Published in final edited form as:

Immunol Rev. 2018 July ; 284(1): 79–90. doi:10.1111/imr.12668.

Bohemian T cell receptors: sketching the repertoires of unconventional lymphocytes

Stefan A Schattgen and Paul G Thomas

MS 351, 262 Danny Thomas Place, St. Jude Children's Research Hospital, Memphis, TN 38105

Summary

Over the last several decades, novel populations of unconventional T cells have been identified; defined by an invariant (or nearly invariant) T cell receptor (TCR) with a fixed specificity to non-canonical antigens and major histocompatibility (MHC) molecules, they form large, functionally monoclonal populations tasked with surveying for their specific antigens. With residence in both lymphoid and non-lymphoid tissues coupled with their ability to rapidly produce a spectrum of cytokines and effector molecules, the unconventional T cells are poised as some of the first responders to infection/damage and are thought to provide critical coverage before more focused, conventional T cell responses are mobilized. However, new technologies for the measurement and characterization of TCR repertoires have identified an underappreciated amount of TCR diversity in the unconventional T cells. In many cases, the specificities of these diverse TCRs converge on the same or similar antigens as their invariant counterparts, while others have yet to be defined. Here, we will review the current knowledge of the TCR repertoires of unconventional T cells and discuss how repertoires might be used as a framework for their organization, and further our understanding of their role not only during an immune response, but also their contribution in maintaining homeostasis.

Keywords

T Cell Receptors; T Cells; Gene Rearrangement; Immune System Ontogeny; Mucosa

Introduction

The immune system relies on a number of receptor families to discriminate non-self or “altered”-self from healthy self, with discretion between these states as the switch maintaining tolerance against autoimmunity and a warranted response against pathogens and cancer. The first point of recognition in the immune response is tasked to ancient families of germline-encoded receptors able to sense conserved molecular patterns generated by tissue damage and inflammation (damage-associated molecular patterns, DAMPs), and/or pathogen infection and replication (pathogen-associated molecular patterns, PAMPs). These molecular products sensed by the pattern-recognition receptors (PRRs) can be found across

all kingdoms of life (i.e. nucleic acids, cell wall components, ATP), and their recognition of the molecules in context initiates inflammatory signaling pathways promoting the initiation of an immune response (1, 2). Another pathway modulated by PRR signaling pathways is antigen presentation - the processing and display of exogenous and self-antigens in major histocompatibility complexes (MHC) for their recognition by a more flexible set of receptors used by the adaptive immune system (3).

Adaptive immunity is a more recent evolutionary development relying on receptor families consisting of somatically rearranged gene products with tremendous potential for variability. The T cell receptor (TCR) is the product of the chromosomal rearrangement of hundreds of mobile genetic elements through the process of VDJ recombination; successful, in-frame rearrangements of variable (V), joining (J), and diversity (D) genes of four TCR loci (TCR α , TCR β , TCR γ , TCR δ), with the heterotypic pairings TCR α with TCR β , and TCR γ with TCR δ , broadly define the two major, though distinct, T cell lineages (i.e. $\alpha\beta$ and $\gamma\delta$ T cells, respectively). This massive amount of receptor diversity endows T cells with the capacity to see nearly any peptide able to be presented in MHC; the theoretical number of potential 9-mer and 14-mer peptides able to bind MHCI and II is estimated at 10^{10} and 10^{17} , respectively (4). Furthermore, it has been estimated a single TCR is able to recognize upwards of 10^6 – 10^7 unique peptides (4). This combination of diverse antigens and receptors able to recognize them results in a flexible compartment of cross-reactive immune cells able to respond to new pathogens, and importantly, able to remember previous encounters. We refer to the vast universe of T cell clones within an individual as being their TCR repertoire. A conventional response by T cells involves an encounter between the host and a pathogen or tumor. New and abundant antigens with PRR-driven inflammation results in the recruitment and clonal expansion of antigen-specific T cells aimed at eliminating the threat through cytotoxic killing (1). The trade-off for this level of specificity however, is slow kinetics, taking several days to weeks between first contact with PRRs and a pool of antigen-specific T cells responding to the agent. While this targeted type of conventional T cell response against peptide presented by MHC in a canonical-manner is certainly the best understood in terms of mechanism, it is only one way the antigen-specificity of the TCR is harnessed by the immune system (5). It is well-appreciated that unconventional T cells with more delimited functions and specificities develop and are elicited in non-canonical ways; more recently we have begun to better understand how these unconventional populations sit on the spectrum of innate and conventional acquired immunity.

Many questions regarding the origin, specificities, and activities of these unconventional T cells remain unanswered. The fact that several types of unconventional T cells are selected on the older, non-classical MHCs presenting a limited array of non-peptide antigens suggests that these unconventional T cells appeared early in the evolution of the lymphocytes. Reflecting the need for recognizing conserved molecules outside the bounds of peptide:MHC, lymphocyte populations with rapid response kinetics using invariant TCRs are selected for their recognition of non-classical MHC class Ib (MHC Ib) molecules, fulfilling their destiny as an innate-like T lymphocyte (6). The MHC Ib genes, are the older, well-conserved members of the MHC family, sharing considerable structural similarity with most requiring β 2-microglobulin (β 2m) for their expression on the cell surface (7). It is unknown what the ligands are, if any, for a number of the MHC Ib proteins, and lesser known

still how they affect tolerance or shape the innate and adaptive specificities and responses, alike. What is clear however, is some of these MHCIIb proteins present small molecules both exogenous and endogenous in origin recognized directly by the invariant TCRs of unconventional lymphocytes, and sizeable pools of the invariant clones capable of responding to their antigens are present and stable as a means of immune surveillance. The unconventional lymphocytes display a diverse array of antigen-driven effector functions, even within the same population, with some producing cytokines and helping B cells similar to CD4 T helper cells, while others acquire cytotoxic capacities like the CD8 and NK cells (8). Clearly, the unconventional lymphocytes play critical roles in bridging the gap between innate and adaptive immunity, but with so many of the MHCIIb ligands unaccounted for, and with some ignorant to positive selection in the thymus, how many more populations of unconventional lymphocytes might be out there? Further complicating matters is that one of the unconventional T cell types (CD8 $\alpha\alpha$ +, double-negative IELs) we will discuss here are selected by both classical and non-classical MHCs; further, after selection via high-affinity interactions with self-peptides, differentiates into a specialized, tolerogenic population.

The repertoires for several of the unconventional lymphocyte populations have been well-identified for some time, however, advances in sequencing technology allowing for a survey of immune repertoires at unprecedented depth and resolution have begun to reveal a world of diverse TCRs targeted to the recognition of non-peptide antigens. In this review, we will highlight and discuss the TCR repertoires of the unconventional lymphocytes and the role they play in shaping and defining the functions of these populations. Patterns of fate, function, and location tied to TCR reactivity are clearly emerging within the unconventional lymphocytes, and we speculate further study of their repertoires will help paint a more complete picture of their place in immune surveillance and responsiveness. We will discuss what is historically known about each population's repertoire, the influences shaping it, how TCR specificity entails a population's lineage fate, and how new tools and techniques are uncovering previously unknown repertoire diversity and tuning within unconventional lymphocytes.

The $\gamma\delta$ T lymphocyte, the founding member of the unconventional lymphocytes

The $\gamma\delta$ T cells are a unique lineage of T cells integral in a number of immune-related processes: tissue function/homeostasis, response to pathogens and commensal microbes, and tumor surveillance. In both mouse and human, $\gamma\delta$ T cells are widely distributed across a number of tissues but enriched within lymphoid and mucosal tissues. Their localization puts $\gamma\delta$ T cells at the frontline for surveillance, and they are able to respond rapidly to damage and infection, helping mediate their repair, secreting antimicrobial peptides, and coordinating the escalation of the immune response if necessary through cytokine/chemokine production. The functions and contributions of $\gamma\delta$ T cells to immune responses have been well-reviewed in a number of excellent articles (9–11). Here, we will focus our discussion on the repertoire landscape of $\gamma\delta$ T cells to try and highlight the emerging pattern whereby the $\gamma\delta$ TCR seemingly directs cell-fate decisions in a clonotypic-manner. (We will use the Tonegawa nomenclature for $\gamma\delta$ TCRs)

Like their $\alpha\beta$ T cell brethren, $\gamma\delta$ T cells develop in the thymus, undergoing the required recombination activating gene (RAG)-dependent VDJ recombination resulting in productive, paired TCR γ and TCR δ chains. During rearrangement the CDR3 γ and CDR3 δ regions are amenable to nucleotide insertion for tuning of antigen recognition. In fact, due to the presence of multiple diversity genes (D) of TCR δ capable of being translated across three reading frames, the theoretical CDR3 diversity of the $\gamma\delta$ TCR due to germline gene rearrangements and excluding N-additions at the CDR3 is $\sim 10^{18}$, higher than that of $\alpha\beta$ T cells ($\sim 10^{16}$) and Immunoglobulins (Ig) ($\sim 10^{11}$) (12), and thus are at no disadvantage for recognizing diverse antigens in this regard. The reality does not reflect this capability, however. Rather a major portion of the $\gamma\delta$ T cell repertoire is occupied by invariant, frequent rearrangements between just a small number of genes within the TCRD/TCRG loci, and the pairings between the TCR δ and TCR γ chains are rather restricted, overall imparting a lower functional diversity compared to $\alpha\beta$ T cells and B cells.

