Review

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Tolerance has its limits: how the thymus copes with infection

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The thymus is required for T cell differentiation; a process that depends on which antigens are encountered by thymocytes, the environment surrounding the differentiating cells, and the thymic architecture. These features are altered by local infection of the thymus and by the inflammatory mediators that accompany systemic infection. Although once believed to be an immune privileged site, it is now known that antimicrobial responses are recruited to the thymus. Resolving infection in the thymus is important because chronic persistence of microbes impairs the differentiation of pathogen-specific T cells and diminishes resistance to infection. Understanding how these mechanisms contribute to disease susceptibility, particularly in infants with developing T cell repertoires, requires further investigation.

The thymus is essential for T cell differentiation

The appearance of adaptive immunity in jawed vertebrates is considered a major evolutionary step, because B and T cells enable the immune system to generate and recall pathogen-specific immune responses [1]. Among lymphocytes, T cells are unique in their expression of the T cell receptor (TCR). TCR-mediated recognition of microbial peptides bound to major histocompatibility complex (MHC) is the principle way that the immune system identifies infected cells. T cell precursors are generated in the bone marrow and become functional after differentiation within the thymus (Figure 1) [1]. The deceptively simple anatomical structure of the thymus belies its sophisticated ability to generate T cells expressing a broad TCR repertoire capable of recognizing virtually any foreign antigen. Thymic selection eliminates most potentially harmful self-reactive T cells. After selection, naïve self-restricted T cells exit the thymus and traffic to secondary lymphoid organs.

T cell differentiation depends on the thymic microenvironment and the cytokine milieu surrounding the differentiating cells [1]. This raises the possibility that during infection, changes in soluble factors or antigens present within the thymus alter T cell differentiation. Indeed, systemic infection has detrimental effects on thymic structure and function (Figure 2). Furthermore, certain bacteria, virus, fungi, and parasites can directly invade the thymus (Table 1). These observations suggest that some pathogens, particularly those that cause chronic infections, interfere with the generation of immune responses designed to fight them by disrupting T cell development and possibly altering central tolerance. Here, we discuss how attitudes about thymic function during infection are changing. It is now clear that diverse pathogens infect the thymus, and because of their local and systemic effects, disrupt its architecture and function. We review recent evidence that the presence of pathogens in the thymus leads to microbe-specific tolerance and impairs host resistance. However, the immune system reacts to such invasions by recruiting immune responses to the thymus. Finally, we suggest that the ability to defend the thymus from infection may be important in maintaining the integrity of the immune system, particularly in situations where the T cell repertoire is being generated or is regenerating, such as in young children or patients with AIDS, respectively.

Thymus: myth and reality

Few studies have addressed whether infection affects thymic function in people, with the exception of studies on HIV infection [2]. In part, this is because of limited availability of thymus from infected patients, because the thymus is difficult to biopsy, and the usefulness of indirect measurements of thymic activity in humans is still controversial. As important are misconceptions that have affected how scientists and physicians view thymic function. Although now largely refuted, these include: (i) the thymus is an immune-privileged site protected from infection and immune responses; and (ii) thymic function is only important during early life and dispensable after puberty.

The notion that the thymus is immune privileged is inseparable from the concept of the blood–thymus barrier; considered for several years to be responsible for an antigen-free thymic microenvironment that prevents immune responses to exogenous antigens [3]. However, it is now clear that the thymus is both a target of infection and a site to where immune responses are recruited (Table 1) [4–7].

Although the thymus is essential for the establishment of a diverse T cell repertoire early in life, it is widely believed to be unnecessary after puberty. This idea is supported by the finding that the peripheral T cell pool can be maintained by thymic-independent mechanisms [8]. Even if this were

