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REVIEW

MHC class II-dependent T–T interactions create a diverse, functional and immunoregulatory reaction circle

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Unlike conventional T cells, innate-like T cells such as natural killer (NK) T cells are selected by homotypic T-cell interactions. Recently, a few reports have shown that T–T CD4$^+$ T cells can be generated in a similar manner to that for NKT cells. These two types of cells share common functional properties such as rapid response to antigenic encounters and the potential for a panoply of cytokine secretion. However, T–T CD4$^+$ T cells differ from NKT cells in that they are restricted by highly polymorphic major histocompatibility complex (MHC) II molecules and have a diverse T-cell receptor repertoire. Additional example of T–T interactions was recently reported in which peripheral T cells re-circulate to the thymus and participate in the thymocyte selection process. In this review, we dissect the cellular mechanisms underlying the production of T–T CD4$^+$ and NKT cells, with particular emphasis on the differences between these two T-cell prototypes. Finally, we propose that T–T CD4$^+$ T cells serve two major functions: one as an acute-phase reactant against viral infection and the other is the generation of anti-ergotypic CD4$^+$ T cells for regulatory purposes. All of these features make it possible to create a diverse set of functional cells through MHC class II-restricted T–T interactions.
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T CELLS SELECTED BY HEMATOPOIETIC CELLS

Innate or non-conventional T cells are a distinct lineage of T cells selected by non-classical major histocompatibility complex (MHC) class Ib molecules, such as H2-M3, Qa-1, TL, MR-1 and CD1d, which are expressed primarily on hematopoietic cells.[1–4] They develop from CD4$^+$CD8$^+$ progenitors in the thymus and acquire effector functions as a result of maturation processes rather than as a consequence of activation following an antigenic encounter in the periphery.[5] With some exceptions, they have several features in common such as upregulation of effector or memory markers, rapid production of cytokines, limited T-cell receptor (TCR) diversity, interleukin (IL)-15 dependence and an absence of memory function.[5,6] On the basis of such properties, these cells would appear to participate in the first line of defence against foreign pathogens. NKT, CD8αα IEL, MAIT and H2-M3-restricted CD8$^+$ T cells all belong to this family.[5] The recently identified Tec kinases ITK and RLK were found to play a crucial role in the decision between conventional versus non-conventional cells derived from the CD8$^+$ T-cell lineage.[7,8] In the absence of this transcription factor, interactions between thymocytes lead to mature CD8$^+$ T cells with innate properties, even when they are restricted by MHC Ia. Again, these innate T cells, such as NKT cells, depend on homotypic SLAM–SLAM and CD28–B7 interactions for their respective lineage development and functional maturation.[9] Altogether, these data indicate that cellular interactions between cells of hematopoietic origin predominantly direct the development of non-conventional lineage T cells.

T–T CD4$^+$ T-CELL DEVELOPMENT IN HUMANS AND MICE

In 1992, human fetal thymocytes were first shown to express MHC class II molecules on their cell surface.[10] The expression level of HLA-DR peaks during the perinatal stage and gradually decreases below detection levels 5 years after birth. On the basis of this observation, we hypothesized that these MHC class II-positive thymocytes might be able to mediate positive selection through T–T interactions. This possibility was subsequently confirmed in our laboratory using an *in vitro* reaggregate culture system, in which MHC class II expression was present only on immature double-positive (DP) thymocytes.[11] In this system, mature CD4$^+$ and CD8$^+$ single-positive T cells were efficiently produced. This was the first documentation that the MHC class II present on immature T cells was able to positively select other immature T cells in the thymic cortex. Collectively, these findings show that T–T interactions might represent an important alternative pathway for T-cell maturation, particularly

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during the neonatal period when the conventional effector and regulatory T-cell network are not fully developed.