Unlike $\alpha\beta$ T cells however, $\gamma\delta$ T cells are not subject to positive selection based on their affinity for class Ia or II MHC proteins, and for a number of $\gamma\delta$ TCRs, their development does not require any sort of antigen-based selection in the thymus. However, this is not to say all $\gamma\delta$ TCRs develop without reactivity against thymic antigens, and in some cases thymic selection is required for the development of specialized subsets of $\gamma\delta$ T cells. It is now well-appreciated that the differential ability of $\gamma\delta$ TCRs to interact with thymic antigens, and the strength of TCR signaling due to this interaction, has a profound effect on the fate of the developing cell and the overall $\gamma\delta$ T cell landscape (13). Broadly, $\gamma\delta$ T cells can be subset by their capacity to make either IL-17 or IFN γ , and the decision to produce one or the other is made during thymic development based on the amount of input received through the TCR. In examining $\gamma\delta$ T cells specific for the non-classical MHC Ib molecules T10 and T22, Jensen et al noted T10/22-specific $\gamma\delta$ T cells that developed in wild-type mice displayed an antigen-experienced phenotype and produced IFN γ in response to TCR stimulation (14), but when developed in mice deficient in $\beta 2m$, an accessory protein required for presentation of T10/22 on the cell surface, T10/22-specific $\gamma\delta$ T cells were still able to develop, but now had an antigen-naïve phenotype and produced IL-17A. Conversely, the specialized V $\gamma 5$ V $\delta 1$ + dendritic epidermal T cells (DETCs) found in mouse epidermis require the expression of Skint1 by thymic epithelial cells to acquire the ability to make IFN γ , and deletion of Skint1 directs DETCs towards the IL-17+ fate (15). These observations determined the default developmental program for $\gamma\delta$ T cells is IL-17-production, but strong TCR signaling diverts the developing cell towards the IFN γ -producing fate. Thus, the ability to recognize thymic antigens does not appear to be required or biasing in selecting $\gamma\delta$ T cells undergoing development, but rather a mechanism of education directing their effector functions.

An interesting framework within the $\gamma\delta$ T cell populations has begun to emerge whereby the highly public, invariant TCRs seemingly provide an orderly distinction between the innate-like $\gamma\delta$ T cells, and other clonotypes more tuned to conventional adaptive immunity. A number of invariant $\gamma\delta$ TCR clonotypes have been discovered across multiple species, and in each case a public, easily-generated $\gamma\delta$ TCR commits the developing T cell to a discrete fate and localization within specific tissues (mucosa, lymphoid organs, dermis) (16). This provides a useful mechanism for sorting different subsets of $\gamma\delta$ T cells to their intended

tissue where they establish residency and provide surveillance and response in tune with the tissues requirements. The high preponderance of semi-invariant, oligoclonal, and public $\gamma\delta$ TCRs found in the IL-17-producing populations, and their ability to respond to unidentified, though presumably conserved antigens, has led their categorization as innate-like lymphocytes. Formation of large clonal pools of $\gamma\delta$ T cells using a fixed repertoire of receptors capable of responding rapidly to a shared molecular structure without the need for prior clonal expansion is reminiscent of the PRRs used by the innate immune system. These invariant $\gamma\delta$ T cell populations are highly public across individuals of a given species, providing a fixed specificity within their repertoire dedicated to recognizing conserved molecular patterns at the organismal-level. This public specificity suggests that the $\gamma\delta$ T cells evolved to address signals of stress elicited, and shared, by many forms of insult including: infection, tumorigenesis, autoimmunity, tissue damage, or any other inflammatory process giving them the flexibility to respond to a broad set of situations rather than restricted to a cognate antigen particular to a certain microbe.

Indeed, the ubiquitous nature of the few known antigens for $\gamma\delta$ TCRs reflect this possibility. For example, the predominant human blood subset of $\gamma\delta$ T cells, V γ 9V δ 2+, are highly public and semi-invariant across individuals (17). They recognize conserved diphosphate structures called phosphoantigens. One known phosphoantigen is isopentenyl pyrophosphate (IPP), an intermediate in the mevalonate pathway used by both eukaryotes and archaeobacteria for cholesterol biosynthesis. Another is hydroxymethyl-but-2-enyl-pyrophosphate (HMBPP), an intermediate of the alternative, non-mevalonate pathway of cholesterol used by some Eubacteria, and importantly, the malaria-causing agent *Plasmodium falciparum* (18). V γ 9V δ 2+ cells rapidly produce IFN γ in response to *P. falciparum*-infected red blood cells (iRBCs), and proliferate, making up to 20% or so of peripheral lymphocytes (19, 20). Individuals clinically tolerant to *P. falciparum* were found to have a lower frequency of V γ 9V δ 2+ T cells along with qualitative loss of their functionality, suggesting they play an important role in immunity to the parasite (21). Presentation of phosphoantigens is not mediated by any classical or non-classical MHCs, rather they require butyrophilin 3A1 (BTN3A1) for their recognition by V γ 9V δ 2+ T cells. BTN3A1 is a ubiquitously expressed cell surface protein containing extracellular immunoglobulin domains, and a B30.2 domain in the intracellular portion. Intracellular IPP and HMBPP directly bind with the B30.2 domain of BTN3A1, and is necessary and sufficient for the activation of V γ 9V δ 2+ T cells, possibly through their recognition of allosteric alterations of BTN3A1's extracellular domains elicited by phosphoantigen binding to the B30.2 domain (22, 23). This mechanism is in many ways analogous to how sensing of pathogens by PRRs stimulates innate immune responses; i.e. increased levels of PAMPs and DAMPs are sensed by BTN3A1, and this in turn stimulates its interaction and activation of V γ 9V δ 2+ T cells. Their conserved specificity to a PRR modified in response to DAMPs/PAMPs, coupled to their rapid responses, makes the case that V γ 9V δ 2+ T cells are more akin to innate cells than what we consider a classic adaptive response.

A classic example of tissue residency as dictated by the TCR specificity, is the IFN γ -producing V γ 5V δ 1+ DETCs (mentioned above). The V δ 1 (V δ 1-D δ 2-J δ 2) chain is a germline rearrangement without nucleotide insertion, which pairs to a near-invariant V γ 5 (V γ 5-J γ 1-C γ 1) chain (24, 25). DETCs are seeded from the earliest waves of thymic

emigrants, developing at embryonic day 14 (E14), and are maintained throughout life through self-renewal. The transmembrane protein Skint-1, expressed exclusively in the thymic epithelial cells and keratinocytes, was identified as being required for the selection and maintenance of the DETCs expressing V γ 5V δ 1+. Importantly, there is no evidence the DETC TCR is able to directly interact with Skint-1, and while no other ligands for it have been identified, damaged keratinocytes are able to stimulate them through the TCR without MHC-restriction. Additionally, and this is a feature shared by a number of $\gamma\delta$ T cell subsets, DETCs express multiple NK cell receptors that provide TCR co-signaling or alternative TCR-independent mechanisms of activation. Thus, DETCs are able to integrate TCR-dependent and independent signals from their environment to provide an appropriate response for the insult; e.g. engagement of junctional adhesion molecule-like (JAML) on DETCs by the coxsackie-virus and adenovirus receptor (CAR) induced in keratinocytes surrounding wound sites solicits DETC production of inflammatory cytokines and keratinocyte growth factor (KGF) needed for efficient wound resolution (26). In an analogous-manner, it was recently found the mouse V γ 7 and human V γ 4+ intraepithelial lymphocytes (IELs) residing in the gut mucosa are positively selected for their fate during early development by another family of transmembrane proteins known as butyrophilin-like (Btl) proteins. Embedded in the epithelium of the gut mucosa, $\gamma\delta$ IELs are positioned to survey and respond to damaged epithelium to mediate its repair, and prevent penetration of commensal microbes into underlying tissues by providing immunomodulatory and antimicrobial factors (27–29). In mice, V γ 7V δ 4 + IELs are positively selected on Btl1 and Btl6 in a wave of prenatal, extrathymic development. Btl1 and Btl6 expression is confined to enterocytes providing continued TCR stimulation, driving their maturation and self-renewal (30). Human BTNL3 and BTNL8 appear to select for V γ 4+ IELs in a similar manner, suggesting a conserved mechanism exists for getting $\gamma\delta$ IELs to the right location. It will be interesting to see if these butyrophilin-like proteins identified as being required for $\gamma\delta$ IEL development use a similar mechanism of intracellular sensing as BTN3A1 for $\gamma\delta$ TCR stimulation.

