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Immunology in the clinic review series; focus on type 1 diabetes and viruses: enterovirus, thymus and type 1 diabetes pathogenesis

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Summary

Thymus dysfunction, especially immune suppression, is frequently associated with various virus infections. Whether viruses may disturb the thymus function and play a role in the pathogenesis of autoimmune diseases is an open issue. Enteroviruses, especially Coxsackievirus B4 (CV-B4), have been largely suggested as potential inducers or aggravating factors of type 1 diabetes (T1D) pathogenesis in genetically predisposed individuals. Several pathogenic mechanisms of enterovirus-induced T1D have been suggested. One of these mechanisms is the impairment of central self-tolerance due to viral infections. Coxsackievirus-B4 is able to infect murine thymus *in vitro* and *in vivo* and to infect human thymus *in vitro*. Thymic epithelial cells and thymocytes are targets of infection with this virus, and several abnormalities, especially disturbance of maturation/differentiation processes, were observed. Altogether, these data suggest that CV-B infection of thymus may be involved in the pathogenesis of T1D. Further investigations are needed to explore this hypothesis.

Keywords: Coxsackievirus B4, human, mouse, thymus, type 1 diabetes

Introduction

Infection of the thymus with viruses is an issue that has been addressed but has been poorly investigated, except in the case of human immunodeficiency virus (HIV) infection [1]. As well as HIV, other viruses can infect the thymus which may have consequences on the architecture and functions of that organ. Marked abnormalities of the thymus and its functions have been reported in the course of viral infections, although the presence of viruses in the thymus has not been evidenced [2].

The thymus is a major part of the immune system, therefore infection of that organ with a virus can facilitate immune tolerance towards viral antigens, and thus may greatly influence the outcome of the infection, with persistence of the virus in the host [3,4]. Thymus being the central site for self-tolerance establishment, it cannot be discounted that a viral infection may lead to thymus dysfunction resulting in disturbed self-tolerance, possibly involved in autoimmune pathogenic processes.

Type 1 diabetes (T1D), also termed 'juvenile diabetes' or 'insulin-dependent diabetes', which is due to selective destruction and/or impairment of pancreatic insulin-producing β cells, is accompanied frequently by severe complications, and is becoming a huge public health problem as its incidence is in continual and dramatic increase all over the world [5,6].

The relationship between enteroviruses, especially type B Coxsackieviruses (CV-B) and T1D, in genetically predisposed individuals has been highlighted largely through epidemiological studies [7–11]. Several mechanisms not mutually exclusive have been suggested to elucidate the viral pathogenesis of T1D [11,12]. One of the possible mechanisms is the disturbance of central tolerance as a result of the infection of thymus with viruses.

Viral infections can disturb the thymus

Clinical evidence and experimental findings show that viral infections are responsible for thymus abnormalities and

dysfunctions, although in some cases the organ has not been reached by the infectious agent.

HIV

HIV, belonging to the *Retroviridae* family, is the virus that has been associated most frequently with thymus disorders in the literature.

HIV has been detected in thymuses of infected individuals [13–16]. In addition, human intrathymic T cell precursors and their progeny, representing many stages of T cell ontogeny, have been demonstrated to be susceptible to HIV-1 infection *in vitro* [17]. The chimeric severe combined immunodeficiency–human (SCID-hu) xenograft mouse model (bearing human T cells derived from transplantation of human thymic fragments and liver tissue under the renal capsule) showed that human thymocytes are also susceptible to HIV infection *in vivo* [18,19]. Moreover, it is likely that HIV infects the thymic microenvironment, as marked disruptions and significant viral loads have been observed in the thymic compartment in HIV-infected SCID-hu mice [19], a finding that was confirmed later by the demonstration of infected thymic dendritic cells [20]. Whether or not the thymic epithelium is also infected by this virus is an issue that deserves further investigation. Indeed, thymic epithelial cells (TEC) were shown previously to contain HIV RNA in human autopsy samples and also in the SCID-hu mouse model, although productive infection of these cells could not be demonstrated definitively [14,19]. Furthermore, the same SCID-hu mouse model showed degenerating TEC even without detection of HIV in these cells [19]. The relevance of TEC infection lies in the fact that these cells play a critical role in the differentiation of T cell precursors, providing a microenvironment with a unique capacity to generate functional and self-tolerant T cells [21,22].

