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# A multilayered immune system through the lens of unconventional T cells

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## Abstract

The unconventional T cell compartment encompasses a variety of cell subsets that straddle the line between innate and adaptive immunity, often reside at mucosal surfaces and can recognize a wide range of non-polymorphic ligands. Recent advances have highlighted the role of unconventional T cells in tissue homeostasis and disease. In this Review, we recast unconventional T cell subsets according to the class of ligand that they recognize; their expression of semi-invariant or diverse T cell receptors; the structural features that underlie ligand recognition; their acquisition of effector functions in the thymus or periphery; and their distinct functional properties. Unconventional T cells follow specific selection rules and are poised to recognize self or evolutionarily conserved microbial antigens. We discuss these features from an evolutionary perspective to provide insights into the development and function of unconventional T cells. Finally, we elaborate on the



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functional redundancy of unconventional T cells and their relationship to subsets of innate and adaptive lymphoid cells, and propose that the unconventional T cell compartment has a critical role in our survival by expanding and complementing the role of the conventional T cell compartment in protective immunity, tissue healing and barrier function.

The selection through evolution of a system as complex as T cell immunity and its requirement for survival leaves no doubt about its role and importance in vertebrates. Conventional T cells, which are the central actors of adaptive immunity, target and clear infectious non-self. Whereas conventional T cells primarily recognize specific peptides that are presented by the classical polymorphic major histocompatibility complex (MHC) class I and class II molecules, the unconventional T cell compartment encompasses subsets of T cells that cover the sensing of a diverse range of self and non-self molecules. These include lipids sensed by natural killer T (NKT) cells, CD1a-, CD1b- or CD1c-restricted T cells and T cell receptor (TCR)γδ T cells; metabolites sensed by mucosal-associated invariant T (MAIT) cells and TCRγδ T cells; peptides sensed by H2-M3-restricted T cells, Qa-1-restricted T cells, HLA-E-restricted T cells and TCRαβ CD8αα intraepithelial T lymphocytes (IELs); and self-surface proteins sensed by various subsets of TCRγδ T cells. Numerous previous reviews have focused on TCRαβ[1–11] and TCRγδ unconventional T cells[12,13]. Because of its highly redundant nature and the difficulty of demonstrating the requirement for any specific unconventional T cell subset, it is essential to discuss the unconventional T cell compartment as a whole with its unique features and functions. Why vertebrates have also evolved an unconventional T cell compartment, and what the importance of this compartment is in health and disease, remain highly debated questions in biology.

In this Review, we will attempt to provide order to a complex unconventional T cell compartment, while drawing comparisons with the more prominent conventional T cell compartment. We will discuss unconventional T cells with regard to their abundance, their niches and their stability, and will position them in terms of where they fit in an immune response, with an emphasis on human immunity. For example, for TCRγδ T cells we will focus on the butyrophilin (BTN)- and butyrophilin-like (BTNL)-reactive subsets. Finally, we will discuss the evolution and timescale of unconventional T cells relative to adaptive immunity, emphasize the value of redundancy as revealed by their level of genetic conservation across individuals, but also highlight aspects of these T cells that are indispensable to survival.

## Classifying unconventional T cells

### Class of ligand

The central requirement for a functional immune response is the ability to respond to foreign agents that breach the barriers of the host. Similar to conventional T cells, some unconventional T cell subsets also engage in non-self recognition. For instance, unconventional HLA-E-restricted T cells in humans[14] recognize foreign peptides[15]. However, non-self recognition by unconventional T cells extends beyond peptides, and includes the recognition of canonical non-self molecules that are present across bacterial

species, including foreign lipid moieties[16–18], bacteria-derived vitamin B metabolites[19,20], bacteria-derived formylated peptides[21,22] and phosphoantigens[23]. Therefore, one can think of the antigens driving these unconventional cells as a form of microbial extended self.

The same set of machinery that facilitates the recognition of non-self is also used for the recognition of self. CD1 molecules can load self-lipids and CD1a autoreactive T cells have been characterized in human skin[24,25]. Fetal Vγ9Vδ2 T cells take on an effector profile[26], suggesting that the recognition of self phosphoantigens is a key part of their biology. Although HLA-E can present foreign peptides in the context of certain viral infections[14,27], under homeostatic conditions it actually serves to present peptides derived from MHC class I leader sequences[28] to primarily regulate T cells[29] and natural killer (NK) cells[30] through the engagement of NKG2–CD94 receptors. However, the potential role of T cells with specificity for HLA-E in immunity remains poorly understood[31]. The recognition of self is of course not reserved to unconventional T cells, as regulatory T cells are known to recognize self-peptides[32].

Moreover, the unconventional T cell compartment can recognize non-polymorphic ligands that have no capacity to present antigens. This is particularly true for the TCRγδ T cell compartment—for example, the biology of a subset of mouse skin-resident TCRγδ T cells depends on recognition of SKINT-1 (ref.[33]), and the biology of a subset of intraepithelial intestine-resident cells depends on the molecules BTNL1 and BTNL6 (hereafter, BTNL1/6) in mice[34] or the human equivalents BTNL3 and BTNL8 (hereafter, BTNL3/8) in the colon[34] and small intestine[35]. TCRγδ T cells can recognize other non-antigen-presenting molecules such as MICA[36], ULBP4[37], T10[38] and T22[39], but the biological implications of these interactions are less clear than those of SKINT-1 and the BTNLs.

Thus, the unconventional T cell compartment as a whole is capable of surveillance through the TCR at all levels of cellular immunity—via the recognition of non-self- and self-derived antigens as well as non-antigen-presenting self-ligands—akin to how innate immunity operates.