Taking a broader, systemic view of the $\gamma\delta$ TCR repertoire has proven successful in identifying novel, innate-like clonotypes and adding new insight to old ones as well. Some of the first $\gamma\delta$ T cells to develop during the early waves of prenatal thymic development are the so-called “natural” T $\gamma\delta$ 17 cells that go on to seed a number of different tissues. Their name comes from their ability to rapidly produce IL-17A in response to a broad array of infectious and non-infectious agents. By sequencing the TCR δ genes paired to V γ 4+ cells, Kashani and colleagues found a high proportion of clones used a germline V δ 5-D δ 2-J δ 1 rearrangement, and this clone acquires an innate-like (IL-17A+ CCR6+ CD27–) phenotype with widespread tissue distribution, categorically placing them into the natural T $\gamma\delta$ 17 cells (31). Consistent with this finding, single-cell TCR sequencing of the IL-23R+ natural T $\gamma\delta$ 17 cells from multiple tissues identified the same V γ 4V δ 5+ clonotypes (32). This report also showed the repertoires of IL-23R+ versus IL-23R– $\gamma\delta$ T cells are remarkably different in their diversity, with the IL-23R+ cells being mainly V γ 4V δ 5+ and V γ 6V δ 1+ cells, while the IL-23R– population is quite diverse. Their invariant, public nature would suggest the V γ 4V δ 5+ and V γ 6V δ 1+ TCR likely recognize well-conserved structures revealed in a number of situations, but for now their antigens remain unknown.

In addition to fixed specificity of the invariant, innate-like $\gamma\delta$ T cells, it has been speculated for some time that $\gamma\delta$ TCRs with diverse gene rearrangements and CDR3 diversification were more adaptive-like in their nature. The finer points of $\gamma\delta$ T cell antigen-specificity have been well reviewed elsewhere (13). With exception to those germline rearrangements of the innate-like $\gamma\delta$ T cells, TCR δ chains in particular show extensive breadth of their CDR3 with clonal lineages appearing uniquely within a given individual, exhibiting what we would call TCRs with private specificity. On the other hand, TCR γ rearrangements are quite well-conserved and are generally public between individuals. Structural studies of the $\gamma\delta$ TCR interacting with its ligands show recognition of ligands occurs through the CDR3 δ , and the CDR3 δ is subject to extensive nucleotide addition making them quite long. While clearly $\gamma\delta$ T cells can acquire antigen-specificity, technological limitations have hampered our understanding of the clonal dynamics within $\gamma\delta$ T cells during antigen-specific responses and at the steady state. One of the outstanding questions regarding $\gamma\delta$ T cell biology is, given that $\alpha\beta$ T cells and B cells are activated and clonally expand upon recognition of their cognate antigens, do $\gamma\delta$ T cells follow the same pattern? Most studies of $\gamma\delta$ T cell repertoires have historically relied on antibody panels targeting specific TCR γ or TCR δ chains, but this method is limiting in that it is unable to identify clonal expansions or evidence of CDR3 selection. Single cell TCR sequencing drastically improved the ability to discern interesting receptor pairs and provide some of the first evidence of selection and expansion for $\gamma\delta$ T cells, but until recent advances in single cell sequencing, it is a rather low-throughput approach with which to probe the repertoire at a population level at any meaningful depth. However, the advent of next-generation sequencing (NGS) technologies now allows one to sequence TCRs from vast numbers of cells for identifying their CDR3 and gene usage, providing a powerful tool for surveying immune repertoires and how they are shaped by antigenic environments.

Two recent works applying NGS methods to probe the $\gamma\delta$ TCR repertoire of humans demonstrated that selection and expansion of particular clonotypes occurs naturally with age, as well as after allogeneic hematopoietic stem cell transplant (alloHSCT). In contrast to the semi-invariant V δ 2 chain discussed above, Davey et al showed human $\gamma\delta$ T cells expressing the V δ 1 chain undergo clonal selection with age, where the V δ 1+ repertoire of neonatal cord blood was shown to be more polyclonal compared to peripheral blood from adults, which was dominated by clonal expansions (17). Clonally-expanded V δ 1+ cells display an effector phenotype (i.e. CD27^{low}, granzymes, IFN γ +) and maintain their TCR sensitivity for a quick response to their cognate antigens. Studies by Ravens and colleagues yielded similar results where they found that while there is a high amount of diversity in $\gamma\delta$ T cells there is also marked oligoclonality in the V γ 9- cells from adult blood, and far fewer clonal expansions in neonatal cord blood (33). When they compared the repertoires of $\gamma\delta$ T cells from humans prior to and after alloHSCT therapy it was found that the repertoires of clonally-expanded $\gamma\delta$ T cells after treatment were reconstituted quickly, stable, and fundamentally distinct from their pre-treatment repertoires. Cytomegalovirus (CMV) is a common human pathogen capable of establishing life-long latency, and is largely asymptomatic, however, CMV reactivation in patients undergoing HSCT therapy is a life-threatening complication. Human V δ 2+ $\gamma\delta$ T cells are known to proliferate and acquire cytolytic capabilities in response to CMV reactivation, and this interaction with CMV can

have profound effects on the $\gamma\delta$ T cell landscape (34, 35). Whether the activation of V δ 2+ $\gamma\delta$ T cells happens in a polyclonal manner with a number of reactive clones being pulled in, or if the repertoire simply becomes dominated by a few reactive clonotypes driven by a selecting antigen was heretofore unknown. Addressing this question, CMV reactivation in alloHSCT recipients induced the expansion of just a few distinct $\gamma\delta$ T cell clonotypes, using mostly V δ 1 and V δ 3, and each expansion appeared privately within an individual making up a substantial frequency of their repertoire (33). This is the first report that the repertoire of $\gamma\delta$ T cells, in part, undergoes selection in response to a virus infection, but where CMV-reactive $\gamma\delta$ T cells fit into the effective immune response against the virus remains to be seen. A study of immune reconstitution in children undergoing HSCT for tumor malignancies found that patients that recovered their $\gamma\delta$ T cell compartment more rapidly suffered fewer infections overall (36); no similar association was observed for $\alpha\beta$ T cell recovery, suggesting an important role for $\gamma\delta$ T cells in preventing mucosal infections in this vulnerable population.

In addition to their roles maintaining homeostasis in response to infection or injury, $\gamma\delta$ T cells participate in the coordinated effort of tumor surveillance provided by the immune system. The presence of $\gamma\delta$ T cells within tumors is associated with a more favorable prognosis for a number of diverse cancers (37). As discussed, they express a range of activating NK cell receptors capable of activating and licensing them for anti-tumor activity, but whether the TCR itself is able to recognize tumor antigens, or if the $\gamma\delta$ TCR repertoire is tuned to recognize certain antigens upregulated in adjacent tissue during tumorigenesis is unknown (10). Some of the first evidence that clonal selection of responding $\gamma\delta$ T cell lineages is occurring in cancer comes from a recent study by Chen et al. (38). Here, the diversity of TCR δ , but not TCR γ , in peripheral blood $\gamma\delta$ T cells from patients with lung carcinoma (LC) was significantly lower compared to the healthy controls due to high amounts of clonal expansion. Interestingly, the CDR3 δ sequences from LC patients were more frequently shared between LC patients versus the controls, suggesting that a conserved set of antigens associated with tumorigenesis might be responsible for their selection. It is tempting to speculate these clonally-expanded responses are selected on intratumoral interactions between $\gamma\delta$ T cells and antigen, but there is currently no evidence to support this possibility, and it cannot be ruled out that signals coming from the tumor might elicit their cognate antigens at peripheral sites, which in turn are the selecting factor. Future studies investigating the repertoires of $\gamma\delta$ TCR+ tumor-infiltrating lymphocytes (TILs) will help to further elucidate the mechanisms and locations of $\gamma\delta$ T cell selection, and better resolve the nature of clones associated with anti-tumor immunity.