HIV infection is generally accompanied by several cytological and histological abnormalities in the thymus network. Indeed, autopsy and biopsy studies have demonstrated that the thymus in HIV-infected individuals is abnormal, with marked involution, effacement of the medulla and cortex, depletion of epithelial elements, thymocyte apoptosis, variable degrees of plasma cell infiltration and fibrosis and absence of Hassall's corpuscles [2,14,23,24]. Studies using the SCID-hu mouse showed similar abnormalities [19].

Damage to the thymic epithelium may alter the thymic microenvironment and contribute to the immune suppression observed in acquired immune deficiency syndrome (AIDS) patients and models. Importantly, it has been observed that thymic epithelial fragments from AIDS children arrest T cell differentiation of normal bone marrow-derived $CD34^+$ stem cells *in vitro* [25]. Similarly, HIV-1 infection has been shown to interrupt thymopoiesis *in vivo* in the SCID-hu mouse model [26].

The thymus releases mature lymphocytes into the periphery of the immune system. This function can be evaluated through analysis of recent thymic emigrants (RTEs) [27], that themselves can be estimated by the presence of T cell receptor excision circles (T_{recs}), circular DNA fragments derived from the rearrangement of TCR genes, that remain within RTEs [28]. T_{rec} analysis in HIV and simian immunodefiency virus (SIV) infections revealed decreased numbers of T_{rec}^+ T lymphocytes in the peripheral blood compared with uninfected individuals [29,30]. Interestingly, specific highly active anti-retroviral therapy seems to correct this defect in AIDS patients [31].

Another important feature is that the thymic secretory function is also affected in HIV-infected individuals, as the blood levels of thymic peptides are abnormal [23]. For example, thymosin α_1 levels are elevated in many patients with AIDS, especially in the early stages [23,32]. In contrast, a consistent and long-term diminution of thymulin secretion has been documented in AIDS patients, in terms of both serum levels and intrathymic contents of the hormone [24,33,34].

Hepatitis viruses

It is known that mouse hepatitis viruses (MHV), which are members of the *Coronaviridae* family, show a tropism to thymic stromal cells [35] and T lymphocytes [36]. Otherwise, thymus involution was described in MHV-A59-infected BALB/c mice [37]. That involution was characterized by a severe transient atrophy resulting from apoptosis of immature $CD4^+CD8^+$ T cells that might be caused by infection of a small proportion of TEC.

Marked thymic involution characterized by striking diminution of thymus weight and cellularity was also observed in CBA mice infected intraperitoneally with MHV-3, together with a significant decrease in thymocyte subpopulations and significant numbers of apoptotic cells [38].

In humans, T_{rec} quantification revealed an impairment of RTEs, reflecting a thymic dysfunction in hepatitis C virus (HCV)-infected patients [39].

Measles

Measles, a member of the *Paramyxoviridae* family, is generally followed by immune suppression with transient lymphopenia and impaired cell-mediated immunity [40,41]. Impaired thymic function seems to contribute to measles virus-induced immune suppression. Indeed, measles virus infects TEC and monocytes in the thymus of humans and monkeys [42,43], leading to a decrease in the size of the thymic cortex [44,45]. In human thymic implants in SCID-hu mice, measles virus infection of TEC causes thymocyte apoptosis [46]. Measles virus replication in human TEC *in vitro* results in terminal differentiation and apoptosis [47]. Surprisingly, with regard to thymic output, an increase

in TREC$^+$ CD4$^+$ T cells has been reported in measles virus-infected children despite severe lymphopenia [48].

Cytomegalovirus (CMV)

Infections with CMV (belonging to the Herpesviridae family) are also immunosuppressive, resulting in poor cellular responses from cultured blood leucocytes, low CD4/CD8 ratios and potential secondary infections [49].

At the thymic level, CMV infection in the SCID-Hu mouse results in high and persistent viral replication in the thymus. The majority of virus-infected cells were localized in the thymic medulla and immunofluorescence analysis identified TEC rather than any haematopoietic cell population as the principal hosts for viral replication [50].

Infection of BALB/c mice with murine (M)CMV decreased the numbers of cells recovered from the thymus by 80–90% after 4–7 days, although fewer than 0·001% were infected productively with the virus. A loss of cortical thymocytes was evident in histological sections and correlated with depletion of CD4$^+$CD8$^+$ cells [51].

Rabies virus

Suppression of cell-mediated immunity is also a common feature of rabies virus infection [52,53]. This phenomenon relies essentially upon thymocyte apoptosis and thymus atrophy (despite no evidence of virus infection), as observed in numerous studies carried out in mice [52,54–56].