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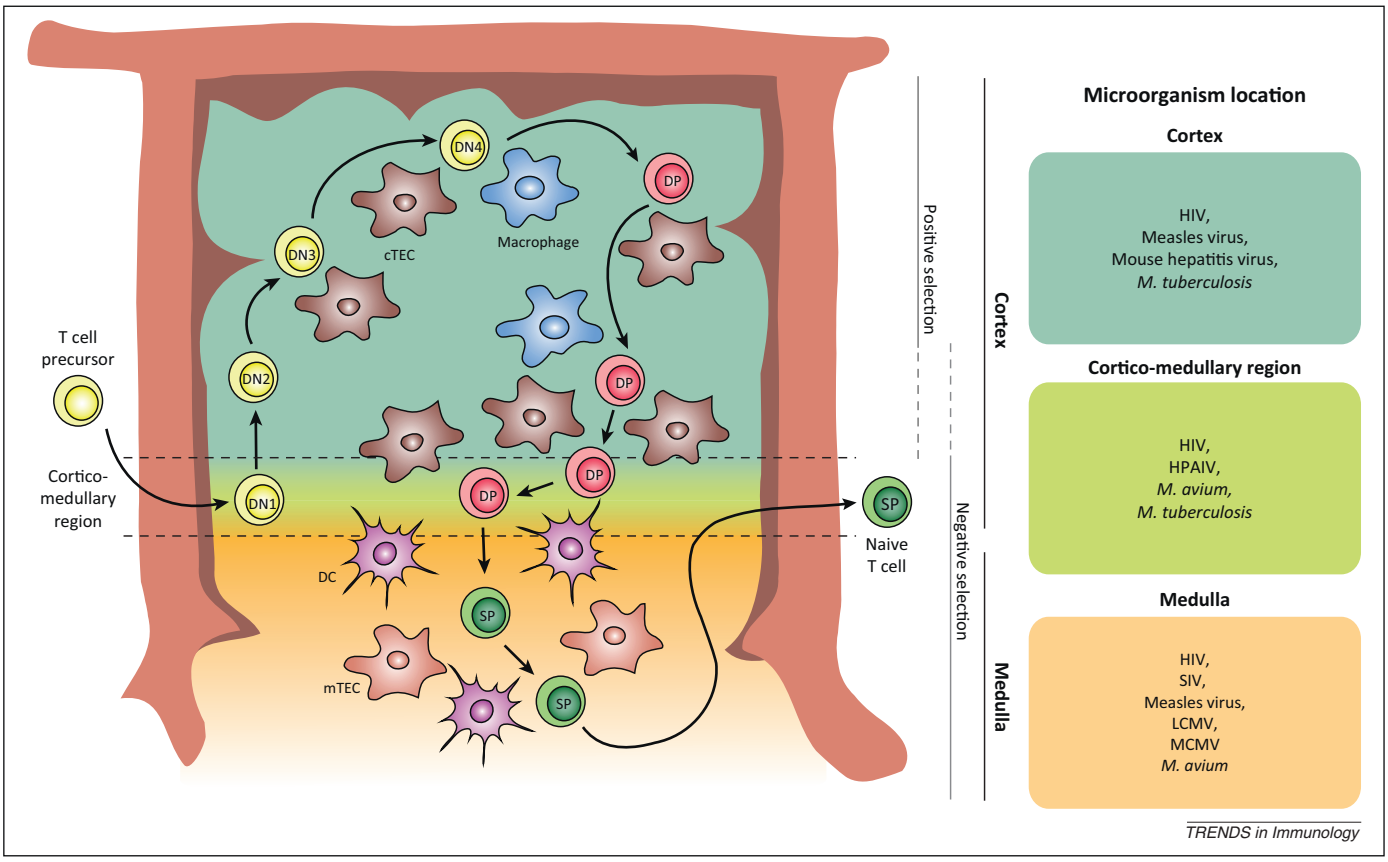

Figure 1. T cell differentiation. Schematic representation of T cell traffic within the thymus and location of the major steps during T cell selection. T cell precursors from the bone marrow enter the thymus in the cortico-medullary region and migrate to the cortex, where they go through the double negative (DN; e.g., $CD4^{-}CD8^{-}$) stages of T cell differentiation and become double positive (DP; e.g., $CD4^{+}CD8^{+}$) cells. DP cells migrate from the cortex to the medulla, interacting with structural components of the thymus in these regions during positive and negative selection. The resulting naïve single positive (SP; e.g., $CD4^{+}CD8^{-}$ or $CD4^{-}CD8^{+}$) cells exit the thymus and migrate to the peripheral lymphoid organs. Several microbes can be detected within the thymus following infection. Examples of microbes that are detected in the cortex, medulla or cortico-medullary region of the thymus following *in vivo* infection are shown. Abbreviations: DC, dendritic cell; DN, double negative thymocytes; DP, double positive thymocytes; SP, single positive thymocytes; cTEC, cortical thymic epithelial cell; mTEC, medullary thymic epithelial cell.

true, it is incredibly shortsighted to ignore the effect of infection on the thymus, because children and young adults under the age of 24 years make up 44% of the world's population, and are particularly vulnerable to infection. In 2011, nearly 6.9 million children under the age of 5 years died, with infection causing more than half of these deaths [9]. Although most of these deaths were due to acute infection (pneumonia, 14%; diarrhea, 10%; measles, 1%), a significant number were due to chronic infection (malaria, 7%; HIV/AIDS, 2%; tuberculosis, 1%) [9]. These numbers increase with age and HIV/AIDS and tuberculosis account for 11% of deaths among young adults (age 10–24 years) worldwide [9]. Both HIV and *Mycobacterium tuberculosis* infect the thymus and cause alterations in T cell output, which could be relevant both in settings of vaccination and during natural immunity to these pathogens [2,10–12].

Although childhood infections are arguably a greater cause of morbidity and mortality than adult infection, particularly in the developing world, recent studies reinforce the idea that the thymus affects resistance to infection during adulthood. Reduced thymic output of T cells is associated with HIV progression to AIDS and the thymus has been implicated in the successful immune reconstitution of AIDS patients in response to antiretroviral therapy [2]. Moreover, thymectomy during early childhood has been linked to accelerated decline in immunologic function, particularly following cytomegalovirus (CMV) infection [13], and work on experimental viral infection models finds that continuous recruitment of naïve T cells from the thymus has a beneficial role in the control of persistent infections [14–16]. The integrity of the adult thymus is also required for other aspects of ongoing immunity including antibody generation [17] and oral tolerance [18]. These observations indicate that an intact and functional thymus is required for optimal immunity to infection throughout life.

How does infection alter thymic function?

There are two ways in which infection can affect the thymus: local and systemic (Figure 2). Local refers to effects of direct infection of the thymus by microbes. Systemic refers to the consequences of infection elsewhere on the thymus. Systemic effects occur when soluble factors, such as glucocorticoids (GCs) and proinflammatory mediators, are released into the blood stream.

