New insights into asthma inflammation: focus on iNKT, MAIT and $\gamma\delta$ T cells

Short title: Innate-like T cells and asthma

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Abstract

Asthma is a chronic immunological disease affecting all age groups, but often starting in childhood. Although it has long been ascribed to a single pathology, recent studies have highlighted its heterogeneity due to the potential involvement of various pathogenic mechanisms. Here, we present our current understanding of the role of innate-like T (ILT) cells in asthma pathogenesis. These cells constitute a specific family mainly comprising $\gamma\delta$ T, invariant Natural Killer (iNKT) and Mucosal-Associated Invariant (MAIT) T cells. They all share the ability to massively secrete a wide range of cytokines in a T cell receptor (TCR)-dependent or -independent manner. ILT cells are prevalent in mucosal tissues, including airways, where their innate and adaptive immune functions consist primarily in protecting tissue integrity. However, ILT cells may also have detrimental effects leading to asthma symptoms. The immune mechanisms through which this pathogenic effect occurs will be discussed in this overview.

Key Words: Asthma, $\gamma\delta$ T cells, iNKT cells, MAIT cells, children, patients, CD1d, MR1

INTRODUCTION

Asthma is now considered an umbrella term encompassing various conditions characterized by wheeze, cough, shortness of breath, chest tightness and variable degrees of airflow limitation. These symptoms are associated with different patterns of inflammation [1-4]. For the sake of clarity, an allergic and a non-allergic type of asthma are commonly distinguished. In the first case, inflammation is primarily caused by type 2 immune responses mediated through the Th2 cytokines IL-4, IL-5, and IL-13 and associated with increased Th2 cells and eosinophils in the airways [5]. By contrast, non-allergic asthma is mainly triggered by an inflammatory response to viral infections with a major neutrophilic component [6]. There is mounting evidence that neutrophilic forms of murine and human asthma are associated with IL-17A (hereafter referred to as IL-17) [7-9]. This cytokine is involved in the induction and persistence of neutrophilic inflammation[10] and can contribute to airway smooth muscle remodeling in association with TGF- β [11].

Asthma can adopt diverse phenotypes associated with a number of endotypes[12,2,4]. Previous reports have implicated Th2 and Th17 cells in mechanisms favoring asthmatic immune endotypes [13]. However, these two populations alone cannot account for the great diversity of symptoms, mainly because other cell types present in the airways can also contribute to the production of Th2- and Th17-type cytokines. The best potential candidates for this purpose are “unconventional” T cells also currently named “Innate-Like T (ILT) lymphocytes” that recognize non-peptide antigens presented by specialized MHC class I-like molecules [14-16].

Innate-like T (ILT) cells are unique unconventional T lymphocytes sharing features of both innate and adaptive immunity. This population recognizes non-peptide antigens presented by specialized MHC class I-like molecules. It includes invariant natural killer T (iNKT), mucosal-associated invariant T (MAIT) and gamma-delta T ($\gamma\delta$ T) cells, all characterized by a conserved and restricted T cell receptor (TCR) [14]. iNKT cells express an antigen-specific semi-invariant TCR, V α 14-J α 18 (TRAV11-TRAJ18) and V α 24-J α 18 (TRAV10-TRAJ18) in mice and humans, respectively [17,16,14]. Similarly, MAIT cells display V α 19-J α 33 (TRAV1-2-TRAJ33) in mice and V α 7.2-J α 33 (TRAV1-2-TRAJ33) in humans [18-20]. The $\gamma\delta$ T cell population is not homogeneous, even though the V δ 2⁺ subset is predominant in human peripheral blood [21]. These cells can produce large amounts of cytokines shortly after stimulation and play an important role in first-line defense against microbial infections [22]. $\gamma\delta$ T, iNKT and MAIT cells are currently divided into distinct subsets according to their cytokine profile (Table 1). They have all been associated with a growing number of inflammatory diseases including asthma.

The next paragraphs will be focused on the development and functional properties of ILT cells and their impact on the immune mechanisms leading to asthma.

INNATE-LIKE T CELLS

$\gamma\delta$ T cells

$\gamma\delta$ T cells constitute a distinct population of T lymphocytes in mice and humans, which upon activation can promptly generate a number of cytokines and chemokines with modulatory activities in innate and adaptive immunity [23-25]. Compared to other unconventional T cells, they can readily perform effector functions in response to tissue stress [22,23]. Furthermore, in epithelial tissues including lungs, $\gamma\delta$ T cells make up the major resident T cell population and their functions, although still controversial, can be related to tissue integrity and maintenance of host homeostasis [22].

