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Non-conventional T cells recognize 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 recognize pathogen-specific antigens but classes of pathogen-derived and stress-induced molecules and thus act as pattern recognition receptors. These features and characteristic TCR rearrangements differ from MHC-restricted TCR. Functionally, our research focuses on two types of non-conventional or innate T cells. Both are defined by their eponymous TCR: The iNKT cells and the Vγ9Vδ2 T cells.

i(nvariant)NKT cells carry TCR with an invariant α chain defined by a Vα14Jα18 re-arrangement. They are effectors with anti-microbial, anti-tumor and immunomodulatory activity. Their major antigens are glycolipids that associate with non-polymorphic MHC I-like CD1d cell surface molecules. Their strongest antigens are bacterial sphingolipids with α-anomeric linked carbohydrates (e.g. α-Galactosyl ceramide: αGalCer). The binding of such ligands to CD1d oligomers are commonly used to directly identify iNKT.

Vδ2 T cells are expanded in many infectious diseases and possess anti-tumor activity. So far they have been found only in humans and higher primates. Their TCR contain characteristic rearrangements of the γ-chain (Vγ9JP), which is paired with Vδ2 containing δ-chains. They recognize pyrophosphorylated metabolites of isoprenoid synthesis, 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 found in many eubacteria and in apicomplexa such as *Plasmodium spp*. HMBPP is ten thousand times more potent than IPP, the central compound of either pathway of isoprenoid synthesis. Increased IPP levels in host cells also lead to Vγ9Vδ2 T cell-activation and are found in some tumours, after administration of aminobisphosphophonates (e.g. zoledronate), and also upon infection.

RESEARCH HIGHLIGHTS

Functional and phenotypic characterisation of rat iNKT cells has revealed remarkable similarities to human iNKT cells. We have recently focused on the molecular parameters that control antigen recognition by rat iNKT cells by comparing iNKT TCR of *in vitro* expanded iNKT cells and mutagenesis of rat, mouse and human iNKT cell receptors. This analysis has identified natural occurring variations at position 93, which flanks the CDR3 of the α-chain and at position 68 in the HV4α region as potent modulators of avidity for CD1d-αGalCer complexes. Both effects result from altering the orientation and rigidity of the CD1d-aGalCer-binding CDR1α and CDR3α (Fig. 1). Position 93 was also found to strongly affect cross-species reactivity of iNKT TCR. We have also performed the first systematic comparison of parameters controlling the *in vitro* loading of CD1d oligomers. Such oligomers are mandatory to directly identify the iNKT cells. Analysis of CD1d oligomers loaded with different antigens revealed unexpected species- and cell-specific effects of the CD1d-oligomers and the detergents used for their *in vitro* loading upon iNKT TCR binding. Finally, functional iNKT cells were identified in cotton rats and their TCR and CD1d characterized. Cotton rats are of especial interest

since they serve as a model organism for human viral diseases such as measles and respiratory syncytial virus.

After identification of butyrophilin 3 A1 (BTN3A1) as a mandatory component for Vγ9Vδ2 TCR-mediated activation we have focused on defining the minimal molecular and genetic requirements for this type of activation. New reporter cells have enabled us to show that in addition to *BTN3A1* at least one other human chromosome 6 encoded gene is required for Vγ9Vδ2 T cell activation by PAg (Fig. 2), while activation using a BTN3-specific agonistic antibody displays no such requirement. In addition, we have found that in contrast to PAg-stimulation, activation by this antibody discriminates between TCR idiotypes and interferes with PAg mediated activation.

Finally, we have continued our analysis of the co-emergence of Vγ9, Vδ2 and BTN3 genes in placental mammals and their possible coevolution. Identification of typical Vγ9Vδ2 TCR rearrangements in alpaca (*Vicugna pacos*) identified this camelid as first candidate for a non-primate species to possess functional Vγ9Vδ2 T cells. We now aim to directly identify these cells in alpaca and have started an analysis of γδTCR and BTN3 genes of the nine-banded armadillo (*Dasypus novemcinctus*), a natural host and model of infection for *Mycobacterium leprae*.