Infection-induced thymic atrophy

Premature thymic atrophy is a common consequence of infection by viruses, bacteria, parasites, and fungi (Box 1 and Figure 2) [19] and can result from local and/or systemic effects. For example, GC levels rise during acute infection, and can induce thymocyte apoptosis, especially among

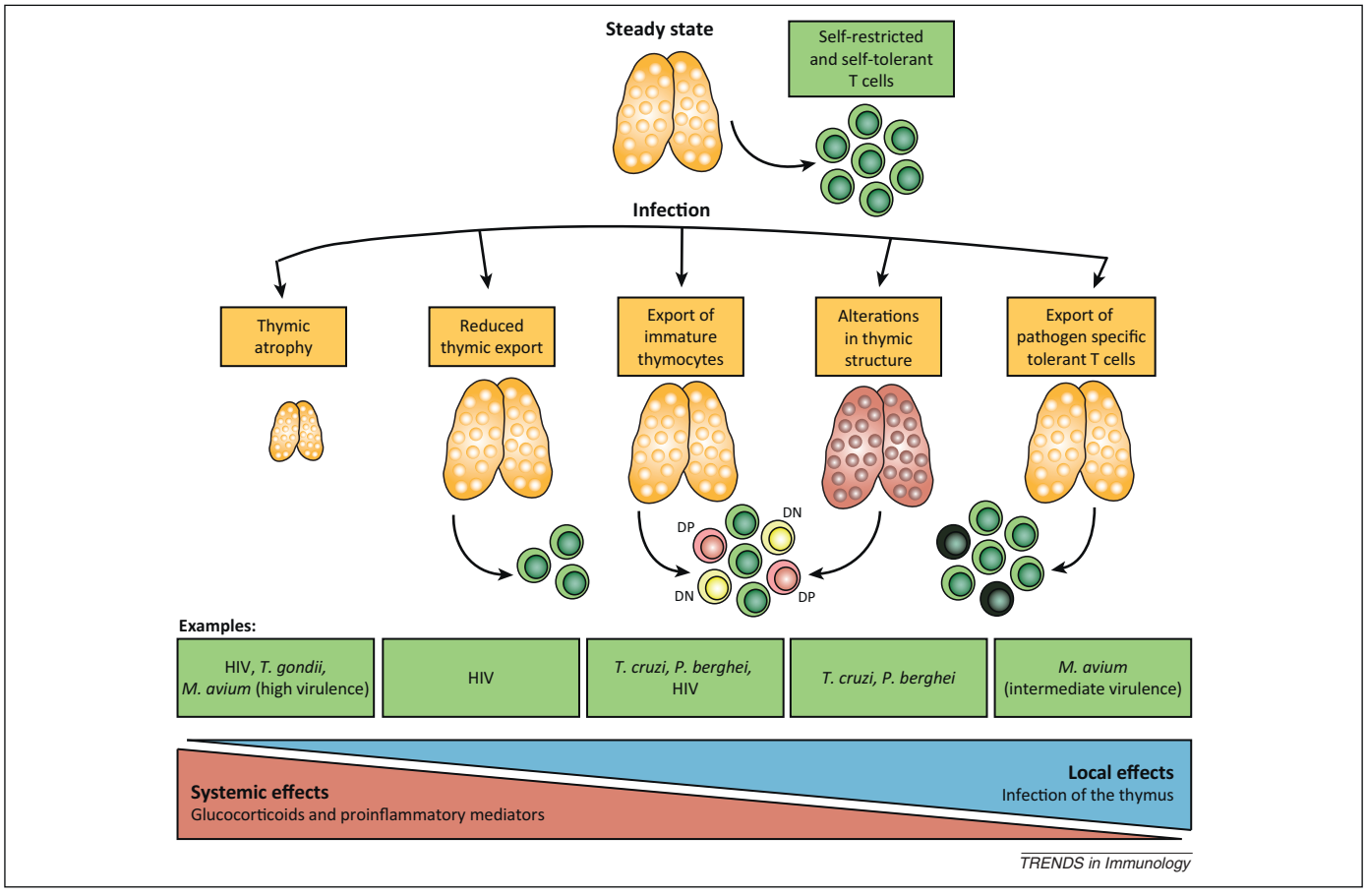

Figure 2. The effects of infection on the thymus. Schematic representation of how infection can affect the thymus through systemic and/or local effects. Glucocorticoids and/or proinflammatory mediators mediate systemic effects, while local effects require the presence of a pathogen within the thymus. Infection-induced alterations include thymic atrophy, modifications in the thymic structure, and alterations in the T cells exported to the periphery. Representative pathogens capable of inducing the different alterations in thymic structure and/or function are indicated. Abbreviations: DN, double negative thymocytes; DP, double positive thymocytes.

Table 1. Pathogens that infect the thymus[a].