These studies of the $\gamma\delta$ T cell repertoire and how it responds to strong selective pressures at the population level provide fascinating new insights into their biology building on the growing evidence the $\gamma\delta$ TCRs can be selected based on specific antigen reactivity and establish a pool of clones targeted for antigen recognition. At face value these data suggest $\gamma\delta$ T cells maintain a diverse pool purposed with recognizing new antigens by virtue of their TCR reactivity, permitting new expansions with continued encounters, and the development of memory to prior infections accumulating along the way. However, it is important to consider where these selection events are taking place in time and space, and the antigens imparting it. With the notable exceptions of those positively selected based on their

reactivity to non-classical MHCIIb molecules (39), the $\gamma\delta$ T cell repertoire is thought to be unbiased towards thymic antigens suggesting that clonal selection begins to occur in the periphery. Few antigens have been identified for $\gamma\delta$ TCRs, and far fewer proven to bind to them, but how big is the universe of potential $\gamma\delta$ target antigens? This is much more difficult to predict than for $\alpha\beta$ T cells, where MHC restriction provides a bound on the potential diversity of antigen recognition. Beyond that initial question, others include, are the antigens targeted foreign or self? Does each clone have one specificity against a unique antigen, or is it the case that multiple $\gamma\delta$ TCRs converge on a handful of antigens? The former case fits with our current model of adaptive responses, but the second possibility falls more in line with what we already know about the innate-like nature of $\gamma\delta$ T cells. This type of selection mechanism would be useful for keeping clones capable of responding to a ligand expressed in response to stress and damage, thus providing a pool able to respond to a broad range of insults. Future studies focused on the biochemistry of antigen recognition will help resolve these ambiguities.

The unconventional $\alpha\beta$ T cells

NKT cells

The natural killer T cells (NKT) are another case where the use of a semi-invariant TCR with a fixed reactivity to a class of antigens presented by non-classical MHCIIb molecules generates a pool of innate-like lymphocytes capable of rapidly producing cytokines. As their name might imply, they express a number of NK cell receptors and a rearranged $\alpha\beta$ TCR, endowing the NKT cells with several mechanisms of immune surveillance. NKTs are subset into invariant (iNKT) and diverse (dNKT) as determined by the nature of their TCR repertoire and specificity, though both types are CD1-restricted and respond to lipid antigen/CD1 complexes through direct interaction. CD1 is an elder of the MHCIIb family, with humans having four isoforms (CD1a, b, c, d) while mice only have CD1d. Specific lipid ligands have been identified for each of the human CD1 isoforms, but the T cell population restricted to each of these have yet to be well-characterized with the exception of CD1d, the most extensively studied of the CD1 family given that it is responsible for the selection and development of iNKT cells (40).

The classical type I, or invariant NKT (iNKT) cells use an invariant TCR α chain (V α 14-J α 18 in mice, V α 24-J α 15 in humans) paired with one of a small number of TCR β chains (V β 7, 8.2 or 2 in mice, V β 11 in humans), and at least in mice, make up a majority of the overall CD1d-restricted repertoire (41, 42). These iNKT TCR specifically recognized the endogenous α -linked monoglycosylceramides, α -galactosyl ceramide (α GalCer) and α -glucosylceramide (α GlcCer) in complex with CD1d. Structural studies have revealed the mouse iNKT TCRs V α 14-V β 7 and V α 14-V β 8.2 are both able to bind α GalCer:CD1d complexes using similar docking orientations, but they are biased in their affinities toward α GlcCer or α GalCer, respectively (43, 44). The mechanism of thymic selection for the iNKT cells is quite unique whereby developing double-positive (DP) thymocytes display lipid:CD1d complexes for recognition by other DP cells destined for the NKT lineage based on their TCR specificity. This trans-DP thymocyte positive-selection is agonistic, as the high affinity interaction between the iNKT TCR and lipid:CD1d stimulates a strong TCR

necessary for NKT development. After exiting the thymus, the NKT cells home to their niches within different tissues where they take up permanent, lifelong residence (45). Intriguingly, despite having their fixed specificity for α GalCer, the iNKTs are quite heterogeneous in their programming with different populations producing IFN γ , IL-4, or IL-17A exclusively, and in a manner similar to helper CD4 T cells are designated as iNKT1, iNKT2, and iNKT17 corresponding to their cytokine profiles. Each of the subsets has a unique gene expression signature enforced by the same master transcription factors (i.e. T-bet, GATA3, ROR γ t) mediating the differentiation of other lymphocyte populations with similar cytokine profiles (i.e. iNKT1 is more similar to Th1-polarized CD4 T cells) (46). The observation that iNKT subsets are not uniformly distributed across tissues, but rather are differentially enriched by tissue even within compartments of the same tissue (e.g. splenic iNKT2 cells reside in the T cell zone whereas iNKT1 are in the red pulp) (47), suggesting tissue-specific cues either educate or select for different subsets, however, the identity of these signals remains to be determined.

While the iNKT cells are limited by their restricted TCR and the specificity thereof, the dNKT cells use a more diverse repertoire able to recognize a broader range of lipid antigens. A number of lipid antigens, both self and non-self in their origin, have been identified as having stimulatory activity following TCR recognition by the dNKT cells (well-reviewed by Dasgupta and Kumar (48)). These include sphingolipids (e.g. sulfatides and β GalCer) and phospholipids (e.g. phosphatidylinositol, phosphatidylglycerol, phosphatidylethanolamine). The dNKT repertoire is described as diverse relative to the iNKT cells, but few studies have been done on this question. In a report from Arrenberg et al. investigating the repertoire of NKT cells from unprimed mice that bound sulfatide:CD1d tetramers it was found they used a only a couple of variable segment genes with N-additions in the CDR3 regions for both TCR α and TCR β chains (49). However, a significant proportion of TCR β chains were germline-encoded VJ rearrangements, and for those CDR3 α and CDR3 β regions with N-additions, the motifs were highly similar between clonotypes with some clones being dominant. It is important to note here that it has been shown most T cells from human peripheral blood able to bind sulfatide:CD1d tetramers are not dNKT cells, or even $\alpha\beta$ T cells for that matter, rather they are V δ 1+ $\gamma\delta$ T cells (50, 51). Whether V δ 1+ $\gamma\delta$ T cells and dNKTs reactive to sulfatide:CD1d overlap functionally as well remains to be seen. Deep sequencing of the TCR β chain of human dNKT cells specific for β -glucosylceramide 22:0 (β GAL1-22) and glucosylsphingosine (LGL1), two sphingolipids that accumulate in Gaucher disease, showed a similar pattern of oligoclonal expansion after in vitro stimulation with comparable variable gene usage between β GAL1-22 and LGL1-reactive clones (Nair et al., 2015).

Taken together, these data suggest oligoclonal pools of dNKT cells are formed based on a shared antigen specificity, raising multiple questions: How many clonal pools are there within an individual and does each correspond with a single antigen? Are these bona fide clonal expansions driven in response to antigen or are these TCRs simply so efficiently rearranged and selected for that fresh thymic emigrants keep the pool full? Are thymic antigens positively selecting the dNKTs? Speculatively, further probing of the dNKT repertoire at the population level may reveal they undergo clonal selection after birth with relatively few clonotypes dominating the landscape similar to the V δ 1+ $\gamma\delta$ T cells described

above. Alternatively, unlike the highly polymorphic classical MHC molecules, CD1d is monomorphic within a species and the determinants governing its interaction with the TCR are likely to be constrained, and this, coupled to the conserved molecular patterns of their lipid antigens, makes it quite plausible that dNKTs have a highly public repertoire. It is unknown whether the dNKT cells are partitioned into different functional subsets by anatomical locations like the iNKT cells. Future study on their repertoire is likely to be fruitful with new insights as to how the type II NKT are functionally organized at a population level.

MR1-restricted T cells

The mucosal-associated invariant T (MAIT) cells are another innate-like $\alpha\beta$ TCR⁺ population positively selected by an MHC Ib molecule, in this case MHC-related molecule 1 (MR1). Like many other non-classical MHCs, MR1 does not bind peptide, rather it presents intermediary molecules of folic acid (Vitamin B9) and riboflavin (Vitamin B2) metabolism, the latter being exclusively produced by microbes. MR1 is an ancient MHC Ib, and highly conserved among species with mouse and human MR1 having ~90% shared sequence, and with this amount of similarity between them, it is not surprising their specificity for folate and riboflavin products are also conserved. MAIT cells are not restricted to the mucosa as their name might imply; though they are enriched in the lung, gut, liver, and other mucosal surfaces, they can be readily found in peripheral blood of humans and primates, where they look similar with regard to their invariant repertoire and functional response (52). For a more comprehensive look at MAIT cells we refer the reader to these recent reviews (53–55), as we will focus our discussion on their repertoire.

Mouse and human MAIT cells use an invariant TCR α chain composed of a single TRAV gene rearranged with a few different TRAJ genes contributing a tyrosine residue in the CDR3 α critical for its interaction with 5-OP-RU:MR1. Human MAIT cells use V α 7.2 rearranged with several J regions (Ja12 and Ja20 being most dominant). The MAIT TCR α associates with a number of TCR β chains (V β 2 or V β 6 being most dominant), with the CDR3 β region constrained to a favorable length for its interaction with MR1 (56). Mouse MAIT cells use a V α 19-J α 33 TCR α paired with V β 8 or V β 6 TCR β chains (57, 58).

