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Human Vγ9/Vδ2 T cells: Innate adaptors of the immune system

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ABSTRACT

Unconventional T cells are gaining center stage as important effector and regulatory cells that orchestrate innate and adaptive immune responses. Human Vγ9/Vδ2 T cells are amongst the best understood unconventional T cells, as they are easily accessible in peripheral blood, can readily be expanded and manipulated *in vitro*, respond to microbial infections *in vivo* and can be exploited for novel tumor immunotherapies. We here review findings that suggest that Vγ9/Vδ2 T cells, and possibly other unconventional human T cells, play an important role in bridging innate and adaptive immunity by promoting the activation and differentiation of various types of antigen-presenting cells (APCs) and even turning into APCs themselves, and thereby pave the way for antigen-specific effector responses and long-term immunological memory. Although the direct physiological relevance for most of these mechanisms still needs to be demonstrated *in vivo*, these findings may have implications for novel therapies, diagnostic tests and vaccines.

Introduction

γδ T cells represent a third lineage of lymphocytes expressing re-arranged antigen receptors that co-evolved with 'classical' B cells and αβ T cells over 400 million years, arguing for a crucial and non-redundant contribution of each lymphocyte to effective immune responses, and hence the evolutionary survival of all jawed vertebrate species [1]. 30 years have passed since the accidental and unexpected cloning of the γδ T cell receptor (TCR) and the subsequent realization that αβ T cells and γδ T cells are fundamentally different with respect to the types of antigens they recognize and the distinct effector functions triggered upon such recognition. However, the manifold contributions of γδ T cells to homeostasis, inflammation, infection and tumor surveillance have historically been neglected and are only beginning to be integrated into comprehensive models of the immune system [1–7].

Abbreviations: APC, antigen presenting cell; BCG, bacillus Calmette–Guérin; BTN3A, butyrophilin 3A (CD277); DC, dendritic cell; DMAPP, dimethylallyl pyrophosphate; α-GC, α-galactosylceramide; HMB-PP, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; ICOS, inducible T cell costimulator; iNKT cell, invariant natural killer T cells; IPP, isopentenyl pyrophosphate; MAIT cell, mucosal-associated invariant T cell; TCR, T cell receptor; T_{FH} cell, follicular B helper T cell; TLR, Toll-like receptor.

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1. Innate-like pattern recognition by human Vγ9/Vδ2 T cells

Like other 'unconventional' T cells carrying an αβ TCR such as mucosal-associated invariant T (MAIT) cells and natural killer T (NKT) cells, γδ T cells are characterized by a markedly restricted TCR usage that allows them to recognize self and non-self molecules in the absence of classical antigen presentation via MHC I or MHC II. Vγ9/Vδ2$^+$ γδ T cells represent the major γδ T cell subset in human peripheral blood where they typically comprise 1–5% in healthy adults [8]. In many microbial infections, Vγ9/Vδ2 T cells increase locally and/or systemically [9,10] and can reach in excess of 50% of all peripheral T cells within a matter of days [11], revealing a fundamental role of this unconventional T cell population in acute disease and suggesting their exploitation for diagnostic and therapeutic purposes [12–14].

Vγ9/Vδ2 T cells uniformly respond to a class of low molecular weight molecules often referred to as 'phosphoantigens' that represent metabolites of the isoprenoid biosynthesis, or synthetic analogs thereof. By far the most potent of these 'phosphoantigens' is (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP), an intermediate of the non-mevalonate pathway that is found in the majority of Gram-negative bacteria and many Gram-positive species as well as apicomplexan parasites such as *Plasmodium falciparum* and *Toxoplasma gondii* [11,15]. In these organisms, HMB-PP is converted into isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), two further 'phosphoantigens' with a

bioactivity approx. 10,000-fold lower than that of HMB-PP [11,16]. In all other bacteria as well as in higher eukaryotes including humans, IPP and DMAPP are generated via the mevalonate pathway that is closely regulated by the action of 3-hydroxy-3-methyl-glutaryl-CoA reductase and downstream enzymes such as farnesyl pyrophosphate synthase. Overproduction of the low bioactivity compounds IPP and DMAPP as a result of a dysregulation of the mevalonate pathway in human cells, be it in metabolically active tissues including tumor cells or through inhibition of farnesyl pyrophosphate synthase by aminobisphophonates such as zoledronate, is thought to render such cells targets of Vγ9/Vδ2 T cells, despite the absence of the high bioactivity metabolite HMB-PP in this context [11,16–18]. Zoledronate and related drugs are therefore receiving substantial attention as Vγ9/Vδ2 T cell-stimulating agents *in vivo* especially with respect to immunotherapies against advanced solid and hematological tumors [19–21].

The recent landmark discovery of butyrophilin 3A (BTN3A/CD277) as the long-sought unconventional 'presenting' molecule for HMB-PP and IPP provided a molecular mechanism that elegantly integrates the action of endogenous and exogenous stimuli via binding of phosphoantigens to the intracellular B30.2 (PRY-SPRY) domain of BTN3A [22–25]. This intracellular recognition of HMB-PP and IPP evokes similar cases of B30.2-mediated innate responses through proteins such as TRIM5α and TRIM21 [26,27], thereby adding BTN3A to the rapidly growing number of innate pattern recognition receptors. Despite this progress, the precise mechanism of how the HMB-PP/BTN3A complex might engage the TCR of Vγ9/Vδ2 T cells awaits further elucidation.