These *in vitro* findings were also confirmed in our own work[12] and that of another independent group[13] using an *in vivo* transgenic mouse system, in which CIITA expression was restricted to thymocytes and mature T cells. In this system, T–T $CD4^+$ T cells showed diverse TCR Vβ usage and were functionally competent. In the periphery, these $CD4^+$ T cells showed a memory phenotype, as revealed by high CD44 and low CD62L expression, and showed rapid activation and vigorous proliferation after antigenic stimulation. They also secreted large amounts of effector cytokines, such as interferon (IFN)-γ and IL-4.[14] Subsequently, the selection pathway of T–T $CD4^+$ T cells was found to be dependent on homotypic SLAM interactions, which are known to be mediated by the SAP–Fyn–NF-κB signalling pathway, similar to NKT cells.[15] In this experiment, peripheral T–T $CD4^+$ T cells were drastically reduced in number and showed no innate properties in the absence of SAP signalling. This SLAM-mediated signalling pathway is particularly important because it is analogous to the human system, and mutations in the *sh2d1a* gene, encoding an adaptor molecule SAP, have been reported.[16] Human X-linked lymphoproliferative disease (XLP) very closely mimics the SAP knockout mouse in that there are no NKT cells[17] and possibly, T–T $CD4^+$ T cells.[15]

PERIPHERAL TO CENTRAL T–T INTERACTIONS; LYMPHOCYTE RE-CIRCULATION TO THE THYMUS

The trafficking of mature T cells from the thymus to peripheral secondary lymphoid organs has generally been considered to be unidirectional.[18] However, several reports have described thymic re-entry of activated peripheral T cells, as well as B cells and dendritic cells (DCs).[19–22] These cells have been reported to localize primarily to the thymic medullary area, and this finding has led a few immunologists to examine their possible role in thymic negative selection.[20] Representative examples include allogeneic double-negative (DN) thymocytes,[23] minor antigen-incompatible $CD8^+$ T cells[24] and antigen-capturing DCs,[21] all of which are capable of inducing intrathymic tolerance. The biological relevance of this phenomenon is thought to be that it is a mechanism by which T cells reactive to peripheral tissue-specific antigens are eliminated by direct import of peripheral antigen-specific or antigen-bearing cells.[25]

Recently, Ceredig and co-workers[25] showed that peripherally injected donor T cells re-circulate into the thymic cortex, mediating positive selection of immature host T cells. Using parabiosis, host OT-I thymocytes were able to be positively selected by donor H-$2K^b$-positive P14 TCR transgenic T cells that had re-entered the thymus. These T cells, if they exhibit innate immune properties, are similar in some respects to $CD8^+$ T cells in LTK- and RLK-deficient mice, in which $CD8^+$ T cells with an innate phenotype are selected by MHC Ia-restricted hematopoietic cells.[9] Detailed analysis of their functional properties, however, was precluded by the very small number of selected OT-I T cells and their poor survival in H-$2K^b$-deficient hosts.[25]

In mice, thymocyte selection by re-circulated peripheral T cells can only be restricted to the $CD8^+$ T-cell population due to the absence of MHC II expression on peripheral T cells and thymocytes. In human and rat systems, however, activated peripheral T cells express considerable amount of MHC II molecules.[26] Therefore, when these cells re-circulate into the thymus, they might also be able to participate in the thymic selection process. On the basis of this report, it seems possible that thymocytes selected by interactions between thymocytes and peripheral activated T cells represent another type of T–T interaction, and it is highly likely that their development and functional maturation is dependent on the SLAM–SAP signalling pathway and B7–CD28 interaction.