Altogether, these data show that viruses belonging to various families can infect the thymus *in vivo* and *in vitro*. Clearly, viruses can impair thymus functions significantly.

Thymus and T1D

Like any autoimmune disease, T1D results from self-tolerance breakdown. Self-tolerance establishment is initiated at the central level within the thymus. Thus, it cannot be excluded that disturbance in thymic architecture and/or function may play a role in the development of autoimmune processes. At the peripheral level, self-tolerance is based on regulatory T cells (T_{reg}), a specialized subset of T cells whose functions include the suppression of autoreactive T cells.

In the case of T1D, pancreatic islet β cells are targeted selectively by the autoimmune destruction process, meaning that there is a defect in the recognition of islet β cell antigens. Anomalies in T_{reg} cells functions and numbers have been associated with autoimmunity towards islet β cells and are thought to play a role in the progression of T1D [57]. At the thymic level, this defect can arise from several aberrations encountered during T cell education through positive and negative selection.

Alteration of the repertoire affecting positive selection

During positive selection, the newly rearranged TCRs expressed on developing thymocytes interact with MHC molecules on cortical TEC; thus, any anomaly in MHC and/or TEC may lead to aberrant positive selection. Diabetes-associated MHC class II molecules [I-A^{g7} in non-obese diabetic (NOD) mice and human leucocyte antigen (HLA)-DQ8 in humans] mediate the selection of a large number of autoreactive T lymphocytes with strong affinity for islet β cell autoantigens [58]. Interestingly, the grafting of purified TEC from embryos of NOD mice to newborn C57BL/6 nude mice results in the development of insulitis, suggesting a functional anomaly in TEC from NOD mice cells [59].

Defect of negative selection

During negative selection, developing T cells interact with thymic epithelium- and bone marrow-derived antigen-presenting cells (APCs), in particular thymic medullary dendritic cells. Thus, aberrant negative selection results essentially from anomalies affecting thymic APCs.

Like the majority of ubiquitous or organ-specific autoantigens, several islet β cell antigens involved in T1D, such as glutamic acid decarboxylase (GAD) and proteins of the insulin family, are expressed promiscuously in the thymus to be presented to thymocytes during education [60,61]. The decreased expression of these antigens can disturb the negative selection of autoreactive T lymphocytes, which may predispose to the development of autoimmunity. In humans, susceptibility to T1D is associated with a polymorphism in the 5′ region of the insulin gene, which influences the rate of expression of peptides derived from insulin by APCs in the thymus. The protective allele is associated with a high level of thymic expression of insulin and the susceptibility allele to a low level [61]. NOD mice which express neither the pro-insulin 2 nor the islet-cell antigen 69 (ICA69) in the thymus develop diabetes rapidly [62,63], as in BioBreeding Diabetes Prone (BBDP) rats, which do not express type 2 insulin-like growth factor (Igf2) in thymus [64]. Furthermore, depletion of Ins2 expression in medullary TEC is sufficient to break central tolerance and induce anti-insulin autoimmunity and rapid diabetes onset in mouse [65].

Interestingly, intrathymic transplantation of pancreatic islet cells reduces autoimmunity towards β cells and prevents diabetes development in NOD/Lt mice [66]. Thus, the thymus could also play a role in acquired tolerance and may be a potential candidate in the therapeutics of autoimmune diseases.

Negative selection might also be affected owing to antigen-processing defects. A defect of peptide presentation can result from the weak affinity of TCR for unstable MHC–peptide complexes and/or from a defect in antigen processing by proteases of thymic APCs [58,67].

Major defects in the architecture of the thymic stroma found in animal models of diabetes are also thought to contribute to a defect in negative selection [58,67]. In NOD mice, for example, medullar TEC are present in the cortex,

and large areas devoid of TEC and expression of MHC molecules are observed in the thymus [68]. Multiple thymocyte migration-related abnormalities have also been observed in the NOD mouse thymus [69].

Because negative selection is based on the apoptosis of autoreactive T lymphocytes, it is possible that a defect of apoptotic factors and/or a genetically determined resistance of thymocytes to apoptosis (as described in the NOD mouse [70]) can contribute to impaired negative selection [58,67].