$\gamma\delta$ T cells can be classified by the expression of their $\gamma\delta$ TCR chains. In humans, the differential expression of δ (δ 1-3) and γ genes (γ 2-5, γ 8, γ 9, and γ 11) has been used to this end, whereas in mice $\gamma\delta$ T cell subsets are defined by the expression of distinct γ chains[26] (Table 1). Contrasting with conventional T cells, which recognize peptides presented by MHC molecules, $\gamma\delta$ T cells can distinguish uncommon antigens, such as stress molecules (MICA and MICB), heat-shock proteins or synthetic phosphoantigens (pAg) [27,28]. It is clear by now that $\gamma\delta$ T cells do not interact directly with pAg but require the presence of butyrophilin BTN3A as an intermediate [29]. This indicates the usage of a fundamentally different mechanism of antigen detection whose molecular basis remains to be elucidated.

Although $\gamma\delta$ T cells can produce several cytokines, two major murine subsets defined by their capacity to produce IL-17 or IFN- γ have been described in recent years [30] (Table 1). Compared to conventional TCR $\alpha\beta$ ⁺ CD4 and CD8 T cells, $\gamma\delta$ T cells are more prompt in producing significant amounts of IFN- γ [31], thus providing an early source of this factor that could be protective in tumor immunity [32-34]. However, in some cases, such as fulminant viral hepatitis in a murine model, the production of IFN- γ by $\gamma\delta$ T cells can become deleterious [35]. $\gamma\delta$ T cells that produce substantial amounts of IL-17 are frequently termed $\gamma\delta$ T17 and display a CD27⁺NK1.1⁺CCR6⁺ phenotype [30,36]. They can take part in tissue surveillance and repair and mediate effective immune responses against bacterial and fungal infections [37-40]. Conversely, they are potential promoters of inflammation in type 1 autoimmune diseases, such as arthritis or colitis [41,42]. According to recent reports, $\gamma\delta$ T17 cells could also promote tumor progression in experimental models [43].

γδT cells in experimental models of asthma

The role of γδT cells in lung inflammation has been debated in the literature for more than 30 years. In 1998, one of the first publications supporting the requirement of γδT cells for promoting IgE production and Th2 airway inflammation in an IL-4-dependent manner came from experiments with γδT cell-deficient mice, which failed to develop the pathology [44]. One year later, another team reported that airway reactivity was increased in the absence of γδT cells, suggesting a protective role of these cells [45]. These controversial data could be explained by the distinct genetic backgrounds, namely BALB/c [44] and C57BL/6 [45] in these γδT cell-deficient mice. Indeed, these strains respond differently in the asthma protocol since BALB/c display more severe AHR than C57BL/6 mice, while airway eosinophilia is more pronounced in the C57BL/6 strain [46].

Further studies using blocking anti-γδTCR mAb administered during the resolution phase of OVA-induced allergic inflammation, provided evidence for an exacerbation of lung injury [47]. However, it turned out that GL3 mAb used in this study did not deplete γδT cells in vivo, but induced the downmodulation of their γδTCR [48].

The complexity of γδT cell regulation of asthmatic inflammation may be explained by the intervention of distinct subsets. In this line of evidence, it has been shown that the effect of γδT cells on IgE production in an OVA-induced allergic asthma model changed according to the phenotype of the γδT cell population involved [49]. Indeed Vγ1 γδT cells promoted an enhanced IgE response, while their Vγ4 counterpart suppressed this activity [50]. These non-overlapping functions of Vγ4 and Vγ1 γδT cell subsets were also observed in terms of AHR [51]. Consistent with this observation, it has been shown more recently that IL-4-producing Vγ1⁺ γδT cells promoted airway eosinophilia and AHR in experimental allergic asthma induced by the dust mite *Blomia tropicalis* [52].

γδT cells also produce IFN-γ and IL-17. It has been proposed that the IFN-γ thus generated might favor allergic inhibition by reducing IgE production [53], while IL-17-producing γδT cells could ameliorate AHR and accelerate the resolution of eosinophilic airway inflammation [47]. Furthermore, a relationship between reduced thymic maturation of IL-17-producing γδT cells and decreased lung inflammation has been described in a murine protocol of OVA-induced allergy [54].