T-CELL DEVELOPMENT THROUGH T–T INTERACTION IN THE THYMUS

Positive selection in the thymus

Natural killer T cells differentiate from DP thymocytes through engagement of their invariant TCR with CD1d molecules expressed on cortical thymocytes.[27–29] More than 80% of them (invariant NKT or type I NKT cells) have a canonical Vα14-Jα18 chain, which is preferentially associated with Vβ2, 7 and 8 chains (Vα24-Jα18-Vβ11 in humans). Type II NKT cells show somewhat more diverse, but still limited, Vα and Vβ gene usage.[30–32] Two transcription factors, RORγt and Runx1, are essential for proper NKT cell development, because they are involved in the survival of DP thymocytes until successive rearrangement of distal Vα-Jα genes and lineage differentiation, respectively.[33] Upon TCR expression, these cells interact with adjacent DP thymocytes in a CD1d-dependent manner to develop into $CD24^{hi}CD44^{low}NK11^-$ stage 0 NKT cells.[34] In this process, isoglobotrihexosylceramide serves as an intrathymic endogenous self-ligand for positive selection in both the mouse and human systems.[35] The biased Vβ usage of NKT cells is due to the inability of Vβ chains other than Vβ 2, 7 and 8 to interact with isoglobotrihexosylceramide-loaded CD1d molecules.[36] In addition, the relative degree of Vβ chain enrichment is proportional to the total avidity of the interactions between CD1d expression levels and individual TCR affinity.[37] For example, under conditions in which the expression of CD1d is downregulated, Vβ7-positive NKT cells can still be selected due to high TCR affinity, as compared to those positive for Vβ 2 and 8.[38] Therefore, the limited TCR repertoire of NKT cells appears to be shaped mainly by cortical positive selection, with little contribution from medullary events under physiological conditions.[38]

Unlike NKT cells, T–T $CD4^+$ T cells show a diverse TCR repertoire (our unpublished data) and are restricted by highly polymorphic MHC II molecules. Although substantial numbers of T cells can be generated even when a single Ep antigen (Eα peptide 52–68) is presented by thymic epithelial cells,[39] the TCR repertoire in these animals seems to be far from normal physiological condition.[40] Therefore, during the process of positive selection, it is essential for TECs to present a broad range of peptide antigens to generate a diverse, functional TCR repertoire. As the protein hierarchy of immature thymocytes is distinct from that of thymic epithelial cells in terms of developmental ontogeny, the nature of positively selecting peptide ligands presented by immature thymocytes is an interesting issue. One possibility is that either the proteins exclusively expressed by T cells such as TCRs or activation molecules—representative examples of which are CD25 and HSP60—would be strong candidates for positively selecting peptides. These two types of proteins could be individually categorized as an idiotope and ergotope and would be a primary source of antigens presented by T cells.

One example, where T-cell idiotope can be presented to T cells is the Qa-1 system. Qa-1 is a member of the MHC Ib family, which are expressed on antigen-presenting cells and activated T cells in mice. Its ligands comprise various peptides such as the MHC class I leader sequence Qdm, preproinsulin, bacterial GroEL, TCR Vβ fragments and endogenous heat-shock protein 60 (hsp60).[41–43] A number of studies have shown that TCR degradation products (idiotope) can be presented by Qa-1 molecules in activated $CD4^+$ T cells to be

eliminated.[41,44,45] Through this mechanism, regulatory $CD8^+$ T cells are able to control the outgrowth of pathogenic $CD4^+$ T-cell clones in a Qa-1-dependent manner,[46,47] This might also be the case in humans, as HLA-E, the human homologue of mouse Qa-1, is expressed on T lymphocytes and is known to present TCR Vβ peptide fragments.[48,49] All these data suggest similar mechanisms by which presentation of either T-cell-specific (idiotope) or activation molecules (ergotope) occur in thymocytes or activated peripheral T cells expressing MHC II on their surfaces.

Negative selection in thymus

Transgenic overexpression of CD1d molecules driven by the $H\text{-}2K^b$ promoter reduced the frequency of NKT cells in the thymus and peripheral lymphoid organs. This process is mediated by bone marrow-derived DCs rather than by thymic epithelial cells,[50] indicating that NKT cells are not inherently resistant to negative selection. However, the physiological relevance of this negative selection in terms of Vβ repertoire choice is not clear because biased Vβ usage of NKT cells is determined by cortical positive selection.[50]

T–T $CD4^+$ T cells in a CIITA knockout background escape negative selection and proliferate in response to autologous splenic antigen-presenting cells in a mixed lymphocyte reaction.[12] This is because medullary hematopoietic cells are also devoid of MHC II expression. However, when plck-CIITA-transgenic mice were backcrossed into a CIITA promoter type IV null background, in which the expression of MHC II molecules is absent only from cortical thymic epithelial cells ($CIITA^{tg}pIV^{-/-}$), T–T $CD4^+$ T cells did not show any signs of activation or proliferation in response to self-antigens (unpublished data). Therefore, it is evident that T–T $CD4^+$ T cells are subject to negative selection by both thymic epithelial cells and hematopoietic cells.

