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Non-conventional T cells recognise non-peptide antigens and act as bridge between the adaptive and innate immune systems. We aim to understand their antigen-recognition and co-evolution of antigen-receptors and antigen-presenting molecules.

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INTRODUCTION

Non-conventional T cells serve as bridge between adaptive and innate immunity. Their T cell antigen-receptors (TCR) do not bind peptide antigens presented by MHC class I or II but recognise classes of pathogen-derived and stress-induced molecules analogous to pattern recognition receptors. The composition of their TCR is characterised by often eponymous gene rearrangements such as in case of the Vγ9Vδ2 T cells which are defined by expression of a Vγ9-containing chain (Vγ9JPCγ1) paired with Vδ2-containing δ-chains. Vγ9Vδ2 T cells are effectors with anti-microbial, anti-tumour and immunomodulatory activity, which are expanded in many infectious diseases (e.g. malaria). So far they have been found only in humans and higher primates showing a unique reactivity to pyrophosphorylated metabolites called phosphoantigens (PAg). The most potent PAg is (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) which is the immediate precursor of isopentenyl pyrophosphate (IPP) in the non-mevalonate pathway of isoprenoid synthesis found in many eubacteria and in apicomplexa such as Plasmodium spp. HMBPP is ten thousand times more potent than isopentenyl pyrophosphate (IPP), the central compound of all isoprenoid synthesis pathways. Increased IPP levels in host cells also lead to Vγ9Vδ2 T cell-activation. They are found in tumours, especially after administration of aminobisphosphophonates (e.g. zoledronate), and also upon infection.

RESEARCH HIGHLIGHTS

We have completed the functional and phenotypic characterisation of rat and cotton rat iNKT cells as well as studies on the molecular basis of antigen recognition by the iNKT TCR. Studies on the non-conventional MHC class II molecules H2E2 and RT1D2 containing Eb2 (mouse) and Db2 (rat) β-chains with a high capacity to present superantigens from *Yersina pseudotuberculosis* (YPM) have also been completed. We are now concentrating on the analysis of Vγ9Vδ2 T cells and investigating their potential for tumour therapy, the molecular mechanism underlying their activation by PAg, the coevolution of Vγ9Vδ2 TCR with butyrophilin 3 (BTN3) and identification and characterisation of Vγ9Vδ2 T cells in non-primate species.

A key player in Vγ9Vδ2 T cell-activation is the cell surface molecule BTN3A1. Its extracellular domain is remarkably similar to members of the B7 family (e.g CD80/86) and the intracellular B30.2 domain binds PAg. Binding of PAg leads to a conformational change of the entire BTN3A1 molecule, which may then allow binding of the Vγ9Vδ2 TCR to the BTN3A1 expressing (primate) cell. We also have investigated biotinylated PAg, which can be chemically crosslinked after photoactivation to PAg binding sites, as new tool to study PAg action. We have also compared the activation of Vγ9Vδ2 TCR transductants by PAg and the BTN3 specific antibody 20.1 and shown an important contribution of Vγ9Vδ2 TCR CDR3 regions to either response and interference rather than synergism of both modes of activation (Fig. 1 outlines a possible mechanism). Currently, we are analysing radiation hybrids to map gene(s) on human chromosome 6, which in addition to the *BTN3A1* gene control Vγ9Vδ2 T cell activation by PAg, and studying the interplay between BTN3 isoforms and PAg activation. This involves testing combinations of BTN3 isoforms, BTN3-chimeras and mutants expressed in BTN3 knock out cells for PAg-"presentation". Finally, we are continuing our analysis of the co-emergence of Vγ9, Vδ2 and BTN3 genes in placental mammals and their possible coevolution. We have identified the alpaca (*Vicugna pacos*) as the first candidate for a non-primate species to possess functional Vγ9Vδ2 T cells and study its HMBPP response with the help of new monoclonal antibodies that are specific for alpaca gδ TCR and alpaca BTN3. We are also investigating VδTCR and *BTN3* genes of the nine-banded armadillo (*Dasypus novemcinctus*), a natural host and model of infection for *Mycobacterium leprae*. We have found that despite the occurrence of a Vγ9 open reading frame, Vγ9 genes were not present among expressed TCRγ-chain rearrangements and identified the armadillo *BTN3* gene family as non-functional. Thus, the armadillo may serve as a link in the emergence of the butyrophilin 3 (BTN3)/Vγ9Vδ2 system with placental mammals but not as a model for Vγ9Vδ2 T cell research.

FUTURE DIRECTIONS

We will continue our studies on Vγ9Vδ2 T cells to finally harness them for tumour and infection control. This work requires the development of new strategies to overcome the unresponsiveness of Vγ9Vδ2 T cells in tumour patients including the evaluation of BTN3 specific agonistic antibodies as activating agents and comparison of TCR usage of PAg and mAb 20.1 activated T cells. In this context we aim to identify the minimal molecular requirements of BTN3 mediated Vγ9Vδ2 T cell activation and to pursue the identification of chromosome 6 encoded gene(s) that are mandatory for PAg-mediated activation. Identification of such gene(s) will help us to manipulate Vγ9Vδ2 T cells and is a prerequisite for the generation of rodent animal models of functional Vγ9Vδ2 T cells. Finally, we will continue our efforts to identify and characterise Vγ9Vδ2 T cells in non-primate species.

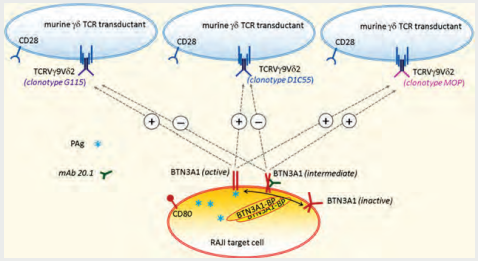

Fig. 1: How do PAg and BTN3 specific antibodies activate via the Vγ9Vδ2⁺ TCR ? At the surface of target cells, BTN3A1 probably exists under different conformations schematically: active, intermediate, and inactive. Intracellular proteins and additional BTN3A1-binding proteins can modulate these conformations. The anti-BTN3A1 mAb 20.1 and PAg may stabilize distinct conformers of BTN3A1, that some clonotypes of TCRVγ9Vδ2 (G115, D1C55) differentiate whereas others (MOP) do not. Murine transductants have a higher differentiation capacity than their human counterpart in 'seeing' the distinct PAg and anti-BTN3A1 stimuli. Picture of an editorial on Starick et al (2017). Source: Franchini DM, Michelas M, Lanvin O, Poupot M, Fournié JJ. (2017) BTN3A1-antibodies and phosphoantigens: TCRVγ9Vδ2 "see" the difference Eur. J. Immunol. 47(6) Pages 954–957 Copyright Wiley-VCH Verlag GmbH & Co. KGaA.

SELECTED PUBLICATIONS

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