γδT cells in asthmatic patients

In humans, peripheral γδT cells were reported to be decreased in difficult-to-control asthmatic patients compared to controls [55]. Further, a higher frequency of IL-4-producing versus a lower incidence of IFN-γ-producing γδT cells was described in patients with allergic asthma, as compared with healthy controls [56].

In another study $\gamma\delta$ T cells were found to be more frequent at the site of inflammation in bronchoalveolar lavage fluid (BALF) of untreated asthmatic patients, as compared with control volunteers [57]. Among the scarce data available on this issue, according to another group, $\gamma\delta$ T cells occurred at similar levels in BALF or lung biopsies, whether the donors were suffering from asthma or not [58], while another research confirmed that the frequency of $\gamma\delta$ T cells in BALF from stable asthmatic subjects was not significantly different from controls [59]. However, after *in vivo* allergen challenge, BALF $\gamma\delta$ T cells increased their capacity to produce Th2-type cytokines, including IL-5 and IL-13, and reduced their secretion of Th1-type cytokines, such as IFN- γ [59]. These findings suggest that the $\gamma\delta$ T cytokine profile is flexible in BALF, ready to be skewed toward the Th2-type in asthmatic patients [59].

Overall, the studies cited here reveal that $\gamma\delta$ T cells exert a highly complex control in lung inflammation, possibly because of the contribution of various subsets whose composition could differ according to the clinical status of asthmatic patients (Fig.1).

invariant Natural Killer T cells

Differentiation and maturation of iNKT cells occur in the thymus, where they appear slightly later than MHC-restricted conventional T cells [60-63]. Thymic iNKT cell differentiation requires a particular rearrangement between V α and J α gene segments in the TCR α locus. This is believed to be a random process that occurs at very low frequency leading to the characteristic iNKT cell invariant TCR α chain: V α 14J α 18 (TRAV11-TRAJ18) in mice and V α 24J α 18 (TRAV10-TRAJ18) in humans [64,65]. The TCR α chain associates with a limited set of TCR β chains [66-68] (Table 1).

Once they have completed a tightly controlled thymic differentiation pathway, iNKT cells migrate to peripheral organs as CD4⁺CD8⁻ and double-negative (CD4⁻CD8⁻) cells. In humans, the CD4⁻CD8⁺ subset does likewise occur [69]. iNKT cells are mostly resident in tissues, where they are believed to act as sentinels of tissue integrity by “patrolling” to identify threats to the body [70]. They are rare in most peripheral sites (0.1-1% among T lymphocytes) but numerous in the liver [16]. The invariant TCR α chains in mice and humans are conserved, enabling iNKT cells to recognize the same glycolipids in both species, as is the case for the glycolipid α -galactosylceramide (α -GalCer) [71,72].

iNKT cells are restricted by lipids and glycolipids loaded onto CD1d molecules present on the surface of APCs [73]. Moreover, their cytokine production along with their cognate interactions, enable them to modulate the activities of different immune cells playing a pivotal role in the orchestration of the immune response [74].

iNKT cells in experimental models of asthma

In the nineties, several studies demonstrated that iNKT cells promptly and massively secreted IL-4 [75-78]. Consequently, it was proposed that their major role was to promote Th2 differentiation via this cytokine [79]. This has led a number of groups to test IL-4-producing iNKT cells in specific experimental models requiring Th2 cells, including allergic asthma models.

Initial studies using iNKT cell-deficient mice, namely β_2 microglobulin (β_2m)^{-/-} and CD1d^{-/-} strains, revealed no major differences in the severity of allergic airway inflammation [80-82]. More recently, other investigators using these same mutants concluded that NKT cells were dispensable for T cell-dependent allergic airway inflammation [83,84]. However, it turned out that both β_2m ^{-/-} and CD1d^{-/-} mice are also deficient for other T cell subsets (CD8⁺ T and CD1d-dependent T lymphocytes, respectively), thus masking a potential effect of iNKT cells. To solve this issue, two groups used more specific iNKT cell-deficient ($J\alpha 18$ ^{-/-}) mice, for which they reported attenuated asthma symptoms, including AHR, airway eosinophilia, Th2 inflammation and OVA-specific anti-IgE production relative to wild-type controls [85,86]. The adoptive transfer of wild type iNKT cells was sufficient to restore asthma severity in $J\alpha 18$ ^{-/-} mice. Of note, adoptive transfers of iNKT cells from IL-4^{-/-} or IL-13^{-/-} mice were much less effective and iNKT cells from double knockout IL-4^{-/-}IL-13^{-/-} mice had no effect at all [86]. This finding demonstrated that iNKT cells enhanced allergic asthma symptoms through the production of IL-4 and IL-13 [85,86].