Several lines of evidence suggest that thymocytes themselves or various cells re-circulated into the thymus are able to mediate negative selection. Intrathymic transfer of semi-allogeneic DN thymocytes into irradiated hosts induces tolerance of host thymocytes and splenocytes to donor-specific MHC I, but not to MHC II, antigens.[23] This phenomenon is called split tolerance. Therefore, it seems clear that murine thymocytes, and possibly circulating lymphocytes, might participate in the deletion process that takes place in the thymic medullary region. Given that mouse T cells and thymocytes do not express MHC II molecules, split tolerance might be due to the lack of MHC II expression on transferred donor cells. However, we cannot completely exclude the possibility that a small number of donor-derived non-lymphoid hematopoietic cells might be responsible for this observation, as DN thymocytes still retain myeloid lineage potential.[51,52]

Minor lymphocyte stimulatory antigen (mls-1) is a super-antigen encoded by the endogenous mouse mammary tumour virus Mtv-7.[53] When mls-incompatible congenic lymphohematopoietic cells were injected intravenously, they re-circulated to the thymus and induced mls-specific tolerance.[24,54,55] More specifically, activated T cells, splenic B blast and thymic $CD5^+$ B cells—but not resting T or B cells—were able to tolerize host thymocytes.[56]

Dendritic cells pick up tissue specific antigens and enter the circulation to carry them to lymphoid organs, including LN, spleen, bone marrow and thymus, where they present antigens to T cells, resulting in tolerance or immunity. Under circumstances in which ovalbumin-loaded exogenous DCs were transferred intravenously, antigen-specific OT-II thymocytes were deleted by DCs that circulated into the thymus.[21] These two experiments provide a mechanism by which tissue-specific self-antigens, which would otherwise be invisible to developing thymocytes and lead to the failure of central tolerance to peripheral tissue antigens, are sampled outside the thymus.

T-cell maturation after positive selection

After positive selection, NKT cells undergo maturation processes in the thymic medulla. This process is divided into four stages according to CD24, CD44 and NK1.1 expression profiles, as shown below.[34,38]

- Stage 0: $CD24^{hi}CD44^{low}NK1.1^-$ with $CD4^+$ or DP^{low} ($CD4^{low}CD8^{low}$)
- Stage 1: $CD24^{low}CD44^{low}NK1.1^-$ with $CD4^+$ or DN ($CD4^-$ $CD8^-$)
- Stage 2: $CD24^{low}CD44^{hi}NK1.1^-$ with $CD4^+$ or DN ($CD4^-$ $CD8^-$)
- Stage 3: $CD24^{low}CD44^{hi}NK1.1^+$ with $CD4^+$ or DN ($CD4^-$ $CD8^-$)

Stage 0 NKT cells are the first detectable developmental intermediate, expressing high levels of CD69 and CD24, surface markers for positive selection and thymocyte immaturity, respectively. They are detected only by α-galactosylceramide-loaded CD1d tetramers and are almost completely absent in CD1d knockout mice.[57] However, this stage 0 population is relatively unaffected in mice deficient in SLAM, SAP, Fyn, PLZF, B7 and CD28, all of which are involved in subsequent maturation processes after the DP stage but are not involved in positive selection itself.[58–62] After positive selection, CD24 expression is downregulated and at stage 2, NKT cells begin to proliferate and upregulate activation marker CD44 before NK receptor expression. During the final maturation process, NKT cells either exit the thymus and begin to express NK1.1 molecules or remain in the thymus without emigration for unexplained reasons.[63] NK1.1 itself is not prerequisite to the functional maturation of NKT cells given that some of the $NK1.1^-$ population in the spleen and liver is functionally competent.[64] For their proper function, NKT cells secrete a panoply of cytokines, such as IFN-γ and IL-4, following an antigenic encounter. Interestingly, cytokine analysis of developmental intermediates shows that they first acquire the ability to transcribe IL-4 at stage 1 and IFN-γ starts to be transcribed later during stage 2, finally skewing to IFN-γ at stage 3. This Th2-to-Th1 conversion with NK1.1 upregulation is accompanied by a thymic output process, suggesting that their regulatory function is developmentally determined.[65]