	Pathogen	Consequences of infection	Refs
Virus	HIV	Atrophy	[2,36–40]
	Simian immunodeficiency virus (SIV)	Atrophy (strain dependent)	[30]
	Influenza virus	Atrophy (strain dependent); immune response in the thymus	[7]
	Lymphocytic choriomeningitis virus (LCMV)	Atrophy; immune response in the thymus; pathogen-specific immune tolerance	[6,62], [63]
	Murine leukemia virus (MLV)	Atrophy; pathogen-specific immune tolerance	[42,43,48]
	Mouse hepatitis virus	Atrophy	[50]
	Human cytomegalovirus (CMV)	Atrophy	[49]
	Measles virus	Atrophy (strain dependent); TEC apoptosis	[29,51]
	Coxsackievirus	Modulation of TEC function	[52]
	Epstein–Barr virus (EBV)	N/A	[71]
	Junin virus	N/A	[72]
	Poliovirus	N/A	[73]
Bacteria	*Mycobacterium avium*	Atrophy (strain dependent); immune response in the thymus; pathogen-specific immune tolerance	[4,11], [12,26]
	Mycobacterium tuberculosis	Immune response in the thymus	[4,5,12]
	Francisella tularensis	Atrophy (strain dependent)	[23]
	Salmonella enterica	Atrophy	[60]
Fungi	*Paracoccidioides brasiliensis*	Atrophy	[74]
	Cryptococcus neoformans	Alterations in thymic architecture	[75]
Parasites	*Trypanosoma cruzi*	Atrophy (*strain dependent*); release of DP/DN/autoreactive T cells; alterations in extracellular matrix	[24,46,47,67]
	Plasmodium berghei	Atrophy (strain dependent); release of DP/DN cells; alterations in extracellular matrix	[44,45]
	Toxoplasma gondii	Atrophy	[22]

[a]Virus, bacteria, fungi and parasites have been documented to infect the thymus, either in humans or experimental animal models. The major consequences of each infection on thymic structure or function are listed, when information is available.

Box 1. Mechanisms of thymic atrophy

Thymic involution is a physiological process that occurs naturally and progressively with aging and also transiently during pregnancy and stress. Atrophy occurs by a gradual and progressive decline in the number of thymocytes, and known mechanisms of physiological atrophy are mediated by either TEC or thymocytes (reviewed by Dooley *et al.* [20]). Because interactions between developing thymocytes and structural components of the thymus are essential for normal T cell differentiation, alterations in TEC can result in diminished functional thymopoiesis and export of naïve T cells, leading to thymic atrophy. For example, thymic atrophy depends on sex hormones in a process that requires the expression of the androgen/estrogen receptors by TEC. By contrast, thymocyte-mediated atrophy is usually the result of alterations in the thymic milieu that induce rapid apoptotic cell death of double positive (DP) thymocytes. This is best exemplified by stress-mediated thymic involution, which occurs by DP thymocyte death in response to elevated levels of circulating GCs.

Several non-physiological stimuli can induce premature thymic atrophy, such as infection. Infection-induced atrophy could occur by: alterations in TECs; induction of apoptosis in thymocytes particularly DP cells; or reduction of thymocyte precursors within the bone marrow. The similarities between the mediators of physiological and infection-induced thymic atrophy suggest that the molecular mechanisms responsible for the decline in thymic cellularity might be common in both settings, and this possibility should be addressed experimentally (Box 2).

Why thymic atrophy should accompany infection is debated. The different hypotheses include: (i) thymic atrophy is a by-product of infection, with no specific advantage for the pathogen or the host; (ii) thymic atrophy is a virulence strategy employed by pathogens to subvert antimicrobial immunity; and (iii) thymic atrophy is a host strategy that reduces thymic activity during infection to prevent disruption of T cell selection and prevent the emergence of central tolerance to the invading organism. In any case, thymic atrophy can impair thymic function and has implications for ongoing immunity.

double-positive (DP) thymocytes [20]. Adrenalectomy prior to infection prevents thymocyte depletion in rabies virus infected mice, which confirms a role for GCs in infection-induced thymic atrophy [21]. Infection-induced premature thymic atrophy also occurs independently of increased systemic GC levels. For example, adrenalectomy prior to *Toxoplasma gondii* infection abolishes peripheral lymphopenia but does not prevent thymocyte loss [22]. In some infections, GCs synergize with other mediators to induce thymic atrophy. These include tumor necrosis factor (TNF) during *Francisella tularensis* [23] and *Trypanosoma cruzi* [24] infection, interferon (IFN)γ during *Salmonella enterica* infection [25], and IFNγ and nitric oxide during *Mycobacterium avium* infection [26]. Moreover, some of these molecules can alter thymic populations independently of GCs. This is the case for TNF, which can lead to the deletion of DP thymocytes, mediated by excessive peripheral T cell activation following antigen injection, even after treatment with antagonists of GC receptors [27].