Recently, a new mouse strain with the deletion of *Traj18* was created on a C57BL/6 genetic background [90]. These *Traj18*-deficient mice lack iNKT cells but have a normal $J\alpha$ -region repertoire. They display significantly less eosinophils and AHR in an airway hyper-reactivity model [90], thereby confirming the implication of iNKT cells in asthmatic airway inflammation. In further support of this conclusion, *Ezh2* (Enhancer of zeste homolog 2)-deficient mice spontaneously developed an asthma-like disease [91] associated with abnormally high iNKT cell counts, increased IL-4 and IL-13 production, AHR, lung inflammation and IgE levels, all consistent with a contribution of iNKT cells to asthma pathogenesis [91].

Even though iNKT cells do not recognize OVA as an antigen, their ability to promote lung inflammation is TCR-dependent since the injection of blocking anti-CD1d antibodies reduced the effect, indicating that endogenous lipidic antigens stimulated the iNKT cells [85]. Indeed, in this setting we cannot exclude that commensal microbial antigens present in the lung could take part in the activation of iNKT cells. In the same line of evidence, an elegant study by Blumberg's team showed that iNKT cells accumulated in the lung and in the colonic lamina propria in germ-free (GF) mice, rendering these

animals more susceptible to OVA-induced asthma and oxazolone-induced colitis [87]. The colonization of neonatal GF mice with a normal flora or *Bacteroides fragilis* decreased the number of iNKT cells and protected the mice against these diseases, clearly establishing a link between iNKT cells and the microbiota [87,88]. Knowing that a bacterial community is present in the airways and that this microbiota could be altered in the case of asthma [89], we postulate that some bacterial antigens might activate iNKT cells in the lung, thus promoting inflammation. Further studies are required to test this hypothesis.

For asthma that is not associated with pro-Th2 allergic immune responses, it has been reported that i.n. administration of α -GalCer activates IL-17-secreting iNKT cells, which, in turn, promote airway neutrophilia [92]. IL-17-secreting iNKT cells are also required for the pathogenic mechanisms leading to AHR after exposure to ozone, a major component of air pollution [93]. Hence, iNKT cells may promote asthma inflammation in different ways, depending on their cytokine profile (Fig. 2).

iNKT cells in asthmatic patients

Analysis of the incidence of iNKT cells in the peripheral blood of adult asthmatic patients, revealed no significant differences when compared to control subjects [94]. Further studies suggested however that IL-4-producing iNKT cells were more frequent in these cases and that they were associated with lung functions [95]. In children with asthma, the percentage of peripheral blood iNKT cells did not differ significantly between asthmatic children classified as exacerbators (1 or more severe exacerbations in the last 12 months) and non-exacerbators [96]. Similarly, no relationship was observed between the frequency of circulating iNKT cells and IL-4- or IFN- γ -producing iNKT cells in one-year-old children and asthma-related clinical outcomes at the age of seven years [97].

In addition to peripheral blood analysis, a number of studies have evaluated iNKT cells in BALF or bronchial biopsies of asthmatic patients. The first data in adult asthmatic patients revealed that about 60% of the pulmonary CD4⁺CD3⁺ T cells in the group with moderate-to-severe persistent asthma were iNKT cells [98]. These results were not reproduced by others [99,100], who found that up to 2% of the T cells recovered from airway biopsies, BALF and sputum induction from subjects with mild or moderately severe asthma were iNKT cells. Umetsu and collaborators reported later that only a small fraction of T cells in the lung of adult asthmatic patients were iNKT cells [101], which accounted for less than 1% of T cells in BALF from children with severe asthma [102]. The discrepancies between these studies could be explained by the limited number of samples, the heterogeneity of the cohorts, or by non-specific staining of cells in BALF, as suggested by the study of Thomas *et al* [100].

Taken together, these findings show that large adult and children cohorts composed of asthmatic patients with well-defined symptoms and grades of severity (controlled, non-controlled and severe asthma) associated with relevant controls are required to definitely assess whether iNKT cells are involved in asthma pathology or not.