## T cell receptors

Unconventional T cells can be classified into three pools on the basis of their TCR usage and diversity. The first pool is characterized by semi-invariant TCR usage and includes the most-characterized unconventional T cell subset—the type I NKT cell—which expresses an invariant Vα14-Jα18 (TRAV11, TRAJ18) and Vα24-Jα18 (TRAV10, TRAJ18) TCR α-chain in mice and in humans, respectively[40], to recognize CD1d loaded with the prototypical lipid antigen α-galactosylceramide[4,41]. Similarly, MAIT cells express an invariant Vα19-Jα33 (TRAV1–2, TRAJ33)[42] and Vα7.2-Jα33–20-12 (TRAV1–2, TRAJ33, TRAJ12, TRAJ20)[43] TCR α-chain in mice and humans, respectively, to recognize the vitamin B precursor 5-OP-RU loaded on MHC class I-related protein (MR1)[19,44]. For both of these unconventional T cell subsets, whereas the use of the TCR α-chain is largely fixed, that of the TCR β-chain is variable but constrained. Similarly, germline-encoded mycolyl-reactive (GEM) T cells express an invariant TRAV1–2 and TRAJ9 TCR α-chain to sense mycobacterial mycolates loaded on human CD1b[45,46]. Notably, TCRγδ T cell subsets also tend to be characterized by their limited TRGV and TRDV gene-segment use,

and this is reflected in their ligand specificities; for example, Vγ5 TCRs are restricted to SKINT-1[33], and TCRs that use the Vγ chains Vγ7 in mice and Vγ4 in humans are restricted to BTNL1/6 and BTNL3/8, respectively[34,35]. However, these TCRγδ T cell subsets exhibit a high CDR3 diversity[35,47]. Given this conserved restriction on TCR–ligand pairs, it is likely that TCRs and their ligands coevolved over time[48].

The second pool of unconventional T cells is characterized by diverse TCR usage with regard to both TCR gene-segment use and CDR3 sequence, and includes subsets such as H2-M3-restricted T cells[49], HLA-E-restricted T cells[50] and TCRαβ CD8αα IELs that recognize class I and II molecules[51,52] and non-classical MHC class I molecules[53]. Of note, this second pool is tailored—similarly to conventional T cells—to the recognition of peptides.

Finally, the third pool of unconventional T cells is characterized by T cells with diverse TCRs that can bind CD1 and MR1. This pool includes type II NKT cells[1], MR1-restricted T cells[54] and TCRγδ T cells[55–57]. The presence of such cells is probably the consequence of the diversity present within the naive TCR repertoire, as well as the diversity of antigens that such ligands can present, which allows for naive T cell clones being selected in the periphery by these monomorphic MHC class I-like molecules.

## Modes of ligand recognition

Since the pioneering studies detailing the first αβTCR–peptide–MHC-Ia structures 25 years ago[58,59], we have learned a lot about the molecular basis that underpins αβTCR engagement. Numerous structural studies have shown that αβTCRs can recognize peptide–MHC-Ia complexes in various docking modes, mostly with a canonical docking polarity[60] (Fig. 1a)—albeit with two notable exceptions[61,62]. Nevertheless, αβTCRs have been universally shown to simultaneously co-recognize the peptide and MHC[63], which represents a central tenet of the MHC-restricted T cell response.

With regard to unconventional TCRs, whether they would adopt the general principles of TCR–peptide–MHC recognition was unknown. The MHC fold has shown remarkable plasticity, having adapted to present lipid- and metabolite-based antigens (by the CD1 family and by MR1, respectively)[64]. Moreover, whereas MHC-Ia is highly polymorphic, MHC-Ib, CD1 and MR1 show extremely limited polymorphism, yet represent targets for αβTCR and γδTCR recognition[65]. Although MHC-Ib molecules are considered primarily a ligand for NK cells[66], they can also be recognized by TCRαβ T cells, in which the αβTCR can bind peptide–MHC-Ib in a similar manner to TCR–peptide–MHC-Ia binding[67] (Fig. 1a). The first insight into how unconventional TCR recognition differs from conventional TCRs came from the structure of the type I NKT TCR–CD1d–lipid complex[68,69]. In this structure, the type I NKT TCR was perched towards the extreme end of the CD1d antigen-binding cleft, adopting a parallel docking mode. The TCR–MR1–metabolite recognition of MAIT cells was more analogous to TCR–peptide–MHC-I recognition. The invariant TCR α-chain bias observed in type I NKT cells and MAIT cells was attributable to specificity contacts with CD1d–lipid and MR1–metabolite, respectively[68,70,71] (Fig. 1a), indicating that germline-encoded recognition is a common feature of type I NKT and MAIT cells—an

observation echoed by GEM TCR recognition of a mycobacterial antigen presented by CD1b[46] (Fig. 1b).

In comparison to MAIT cells and type I NKT cells, a more diverse unconventional TCR repertoire directed against MR1 and CD1 has been observed, which has manifested in more-varied docking strategies atop their respective antigen-presenting molecules, with many features analogous to that of conventional TCR–peptide–MHC recognition[72–74] (Fig. 1a). Some CD1 family members and MR1 also represent ligands for γδTCRs. γδTCR recognition of CD1d–lipid demonstrated how the γδTCR bound the CD1d molecule and co-recognized the lipid[55,56] (Fig. 1c), whereas a subset of γδTCRs was shown to bind 'down under' the antigen-binding platform of MR1, and thereby not interact with the metabolite-based antigen[57] (Fig. 1c). This break of the TCR co-recognition paradigm also appears as a feature of autoreactive αβTCRs towards CD1a and CD1c, whereby the αβTCR sat atop the antigen-binding platforms but nevertheless, via distinct mechanisms, did not contact the lipid[25,75] (Fig. 1b). Thus, for some unconventional TCRs, a distinguishing feature is the lack of requirement for the co-recognition of antigen and antigen-presenting molecule, which raises questions relating to thymic selection, specificity of response and whether such features could be exploited for therapeutic purposes.

## Development and gain of effector programs

Conventional T cell development takes place in the thymus, in which cells are positively selected on self-peptide–MHC complexes, whereas the acquisition of effector programs occurs in the periphery on foreign-peptide–MHC complexes. The unconventional T cell compartment does not fit into this paradigm. By focusing on where and how a given T cell subset acquires its effector program, we can divide unconventional T cells into three groups (Fig. 2).