Deep sequencing of the human TCR β chains of V α 7.2+ MAIT cells revealed their repertoire is defined by oligoclonal expansions, with considerable publicity between individuals, and stable over time (59). A thorough ontogenetic study of human MAIT found a similar scenario to the dNKT and V δ 1+ T cells; TCR β diversity is high in cord blood, with the V α 7.2+ population phenotypically naïve, but during childhood a few dominate clones are selected by antigenic stimulation, acquire effector memory, and stabilize (60). MAIT cells account for ~1–10% of all $\alpha\beta$ T cells in peripheral blood making for a large pool of innate-like lymphocytes focused at one, conserved molecular pattern (mouse MAITs are considerably lower in frequency). Analysis of the TCR β chains paired with the TCR α chains of MAIT cells from individuals found them to be oligoclonal and private, and intriguingly, MAIT stimulation with *M. smegmatis*, *S. typhimurium*, or *C. albicans* resulted in the differential expansion of particular clones, suggesting perhaps the TCR β may contribute to the fine tuning of specificity (61). A recent study looking at the effect of

Salmonella Paratyphi A infection on the MAIT TCR β repertoire in human volunteers showed that, in those developing enteric fever, the number of active MAIT cells increased, and the clones dominating before infection contracted slightly with new clones having better functional avidity taking their place (62). MAIT activation is commonly observed in a number of immunological scenarios including infection, autoimmunity, and tumor immunity, but their role in these responses are still being elucidated. Similar to the iNKT cells, MAIT cells are heterogeneous with regard to their cytokine response differentiating into Th1, Th2, and Th17-like profiles depending on the context rather than their TCR specificity.

The development of MR1 tetramers loaded with folic acid (Ac-6-FP) and riboflavin (5-OP-RU) ligands have greatly expedited efforts to understand the role of MAIT cells in the immune response, but fortuitous observations have identified an entirely independent population of MR1-restricted $\alpha\beta$ T cells using a more diverse repertoire. In a nice pairing of structural and repertoire studies, Gherardin and colleagues found that a portion of $\alpha\beta$ T cells able to bind 5-OP-RU:MR1 tetramer did not express the MAIT TCR, but instead use a diverse selection of TRAV and TRBV genes with additions at the CDR3 regions (63). The classical MAIT TCRs are cross-reactive to both Ac-6-FP and 5-OP-RU, with the latter being the dominant ligand, while the non-classical, diverse MR1-restricted TCRs (referred to as MR1T cells) showed a more differential tuning of their specificity towards one or the other ligand and are also capable of reacting to MR1-expressing cells without microbial ligands, suggesting they have greater specificity for endogenous ligands. The MR1T TCRs specific to autoantigen and folic-acid derivatives used different mechanisms for docking and interacting with the ligand:MR1 complex. Co-culturing of PBMCs with cell lines expressing low levels of MR1 in the absence of microbial ligands identified other MR1T TCRs using diverse rearrangements (64). All together these findings highlight MR1-restriction and specificity can happen in more ways than one, but with MAIT cells far out numbering the MR1T cells. It will be interesting to see whether or not the MR1T cells are found to be enriched during cases of sterile inflammation as part of an autoreactive response, and if not players in that process, what other scenarios they might have a part in.

IELs

Up until this point all the cell types we have discussed are restricted, at least in part, to MHCIIb selection. TCR $\alpha\beta$ + IELs, however, are a marked departure from these scenarios as they are subject to conventional positive selection on peptide:MHC, though with some exceptions noted below. IELs are a specialized and heterogeneous population of T lymphocytes residing within the epithelial layer of the intestinal mucosa. This unique anatomical location places IELs in close contact with gut luminal contents and a stream of microbial and dietary antigens nearly infinite in its diversity, with only a mucus buffer between them. Both $\gamma\delta$ TCR+ and $\alpha\beta$ TCR+ IELs, and TCR $^-$ ILCs, share the same space at the epithelium; $\gamma\delta$ IELs are seeded by the first waves of neonatal lymphocyte development, while $\alpha\beta$ IELs come later in response to antigens. $\gamma\delta$ IELs do not require gut microbiota or food antigen for their development as they are selected for recognition of Btl proteins (discussed above) and require only the aryl hydrocarbon receptor (Ahr) ligands from dietary sources (65). The $\alpha\beta$ TCR+ IELs, on the other hand, while present in germ-free mice,

expand in response to microbial colonization, eventually taking up a significant proportion of the compartment.

Antigens imparting the selection and activation of $\alpha\beta$ IELs under normal, resting conditions have yet to be identified, but tracking responses to pathogen-derived epitopes and model antigens has revealed differential mechanisms in their development, delineating a few distinct subsets. The “induced” $\alpha\beta$ IELs are CD4⁺ (CD4-IELs) and CD8 $\alpha\beta$ ⁺ (CD8-IELs) T cells developing by way of conventional $\alpha\beta$ T cell selection on peptide-loaded class II or class I MHC, respectively. Activation by their cognate gut-derived antigens in the periphery ends with their residence in the epithelium. CD8-IELs and CD4-IELs display phenotypes consistent with tissue resident memory T cells (Trm); markers of activation on the cell surface (i.e. CD5⁺, CD69⁺, CD103⁺, Thy1), production of Th1-cytokines, and cytotoxic capacities. One interesting addition to the surface for a portion of all the IEL subsets ($\gamma\delta$ TCR⁺ or $\alpha\beta$ TCR⁺) is the CD8 $\alpha\alpha$ homodimer implicated in the negative regulation of signals coming through the TCR. Ahr-dependent sensing of tryptophan derivatives produced by a particular *Lactobacillus* species (*L. reuteri*) induced expression of CD8 $\alpha\alpha$ in CD4-IELs as a form of commensal education (66). How exactly the CD4-IELs and CD8-IELs contribute in the development or resolution of gut inflammation is largely unclear. Viral and bacterial infection results in the accumulation of pathogen-specific CD8-IELs at the mucosa displaying an activated, memory-like phenotype, including the production of IFN γ , granzymes, and perforin. CD4-IELs acquire a Th1-like phenotype and are implicated in contributing to the inflammation-mediating damage in celiac disease, ulcerative colitis, and Crohn’s disease, but precisely how is unknown (67, 68). It was recently shown that somatic cell nuclear transfer of a TCR derived from a lamina propria Treg cell drove not only differentiation into a peripheral Treg fate, but into CD4-IELs as well, suggesting there is some level shared specificity between them (69).

Another fascinating subset of $\alpha\beta$ IELs are known as the “natural” IELs. These natural IELs are the first of the $\alpha\beta$ IELs to arrive after birth, though the recruitment and expansion of the CD8-IELs and CD4-IELs later drives their frequency downward. They do not express the CD8 $\alpha\beta$ or CD4 co-receptors (and thus we refer to them as double-negative, “DN”-IELs), but are nearly all CD8 $\alpha\alpha$ ⁺, and lack canonical markers of T cell activation (CD2[−], CD5[−], CD69[−], Thy1[−]). These qualities are imprinted in DN-IELs during thymic development in a fate decision made by strong, agonistic TCR signaling triggered by high affinity interaction with self-antigen:MHC (70, 71). TCRs with high affinities to self-antigen:MHC are used to generate regulatory T cells (Tregs) and iNKTs as well, further cementing agonistic selection as a mechanism for generating specialized populations. With regard to their MHC-restriction, DN-IELs require β 2m-dependent class I and Ib MHCs for their development, and individual TCRs have differential requirements and specificity towards β 2m partners (72). DN-IELs require the Transporter associated with Antigen Processing 1 (TAP1) for their development, meaning they are likely selected on peptides presented by MHC class I; intriguingly, though, their numbers are unaffected by the simultaneous loss of H2-K and H2-D (73). Their magnitude is also not dependent on Qa2, CD1d, or thymus leukemia antigen (TL), another MHC Ib molecule that is unable to load peptide, but directly interacts with CD8 $\alpha\alpha$ (74–76). One possible interpretation of these data is that while these disruptions leave the magnitude of the DN-IEL compartment intact,

perhaps there is a concomitant loss of diversity associated with deletion of each individual MHCIIa or MHCIIb proteins, with the remaining, fewer, clones expanding to fill the space; this has yet to be tested, however. The antigens selecting DN-IELs have not been identified, but it has been hypothesized that they are self-reactive, perhaps reacting to stress-induced antigens expressed by intestinal epithelial cells. They also express a number of NK cell receptors suited for sensing self, or the lack thereof, as well. However, due to the lack of knowledge of their antigens, or how they respond to conditions in the gut, the role of DN-IELs in maintaining gut homeostasis remains elusive.