Interestingly, infection-induced thymic atrophy often correlates with strain virulence, as observed for *T. cruzi* [28], *F. tularensis* [23], *M. avium* [26], measles virus [29], highly pathogenic avian influenza viruses (HPAIVs) [7], and simian immunodeficiency virus (SIV) [30]. These data suggest that specific microbial factors directly promote thymocyte death. This is true for bacterial factors such as lipopolysaccharide [31], *Escherichia coli* enterotoxin [32], and mycobacterial cord factor [33], and has been confirmed with the fungal virulence factors gliotoxin [34] and toxin T-2 [35]; all of which directly induce thymocyte apoptosis when administered to mice. Local thymic effects are also observed after HIV infection [2]. HIV thymotropic viral variants are detected *in vivo* [36], raising the possibility that these strains would be more likely to affect thymic function. The infected cell type depends on viral tropism for chemokine CXC receptor (CXCR)4 and chemokine CC receptor (CCR)5, but most thymocyte subsets can become infected [2]. HIV also infects different thymic stromal cells, including macrophages, conventional dendritic cells (cDCs), plasmacytoid DCs (pDCs), and thymic epithelial cells (TECs) [37–39]. HIV affects the fate of infected cell types differently. For example, CD4 single positive (SP) thymocyte depletion results from direct infection, killing of progenitor cells, and apoptosis induction of uninfected cells by viral products [40]. HIV infection also induces DC and TEC death [37,39]. In the case of DCs, cell death is associated with IFNα production by pDCs but not cDCs [37]. Thus, in addition to inducing thymocyte death, HIV disrupts thymic function by altering the local microenvironment. Collectively, these data show that HIV infection induces thymic atrophy and impacts thymic function, leading to a decline in the export of newly differentiated T cells [2,41].

Similar to HIV, murine leukemia virus (MLV) has specific long terminal repeat region sequences that affect viral infection and replication in DP and double negative (DN) thymocytes and in other thymic populations [42]. MLV induces apoptosis of infected cells within the thymus even before the leukemic period. Apoptosis is induced by the accumulation of Env protein precursors, triggering endoplasmic reticulum stress [43]. Thus, apoptosis is a consequence of local infection, and infected DP thymocytes die more than uninfected cells within the thymus.

Thymic atrophy is also detected during infection of mice with HPAIV [7]. These viruses cause severe human disease accompanied by profound lymphopenia. After intranasal challenge of mice, influenza-infected DCs are present in the corticomedullary region of the thymus. Thymic atrophy is strain dependent and occurs only after infection with highly pathogenic virus. HPAIV interferes with thymic function, inducing loss of DP thymocytes and diminished export of naïve T cells to the periphery, leading to severe lymphopenia. It is unknown what role GCs play during this acute infection and the relative contribution of local versus systemic factors to thymic atrophy cannot be ascertained.

These studies demonstrate that local and systemic effects can induce thymic atrophy during infection, and are not mutually exclusive.

Thymic structure is altered by infection

Infection induces thymic structural alterations other than atrophy (Figure 2). For example, both *T. cruzi* and *Plasmodium berghei* directly infect the thymus and induce significant changes in the extracellular matrix [19,28,44–46]. *T. cruzi* increases fibronectin and laminin deposition and chemokine CXC ligand (CXCL)12 and chemokine CC ligand (CCL)4 production within the thymus [46,47]. During *T. cruzi* infection, expression of the fibronectin and laminin receptors [very late antigen (VLA)-4, VLA-5 and VLA-6] and CXCR4 and CCR5 is augmented on thymocytes and

intrathymic thymocyte migration of DP cells is enhanced [46,47]. *P. berghei* affects thymocyte migration by inducing CXCL12 and CXCR4 and reducing CCL25 and CCR9 production within the thymus [45]. When analyzed *ex vivo*, both DN and SP cells from infected thymi migrate faster than control populations towards extracellular matrix components [45]. In both cases, these changes affect peripheral T cell subsets. At the peak of *T. cruzi* infection, a higher frequency of immature and VLA^{hi} DP T cells are found in the periphery [46]. Similarly, increased numbers of DN and DP T cells are found in the periphery of *P. berghei*-infected mice [44].

Viral infections also induce significant changes in thymic structure by infecting stromal cells. HIV can infect TECs *in vivo* and lead to degeneration of these cells [39]. *In vivo* infection of TECs has also been described using MLV [48] and CMV [49]. Mouse hepatitis virus (MHV) [50], measles virus [51], and type-B coxsackievirus [52] have been shown to infect TECs *in vitro*. Interestingly, *in vitro* infection of human TECs with measles virus results in terminal differentiation and apoptosis of these cells [51]. By contrast, type-B coxsackievirus infection of human TECs *in vitro* does not cause damage but modulates cell function, leading to increased production of interleukin (IL)-6, granulocyte–macrophage colony-stimulating factor (GM-CSF), and leukocyte migration inhibition factor (LIF) [52]. The observation that viruses infect TECs, alter their function, and in some cases induce cell death, is especially important given the crucial role these cells play in T cell differentiation [53]. Finally, the changes in thymic cellularity observed during viral infection may be caused by depletion of TECs and secondary decline in thymocyte number.

These data show that structural alterations of the thymus caused by infection modify the characteristics of differentiating T cells and affect T cell export.

Alterations in thymic export

The appearance of DP thymocytes in the periphery, as described following *T. cruzi* and *P. berghei* infection, is also a hallmark of infections caused by HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) [54,55]. MHV can be detected within the thymus [56] (Table 1), and although the origin of peripheral DP cells is unknown, one possibility is that they are released from the thymus due to local infection (Figure 2). Alternately, activated T cells may acquire a DP phenotype as observed during HIV and other infections [57]. Whether peripheral DP cells are an indication of altered thymic function or whether they represent activated T cells likely varies according to the infecting agent and requires experimental investigation.