The first group is characterized by cells that are selected in the thymus and acquire effector programs as a consequence of recognition of their ligand(s) in the thymus. This includes type I NKT cells and MAIT cells, which undergo positive selection on haematopoietic cells[76,77], and dendritic epidermal T cells (DETCs), which are selected on thymic epithelial cells[33]. The acquisition of effector programs in the thymus for NKT and MAIT cells requires the transcription factor PLZF (encoded by *Zbtb16* in mice)[78,79], expression of the ligand on double-positive thymocytes[76,77] and co-signals provided by signalling lymphocytic activation molecule (SLAM)-associated protein (SAP)[80]. Similarly, H2-M3-restricted T cells that acquire effector programs in the thymus are selected on haematopoietic cells[81] and require SAP[82]. Finally, unconventional mouse TCRαβ CD8αα IELs also acquire effector programs in the thymus through selection on a diverse set of classical and non-classical MHC molecules[53,83], and do not require PLZF for their development[78]. Notably, although expression of the ligand in the periphery is not required for the expansion and effector function of NKT cells[84], it is required in mice for unconventional TCRαβ CD8αα IELs[85] and probably MAIT cells[86]. Acquisition of effector functions in the thymus and the hardwiring for particular functional outputs has led this group of unconventional T cells to be dubbed 'preset' T cells that are able to colonize tissues early in life and respond rapidly to stimuli.

The second group consists of cells that are unique to the unconventional T cell compartment. These cells exit the thymus in a naive state but acquire effector programs early in life in tissues that express cognate self-ligands. This has been best described for the mouse Vγ7 subset, which acquires a unique effector profile within the first weeks of life once it engages the self-ligand BTNL1/6 in the intestinal epithelium[34]. In humans this is associated with the acquisition of a unique NK-cell-like program, which endows Vγ4 T cells with specific innate properties[35].

The third group fits more in line with classical T cells in which effector programs are acquired in the periphery in response to engagement with cognate foreign antigen. This includes HLA-E- and Qa-1-restricted T cells[87,88] and may include subsets of CD1- and MR1-reactive T cells that exhibit diverse TCR usage[1,54].

## Tissue-specific niches and stability

A defining feature of the majority of unconventional T cells is their intimate relationship and localization within tissues, especially mucosal sites. As was detailed above, for SKINT-1-reactive DETCs[33,89] and BTNL-reactive TCRγδ T cell subsets[34,35], expression of their selecting ligand determines their enrichment in the skin and in the intestine, respectively. Studies in mice have shown that NKT cells and MAIT cells exhibit enrichment in tissues such as the liver[90,91], and in particular for MAIT cells in the skin[86]. This is presumably due to the enrichment of lipid and metabolite antigens at mucosal sites where bacteria interface with the host.

Space in tissues is limited and as a host ages, exposure to insults leads to increased occupation by adaptive tissue-resident lymphocytes[92,93]. Therefore, it is unsurprising that the niches that unconventional T cell subsets occupy exhibit temporal restrictions. This has been shown both for MAIT cells in the skin[86] and for BTNL1/6-reactive TCRγδ T cells in the intestine[34], whereby exposure to cognate ligand within the first weeks of life is required for T cell expansions and the establishment of sizeable tissue-resident niches in mice. Of note, the size of the type I NKT cell niche in the colonic lamina propria is limited by microbial signals such that germ-free mice exhibit expansions of these cells, which can only be normalized by colonization with microbiota early (and not late) in life[94].

Another critical question is the stability of the unconventional T cell niche with age and in conditions of inflammation, and whether it requires the ongoing expression of ligands. NKT cells, MAIT cells and BTNL3/8-reactive TCRγδ T cells can all be found in healthy adult tissues[35,95,96], which highlights the stability and longevity of these compartments. However, closer inspection in the context of specific acute or chronic perturbations to these niches reveals that in the context of acute viral infection, skin-resident DETCs are locally displaced at the site of infection by conventional tissue-resident CD8 T cells generated against the virus[97]. Furthermore, in the context of chronic inflammation associated with coeliac disease, loss of BTNL3/8 expression in the small intestine and an expansion of TCRγδ T cells with new specificity and function is associated with a permanent displacement of Vγ4 BTNL3/8-reactive TCRγδ IELs[35]. Together, these examples illustrate the complexity of lymphocyte–tissue dynamics and how cross-talk and competition between different unconventional T cell subsets ultimately shapes specific niches.

## Preset functional niche

The unconventional T cell compartment as a whole covers the full spectrum of T cell effector responses including chemokine-driven immune cell recruitment[35,98,99], helper cytokine responses[4,100], cytotoxic responses[4,35,100,101] and wound healing responses[35,49,86,99,102].

It is not so much the nature of the effector function mediated by unconventional T cells that sets them apart from the adaptive T cell compartment, but the nature of the ligands that drive their activation, their effector status at homeostasis and their ability to colonize tissues and respond to insults early in life (Fig. 3). The conventional T cell compartment, by generating antigen-specific 'adaptive' memory responses against pathogens, is best suited for sterilizing adaptive immunity, because of both its exquisite specificity and adaptability (covering all classes of microorganisms). However, this capacity is also intrinsically linked to the requirement for a T cell of a given specificity to undergo massive expansion. By contrast, the majority of unconventional T cell subsets exist as pre-expanded populations at steady state[8] (Fig. 3), that recognize conserved microbial antigens[19,21,22,86], constitutively expressed self-ligands such as BTNL3/8[34,35] or stress-induced self-ligands such as MICA[36,103], which can further facilitate the establishment and maintenance of homeostasis.

The engagement at steady state by self-ligands and microbial extended-self-ligands has two major consequences. First, unconventional T cells—unlike conventional T cells that only exert effector functions at the time their TCR is engaged by a specific foreign microbial antigen—have the capacity to mediate functions at homeostasis that are important for the initiation and amplification of protective immune responses[17,18,104–106], as well as for tissue healing[13,49,86,102,107]. A role for TCRγδ T cells in wound healing in mice[107] and humans[102] was proposed early on before the tissue-resident T cell field had gained traction. This has been more recently highlighted in two mouse models of wound healing that showed that wounds heal faster in mice that establish skin-resident commensal-specific MAIT cell[86] and H2-M3-restricted T cell responses[49] before tissue injury. Second, unconventional T cells are expanded at homeostasis, colonize tissues early in life and have the capacity to respond to innate immune signals; they can therefore have a key role in the protection of tissues against pathogens (Fig. 3). Although tissue-resident conventional CD8 T cells are also critically regulated by innate signals[108], the inherent capability of innate-like T lymphocytes to respond to alarmins and stress ligands without requiring strong TCR engagement (Fig. 3) allows them not only to respond rapidly, but also to instruct the adaptive immune system as to the health status of the tissue. This role is certainly critical in the context of viral infections associated with the downregulation of MHC class I molecules[109]. The prominent role of innate signals alone or in combination with the TCR in the switch to protective functions and the activation of tissue-resident MAIT cells[110,111], NKT cells[112,113] and TCRγδ T cells[114,115] has been demonstrated in mice and humans (Fig. 3). Similarly, in humans, BTNL-reactive TCRγδ T cells[35] and Vδ1 TCRγδ T cells present at tumoral sites[116] were shown to require IL-15 and/or the engagement of activating NK receptors to exert their full cytolytic potential. Finally, the role for innate-like T lymphocytes in the initiation of adaptive immunity and its amplification is illustrated by the adjuvant role that NKT cells can have by initiating a high-speed communication network between the innate

