus (CVB3) have been identified and these peptides are indeed pathogenic in rats [14]. These observations suggest that antigenic mimicry might be responsible for autoimmune myocarditis in individuals previously infected with these pathogens.

In mice, however, the most pathogenic peptides inducing autoimmune myocarditis do not correspond to regions that exhibit homology to coxsackievirus B3 (CVB3) proteins [10]. Thus, cross-reactivity between pathogen-derived and self-derived antigens is not absolutely required for cardiac autoimmunity. Alternatively, it has been suggested that infections provide an unspecific 'adjuvant-like' effect for induction of autoimmunity [20]. In fact, we recently found that myocarditis can be induced in naïve mice by injection of dendritic cells, which were before loaded with myosin peptide and stimulated with toll-ligands such as LPS, CpG, or double-stranded RNA *in vitro* [17]. This suggests that release of self-antigen after cellular damage and activation of dendritic cells by a 'danger signal' can break immunotolerance and induce autoimmune myocarditis (Fig. 2). Nevertheless, this possibility does not exclude a role for antigenic mimicry in putting the organism at risk for autoimmune heart disease.

Development of heart-specific autoimmunity

Autoantibodies are not essential for development of experimental autoimmune myocarditis [21], although they might be of functional relevance in other models of cardiac autoimmunity. Autoantibodies against extracellular cardiac tropomyosin, for example, appear to be involved in the development of spontaneous, non-viral heart failure in mice lacking the programmed cell death (PD-1) receptor [22].

By contrast, there is little doubt that T cells are crucial for coxsackie B3-induced chronic myocarditis [23]. So far, three distinct types of T-cell responses have been found in coxsackievirus B3-induced murine myocarditis. An early innate and CD1d-restricted $V\gamma4^+$ T-cell response enhances an adaptive $CD4^+$ γδ T-cell response, which finally promotes $CD8^+$ αβ T-cell receptor $(TCR)^+$ cytotoxic T-cell-mediated cardiac damage [24]. However, naïve $CD8^+$ T cells specific for ovalbumin (OVA) induce cardiac autoimmunity in transgenic mice that express cardiac myocyte-restricted membrane-bound OVA only after inection with OVA expressing vesicular stomatitis virus (OVA–VSV) [25]. By contrast, it has been demonstrated that $CD4^+$ T cells largely mediate autoimmune myocarditis [17].

The T helper 1 (Th1)–T helper 2 (Th2) concept fails to satisfactory explain autoimmune heart disease

$CD4^+$ T-cell responses can be subdivided in Th1 and Th2 responses, which are crossregulated and probably evolved to protect mammals from infection with intracellular pathogens and nematodes, respectively. The Th1–Th2 concept has also been used to explain regulation of organ-specific autoimmune diseases. It has been widely believed that an inflammatory Th1 type immune response is responsible for autoimmune pathogenicity, whereas Th2 type reponses inhibit disease development. The cytokine interferon gamma (IFN-γ) has long been considered as essential for the expansion and effector function of autoreactive Th1 $CD4^+$ T cells. Data from studies with patients show predominantly IFN-γ producing Th1 cells in target organs of patients with organ-specific autoimmunity. Recent findings in animal models, however, challenge this view (paradigm/dogma). In fact, mice lacking IFN-γ or IFN-γ receptor develop an increased severity of myocarditis with a high prevalence of dilated cardiomyopathy and mortality [26]. Furthermore, ectopic expression of IFN-γ in the pancreas of mice confers resistance to myocarditis following lethal coxsackievirus B3 (CVB3) infection [27]. These results demonstrate that IFN-γ can protect against autoimmune and viral myocarditis. The mechanisms for IFN-γ-mediated inhibition of autoimmune pathogenesis are not known, but can involve induction of T-cell apoptosis [28]. Conflicting reports have been published with respect to the role of the prototypic Th2 cytokine interleukin 4 (IL-4) in autoimmune myocarditis. It has been suggested that autoimmune myocarditis in mice is a Th2-mediated disease [29]. Indeed, treatment of A/J mice with an anti-IL-4 antibody markedly reduced disease severity in a disease characteristic of a Th2 response [29]. By contrast, IL-4-receptor-deficient mice on the BALB/c ($H2^d$) background are not protected from autoimmune myocarditis [30]. Furthermore, the most pathogenic T-helper-cell population