Heat-stable antigen (CD24) is downregulated immediately before exit of mature thymocytes from the thymus. In conventional αβ-thymocytes, therefore, almost all DP thymocytes and the majority of single-positive thymocytes remain in the $CD24^{hi}$ stage. However, most of the α-galactosylceramide-loaded CD1d tetramer-positive NKT cells in the thymus are $CD24^{low}$ due to downregulation of CD24 during stage 1 and subsequent cell expansion during stage 2. This indicates that, unlike conventional αβ-T cells, NKT cells require further maturation after positive selection by acquisition of activation markers, cytokine secretion profile transitions and PLZF upregulation.[60,61] It is of interest that the expression profile of CD24 in NKT and T–T $CD4^+$ T cells is almost identical (Figure 1).[12] In $CIITA^{tg}$ mice, the $CD24^{low}$ fraction is increased compared to littermate controls and most T–T $CD4^+$ T cells in plck-$CIITA^{tg}CIITA^{-/-}$ mice, where MHC class II expression is strictly restricted to T cells, express very low level of CD24.[12] In the CIITA-transgenic mice, T cells develop through both T–T and T–E interactions, while T–T interactions are the only selection pathway in the plck-$CIITA^{tg}CIITA^{-/-}$ mice. Therefore, the accumulation of $CD24^{low}$ thymocytes in the thymic medulla might reflect clonal expansion and the presence of additional maturation processes similar to those present in NKT cells. A few questions remain with regard to why T–T $CD4^+$ T cells follow the expansion and maturation patterns observed for NKT cells. These questions include

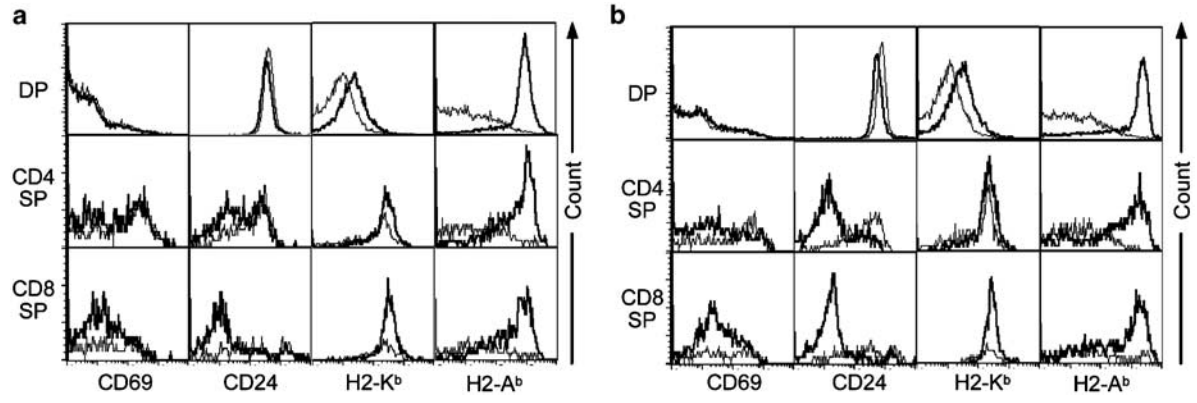

Figure 1 CD24 expression profile of T–T CD4+ thymocytes. Single-cell suspension of thymocytes from CIITA^{tg} (**a**) and CIITA^{tg}×CIITA$^{-/-}$ mice (**b**) were stained for CD69, CD24, MHC I (H2-K^b) and MHC II (I-A^b). The single-colour histogram shows the relative expression level of CD24 (thick line) in comparison with wild-type littermate controls (thin line). Figures are from reference[12] supplement (Supplementary Figures S1a and S2).