and the adaptive immune system[117]. Together, the unique development, expansion and restriction of unconventional T cells endows them with the capacity to colonize tissues early in life; form a first line of defence against pathogens before adaptive tissue-resident T cells colonize tissues; and exert important homeostatic, innate, protective and healing functions at tissue sites. Their functional properties and conservation across individuals has also made them an attractive tool for immunotherapy, especially in the treatment of cancer[118,119].

## Evolution and redundancy

### Perspective on evolution

Unconventional T cell immunity is generally thought of as the primitive form of conventional 'adaptive' T cell immunity. A common argument presented is that limited gene diversity existed initially in the TCR locus and this limited diversity was best suited for the recognition of a limited set of non-polymorphic MHC-like molecules[120]. Under this premise, the unconventional T cell compartment was insufficient and thus the adaptive immune system that encompasses conventional MHC-Ia-restricted T cells evolved to support it. This is certainly a plausible scenario, but here we would like to entertain the alternative possibility whereby conventional T cell immunity evolved first and was later supplemented at different moments over the course of evolution by a variety of unconventional T cell compartments. This occurred, we suggest, because the addition of unconventional T cell compartments added both resilience—by providing the means to ensure early-life protective immunity, tissue homeostasis and barrier function—and robustness, by informing and amplifying the adaptive immune response.

The first argument can be made by studying the occurrence of conventional versus unconventional T cell immunity across species. To our knowledge, no species has been studied to date that has evidence of unconventional T cell immunity in the absence of conventional T cell immunity in terms of both ligands and TCRs, meaning that MHC and MHC-like molecules are always found together and semi-invariant TCRs are not found in the absence of diverse TCRs. There are mammalian species that lack MR1 and CD1d and which therefore lack MAIT and NKT cells[120–122], and the self-ligand SKINT-1 that selects the TCR$\gamma\delta$ T cell subset of DETCs in mice is not well-conserved[123]. When comparing mice and humans, subsets such as H2-M3-restricted T cells in mice are absent in humans, whose genomes do not contain the H2-M3 gene[124], whereas humans have CD1a-, CD1b- and CD1c-restricted T cell subsets that are absent in mice[48,65,125]. Thus, it is possible that each species evolved or maintained the ligands required for the selection of particular unconventional T cells on the basis of the pressures imparted by their unique lifestyle.

In addition to the evidence provided at the species level, another approach to discussing the origins of unconventional T cell immunity is to consider the nature of the antigens that are recognized. Notably, unconventional T cell subsets such as NKT, MAIT and H2-M3-restricted T cells are all geared towards the recognition of conserved bacterial products, and have a blind spot for the direct recognition of virus-derived antigens. Although there are studies that implicate these subsets in antiviral responses[106,126], the evolutionary benefit is more likely to arise from the recognition of universal bacterial products—which in some cases go as far as to guide their development, as in the case of MAIT cells[127]. Such a

recognition could provide the host with the ability to gauge the overall bacterial load at mucosal surfaces and provide broad protective immunity early in life. However, no single unconventional T cell subset is able on its own to ensure effective protective immunity against a variety of pathogens, and adaptive immunity was shown to be required for survival in a pathogen-rich environment[128]. Indeed, a system favouring the broad recognition of protein-derived peptides would thus have been the more suitable first choice as it would allow the host to respond to all classes of pathogens, including bacteria, viruses, fungi, helminths, parasites and protozoans. In this framework, conventional T cell immunity would have evolved first to ensure a broad coverage of classes of pathogens, before focusing on supplementing mucosal barriers and innate immunity in an effort to maintain the optimal homeostatic relationship with the microbiota.

## Framing redundancy within T cells

The majority of studies in animal models that have attempted to demonstrate a critical role for a given unconventional T cell subset in protective immunity have failed or suggested it using contrived systems. An alternative approach to gain insights into the requirement for the unconventional T cell compartment is to take a human genetics approach[129,130] and query how constrained deleterious mutations are in genes that encompass the building blocks of all T cell responses (Fig. 4a). In healthy individuals, loss-of-function mutations are found in CD1d, MR1[131] and BTNL3/8, but also, strikingly, in classical MHC molecules such as HLA-A and HLA-B (Fig. 4a), suggesting that for both unconventional and conventional T cells there is a substantial degree of redundancy when considering a T cell subset with a given ligand specificity. This observation probably reflects the notion of a layered immune system, whereby the same effector functions can be attained through both unconventional and conventional T cells and therefore the loss of any individual component might be tolerated (Fig. 4b). By contrast, null mutations in *ZAP70*, encoding a signalling molecule required for the differentiation and activation of all T cell subsets, are very rare (Fig. 4a). Other genes that are intolerant to loss-of-function mutations encode proteins that are shared not only by all T cell subsets but also by non-T cell subsets. This can be observed at the level of cell programming; for example, for transcription factors such as TBX21 that are associated with T cell differentiation, and effector molecules such as interferon-γ (IFNγ), which are relevant for both conventional and unconventional T cells as well as innate lymphoid cells. Notably, for effector molecules involved in type 2 immunity, in which IL-4, IL-5 and IL-13 may be able to compensate for one another, loss-of-function mutations are more common (Fig. 4a). The tissue-repair-associated molecule amphiregulin exhibits a strong selective constraint (Fig. 4a), which is particularly interesting when considering that unconventional T cells may be best suited for wound healing in tissues as described above.