Nearly 25 years ago, the $\alpha\beta$ IELs were first described as being oligoclonal with the clonal expansions being unique between different individuals (77–80). Unlike the invariant unconventional lymphocytes, the N-additions into the CDR3 α and CDR3 β regions across all three types of $\alpha\beta$ IELs suggest their specificities are finely tuned for antigen recognition. Beyond this, little else is known about the repertoire of IELs, though its further study might provide some fresh insights into the functions of these unique cells and what they see. Is the IEL repertoire highly degenerate, or is there tuning of their specificity for clones of higher avidity responding to gut inflammation? What are the antigens selecting the IELs? Are they dietary, microbial, or endogenous? Are the DN-IELs selected by a set of self-antigens targeted for their development? How do their TCR specificities integrate with other environmental cues for their function? We are currently engaged in an extensive survey on the repertoires of mouse $\alpha\beta$ + IELs, and confirming previous studies, the IELs are clonally expanded, and while dominant clones are unique to individuals, the repertoires of CD8-IELs, CD4-IELs, and DN-IELs are quite public, with the DN-IELs the most diverse of the bunch (SS and PT, manuscript in preparation).

Conclusion

Studies of unconventional lymphocytes have demonstrated they arise during the first waves of T cell development due to a high affinity for self-antigen, and upon experiencing their antigens, expand to form large, stable pools of clones poised for responding quickly to conserved molecular structures as part of the immune response to infection and damage (Table 1). In the case of CD1- and MR1-restricted T cells, α GalCer and 5-OP-RU represent the immunodominant epitopes recognized by the invariant TCRs of iNKT and MAIT cells, respectively. The invariant TCRs are efficiently generated and optimized for their antigen specificity, and if not for the process of VDJ recombination required for their generation, they might easily be mistaken for a conventional PRR. On the other hand, deep sequencing the repertoires of MR1- and CD1-restricted T cells differing in their affinity to variants of the prototypical ligands, as well $\gamma\delta$ T cells without known antigens, has found that invariant TCRs are not the only way T cells are able to gain specificity to non-peptide antigens. Rather, a proportion of lymphocytes restricted by a particular MHCIIb molecule display a more diverse TCR repertoire better fitted to recognizing one variant over another with sufficient affinity and avidity to drive their differentiation into an unconventional lineage. This raises the question, with such large and dominating pools of MAITs and iNKTs, why bother? The fact the MR1T and dNKT cells are able to compete at all with their invariant counterparts for space might be telling of their importance – clones tuned to seeing variations of MR1 and CD1d ligands ensure functional avidity is maintained within the

population. What now remains for the field is to develop better understanding of the *in vivo* pathological and physiological conditions leading to differential activation of the invariant and diverse unconventional lymphocytes *in vivo*, and how this contributes to either the resolution or continuation of those conditions. With new technologies allowing for paired transcriptome analysis coupled to TCR identity of a single cell, it is likely that new populations of unconventional lymphocytes endowed with targeted specificity will be discovered as we continue to fill in the atlas of TCR repertoires.

Acknowledgments

This work was supported in part by NIH grants AI107625 and AI121832 (PT), the Neoma Boadway Postdoctoral Fellowship (SS), and ALSAC.

References

1. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat Immunol.* 2015; 16:343–353. [PubMed: 25789684]
2. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunol Rev.* 2012; 249:158–175. [PubMed: 22889221]
3. Trombetta ES, Mellman I. Cell biology of antigen processing *in vitro* and *in vivo*. *Annu Rev Immunol.* 2005; 23:975–1028. [PubMed: 15771591]
4. Mason D. A very high level of cross reactivity is an essential feature of the T-cell receptor. *Immunol. Today.* 1998; 19:395–404. [PubMed: 9745202]
5. Yin L, Scott-Browne J, Kappler JW, Gapin L, Marrack P. T cells and their eons-old obsession with MHC. *Immunol Rev.* 2012; 250:49–60. [PubMed: 23046122]
6. Gascoigne NRJ, Rybakina V, Acuto O, Brzostek J. TCR Signal Strength and T Cell Development. *Annu. Rev. Cell Dev. Biol.* 2016; 32:327–348. [PubMed: 27712102]
7. Rodgers JR, Cook RG. MHC class Ib molecules bridge innate and acquired immunity. *Nat Rev Immunol.* 2005; 5:459–471. [PubMed: 15928678]
8. Pasman L, Kasper DL. Building conventions for unconventional lymphocytes. *Immunol Rev.* 2017; 279:52–62. [PubMed: 29266291]
9. Nielsen MM, Witherden DA, Havran WL. $\gamma\delta$ T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol.* 2017; 17:733–745. [PubMed: 28920588]
10. Chitadze G, Oberg H-H, Wesch D, Kabelitz D. The Ambiguous Role of $\gamma\delta$ T Lymphocytes in Antitumor Immunity. *Trends Immunol.* 2017; 38:668–678. [PubMed: 28709825]
11. Vantourout P, Hayday A. Six-of-the-best: unique contributions of $\gamma\delta$ T cells to immunology. *Nat Rev Immunol.* 2013; 13:88–100. [PubMed: 23348415]
12. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature.* 1988; 334:395–402. [PubMed: 3043226]
13. Chien Y-H, Meyer C, Bonneville M. $\gamma\delta$ T Cells: First Line of Defense and Beyond. *Annu Rev Immunol.* 2014; 32:121–155. [PubMed: 24387714]
14. Jensen KDC, Su X, Shin S, et al. Thymic Selection Determines $\gamma\delta$ T Cell Effector Fate: Antigen-Naive Cells Make Interleukin-17 and Antigen-Experienced Cells Make Interferon γ . *Immunity.* 2008; 29:90–100. [PubMed: 18585064]
15. Turchinovich G, Hayday AC. Skint-1 identifies a common molecular mechanism for the development of interferon- γ -secreting versus interleukin-17-secreting $\gamma\delta$ T cells. *Immunity.* 2011; 35:59–68. [PubMed: 21737317]
16. Nielsen MM, Witherden DA, Havran WL. $\gamma\delta$ T cells in homeostasis and host defence of epithelial barrier tissues. *Nat Rev Immunol.* 2017; 17:733–745. [PubMed: 28920588]
17. Davey MS, Willcox CR, Joyce SP, et al. Clonal selection in the human V δ 1 T cell repertoire indicates $\gamma\delta$ TCR-dependent adaptive immune surveillance. *Nature Communications.* 2017; 8:14760.

18. Morita CT, Jin C, Sarikonda G, Wang H. Nonpeptide antigens, presentation mechanisms, and immunological memory of human V γ 2V δ 2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. *Immunol Rev.* 2007; 215:59–76. [PubMed: 17291279]
19. Ho M, Tongtawe P, Kriangkum J, et al. Polyclonal expansion of peripheral $\gamma\delta$ T cells in human *Plasmodium falciparum* malaria. *Infection and Immunity.* 1994; 62:855–862. [PubMed: 8112855]
20. Teirlinck AC, McCall MBB, Roestenberg M, et al. Longevity and Composition of Cellular Immune Responses Following Experimental *Plasmodium Falciparum* Malaria Infection in Humans. *PLoS Pathog.* 2011; 7:e1002389. [PubMed: 22144890]
21. Jagannathan P, Kim CC, Greenhouse B, et al. Loss and dysfunction of V δ 2⁺ $\gamma\delta$ T cells are associated with clinical tolerance to malaria. *Sci Transl Med.* 2014; 6:251ra117.
22. Vavassori S, Kumar A, Wan GS, et al. Butyrophilin 3A1 binds phosphorylated antigens and stimulates human $\gamma\delta$ T cells. *Nat Immunol.* 2013; 14:908–916. [PubMed: 23872678]
23. Sandstrom A, Peigné C-M, Léger A, et al. The Intracellular B30.2 Domain of Butyrophilin 3A1 Binds Phosphoantigens to Mediate Activation of Human V γ 9V δ 2+ T Cells. *Immunity.* 2014; 40:490–500. [PubMed: 24703779]
24. Havran WL, Grell S, Duwe G, et al. Limited diversity of T-cell receptor gamma-chain expression of murine Thy-1+ dendritic epidermal cells revealed by V γ 3-specific monoclonal antibody. *Proc Natl Acad Sci.* 1989; 86:4185–4189. [PubMed: 2726770]
25. Asarnow DM, Kuziel WA, Bonyhad M, et al. Limited diversity of $\gamma\delta$ antigen receptor genes of thy-1 dendritic epidermal cells. *Cell.* 1988; 55:837–847. [PubMed: 2847872]
26. Witherden DA, Verdino P, Rieder SE, et al. The Junctional Adhesion Molecule JAML Is a Costimulatory Receptor for Epithelial T Cell Activation. *Science.* 2010; 329:1205–1210. [PubMed: 20813954]
27. Ismail AS, Severson KM, Vaishnava S, et al. $\gamma\delta$ intraepithelial lymphocytes are essential mediators of host-microbial homeostasis at the intestinal mucosal surface. *Proc Natl Acad Sci.* 2011; 108:8743–8748. [PubMed: 21555560]
28. Ismail AS, Behrendt CL, Hooper LV. Reciprocal interactions between commensal bacteria and gamma delta intraepithelial lymphocytes during mucosal injury. *J Immunol.* 2009; 182:3047–3054. [PubMed: 19234201]
29. van Konijnenburg DPH, Reis BS, Pedicord VA, et al. Intestinal Epithelial and Intraepithelial T Cell Crosstalk Mediates a Dynamic Response to Infection. *Cell.* 2017; 171:783–794. [PubMed: 28942917]
30. Di Marco Barros R, Roberts NA, Dart RJ, et al. Epithelia Use Butyrophilin-like Molecules to Shape Organ-Specific $\gamma\delta$ T Cell Compartments. *Cell.* 2016; 167:203–218. [PubMed: 27641500]
31. Kashani E, Föhse L, Raha S, et al. A clonotypic V γ 4J γ 1/V δ 5D δ 2J δ 1 innate $\gamma\delta$ T-cell population restricted to the CCR6 CD27– subset. *Nature Communications.* 2015; 6:3076.
32. Wei Y-L, Han A, Glanville J, et al. A Highly Focused Antigen Receptor Repertoire Characterizes $\gamma\delta$ T Cells That are Poised to Make IL-17 Rapidly in Naive Animals. *Front. Immunol.* 2015; 6:118. [PubMed: 25852688]
33. Ravens S, Schultze-Flore C, Raha S, et al. Human $\gamma\delta$ T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection. *Nat Immunol.* 2017; 18:393–401. [PubMed: 28218745]
34. Déchanet J, Merville P, Lim A, et al. Implication of $\gamma\delta$ T cells in the human immune response to cytomegalovirus. *J Clin Invest.* 1999; 103:1437–1449. [PubMed: 10330426]
35. Knight A, Madrigal AJ, Grace S, et al. The role of V δ 2-negative $\gamma\delta$ T cells during cytomegalovirus reactivation in recipients of allogeneic stem cell transplantation. *Blood.* 2010; 116:2164–2172. [PubMed: 20576814]
36. Perko R, Kang G, Sunkara A, et al. $\gamma\delta$ T cell reconstitution is associated with fewer infections and improved event-free survival after hematopoietic stem cell transplantation for pediatric leukemia. *Biol. Blood Marrow Transplant.* 2015; 21:130–136. [PubMed: 25445640]
37. Gentles AJ, Newman AM, Liu CL, Bratman SV, Feng W, Kim D, Nair VS, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med.* 2015; 21:1–12. [PubMed: 25569538]