Mucosal-Associated Invariant T cells

MAIT cells are restricted by the MHC class I-related molecule MR1 and recognize microbial-derived vitamin B2 (riboflavin) metabolites [103] (Table 1). Riboflavin metabolites are not present in mammalian cells but occur in several bacteria and yeast [104], which suggests that MAIT cells can sense antigens displayed by a limited number of microbes.

MAIT cells develop in the thymus, where they become functionally competent and able to promptly produce Th1 (IFN- γ , TNF- α) and Th17 (IL-17 and IL-22) cytokines in response to stimulation [105]. In contrast, MAIT cells produce only low to moderate levels of Th2 (IL-4 and IL-13) cytokines [106-109]. Similarly to iNKT cells, MAIT precursors undergo a positive selection of MR1⁺ double-positive (CD4⁺CD8⁺) thymocytes [110,111]. Previous reports have stressed the key role of the microbiota in MAIT cell expansion since these cells are nearly absent in germ-free mice [20]. Recently, Lantz's team made a major breakthrough in this field by demonstrating that microbial metabolites control the thymic development of MAIT cells [112]. This finding paved the way for revisiting the concept of exogenous and self-antigens.

Human MAIT cells express high levels of CD161 and IL-18R α and are mainly CD8⁺ [113]. A smaller proportion among these is double-negative (CD4⁻CD8⁻) and some CD4⁺ cells have been described in the literature [114]. MAIT cells can be activated in a TCR-dependent and -independent manner. They account for up to 10% of peripheral blood T cells and are numerous in the gut, lung, and liver [115,113,20]. Their abundance in mucosal tissues supports their involvement in antimicrobial responses [20].

Because of the scarce pieces of evidence for a role of MAIT cells in experimental models of asthma, we will not discuss this issue, but pass on directly to studies with asthmatic patients.

MAIT cells in asthmatic patients

As indicated by their name, MAIT cells are preferentially associated with mucosal tissues including lungs, where they promote antimicrobial defenses through the secretion of IFN- γ , TNF- α , IL-17, perforin and granzyme B [116-120]. Evidence for MAIT cell expansion in the lungs of MAIT cell-enriched V α 19iC α ^{-/-}MR1^{+/+} mice experimentally infected with

Mycobacterium tuberculosis supports their role during pulmonary infection [121]. This proliferative response was rapid and depended on the MR1 presentation of riboflavin biosynthesis-derived bacterial ligands [121]. In line with these experimental data it has been reported that adult patients suffering from active pulmonary tuberculosis (TB) had more MAIT cells in BALF and lung parenchyma than uninfected controls [122]. However, in children with similar symptoms, reduced MAIT cell counts were found both in peripheral blood and BALF [123]. This discrepancy might be due to clinical differences between pediatric and adult active TB, calling for further studies to ascertain the role of MAIT cells in this infectious disease.

The data gained from lung infections prompted several groups to address the involvement of MAIT cells in other lung pathologies like asthma. Pioneer studies by Hinks et al. reported that the frequency of MAIT cells was significantly lower in peripheral blood, sputum and bronchial biopsy samples of asthmatic patients, relative to control subjects [124]. This deficiency was correlated with clinical severity and the use of oral corticosteroids [124].

A recent study provided evidence for a relationship between a high frequency of circulating MAIT cells in one-year-old children and a lower risk of developing asthma by the age of seven years [97]. Furthermore, these high MAIT cell levels were correlated with higher frequency of IFN- γ -producing CD4⁺ T cells, suggesting a possible protective effect of MAIT cells as children grow older [97]. IL-17 production by MAIT cells did not correlate with asthma in this study. In children with mild asthma, the frequency of circulating MAIT cells did not differ between exacerbators and non-exacerbators [96]. However, the proportion of MAIT cells producing IL-17 correlated positively with the number of severe exacerbations and negatively with the asthma control test score in these children, which was not the case for their IFN- γ -producing counterpart [96]. More recently, the same team showed that MAIT cells, including their IL-17-producing subset, were present in BALF from children with severe asthma [125]. It is noteworthy that when this group was divided into two according to the frequency of their IL-17-producing MAIT cells in BALF into MAIT17-low and MAIT17-high, the latter presented more severe exacerbations than their MAIT17-low counterpart [125].

Overall, the results of these studies suggest that IL-17-producing MAIT cells may be associated with asthma symptoms, whereas the pro-Th1 subset might provide protection [97,96,125] (Fig. 3).