To formally quantify the levels of selective constraint in unconventional T cells, we analysed whether null mutations were allowed for *ZBTB16* (also known as *PLZF*) and *HIVEP3*, which encode two critical regulators for NKT and MAIT cell differentiation[78] and or expansion[132]; PLZF also has a role in the development of innate lymphoid cells[133]. Strikingly, in contrast to *MR1* and *CD1D*, there is evidence for a strong selection constraint for *PLZF* and *HIVEP3*, suggesting that although NKT and MAIT cells may be dispensable individually, the loss of all PLZF- and HIVEP3-dependent unconventional

T cell subsets may not be, as this would result in the loss of a functional niche that recognizes 'microbial extended self'. Although we cannot formally eliminate that the lack of these transcription factors may have a critical non-immunological biological role, the strong selection constraint seen in humans and the observation that mice can reproduce and survive in the absence of these transcription factors provide evidence that contradict the notion that the unconventional T cell compartment is dispensable. Using approaches that can target multiple unconventional T cells simultaneously would help to ascertain to what extent redundancy exists within the adaptive and unconventional T cell compartments, and at what levels.

An unexpected observation is the constraint on loss-of-function mutations in HLA-E (Fig. 4a). Many viral infections result in the downregulation of MHC class I as a means to subvert conventional T cell responses[109]. In such settings, the leader peptide classically loaded on HLA-E can be substituted with virus-derived peptides to generate virus-specific T cells[14] that provide the immune system with a solution to the riddle of the virus-mediated downregulation of MHC class I. This would be an example of a case in which HLA-E-restricted T cells are not redundant in the presence of conventional T cell immunity.

There is also some value in approaching the discussion of redundancy from the perspective of how many different unconventional T cells can recognize the same pathogen. An example is *Mycobacterium tuberculosis*, for which a variety of unconventional T cell subsets have been associated with the response, including CD1-restricted T cells[45], HLA-E-restricted T cells[134], Vγ9/Vδ2 γδ T cells[135] and MAIT cells[136]. Given the broad array of unconventional T cells that are able to sense *Mycobacterium tuberculosis*—many with overlapping effector functions—it is unsurprising that in isolation each might not be essential for survival. Yet, the synergy between them is likely to provide the host with the best chance of fighting the infection, which is compatible with the high level of conservation of these molecules across species.

In summary, if the full spectrum of subsets were truly redundant it would be unlikely that such a diversity would have been conserved over time[137]. Moreover, there is an inherent advantage in being able to sample pathogens in many distinct ways and/or to survey and respond to internal changes, especially when considering that pathogens are also constantly evolving to subvert immunity.

## Conclusion and future perspectives

Unconventional T cells can engage a broad spectrum of molecules that span peptides, lipid moieties, metabolites and phosphoantigens, and this recognition has been divided up across a multitude of cell subsets.

Many questions remain when thinking about individual unconventional T cell subsets. Why did we evolve so many? Why did mammals develop a T cell subset that can recognize vitamin $B_2$ metabolites in particular, and what other metabolite-sensing T cell subsets exist? Why was the adaptive immune T cell response selected on the basis of peptide recognition and not that of lipids or sugars? Was it because the key driving force was the need for

protection against viruses? There is a clear case for unconventional T cells regulating the response to bacteria through the recognition of canonical antigens. Is this system in place to enable tissues to sense alterations in the intestinal or skin microbiota? Is there such a system in place to target fungi, protozoa, parasites and helminths, or to recognize toxins and allergens? Finally, does displacement of unconventional T cell subsets by chronic inflammation contribute to dysfunctional protective and anti-tumoral immune responses in tissues?

Most importantly, we should not think of unconventional T cells as a rudimentary attempt at adaptive immunity. Instead, they offer unique additions to the mosaic that is the immune system. Whereas conventional T cells have a critical role in sterilizing immunity, unconventional T cell subsets are well-suited for local responses, as they seed and mature with tissues from early in life and are more adapted to promote homeostasis and tissue healing. It is also more cost-effective for the host to rely as much as possible on preset defences rather than having to call on de novo conventional T cell responses constantly. Their role may be especially critical early in life, under periods of extended stress such as nutrient deprivation, and during chronic inflammation and ongoing infection to protect from additional insults. It also remains unclear how all the different arms of T cell immunity work together during the course of an infection and in different tissues. Future studies may want to consider looking at common hubs and targeting several unconventional T cell subsets simultaneously, through the deletion of *PLZF*, for example. Overall, a more systems-based approach that considers redundancy and the kinetics of immune responses, as well as niche formation and stability, will be necessary to truly appreciate the role and complexity of unconventional T cells and their relationship to adaptive immunity. Redundancy, rather than pointing to the trifling nature of these cells, may actually point to their importance in the defence and preservation of tissues.