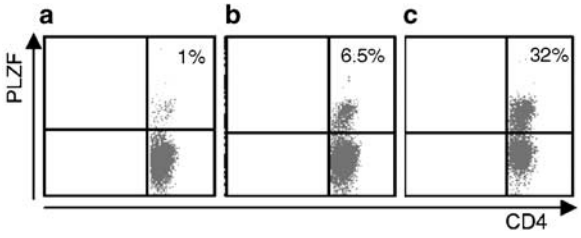

Figure 2 PLZF expression in T–T CD4+ T cells. Single-cell suspensions of thymocytes from wild-type littermate (**a**), plck-CIITA^{tg} (**b**), and CIITA^{tg}pIV$^{-/-}$ (**c**) mice were stained for CD4, CD8, and PLZF as described previously.[57] Numbers indicate the expression percentage of PLZF-positive cells (gated CD4$^+$CD8$^-$ single-positive thymocytes).

whether all T–T CD4$^+$ T cells have equal innate immune properties, whether the expansion profile is even between individual clones or is skewed toward a particular repertoire, and whether all of T cells emerging in the periphery react with a single type of cell, either T or DCs, or some T cells interact with T cells and others with DCs.

Requirement of transcription factors during maturation after positive selection

Recently, two independent research groups found that promyelocytic leukaemia zinc-finger protein, PLZF (also known as zbtb16), controls the development of mouse and human NKT cells.[60,61] Expression of PLZF was restricted to NKT cells and to MAIT and CD4$^-$CD8$^-$CD45RO$^+$ T cells in human peripherial blood mononuclear cells. Mice lacking PLZF due to spontaneous (luxoid mouse) or targeted mutations showed selective depletion of peripheral and thymic CD24^{low} stage NKT cells, whereas the intrathymic CD24^{hi} population remained relatively unaffected. This phenotype is quite similar to situations in which signalling molecules essential for NKT cell development are deficient (for example, SAP and Fyn). The expression of these molecules, however, appears to be independently regulated, as SAP- or Fyn-deficient stage 0 NKT cells exhibit normal PLZF expression. Small number of NKT cells in the periphery of PLZF-deficient hosts do not retain their innate characteristics, including localization to non-lymphoid tissue and cytokine secretion properties. In contrast, transgenic overexpression of PLZF converts naive CD4$^+$ thymocytes into effector memory phenotype T cells. Thus, it seems that PLZF expression is necessary and sufficient for T cells to acquire innate functions.

PLZF is also upregulated in T–T CD4$^+$ T cells (Figure 2, manuscript in preparation), and it is highly probable that PLZF-deficient T–T CD4$^+$ T cells could be arrested at CD24^{hi} as observed for NKT cells. The key question, however, has to do with what kind of cellular interactions are able to specifically upregulate PLZF, leading to the acquisition of innate characteristics.