Thymus and Coxsackievirus B4

Epidemiological studies have clearly shown an association between enterovirus infections, especially CV-B and T1D, and strongly support the role of these viruses as potential triggers of that disease in genetically predisposed individuals [7–10]. Experimental investigations suggest that several pathogenic mechanisms of CV-B4 infection may be involved in the impairment of pancreatic β cells [7–10]. Our group has investigated the hypothesis of virus-induced disturbance of thymus in the development of autoimmunity against these cells (see Fig. 1).

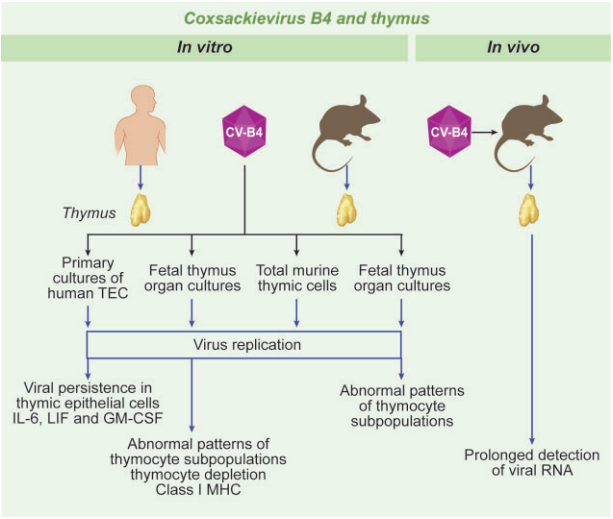

Fig. 1. Coxsackievirus B4 (CV-B4) and thymus. Overview of experimental studies performed *in vitro* in human and mouse systems and *in vivo* in mice. Thymus fragments, obtained from children undergoing corrective cardiovascular surgery for congenital cardiopathies, were processed for isolating thymic epithelial cells (TEC). Human and mouse fetal thymus organ cultures were performed and total thymocytes, isolated from mouse thymus, were cultured. CV-B4 can infect thymocytes and TEC, which results in abnormal patterns of thymocyte populations, overproduction of cytokines and increased expression of class I major histocompatibility complex (MHC). The virus can replicate and persist in TEC. Oral inoculation of CV-B4 to 3–4-week-old Swiss albino mice results in persistence of viral RNA (up to 70 days) in thymus.

It was observed that both CV-B4 diabetogenic (E2) and prototype (JVB) strains can replicate and persist in human TEC *in vitro* with increased production of interleukin (IL)-6, leucocyte migration inhibition factor (LIF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) [71]. In fragments of human fetal thymus, the virus principally infects $CD4^+CD8^+$ immature thymocytes and induces increased expression of MHC class I molecules and a severe thymocyte depletion [72].

Because CV-B4 was also able to infect TEC and immature thymocytes, it was hypothesized that the virus was potentially susceptible to modulate the thymic function. To explore this hypothesis more effectively, and due to the difficulty of undertaking experiments in the human system, further studies were performed in a murine model.

It was demonstrated that the diabetogenic strain CV-B4 E2 can reach the thymus *in vivo* in the course of a systemic infection of outbred Swiss albino mice inoculated through the oral route, the natural contamination route in humans [73]. The infection was characterized by a prolonged detection [until 70 days post-infection (p.i.)] of viral RNA by reverse transcription–polymerase chain reaction (RT–PCR) in the thymus.

When primary cultures of total murine thymic cells were inoculated with CV-B4 E2 and CV-B4 JVB, both viral strains infected and replicated in these cells, as attested by the detection of intracellular negative-strand viral RNA and release of infectious particles in culture supernatants [74]. These findings suggest that thymic cells can play a role in virus dissemination, and therefore in the pathophysiology of CV-B4 infections.

The infection of murine fetal thymus organ cultures was then investigated [75]. It was shown that CV-B4 E2 could replicate within this system, as attested by the detection of intracellular negative-stranded viral RNA by real-time quantitative RT–PCR and infectious particles in culture supernatants. As evidenced by flow cytometry analysis, CV-B4 E2 lead to abnormal patterns of thymocyte populations: a marked increase in the percentages of $CD4^-CD8^-$, $CD4^+$ and $CD8^+$ cells and a decrease in the percentage of $CD4^+CD8^+$ cells.

The increased proportion of $CD4^-CD8^-$ thymic T cells observed *in vitro* is reminiscent of previous data obtained *in vivo* in a murine model of CV-B4 E2-induced T1D [76]. Indeed, in that study the virus, inoculated through the intraperitoneal route, was cleared rapidly from the thymus but led to a significant increase in $CD4^-CD8^-$ thymic T cells preceeding the onset of hyperglycaemia.