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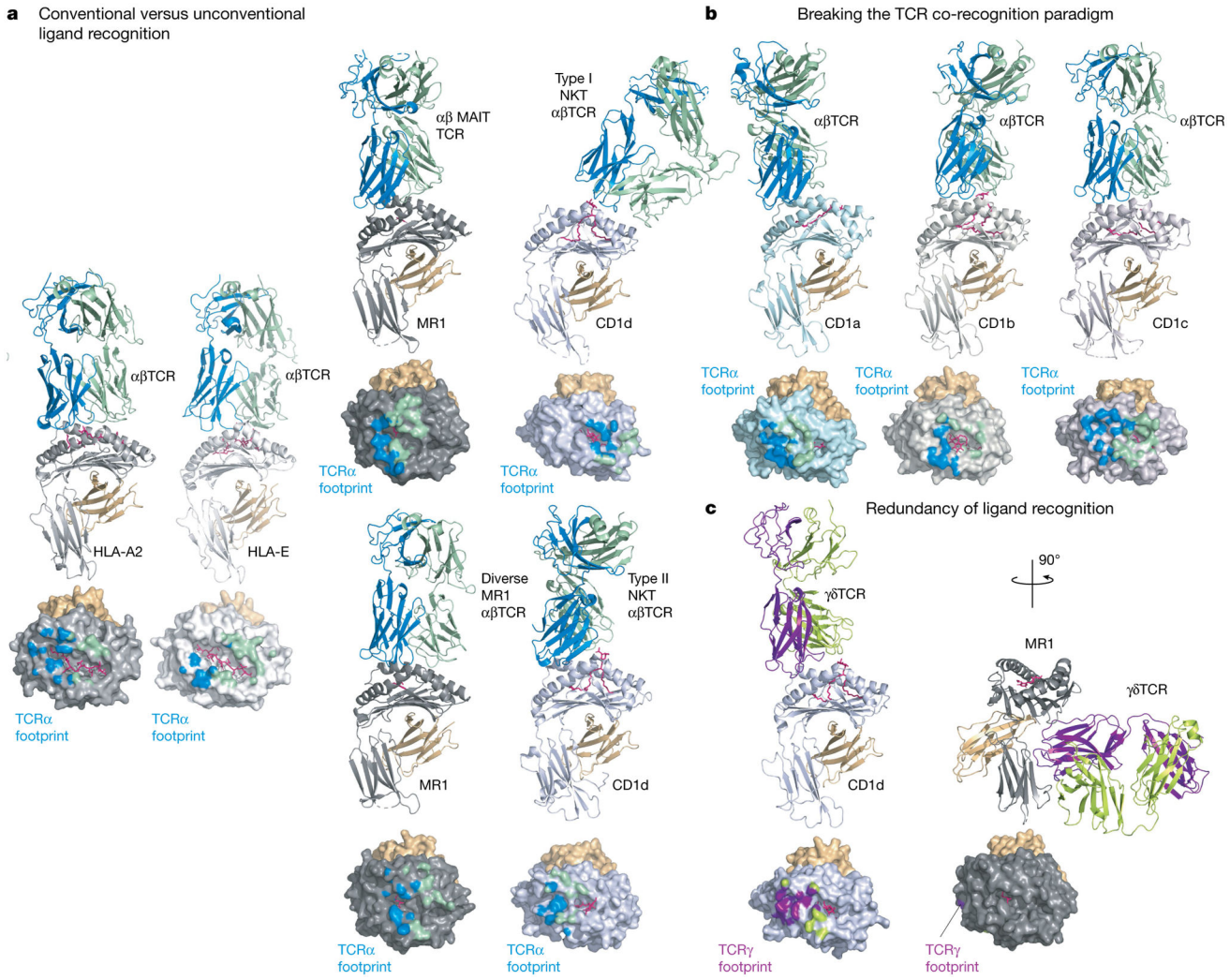


**Fig. 1 |. Comparison of TCR docking modes.**

Experimentally determined TCR-binding modes are shown as cartoon representations for a selection of MHC class I or class I-like antigen-presenting molecules. In each panel the MHC-I subunit or equivalent is coloured as follows: light grey (HLA-A2), white (HLA-E), dark grey (MR1), light blue (CD1a), steel (CD1b), light pink (CD1c) or blue-white (CD1d). The respective antigens are coloured pink and associated β2-microglobulin orange; the interacting TCR subunits are coloured either blue (α-subunit) and green (β-subunit) or purple (δ-subunit) and lemon (γ-subunit). Below each structure is a surface representation of the antigen-presenting MHC-I molecules coloured according to their TCR subunit-recognition surfaces. **a**, Conventional versus unconventional ligand recognition. From left to right: a tumour-associated MART-peptide antigen in complex with HLA-A2 or HLA-E in complex with an αβTCR[67,138]; MR1-presenting vitamin metabolites recognized by a MAIT TCR[70], a diverse αβTCR[74] and CD1d in complex with type I[139] and type II[72] NKT TCRs. **b**, Breaking the TCR co-recognition paradigm. From left to right: an autoreactive αβTCR in complex with a self-lipid presented by CD1a[25]; CD1b in complex with a mycobacterial lipid recognized by a GEM TCR[46]; and an autoreactive TCR recognizing CD1c[75]. **c**, Redundancy

of ligand recognition by alternative unconventional T cell subsets. The diversity of γδTCR recognition is shown with a CD1d-reactive γδTCR using a relatively standard docking mode[55] (left) or the more radical recognition of the underside of MR1 (right)[57].