38. Chen H, Zou M, Teng D, et al. Profiling the pattern of the human T-cell receptor $\gamma\delta$ complementary determinant region 3 repertoire in patients with lung carcinoma via high-throughput sequencing analysis. *Cell Mol Immunol*. 2018; 14:1–10.
39. Fahl SP, Coffey F, Kain L, et al. Role of a selecting ligand in shaping the murine $\gamma\delta$ -TCR repertoire. *Proc Natl Acad Sci*. 2018; 115:1889–1894. [PubMed: 29432160]
40. Cohen NR, Garg S, Brenner MB. Antigen Presentation by CD1 Lipids, T Cells, and NKT Cells in Microbial Immunity. *Adv. Immunol*. 2009; 102:1–94. [PubMed: 19477319]
41. Park S-H, Weiss A, Benlagha K, et al. The Mouse Cd1d-Restricted Repertoire Is Dominated by a Few Autoreactive T Cell Receptor Families. *J Exp Med*. 2001; 193:893–904. [PubMed: 11304550]
42. Cardell S. CD1-restricted CD4 T cells in major histocompatibility complex class II-deficient mice. *Journal of Experimental Medicine*. 1995; 182:993–1004. [PubMed: 7561702]
43. Pellicci DG, Patel O, Kjer-Nielsen L, et al. Differential recognition of CD1d- α -galactosyl ceramide by the V β 8.2 and V β 7 semi-invariant NKT T cell receptors. *Immunity*. 2009; 31:47–59. [PubMed: 19592275]
44. Wun KS, Cameron G, Patel O, et al. A molecular basis for the exquisite CD1d-restricted antigen specificity and functional responses of natural killer T cells. *Immunity*. 2011; 34:327–339. [PubMed: 21376639]
45. Thomas SY, Scanlon ST, Griewank KG, et al. PLZF induces an intravascular surveillance program mediated by long-lived LFA-1–ICAM-1 interactions. *J Exp Med*. 2011; 208:1179–1188. [PubMed: 21624939]
46. Lee YJ, Starrett GJ, Lee ST, et al. Lineage-Specific Effector Signatures of Invariant NKT Cells Are Shared amongst $\gamma\delta$ T, Innate Lymphoid, and Th Cells. *J Immunol*. 2016; 197:1460–1470. [PubMed: 27385777]
47. Lee YJ, Wang H, Starrett GJ, et al. Tissue-Specific Distribution of iNKT Cells Impacts Their Cytokine Response. *Immunity*. 2015; 43:566–578. [PubMed: 26362265]
48. Dasgupta S, Kumar V. Type II NKT cells: a distinct CD1d-restricted immune regulatory NKT cell subset. *Immunogenetics*. 2016; 68:665–676. [PubMed: 27405300]
49. Arrenberg P, Halder R, Dai Y, Maricic I, Kumar V. Oligoclonality and innate-like features in the TCR repertoire of type II NKT cells reactive to a β -linked self-glycolipid. *Proc Natl Acad Sci*. 2010; 107:10984–10989. [PubMed: 20534460]
50. Bai L, Picard D, Anderson B, et al. The majority of CD1d-sulfatide-specific T cells in human blood use a semiinvariant V δ 1 TCR. *Eur J Immunol*. 2012; 42:2505–2510. [PubMed: 22829134]
51. Luoma AM, Castro CD, Mayassi T, et al. Crystal structure of V δ 1 T cell receptor in complex with CD1d-sulfatide shows MHC-like recognition of a self-lipid by human $\gamma\delta$ T cells. *Immunity*. 2013; 39:1032–1042. [PubMed: 24239091]
52. Greene JM, Dash P, Roy S, et al. MR1-restricted mucosal-associated invariant T (MAIT) cells respond to mycobacterial vaccination and infection in nonhuman primates. *Mucosal Immunol*. 2017; 10:802–813. [PubMed: 27759023]
53. Mori L, Lepore M, De Libero G. The Immunology of CD1- and MR1-Restricted T Cells. *Annu Rev Immunol*. 2016; 34:479–510. [PubMed: 26927205]
54. Lantz O, Legoux F. MAIT cells: an historical and evolutionary perspective. *Immunol Cell Biol*. 2017; 33:511–9.
55. Meermeier EW, Harriff MJ, Karamooz E, Lewinsohn DM. MAIT cells and microbial immunity. *Immunol Cell Biol*. 2018; 178:1–11.
56. Reantragoon R, Corbett AJ, Sakala IG, et al. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *J Exp Med*. 2013; 210:2305–2320. [PubMed: 24101382]
57. Tilloy F, Treiner E, Park S-H, et al. An Invariant T Cell Receptor α Chain Defines a Novel TAP-independent Major Histocompatibility Complex Class Ib-restricted $\alpha\beta$ T Cell Subpopulation in Mammals. *J Exp Med*. 1999; 189:1907–1921. [PubMed: 10377186]
58. Rahimpour A, Koay HF, Enders A, Clanchy R, Eckle SBG, Meehan B, Chen Z, et al. Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. *J Exp Med*. 2015; 212:1095–1108. [PubMed: 26101265]