BIOLOGICAL RELEVANCE OF HUMAN T–T CD4$^+$ T CELLS

T–T CD4$^+$ T cells are a unique subset of CD4$^+$ T cells. Unlike conventional CD4$^+$ T cells, they share several features with innate-like T cells. They are selected by thymocytes in a SLAM–SAP-dependent manner and are immediately able to produce cytokines after TCR-CD3 stimulation.[14] In mice, a large number of studies have documented the responses of innate T cells during specific infections. One major theme emerging from these studies is that innate T-cell subsets commonly respond to bacterial infections.[5] For example, NKT cells are dominant during responses to intracellular bacteria such as *Mycobacterium tuberculosis*, while H2-M3-specific innate T cells represent a well-characterized component of the response to *Listeria monocytogenes*.[5] More recently, MR1-specific T cells have been proposed to respond to antigens induced by, or derived from, commensal bacteria present in the gut.[5] Unlike known innate-like CD8$^+$ cells and NKT cells, T–T CD4$^+$ T cells show more diverse TCR Vβ usage. In terms of their repertoire of antigenic peptides eliciting T-cell recognition, viral antigens seem to be even more diverse than bacterial antigens. Thus, T–T CD4$^+$ T cells would be most likely to be naturally more effective in response to viral infections compared with innate-like CD8$^+$ T cell and NKT cells. One important clinical example in support of this suggestion is a phenomenon observed in XLP patients. XLP syndrome is a rare but fatal inherited immunodeficiency characterized by an inability to respond properly to infection caused by the nearly ubiquitous Epstein–Barr virus.[17] This condition is caused by mutation of the *sh2d1* gene, which encodes the SAP protein.[66] Because mutation of the *sh2d1* gene induces defects in the development of NKT cells, it has been suggested that the absence of NKT cells contributes to the abnormal anti-viral immunity observed in XLP patients.[17] Considering the fact that SAP is also a crucial regulator of T–T CD4$^+$ T-cell ontogeny in a mouse model, however, the development and functional activity of T–T CD4$^+$ T cells should also be defective in XLP patients. On the basis of this, we propose that XLP is caused primarily by a deficit of T–T CD4$^+$ T cells rather than NKT cells alone, and that T–T CD4$^+$ T cells might represent the primary acute-phase effector cells necessary for anti-viral immunity. Until very

recently, NK cells have been known as the main responders to viral infection.[5] However, NK cells lack diverse antigen specificity, whereas T–T CD4$^+$ cells have diverse antigen-specific TCRs. Thus, T–T CD4$^+$ T cells might serve as a more effective first line of defence against certain viral pathogens owing to their rapid effector function and antigen specificity. This would provide a strong tool for resistance before the establishment of sufficient memory pool in adaptive immune response elicited by conventional T cells.

An additional characteristic of T–T CD4$^+$ T cells that is distinct from conventional CD4$^+$ T cells is the nature of selecting ligand during thymic education. In contrast to conventional T cells, T–T CD4$^+$ T cells are selected by antigenic peptides generated in thymocytes rather than by thymic epithelial cells. As mentioned above, a randomly rearranged set of TCR peptides (idiotope) or activation markers (ergotope) are plausible candidates as positively selecting ligands for T–T CD4$^+$ T cells. In the thymic medulla, anti-idiotypic or anti-ergotypic T–T CD4$^+$ T cells would escape negative selection mediated by medullary thymic epithelial cells or bone marrow-derived DCs because they do not express these two kinds of molecules. For this reason, T–T interactions through MHC class II–TCR interaction in the thymus may support the development of both anti-idiotypic and anti-ergotypic T cells. Anti-idiotypic T cells recognize specific effector clones by their unique TCR CDR3 peptides. Anti-ergotypic T cells, unlike anti-idiotypic T cells, do not recognize the clonal identity of effector T cells; rather, anti-ergotypic T cells recognize the activation state of target effector T cells, irrespective of their TCR specificity.[67] In humans, activated T cells express MHC class II molecules on their surface; thus, both anti-idiotypic and anti-ergotypic T cells are able to predominantly downregulate their activated target T cells.[67] These interactions represent one of the immune regulation mechanisms. This immunoregulatory role of T–T CD4$^+$ T cells has been well documented in studies using animal models of allergen-induced airway inflammation[14] and experimental allergic encephalitis.[68]