Once activated, Vγ9/Vδ2 T cells exert a range of different effector functions by killing infected and stressed target cells, driving inflammatory and wound healing processes, promoting survival of monocytes and neutrophils, inducing maturation of dendritic cells (DCs), providing B cell help, and priming $CD4^+$ and $CD8^+$ T cells [1–7]. In the following chapters we will review findings showing that Vγ9/Vδ2 T cells, and possibly other unconventional human T cells, play an important role in bridging innate and adaptive immunity by promoting the activation and differentiation of various types of antigen-presenting cells (APCs) and even turning into APCs themselves, and thereby pave the way for antigen-specific effector responses and long-term immunological memory (Fig. 1).

2. Maturation of DCs: provision of fully functional APCs

Dendritic cells (DCs) as the prototype of professional APCs play a crucial role in initiating adaptive responses by presenting antigens to conventional αβ T cells. In this context, the process of DC maturation is a critical component of mounting an effective immune response, and involves several distinct stages; the upregulation of antigen presenting and co-stimulatory molecules, the secretion of distinct sets of cytokines/chemokines, a switch in the migratory profile, the reduction of endocytic and phagocytic ability, and the stabilization of MHC/peptide complexes on the cell surface [28]. DC maturation typically occurs upon recognition of danger signals via pattern recognition receptors, the most well defined of which being Toll-like receptors (TLRs). However, host-derived factors are also capable of inducing DC maturation, such as the cytokines IFN-γ and TNF-α [29].

Unconventional T cells including Vγ9/Vδ2 T cells represent a significant source of IFN-γ and TNF-α upon stimulation and are well placed to encounter immature DCs in the blood or periphery to promote DC maturation [30–32] (Fig. 1). In return, DCs have been shown to activate Vγ9/Vδ2 T cells via TCR-independent mechanisms, predominantly by the release of type I IFNs [33–35]. Effects on Vγ9/Vδ2 T cells include the induction and enhancement of proliferation [36] and the expression of cytotoxic and pro-inflammatory mediators such as perforin, IFN-γ and TNF-α [34,37,38], thereby forming a positive feedback loop for mutual stimulation of both cell types.

Upregulation of APC markers and co-stimulatory molecules is an important step in the DC maturation process, due to their vital role in triggering appropriate adaptive responses to any given challenge. Ismaili and colleagues [39] were the first to report that activated Vγ9/Vδ2 T cells stimulate the upregulation of HLA-DR, CD86 and CD83 on immature DCs, in the absence of other stimuli. Further Vγ9/Vδ2 T cell-induced APC markers on DCs may include MHC class I, CD25, CD40, CD80 and others, depending on the culture conditions [37,40–42], suggesting a certain degree of plasticity under the influence of the microenvironment, albeit with unclear physiological implications. Besides inducing DC maturation on their own, Vγ9/Vδ2 T cell-derived factors also synergize with other signals and as such enhance DC maturation triggered by TLR ligands [40,41,43]. Irrespective of *de novo* maturation of DCs or enhancement of TLR-mediated maturation, the upregulation of several costimulatory molecules was identified as predominantly TNF-α mediated, with IFN-γ having little effect [39,43]. In contrast to mice where γδ T cells have been shown to induce DC maturation via CD40L [44], contact-dependent mechanisms have not yet been described in human γδ-DC interactions.

Upon maturation, DCs exhibit a switch in the expression of chemokine receptors, thereby allowing these cells to progress from inflammatory homing iDCs expressing CCR5 to DCs capable of transporting antigens to secondary lymphoid tissues to initiate T cell responses via expression of the lymph node homing receptor CCR7. Indeed, culture of Vγ9/Vδ2 T cells with immature DCs leads to the upregulation of CCR7 and downregulation of CCR5 surface expression, either alone or with the addition of TLR ligands [40–42].

Maturation of DCs is also accompanied by the loss of endocytic and phagocytic capacity. In support, the Vγ9/Vδ2 T cell-mediated maturation of DCs leads to a reduced ability of DC to take up soluble antigen in comparison with immature DCs, exhibiting a similar effect as LPS-triggered maturation [43]. Alongside generally impaired antigen uptake, DCs display improved cross-presentation of antigens upon maturation. NK cells were recently shown to stimulate the cross-presenting capacity of DCs via secretion of IFN-γ and TNF-α [45]. Given that γδ T cells represent a significant source of these two cytokines, it is likely that Vγ9/Vδ2 T cells are able to mediate this process as well.

A number of studies investigated the maturation of DCs pre-infected with HMB-PP producing pathogens that possess the capacity to activate Vγ9/Vδ2 T cells. In this context, DCs infected with *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) undergo maturation by Vγ9/Vδ2 T cells, activated either with zoledronate/phosphoantigens or by the BCG infected DCs themselves [37,46]. Meraviglia et al. [47] reported that DCs, partially matured upon infection with *Mycobacterium tuberculosis*, were able to activate Vγ9/Vδ2 T cells and become fully matured in return. Lastly, Ni et al. [48] described a system whereby DCs infected with *Brucella* exhibited an inhibition of maturation, but upon co-culture with Vγ9/Vδ2 T cells underwent full maturation with regard to upregulation of costimulatory molecules.

Secretion of polarizing cytokines by mature DCs is essential for the skewing of naive $CD4^+$ T cell responses in the lymph nodes. Activated Vγ9/Vδ2 T cells are able to induce the production of IL-12 by mature DCs, either alone or in combination with TLR stimulation [32]. In striking contrast to the differential effect of Vγ9/Vδ2 T cell-derived cytokines on the upregulation of costimulatory molecules, induction of IL-12 is mediated almost exclusively by IFN-γ, and not by TNF-α [39,40,48]. Unlike IL-12, IL-10 is not induced upon Vγ9/Vδ2 T cell mediated DC maturation, and Vγ9/Vδ2 T cell co-culture actually suppresses the LPS-mediated IL-10 expression