mediating cardiac damage after CVB3 infection of BALB/c ($H2^d$) mice display a Th1 phenotype [24]. In rats, recovery from myocarditis is associated with Th2 responses, whereas dominant Th1 responses correspond to more severe diseases [31]. In conclusion both, Th1 and Th2 $CD4^+$ T cells can contribute to immune-mediated heart disease. The question regarding Th1 versus Th2 is extremely complex, and depends on species, genetic background, and the concomitant infectious or inflammatory agent inducing autoimmunity.

There is strong evidence that the cytokine IL-12, a key factor for Th1 development, is critically involved in the development of autoimmune diseases mediated by T cells [32]. Interleukin 12 (IL-12p70) is a heterodimer consisting of IL-12p40 and IL-12p35 subunits, which signals by binding to the IL-12 receptor (IL-12R) heterodimer composed of IL-12Rß1 and IL-12Rß2. Upon receptor engagement, signal transduction is mediated by recruitment of Janus family tyrosine kinase 2 (JAK2) and a signal transducer and activator of transcription (STAT) 4. Administration of IL-12p70 to rats exacerbates EAM [32], whereas absence of IL-12p40, IL-12Rß1, or STAT4 in genetically deficient mice completely protects from EAM [30].

Furthermore, mice lacking the IL-12Rß1 chain are also protected from coxsackievirus B3-mediated cardiac inflammation possibly due to increased viral clearance [33]. These results indicate that IL-12 is crucial for the development of autoimmune myocarditis. However, recent findings promote re-interpretation of the above data. IL-23, a novel cytokine of the expanding IL-12 like cytokine family, is composed of a unique p19 subunit and the p40 subunit shared with IL-12. Moreover, the IL-23 receptor shares the ß1 chain with the IL-12R and another transmembrane protein [34]. Therefore studies in mice lacking IL-12p40 or IL-12Rß1 do not enable a clear interpretation with respect to distinct activities of IL-12 and IL-23. In fact, recent studies demonstrate that IL-23 rather than IL-12 is responsible for autoimmune encephalitis and arthritis [34,35]. Whether IL-23 and not IL-12 is the factor inducing autoimmune myocarditis is under investigation.

Heart resident dendritic cells

Induction of autoimmune myocarditis requires priming of autoreactive T-cells and their migration to the target organ, the heart, where they interact with resident tissue cells expressing self-antigen loaded MHC class II molecules. Expression of activation markers on tissue resident dendritic cells always precedes inflammatory infiltrates in the course of autoimmune myocarditis [19,36,37]. Tissue resident dendritic cells exhibiting dendritic cell morphology are bone marrow-derived [36] and express self-antigen even in the healthy heart [19]. Their role in the healthy heart is not clear but they might scavenge for physiologic cell debris or mediate a role in the maintenance of tolerance against potential heart-specific autoreactive responses. So far, there is only indirect evidence on the role of dendritic cells in the myocardium, and in the induction and maintenance of myocarditis. As antigen-presenting cells, they might be directly involved in the generation or exacerbation of autoimmune responses after activation. Furthermore, these cells might be crucial for the attraction and recruitment of T cells, as well as other inflammatory cells. Various chemokines and cytokines are capable of fine-tuning the functional role of heart resident dendritic cells. Tumor necrosis factor (TNF)-α, for example, has been recognized as a key cytokine mediating activation of tissue resident dendritic cells. In the context of autoimmune myocarditis, mice lacking the TNF-receptor p55 are protected from disease [37]. Similarly, signaling through the IL-1 receptor type 1 is essential for myocarditis development in female mice. It appears that IL-1β plays a central role in the activation of self-antigen presenting dendritic cells [38].

Heart resident dendritic cells seem to play an important role in the pathogenesis of heart-specific inflammation. Activation of these cells is mediated by specific cytokines and required for both, recruitment and *in loco* expansion of autoreactive T cells.