We propose two main functions of T–T CD4$^+$ T cells. One is a rapid anti-viral immediate effector function; the other is an immunoregulatory function. The mechanisms underlying the regulatory or effector functions of T–T CD4$^+$ T cells are not well understood. Analogies can be drawn, however, from NKT cells, which also play a dual role in the immune system. The CD40–CD40L interaction in NKT cells results in IFN-γ secretion and homotypic SLAM or integrin–LFA-1 interactions, promoting IL-4 production.[69] As a proinflammatory Th1-type cytokine, IFN-γ is associated with the function of adjuvants in enhancing immune responses against microbes or tumour cells. On the other hand, Th2-type cytokines such as IL-4 and IL-10 induce tolerance and ameliorate autoimmune disease in various animal models, including type I diabetes mellitus, multiple sclerosis and rheumatoid arthritis.[69] Because activated T cells express high levels of SLAM family molecules, whereas CD40 is generally thought to be absent or sparse,[70] peripheral T–T interactions might skew responding T cells towards a Th2-type phenotype to secret large amounts of IL-4 and IL-10, which are important for immunoregulation. When mature DCs present peptides derived from foreign pathogens, they upregulate their surface expression of CD40 as well as SLAM[71] to induce an antigen-specific immune response. In this case, T–T CD4T cells develop into effector T cells and participate in early phase of the host defence system due to their innate properties, such as rapid activation and robust cytokine secretion.

The biological functions of T–T CD4$^+$ T cells proposed here are based mainly on data obtained from NKT cells and T–T CD4$^+$ T cells in mice. In humans, it will be fairly difficult to acquire more solid data until T–T CD4$^+$ T-cell markers are available.

Table 1 Differences and similarities between NKT and T–T CD4$^+$ T cells

	NKT cell	*T–T CD4$^+$ T cell*	*Conv. CD4$^+$ T cell*
TCR repertoire	Canonical	Diverse	Diverse
MHC restriction	CD1d	MHC II	MHC II
Selecting ligand	Glycolipid (iGb3)	Idiotype/ergotype?	Self-peptide
Positive selection	Thymocyte	Thymocyte	Epithelial cell
Negative selection	HPC	TEC and HPC	TEC and HPC
PLZF expression	Yes	Yes	No
SALM–SAP dependency	Yes	Yes	No
Conv. B-cell help	No[a]	Yes	Yes
Conv. CD8$^+$ T-cell help	No[a]	Yes	Yes

Abbreviations: Conv., conventional; HPC, hematopoietic cell; MHC, major histocompatibility complex; NKT, natural killer T cell; TCR, T-cell receptor.
[a]NKT cells can provide help to B cell in CD1d-dependent antigen and CD8$^+$ T cell in the presence of α-Gal.[73,74]

CONCLUSION

In this paper, we have discussed a novel CD4$^+$ T-cell population generated by T–T interactions in the thymus. These cells share several features with previously described T cells that are selected by hematopoietic cells. The SLAM–SAP signalling pathway is essential for their development and functional maturation, as shown for NKT cells and innate-like CD8$^+$ T cells. At the same time, T–T CD4$^+$ T cells and innate-like T cells, along with NK cells,[72] can secret large amounts of effector cytokines during early phase of antigen exposure. However, the major difference between other innate T cells and T–T CD4$^+$ T cells lies in their diverse TCR repertoire, which enables them to respond specifically to invading pathogens. On the basis of these distinct characteristics, we propose that once T–T CD4$^+$ T cells enter the peripheral immune system, they provide two major functions. First, T–T CD4$^+$ T cells function as rapid effector cells in response to viral infection. Their highly diverse TCR repertoire, as well as their rapid effector function allow for specific recognition of viral antigens and provide a first line of defence against certain viral infections. Second, selection by thymocytes could lead to skewing of the T-cell repertoire. In particular, it is possible that encountering T-cell-specific peptides presented on MHC class II molecules in the thymus supports the generation of anti-idiotypic and ant-ergotypic T cells. These anti-idiotypic and ant-ergotypic T cells enhance the ability of the regulatory T–T network to control immune responses.

T–T CD4$^+$ T cells are also able to assist B cells in the production of immunoglobulins in response to T-cell-dependent antigens and to aid CD8$^+$ T cells in generating memory responses to conventional protein antigens, just similar to conventional T cells (unpublished data). Thus, their similarities and differences (Table 1) with innate and conventional T cells in terms of TCR diversity, helper functions and innate characteristics position them as a bridge between innate T cells and conventional T cells. These properties seem to be particularly important during defence against viral infections and regulation of the immune system, as seen in XLP patients.

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