FUTURE DIRECTIONS

We will further collaborate with Stefan Niewiesk, Ohio State University, Columbus on cotton rat iNKT cells and aim to test their role in virus infection and vaccination. We will also continue with our work on the non-conventional MHC class II molecules Eb2 (mouse) and Db2 (rat). A future focus will be on Vγ9Vδ2 T cells to finally harness these cells for tumor and infection control. This will also involve the search for Vγ9Vδ2 T cells in non-primate species as well as for chromosome 6 encoded gene(s) that are mandatory for PAg-mediated activation. Identification of such gene(s) will help to manipulate Vγ9Vδ2 T cells and is a prerequisite for generation of rodent animal models of functional Vγ9Vδ2 T cells.

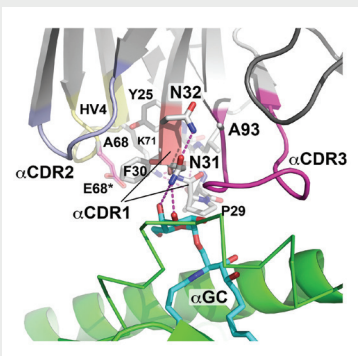

Fig. 1: HV4 modulates iNKT TCR binding to CD1d-antigen complexes. Homology model of the interface between the galactosylceramide-loaded rat CD1d and rat iNKT TCR highlighting the recognition and interaction of the TCR with the ceramide moiety (C-atoms colored in cyan). Alanine 68 in the HV4 element stabilizes CDR1ć by hydrophobic interactions with Tyr25 and Phe30 located in CDR1α. Mutation of Ala68 to glutamate (indicated as transparent sticks with the C-atoms colored in magenta) leads to a steric clash of the introduced glutamate residue with Phe30 thereby altering the conformation of CDR1α. Modified from (Paletta et al 2015)

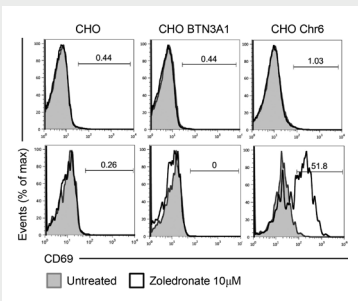

Fig. 2: Zoledronate pulsed CHO cell derivatives expressing BTN3A1 or bearing Chr6 differ in their capacity to activate primary humanVγ9Vδ2 T cells. FACS staining of Vδ2+ T cells after over-night co-culture of human PBMC with untreated or zoledronate-pulsed stimulator cells. Numbers indicate proportion Vγ9Vδ2 T cells positive for the activation marker CD69. Modified from (Riano et al 2014).

SELECTED PUBLICATIONS

Monzón-Casanova E, Rudolf R, Starick L, Müller I, Söllner C, Müller N, Westphal N, Miyoshi-Akiyama T, Uchiyama T, Berberich I, Walter L, **Herrmann T** (2016) *The Forgotten: Identification and Functional Characterization of MHC Class II Molecules H2-Eb2 and RT1-Db2.* **Journal of Immunology** 196:988-99

Fichtner AS, Paletta D, Starick L, Schumann RF, Niewiesk S, **Herrmann T** (2015) *Function and expression of CD1d and invariant natural killer T-cell receptor in the cotton rat (Sigmodon hispidus).* **Immunology** 146:618-629

Paletta D, Fichtner AS, Hahn AM, Starick L, Beyersdorf N, Monzon-Casanova E, Mueller TD, **Herrmann T** (2015) *The hypervariable region 4 (HV4) and position 93 of the alpha chain modulate CD1d-glycolipid binding of iNKT TCRs.* **European Journal of Immunology** 45:2122-2133

Karunakaran MM, Gobel TW, Starick L, Walter L, **Herrmann T** (2014) *Vgamma9 and Vdelta2 T cell antigen receptor genes and butyrophilin 3 (BTN3) emerged with placental mammals and are concomitantly preserved in selected species like alpaca (Vicugna pacos).* **Immunogenetics** 66:243-254

Karunakaran MM, **Herrmann T** (2014) *The Vgamma9Vdelta2 T Cell Antigen Receptor and Butyrophilin-3 A1: Models of Interaction, the Possibility of Co-Evolution, and the Case of Dendritic Epidermal T Cells.* **Frontiers in Immunology** 5:648

Riano F, Karunakaran MM, Starick L, Li J, Scholz CJ, Kunzmann V, Olive D, Amslinger S, **Herrmann T** (2014) *Vgamma9Vdelta2 TCR-activation by phosphorylated antigens requires butyrophilin 3 A1 (BTN3A1) and additional genes on human chromosome 6.* **European Journal of Immunology** 44:2571-2576