Development and maintenance of heart-specific tolerance

Myocarditis induction always requires the combined release of self-antigen and non-specific activation of the immune system. In the absence of a non-specific inflammatory response, disease does not develop. Thus, it is conceivable that protective or down-regulatory mechanisms exist that protect from spontaneous autoimmune responses after tissue damage. If such protective mechanisms involve suppressive T cells it should be possible to induce self-antigen-specific tolerance by selective expansion of suppressor cell populations. In fact, mice can be vaccinated against autoimmune myocarditis by application of myosin-loaded splenocytes [39]. Tolerance can also be induced in mice by repetitive intranasal exposure to myosin [40]. These findings are promising, but the mechanisms of tolerance induction are not known. Growing evidence suggests that dendritic cells not only induce autoimmune responses, but are also critically involved in the maintenance of peripheral tolerance probably by induction of suppressive T cells. Whether peripheral tolerance is antigen-specific is still a matter of debate. Extrapolating recent findings to autoimmune myocarditis, we suggest that tissue resident dendritic cells in the heart constitutively expressing self-antigen, maintain tolerance in the absence of activation stimuli exceeding a threshold. This concept is of great interest for potential clinical application. If post-infectious myocarditis emerges from the coincidence of tissue damage and TLR activation by microbial or endogenous products, we expect the following: pre-vaccination with non-activated or minimally (partially) activated (semi-mature), myosin-peptide loaded dendritic cells might protect from autoimmunity by expanding a pool of myosin-specific

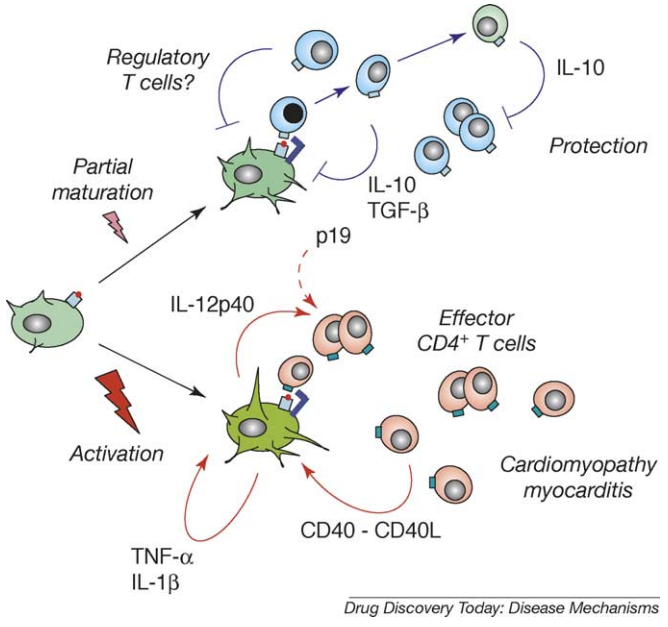

Figure 3. Cytokines are not only involved in priming of autoreactive T cells, but most probably also in tolerance development and downregulation of ongoing inflammatory responses. Dendritic cells appear to be crucial for both induction of autoreactivity after excessive activation and downregulation of ongoing inflammation after partial activation. IL-1β, IL-12, and most probably IL-23, which consists of the IL-12p40 and the p19 subunits, promote the development of autoreactive T cells. IL-10, and probably also Transforming growth factor (TGF)-β play a down-regulatory disease-limiting role. The role of regulatory T cells (Treg) in regulation of autoimmune myocarditis remains to be investigated.

regulatory T cells. Importantly, this approach would not reduce the efficacy of a virus-specific immune response (Fig. 3).

Conclusion

In conclusion, recent clinical and experimental research greatly expanded our knowledge of the pathogenesis of post-viral heart disease. Clinical observations and animal models suggest that autoimmune mechanisms play a crucial role in the development of myocarditis and progression to heart failure. In particular, clinical studies defined subgroups of patients that might respond to immunosuppression or specific cytokine targeting according to immunologic, genetic and molecular criteria. Animal models helped us to understand the process of priming and expansion of heart-specific T cells, as well as the role of various specific inflammatory mediators. We predict that our current view on the mechanisms provides a basis for the development of future therapies against the devastating and often fatal course of inflammatory cardiomyopathy.

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