Alterations in thymic structure due to infection can alter T cell export in other ways. A major consequence of HIV infection is reduced export of recent thymic emigrants; an effect confirmed by T cell rearrangement excision circle (TREC) analysis [2,41]. As thymic activity is essential to maintain or reconstitute a functional peripheral T cell pool, interventions that enhance this process may potentiate the beneficial effects of antiretroviral therapy on immunity [58].

Antigen-driven responses in the thymus can also suppress T cell export. Although not formally demonstrated in the context of infection, intravenous or intrathymic injection of foreign antigen leads to a significant reduction in the amount of recent thymic emigrants in the periphery [59]. This is not due to increased negative selection of developing thymocytes, but rather from retention of medullary thymocytes within the thymus [59].

Although it is intuitive that reduced thymic T cell export is a consequence of infection-induced thymic atrophy, this is not always the case. During *Salmonella*-induced thymic atrophy, T cell export is maintained [60], suggesting that atrophy and a decline in thymic function might be independent. Nevertheless, maintaining thymic export of recent thymic emigrants during infectious episodes is important, because it positively impacts ongoing immunity [14–18].

Generation of T cells tolerant to pathogens

Infection alters thymic structure and affects export of immature and naïve T cells. Another important question is whether infection of the thymus and the presence of microbial antigens in the thymus, particularly during persistent infection, leads to pathogen-specific tolerance (Figure 2). This question was investigated using a model of mycobacterial infection. Infection of mice with *M. avium* of intermediate virulence leads to chronic persistent infection. During this infection, *M. avium* disseminates to, and persists in, the medulla and corticomedullary region of the thymus [11,12]. No thymic atrophy occurs and the chronically infected thymus continues to support T cell differentiation. However, T cells that mature within infected thymi are abnormal because they suboptimally respond to *M. avium* antigens compared to T cells that differentiate in uninfected thymi. Importantly, T cells that differentiate in *M. avium*-infected thymi respond normally to unrelated antigens, indicating that the defect is specific for the invading pathogen [11]. Although the precise mechanisms responsible for this difference is still being elucidated (Box 2), these data demonstrate that thymic infection can induce pathogen-specific T cell tolerance.

Whether tolerance also emerges in the setting of chronic *M. tuberculosis* infection is a matter of debate. At various times after *M. tuberculosis* infection, Reiley *et al.* treated mice with the myeloablative drug busulfan and then infused bone marrow from ESAT6-specific TCR transgenic mice [5]. ESAT6 is a protein secreted by *M. tuberculosis* and is an immunodominant antigen recognized by human and murine T cells. This established mixed bone marrow chimeras containing a population of congenically marked ESAT6-specific naïve $CD4^+$ T cells. When performed 30 days after infection, a significant population of congenic ESAT6-specific $CD4^+$ T cells appeared in lungs within 49 days after reconstitution and gradually increased during the next 3 months. However, if the bone marrow infusion was delayed until 3 months after infection, few if any ESAT6-specific $CD4^+$ T cells could be detected in the lung 49 and 63 days after reconstitution, and only began to appear 84 days after infection, reaching steady-state levels by 140 days. This result was interpreted as indicating that recent thymic emigrants help maintain the peripheral T cell response to *M. tuberculosis*; central tolerance to *M. tuberculosis* antigens does not occur. The authors suggested that the decline in the expansion of congenic recent thymic emigrants after day 90 was due to diminished priming and/or expansion of

naïve T cells during chronic infection. However, an alternative interpretation of their data is that *M. tuberculosis* colonization of the thymus leads to tolerance. Few bacteria are present in the thymus during the first month after infection; by contrast, nearly all infected mice have a significant bacterial load by 3 months [4,5,12]. The diminished expansion of primed ESAT6-specific $CD4^+$ T cells late after infection (d90) can be explained by the progressive development of tolerance during chronic mycobacterial infection, which occurs only when there is a significant amount of mycobacterial antigen in the thymus. Clearly, whether infection-induced central tolerance contributes to the inability of recent thymic emigrants to integrate the immune response during chronic infection with *M. tuberculosis* requires further investigation.

Tolerance to invading pathogens is also observed during viral infection. Neonatal MLV infection leads to infection of thymocytes as well as thymic stromal cells, and renders T cells tolerant to viral antigens [61]. Similarly, congenitally acquired lymphocytic choriomeningitis virus (LCMV) infection is a model of immune tolerance: mice infected *in utero* or at birth show high viral titers in most organs, including the thymus, and have a selective defect in LCMV-specific T cell immunity [62]. LCMV infection of the thymus starts at the fetal stage in DN cells, and transitions to CD4 SP cells in the adult thymus. By contrast, $CD8^+$ T cell infection is minimal [63], and transferred virus-specific cytotoxic T lymphocytes (CTLs), from immunized mice, infiltrate the thymus and eliminate the infection [6,62]. In this model, viral clearance is associated with reacquisition of LCMV-specific CTL responses, suggesting that continuous presence of the antigen in the thymus is required to maintain tolerance, not only during fetal development but in adult animals as well.

Viral hepatitis also induces central tolerance to HBV. Although acute infection in adults is readily resolved, HBV infection *in utero* induces tolerance to viral proteins [64], and infants born to HBV-infected mothers are more likely to become chronic carriers of HBV [65]. One possibility is that viral proteins in the neonatal thymus induce HBV-specific T cell tolerance. This hypothesis is supported by the observation that both MLV and HBV infect TECs [50,61], which could explain why these infections induce T cell tolerance while others do not. TEC turnover is rapid [66], therefore, this hypothesis implies that these viruses can continually infect new TECs or reside in thymic epithelial stem cells.