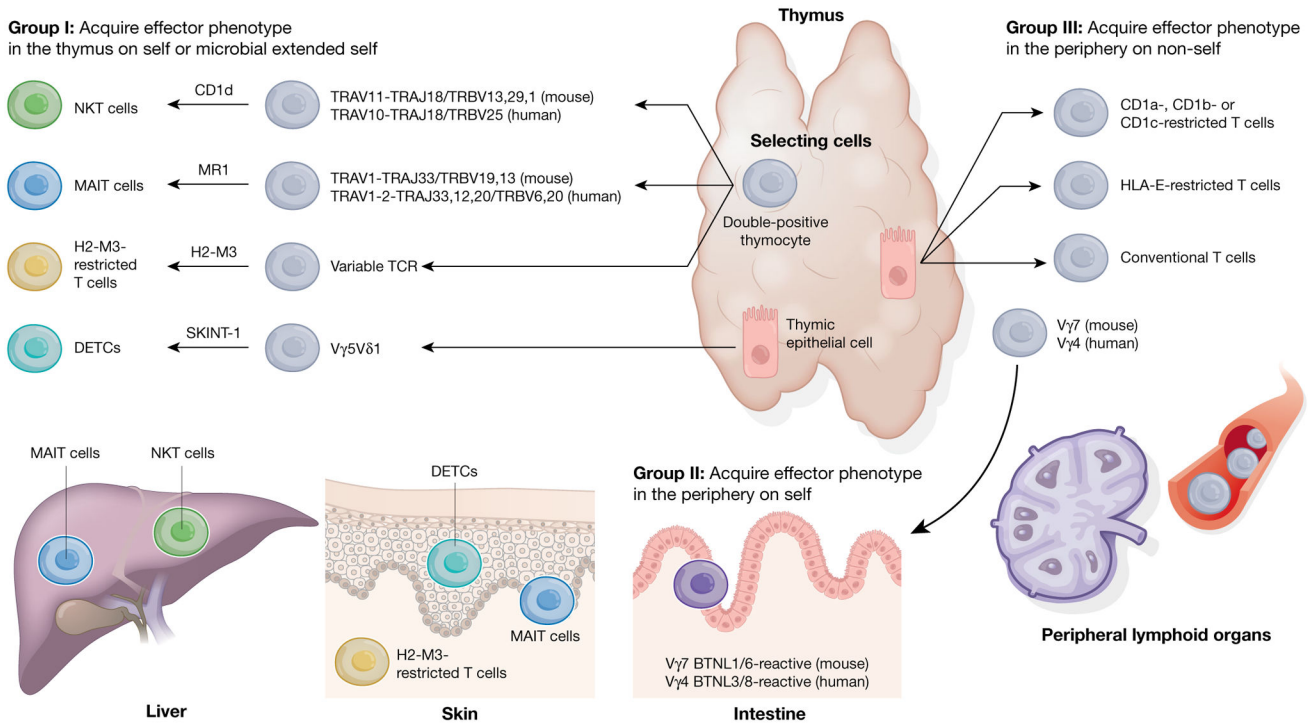


**Fig. 2 |. Classification of non-classical T cells on the basis of central or peripheral development.**

Unconventional T cells can be broadly separated into three groups largely based on their selection and differentiation patterns and how that affects their acquisition of effector programs. Group I unconventional T cells, which are classified by their acquisition of effector functions in the thymus, include NKT, MAIT and H2-M3-restricted T cells, and DETCs. Uniquely for NKT, MAIT and H2-M3-restricted T cells, this process takes place on double-positive thymocytes and requires the SAP pathway. These cells ultimately seed tissues such as the skin and liver, in which they exert their effector functions. Group II unconventional T cells include BTNL-reactive TCRγδ T cells, which leave the thymus naive and acquire effector functions in the periphery on tissue-specific self-ligands. Finally, group III unconventional T cells follow the conventional T cell path by leaving the thymus naive and only acquire effector functions once they encounter their cognate foreign antigen in the periphery. Grey cells represent naive T cells; different coloured cells represent effector T cells.

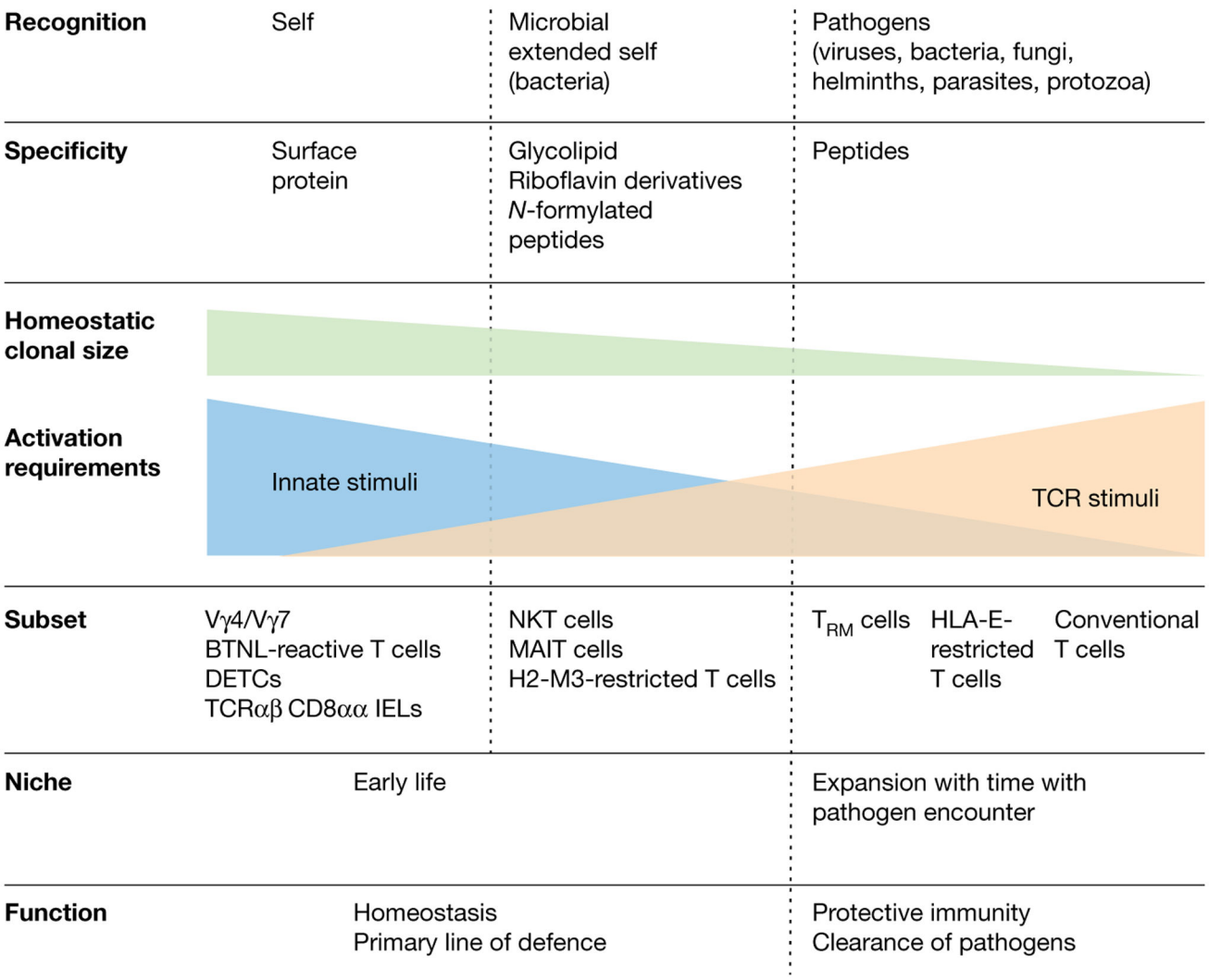


**Fig. 3 |. Functional niche of unconventional T cells.**

The T cell compartment is shown on a gradient from conventional T cells (right) to unconventional T cell subsets (left) according to the classifiers in bold. Classical adaptive T cells occupy a specific niche in terms of the antigenic universe they recognize (that is, MHC–peptide complexes), and they colonize tissues as tissue-resident memory T cells ($T_{RM}$) only after being activated and having expanded in peripheral lymph nodes in response to an infection. By contrast, unconventional T cells recognize a broad spectrum of antigens ranging from self-molecules, to microbial extended self and non-self, to formylated peptides and to peptides. The clonal size at homeostasis for unconventional T cell subsets like NKT, MAIT and BTNL-reactive TCRγδ T cells is large, as these cells expand in tissues early in life and in the case of NKT and MAIT cells can occupy multiple tissues. The role of innate immune signals versus TCR-mediated signals varies in the activation of the different unconventional T cell subsets; innate signals have a more critical role in the innate-like unconventional T cells that expand and acquire an effector phenotype either in response to self in the periphery (BTNL-reactive TCRγδ T cells) or in the thymus during their development (MR1-, CD1d- and H2-M3-restricted T cells) than in HLA-E-restricted T