CONCLUDING REMARKS

Much progress has been achieved in understanding the physiological and pathological role of $\gamma\delta$ T, iNKT and MAIT cells, which have by now acquired a lasting position among the complex immune mechanisms leading to distinct asthma phenotypes and endotypes. Nonetheless, major uncertainties remain. For instance, asthma often begins early in childhood, at a time when the amount and functional properties of lung $\gamma\delta$ T, iNKT and MAIT cells are about to be established. However, very few studies have addressed the evolution of these populations in the airways with age. The cross-talk between lung $\gamma\delta$ T, iNKT and MAIT cells needs likewise to be investigated in more detail, as well as the influence of microorganisms present in the lung. In sum, studies of experimental models and pediatric asthma will be crucial for the comprehension of these complex interactions and the development of new therapeutic approaches.

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Compliance with Ethical Standards

Conflict of Interest. The authors declare that they have no conflict of interest.

Ethical Approval and Informed Consent

No approvals or informed consents were obtained, as this manuscript does not contain primary research data.

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Figure Captations

Figure 1: $\gamma\delta$ T cell immune response in asthma. In experimental asthma models (left) IL-4-producing $\gamma\delta$ T cells could enhance Th2 inflammation, IgE production, airway eosinophilia and airway hyperreactivity (AHR), while IL-17-producing $\gamma\delta$ T17 cells could have an opposite effect. In BALF (bronchoalveolar lavage fluid) from asthmatic patients (right), the frequency of $\gamma\delta$ T cells was elevated. These $\gamma\delta$ T cells improved their ability to secrete IL-5 and IL-13 following allergen challenge. The main effects resulting from the activation of $\gamma\delta$ T are listed within the lung figure.

↑= induction/augmentation; ↓= reduction/inhibition.

Figure 2: iNKT cell immune response in asthma. In experimental asthma models (left) IL-4-and IL-13-producing iNKT2 cells will enhance IgE production, airway eosinophilia and airway hyperreactivity (AHR), while IL-17-producing iNKT17 cells could promote airway neutrophilia and AHR. The frequency of iNKT cells in BALF (bronchoalveolar lavage fluid) from asthmatic patients (right) rang from 0.1% to 10%. The main effects resulting from the activation of iNKT cells are listed within the lung figure.

↑= induction/augmentation; ?= no functional effects described.

Figure 3: MAIT cell immune response in asthma. The studies concerning the implication of MAIT cells in experimental asthma models (left) are currently in progress. IL-17-producing MAIT cells are associated with severe exacerbations in asthmatic patients (right) while their IFN γ -producing counterpart maybe involved in protection.

?= no functional effects described.

Figure 1

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Figure 2

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Figure 3

Table 1. Major features of $\gamma\delta$ T, iNKT and MAIT cells.

	$\gamma\delta$T		iNKT			MAIT	
TCR	<ul style="list-style-type: none"> • Mice: Vγ1, Vγ4, Vγ5, Vγ7 (TRGV1, 4, 5, 7). • Humans: Vδ1-3 (TRDV1-3), Vγ2-5, Vγ8, Vγ9, Vγ11 (TRDV2-5, 8, 9, 11). 		<ul style="list-style-type: none"> • Mice: Vα14-Jα18 (TRAV11-TRAJ18). • Humans: Vα24-Jα18 (TRAV10-TRAJ18). 			<ul style="list-style-type: none"> • Mice: Vα19-Jα33 (TRAV1-2-TRAJ33). • Humans: Vα7.2-Jα33 (TRAV1-2-TRAJ33). 	
Antigen-presenting molecules	BTN3A (as an intermediate)		CD1d			MR1	
Antigens	stress molecules (MICA and MICB), heat-shock proteins or synthetic phosphoantigens (pAg)		glycolipids (α -GalCer, iGb3, others)			Riboflavin derivatives (5-OP-RU)	
Major subsets	$\gamma\delta$T IFN-γ⁺	$\gamma\delta$T17	iNKT1	iNKT2	iNKT17	MAIT1	MAIT17
Transcription factors	Tbet	RORC	Tbet, GATA-3	GATA-3	RORC	Tbet	RORC
Major cytokines produced	IFN- γ	IL-17	IFN- γ , IL-4	IL-4, IL-13	IL-17	IFN- γ	IL-17