These data suggest that direct infection of the thymus by viruses and bacteria alter T cell selection and induce tolerance against the invading pathogen, with the potential to impair ongoing immunity. An interesting question is whether the mechanism of tolerance induced by thymic infection involves the generation of pathogen-specific regulatory T cells, T cell anergy, or negative selection of differentiating pathogen-reactive T cells (Box 2).

Autoimmunity induced by thymic infection

Pathogens that disrupt thymic function may also disrupt the development of central tolerance. Autoreactive T cells that are normally negatively selected in the thymus might escape death because of thymic dysfunction, emigrate to the periphery, and trigger autoimmunity. Although this possibility remains theoretical, some data support it. During experimental *T. cruzi* infection, T cells expressing 'forbidden' TCRs that are normally deleted in the thymus, particularly those belonging to the Vβ5 and Vβ12 families, survive and can be detected in peripheral lymph nodes [67]. Thymic SP cells from infected mice are not enriched in those TCRs. These data indicate that the appearance of these forbidden TCRs in the periphery is not due to defective negative selection but a consequence of abnormal migration of immature cells [67]. In addition, anti-thymus antibodies and myocardium-specific autoreactive T cells are detected following *T. cruzi* infection [28], raising the possibility that infection-induced thymic alterations potentiate autoimmunity.

Box 2. Outstanding questions

- How universal is thymic infection? Do other pathogens infect the thymus?
- How does the thymus get infected? Do pathogens directly target the thymus, or do they disseminate inside recirculating cells?
- Why does atrophy accompany thymic infection so frequently? Is atrophy beneficial for the host or the bug? What are the mechanisms responsible for infection-induced thymic atrophy?
- What are the mechanisms responsible for T cell tolerance? Does the presence of microbial antigens lead to negative selection of developing T cells? Do microbe-specific regulatory T cells emerge following thymic infection? Are developing T cells anergic to the infectious agent?
- How does infection of the thymus impact ongoing immunity? Does thymic infection during childhood impact immunity later in life? Is thymic infection relevant during vaccination with live microorganisms?
- How important are newly generated T cells during chronic infections and during immune reconstitution?

Thymic infection: the beginning and the end

How are various microbes able to reach the thymus and establish infection? Furthermore, if dissemination to the thymus occurs commonly during infection, has the immune system evolved mechanisms to respond to pathogens invading the thymus?

How do microorganisms reach the thymus?

Two scenarios are possible when considering the origin of thymic infection during hematogenous spread of infection (Figure 3; Box 2). First, circulating pathogens can enter the thymus and infect cells in a targeted manner, as represented by thymotropic variants of HIV [36] and MLV [42]. Alternately, there is the 'Trojan Horse' model. The trafficking of several cell types between the periphery and the thymus make this possible. T cells circulate from the periphery to the thymus [68], and if infected, could seed the thymus with pathogens that target T cells (e.g., HIV). Similarly, certain DC subsets (e.g., $Sirp\alpha^+$ cDCs) migrate from the periphery to the thymus and modulate T cell tolerance [69]. DCs infected with influenza virus or *M. avium* can be detected within the thymus, raising the possibility that infected DCs spread the infection [7,11]. DCs are responsible for disseminating *M. tuberculosis* from the lung to the draining lymph nodes [70], and it is possible that dissemination to the thymus occurs by a similar mechanism. Together, these data support the idea that cells circulating to the thymus can carry infectious agents