cells that become activated in the periphery, similarly to conventional T cells. Of note, tissue-resident memory T cells also acquire the ability to respond to innate signals after establishing residence in tissues and are distinct in that regard from circulating memory and effector memory T cells. Finally, the unconventional T cell compartment constitutes a primary line of defence and also has an important role in tissue homeostasis and healing.

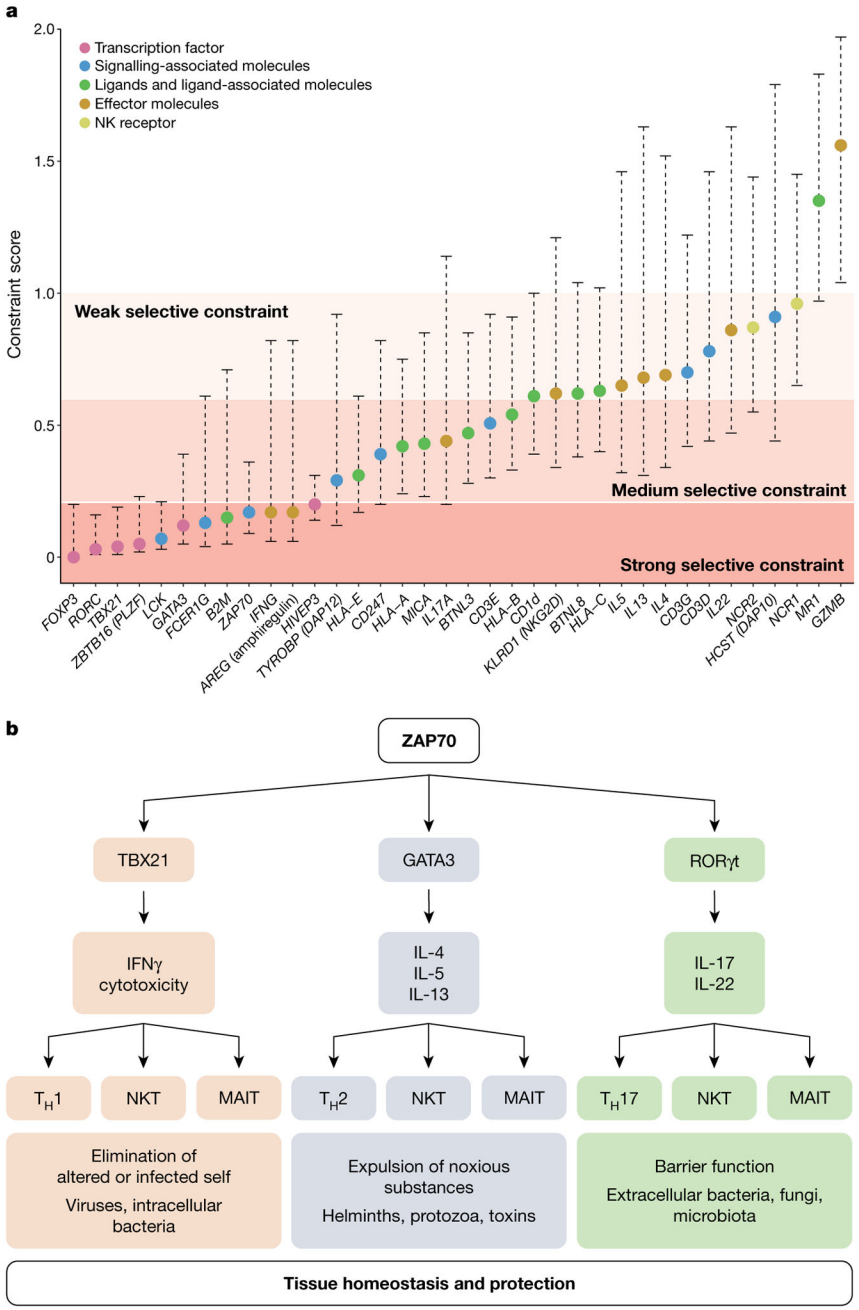


**Fig. 4 |. Conservation and redundancy within the T cell compartment.**

**a**, The selective constraint score shown (filled circles) is the ratio of the observed versus the expected (o/e) number of loss-of-function variants in that gene in the general population. The o/e metric comes with a 90% confidence interval, which is shown by the dashed lines. When a gene has a low o/e value, it is under stronger selection against loss-of-function mutations than a gene with a higher value. Genes were grouped into five major biological groups on the basis of their function and ranked from the most selectively constrained to the least constrained. The scores were obtained from the Genome Aggregation Database (gnomAD, v.2.1.1) and are based on sequencing data from 25,748 exome sequences and

15,708 whole-genome sequences from unrelated individuals. **b**, This figure shows that ZAP70 has a central role in the signalling hub of all T cells and, using MAIT and NKT cells as an example of unconventional T cell subsets, illustrates the multifaceted nature of the immune system that has evolved to have multiple conventional and unconventional T cell subsets that mediate the same key effector functions. Although there is redundancy at this functional level, these T cell subsets have different modes of recognition and are regulated by different stimuli, thereby increasing the robustness and resilience of the immune system. This property of the immune system also underlies the difficulty of showing a requirement for any given unconventional T cell subset. $T_H1$, T helper 1 cell; $T_H2$, T helper 2 cell; $T_H17$, T helper 17 cell.