Conclusion and perspectives: thymus, enterovirus and the pathogenesis of T1D

CV-B4 infection of the thymus has been described in human tissue *in vitro*, and in mice *in vivo* and *in vitro*, and the

infection results in the disturbance of T cell differentiation/maturation processes [71–76].

The role of alterations in T lymphocyte subsets in the development of T1D cannot be excluded in so far as they have been observed already in NOD mice [77], in BB rats [78] and also in diabetic patients [79,80]. Whether enterovirus-induced disturbances of thymic cells can play a role in T1D pathogenesis by impairing T cell differentiation and/or central self-tolerance establishment should be investigated further in experimental models *in vitro* and/or *in vivo*.

For a clearer understanding of the complex interplay between enterovirus and the thymus in the viral pathogenesis of T1D, the link remains to be made between thymus infection and the development of the disease in human beings. Interestingly, in a recent study macrophages infected with an enterovirus (poliovirus) were evidenced in thymus of some patients with myasthenia gravis, suggesting a viral contribution to the intrathymic alterations leading to the disease [81]. Furthermore, CV-A and CV-B have already been found in human perinatal and neonatal thymus in favour of vertical transmission of the viral infection [82,83]. Whether enteroviruses are present in the thymus of patients with T1D or patients in the preclinical stages of the disease merits further study.

In T1D, the tolerance of immune system towards β cells is disturbed at the peripheral level through T_{reg} dysfunction [57]. A disturbance of tolerance at the central level through the infection of thymus with enteroviruses cannot be discarded, and could play a role in the pathogenesis of T1D (see Fig. 2).

The potential role of thymus dysfunction in the pathogenesis of T1D opens the possibility of targeting this organ for preventive and therapeutic strategies. Indeed, there are increasing promising insights towards intrathymic manipulation. On the basis of the close homology and cross-tolerance between insulin, the primary T1D autoantigen and Igf2, the dominant thymic self-antigen of the insulin family, a novel type of vaccination, so-called 'negative/tolerogenic selfvaccination', is currently being developed for the prevention and cure of T1D [84]. Conversely, intrathymic manipulation also offers a potential way of enhancing the ability of T cells to control infection by increasing the numbers of positively selected thymocytes able to recognize a given molecule of the corresponding infectious agent. This concept of 'thymic vaccination' is based on the fact that slightly altered viral peptides bearing lower affinity to the corresponding TCR, rather than to the natural cognate ligand, may induce positive selection of this molecule when injected intrathymically, leading to antigen-specific T cell export from the thymus [2,85,86].

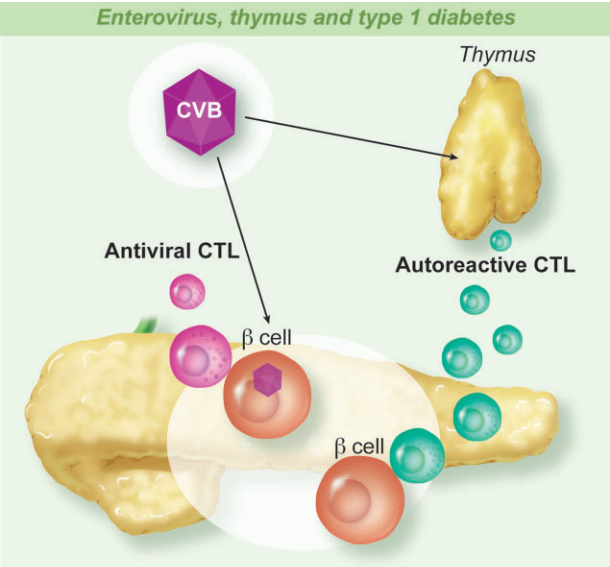

Fig. 2. Enterovirus, thymus and type 1 diabetes. Enteroviruses especially coxsackievirus B (CVB) infect β cells in pancreas islets. Persistent and/or successive infections of β cells results in activation of the innate immune system, influenced by the genetic background, followed by activation of the adaptive immune response which produces anti-viral cytolytic T lymphocytes (CTL) able to damage infected β cells that release self-antigens. In this context, β cell antigens activate anti-β cell autoreactive CTL through bystander activation and molecular mimicry. Enterovirus infections of the thymus can disturb the function of that organ involved in the tolerance to self-antigens, which may result in the production of autoreactive CTL that will be activated in the context of the response to the infection of β cells with enteroviruses.

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Disclosure

None.

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