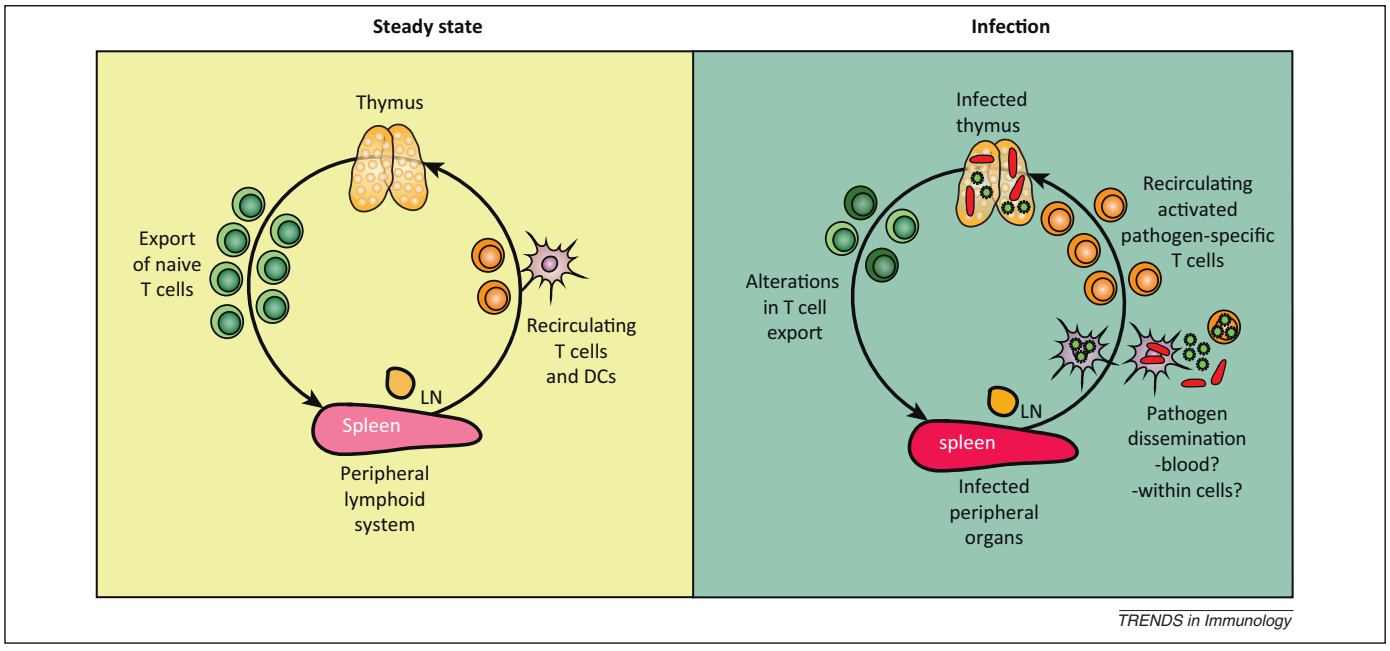

Figure 3. Immune response in the thymus. Schematic representation of microbial dissemination and recruitment of an immune response to the thymus. Under normal conditions, mature T cells and DC circulate from peripheral lymphoid organs to the thymus (*left panel*). Following infection, pathogens disseminate from the periphery to the thymus, either extracellularly or within re-circulating cells. The infected thymus produces chemokines, such as CXCL9 and CXCL10, which recruit CXCR3-expressing antigen-specific T cells from the peripheral tissues back to the thymus to fight infection (*right panel*). Abbreviation: LN, lymph node.

and seed thymic infection (Figure 3). An important corollary is that the route and duration of infection may modify the risk that the thymus becomes infected by different pathogens.

Immunity within the thymus

If the thymus is a site of infection, it may recruit an immune response against invading pathogens. As discussed, during systemic LCMV infection, LCMV-specific CTLs traffic to the thymus, establish an immune response, and eliminate LCMV from the thymus [6,62]. Similarly, during influenza infection, functional influenza-specific CTLs are detected within the thymus [7]. Finally, antigen-specific $CD4^+$ and $CD8^+$ T cells are detected in the thymus following *M. avium* and *M. tuberculosis* infection, as part of the immune response against persistent bacteria [4,5]. The responding T cells in the thymus are not newly differentiated mature thymocytes but instead are activated T cells that circulate from peripheral organs to the thymus to fight infection (Figure 3) [4]. Under these conditions, the recruitment of activated T cells is associated with increased expression of T helper 1 chemokines and an enrichment of $CXCR3^+$ mycobacteria-specific T cells within the thymus [4]. These results confirm that the thymus is not only a site of infection, but suggest that it is actively surveyed by the immune system.

Concluding remarks

The recent studies reviewed here show that the thymus is a site of infection that has important immunological consequences. Pathogens disrupt thymic structure and function, and alter T cell selection and export. These changes affect the peripheral T cell pool and affect ongoing and future immune responses.

These data suggest a model in which the effect of infection on the thymus depends on the type of microbe, the severity of infection, and the ability of the pathogen to infect and persist within the infected thymus (Figure 2). In this scheme, acute infection is characterized by increased GCs and proinflammatory mediator levels, which can lead to thymic atrophy. This effect is more pronounced in DP cells and can occur even in the absence of the pathogen in the thymus. Local thymic infection can exacerbate atrophy, through remodeling of extracellular matrix, production of virulence factors, or direct infection of thymic cells. These structural changes affect thymic function; particularly T cell export, leading to the release of immature (DP/DN) or autoreactive T cells into the periphery. Despite the profound effects of acute infection on the thymus, the impact of thymic dysfunction on immunity is predicted to be limited and transient because the peripheral T cell pool should include pre-existing pathogen-specific T cells. By contrast, local thymic infection may have severe repercussions, particularly for: (i) infections acquired during childhood – when the T cell repertoire is still developing; (ii) in the setting of persistent infection (e.g., tuberculosis), which may induce T cell tolerance; and (iii) infections associated with severe lymphopenia (e.g., HIV), when lymphoid reconstitution is required. In these cases, emergence of central tolerance to the infectious agent may impair deployment of pathogen-reactive T cells in the naïve repertoire. Such a scenario could favor the microbe, because impairment of T cell immunity would contribute to pathogen persistence. In order to minimize such consequences, mechanisms exist to respond to direct infection of the thymus (Figure 3). Just as in other tissues, these rely on the trafficking of peripheral T cells from secondary lymphoid tissue back to the thymus. Although required for protection, circulation of cells back to the thymus could allow some pathogens access to the thymus.

Altogether, preserving a sterile thymic environment is important to maintain thymic integrity, both structurally

and functionally, sustain optimal T cell differentiation and export, and prevent the emergence of tolerance to invading pathogens. Therefore, the thymus should be regarded as an active player during infectious episodes and the contribution of this organ for ongoing immunity should be addressed in future studies (Box 2).

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