59. Lepore M, Kalinichenko A, Kalinichenko A, et al. Parallel T-cell cloning and deep sequencing of human MAIT cells reveal stable oligoclonal TCR β repertoire. *Nature Communications*. 2014; 5:3866.
60. Ben Youssef G, Tourret M, Salou M, et al. Ontogeny of human mucosal-associated invariant T cells and related T cell subsets. *J Exp Med*. 2018; 215:459–479. [PubMed: 29339446]
61. Gold MC, McLaren JE, Reistetter JA, et al. MR1-restricted MAIT cells display ligand discrimination and pathogen selectivity through distinct T cell receptor usage. *J Exp Med*. 2014; 211:1601–1610. [PubMed: 25049333]
62. Howson LJ, Napolitani G, Shepherd D, et al. MAIT cell clonal expansion and TCR repertoire shaping in human volunteers challenged with *Salmonella Paratyphi A*. *Nature Communications*. 2018; 9:253.
63. Gherardin NA, Keller AN, Woolley RE, et al. Diversity of T Cells Restricted by the MHC Class I-Related Molecule MR1 Facilitates Differential Antigen Recognition. *Immunity*. 2016; 44:32–45. [PubMed: 26795251]
64. Lepore M, Kalinichenko A, Calogero S, et al. Functionally diverse human T cells recognize non-microbial antigens presented by MR1. *eLife*. 2017; 6:1633.
65. Li Y, Innocentin S, Withers DR, et al. Exogenous Stimuli Maintain Intraepithelial Lymphocytes via Aryl Hydrocarbon Receptor Activation. *Cell*. 2011; 147:629–640. [PubMed: 21999944]
66. Cervantes-Barragan L, Chai JN, Tianero MD, et al. *Lactobacillus reuteri* induces gut intraepithelial CD4⁺CD8 $\alpha\alpha$ ⁺ T cells. *Science*. 2017; 357:806–810. [PubMed: 28775213]
67. Cheroutre H, Lambolez F, Mucida D. The light and dark sides of intestinal intraepithelial lymphocytes. *Nat Rev Immunol*. 2011; 11:445–456. [PubMed: 21681197]
68. Olivares-Villagómez D, Van Kaer L. Intestinal Intraepithelial Lymphocytes: Sentinels of the Mucosal Barrier. *Trends Immunol*. 2018; 39:264–275. [PubMed: 29221933]
69. Bilate AM, Bousbaine D, Mesin L, Agudelo M. Tissue-specific emergence of regulatory and intraepithelial T cells from a clonal T cell precursor. *Science Immunology*. 2016; 1:eaaf7471. [PubMed: 28783695]
70. Yamagata T, Mathis D, Benoist C. Self-reactivity in thymic double-positive cells commits cells to a CD8 $\alpha\alpha$ lineage with characteristics of innate immune cells. *Nat Immunol*. 2004; 5:597–605. [PubMed: 15133507]
71. Leishman AJ, Gapin L, Capone M, et al. Precursors of functional MHC class I- or class II-restricted CD8 $\alpha\alpha$ ⁺ T cells are positively selected in the thymus by agonist self-peptides. *Immunity*. 2002; 16:355–364. [PubMed: 11911821]
72. Mayans S, Stepniak D, Palida SF, et al. $\alpha\beta$ T cell receptors expressed by CD4(–)CD8 $\alpha\beta$ (–) intraepithelial T cells drive their fate into a unique lineage with unusual MHC reactivities. *Immunity*. 2014; 41:207–218. [PubMed: 25131531]
73. Gapin L, Cheroutre H, Kronenberg M. Cutting edge: TCR $\alpha\beta$ +CD8 $\alpha\alpha$ ⁺ T cells are found in intestinal intraepithelial lymphocytes of mice that lack classical MHC class I molecules. *J Immunol*. 1999; 163:4100–4104. [PubMed: 10510343]
74. Park S-H, Guy-Grand D, Lemonnier FA, et al. Selection and Expansion of CD8 $\alpha\alpha$ ⁺ T Cell Receptor $\alpha\beta$ ⁺ Intestinal Intraepithelial Lymphocytes in the Absence of Both Classical Major Histocompatibility Complex Class I and Nonclassical Cd1 Molecules. *J Exp Med*. 1999; 190:885–890. [PubMed: 10499927]
75. Das G, Gould DS, Augustine MM, et al. Qa-2-dependent selection of CD8 $\alpha\alpha$ ⁺ T Cell Receptor $\alpha\beta$ ⁺ cells in murine intestinal intraepithelial lymphocytes. *J Exp Med*. 2000; 192:1521–1528. [PubMed: 11085754]
76. Olivares-Villagómez D, Mendez-Fernandez YV, Parekh VV, et al. Thymus leukemia antigen controls intraepithelial lymphocyte function and inflammatory bowel disease. *Proc Natl Acad Sci*. 2008; 105:17931–17936. [PubMed: 19004778]
77. Regnault A. Oligoclonal repertoire of the CD8 $\alpha\alpha$ and the CD8 $\alpha\beta$ TCR- $\alpha\beta$ murine intestinal intraepithelial T lymphocytes: evidence for the random emergence of T cells. *Journal of Experimental Medicine*. 1994; 180:1345–1358. [PubMed: 7931068]
78. Regnault A, Levraud JP, Lim A, et al. The expansion and selection of T cell receptor $\alpha\beta$ intestinal intraepithelial T cell clones. *Eur J Immunol*. 1996; 26:914–921. [PubMed: 8625988]

79. Blumberg RS, Yockey CE, Gross GG, Ebert EC, Balk SP. Human intestinal intraepithelial lymphocytes are derived from a limited number of T cell clones that utilize multiple V β T cell receptor genes. *J Immunol.* 1993; 150:5144–5153. [PubMed: 8496608]
80. Van Kerckhove C, Russell GJ, Deusch K, et al. Oligoclonality of human intestinal intraepithelial T cells. *J Exp Med.* 1992; 175:57–63. [PubMed: 1730926]

Table 1

TCR repertoires, antigens, and functions of unconventional lymphocyte subsets

Cell type	TCR repertoire	Antigens	MHC restriction	Location	Phenotype/Cytokine profile	Specialized Functions
Invariant TCR						
Vγ9Vδ2+	V γ 9V δ 2	phosphoantigens	none; require BTN3A1	peripheral blood	antigen-experienced, IFN γ ⁺ , NK receptors	rapid IFN γ production elicited by parasitic infection, immune surveillance?
T$\gamma$$\delta$17	V γ 6V δ 1 and V γ 4V δ 5 (mouse)	?	none	widespread (lymphoid, adipose, lung, liver, blood, etc)	immature, IL-17A ⁺ , NK receptors	rapid IL-17A production in response to broad range of pathogens and stimuli
DETCs	V γ 5V δ 1 (mouse)	Skint-1?	none	skin	dendritic morphology, antigen-experienced, IFN γ ⁺ , NK receptors	produce antimicrobial peptides and factors/cytokines aiding resolution of wound repair
$\gamma$$\delta$ IELs	V γ 7V δ 4 (mouse) V γ 4+ (human)	Butyrophilins and Butyrophilin-like proteins	none	intestinal epithelium	antigen-experienced, IFN γ ⁺ , NK receptors	produce antimicrobial peptides and factors/cytokines aiding resolution of gut inflammation
iNKT	V α 14-J α 18 with V β 7, 8.2, or 2 (mouse) V α 24-J α 15 with V β 11 (human)	α GalCer and α GlcCer	CD1d	widespread (lymphoid, adipose, lung, liver, blood, etc) subsets enriched at different locations	antigen-experienced, IFN γ ⁺ , IL-17A ⁺ , IL-4 ⁺ , NK receptors	early cytokine response in line with T helper profiles, B cell help, others?
MAIT	V α 19-J α 33 with V β 6 or 8 (mouse) V α 7.2-J α 22/20 with V β 2 or 6 (human)	modified riboflavin and folic acid intermediaries	MR1	widespread (lymphoid, adipose, lung, liver, blood, etc)	antigen-experienced IFN γ ⁺ , IL-17A ⁺ , IL-4 ⁺	early cytokine response and cytotoxicity, others?
Diverse TCR						
Vδ1+ $\gamma$$\delta$ T cells	diverse CDR3 δ paired with multiple TCR γ chains, oligoclonal	?, sulfatide	multiple?, CD1d	peripheral blood, others?	antigen-experienced, IFN γ ⁺	CMV immunity, immune surveillance?
dNKT	diverse TCR α and TCR β , oligoclonal	sulfatide, β GAL1-22, LGL1	CD1d	peripheral blood, others?	antigen-experienced, IFN γ ⁺ , NK receptors	?
MR1T	diverse TCR α and TCR β , oligoclonal	modified riboflavin and folic acid intermediaries	MR1	peripheral blood, others?	antigen-experienced, IFN γ ⁺	?
DN $\alpha\beta$ IELs	diverse TCR α and TCR β , oligoclonal	self-antigen?	Mixed. Require β 2m and Tap1	intestinal epithelium	“activated yet resting”, IFN γ ⁺ , cytotoxic, NK cell receptors	poorly understood, immune regulatory activity?
CD8 $\alpha\beta$ IELs	diverse TCR α and TCR β , oligoclonal	peptide antigens from pathogens, self-antigen?	MHC Ia	intestinal epithelium	effector memory, IFN γ ⁺ , cytotoxic, NK cell receptors	memory-like, cytotoxic
CD4 $\alpha\beta$ IELs	diverse TCR α and TCR β , oligoclonal	self-antigen?	MHC II	intestinal epithelium	effector memory, IFN γ ⁺ , NK receptors	memory-like, poorly understood

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Abbreviations: TCR, T cell receptor; MHC, major histocompatibility complex; BTN3A1, butyrophilin 3A1; DETCs, dendritic epidermal T cell; IEL, intraepithelial lymphocyte; NK, natural killer; iNKT, invariant natural killer T; α GalCer, α -galactosyl ceramide; α GlcCer, α -glucosylceramide; dNKT, diverse natural killer T; MAIT, mucosal-associated invariant T; MR1, MHC-related molecule-1; MR1T, MR1-restricted T; DN, double-negative; β GL1-22, β -glucosylceramide 22:0; LGL1, glucosylsphingosine

TCR γ and TCR δ V regions listed here follow the Tonegawa nomenclature