



[haematologica reports]
2006;2(3):4-5

WESCH D
BEETZ S
KABELITZ D

Institute of Immunology,
University Hospital of
Schleswig-Holstein, Campus
Kiel, Germany

TLR3 ligand induces a direct costimulatory effect on phosphoantigen stimulated human $\gamma\delta$ T cells, but not on aminobisphosphonate-stimulated human $\gamma\delta$ T cells

$\gamma\delta$ T cells expressing V γ 9 paired with V δ 2 account for up to 95% of $\gamma\delta$ T cells in the peripheral blood.¹ These cells recognize phosphorylated, nonproteinaceous intermediates of the non-mevalonate pathway of the bacterial isoprenoid biosynthesis pathway (*phosphoantigens*).² Additionally, the same subset of V γ 9V δ 2 T cells has been shown to be activated by therapeutically used aminobisphosphonates.³ Moreover, V γ 9V δ 2 T cells express MHC class I- or MICA/B (MHC class I related molecules)-specific natural killer-inhibitory receptors such as NKG2A and natural killer-activating receptors such as NKG2D, which can modulate reactivity towards e.g. phosphoantigens.^{4,5} $\gamma\delta$ T cells activate innate immune cells, facilitate adaptive immune responses by $\alpha\beta$ T cells, and play a not precisely defined role during antiviral immunity.⁶⁻⁸

Toll-like receptors (TLR) represent pattern recognition receptors that are involved in the regulation of innate immune responses to infection and the modulation of adaptive immune responses. Thus far, TLR3 is considered to be expressed mainly in immature myeloid dendritic cells (DC), natural killer (NK) cells, fibroblasts, and intestinal epithelial cells. We observed TLR3 mRNA expression and intracellular TLR3 protein in human $\gamma\delta$ and $\alpha\beta$ T lymphocytes. We did not detect TLR3 on the cell surface of resting cells, but it was upregulated after short-term stimulation with phosphoantigens, and even more with phosphoantigens in the presence of TLR3 ligand polyinosinic-polycytidilic acid (poly(I:C)) (Figure 1). TLR3 binds double-stranded viral RNA and the synthetic analog poly(I:C).⁹ We used poly(I:C) as a surrogate TLR3 ligand to investigate functional consequences of TLR3 stimulation in T cells. We observed that poly(I:C) did not exert any effect by itself but drastically increased the T cell receptor (TCR)-stimulated IFN- γ production of freshly isolated, highly purified $\gamma\delta$ T-lymphocytes in the absence of other TLR3-expressing cells (Figure 2). Moreover, anti-TLR3 antibodies

partially inhibited IFN- γ production, presumably by antagonizing TLR3 on the cell surface. In these assays, anti-TCR $\gamma\delta$ mAb or phosphoantigens such as bromohydrin pyrophosphate (BrHPP) were used as TCR stimuli. In contrast, poly(I:C) did not enhance IFN- γ production of aminobisphosphonate-stimulated V γ 9V δ 2 T cells in the absence of APC. This fits well with the observation by others that $\gamma\delta$ T cells do not directly recognize aminobisphosphonates but rather respond to ligands produced by e.g. monocytes following treatment with aminobisphosphonates.¹⁰

In line with the studies of Kunzmann and coworkers,¹¹ we observed that phosphoantigen-activated $\gamma\delta$ T cells cultured in the presence of APC are also stimulated indirectly via TLR3-mediated activation of myeloid DC. However, we observed that poly(I:C) actually inhibits the aminobisphosphonate-stimulated $\gamma\delta$ T cell expansion within unfractionated PBMC (thus in the presence of APC). Our preliminary results suggest that this is due to a delayed expression of costimulatory molecules (e.g. CD80, CD86 or NKG2D-ligands such as ULBP-2, ULBP-3) on APC after aminobisphosphonate stimulation compared to the phosphoantigen stimulation of unfractionated PBMC. Further investigations are required to elucidate the cellular and molecular basis of the differential effect of TLR3 ligand poly(I:C) on the activation of $\gamma\delta$ T cells by microbial phosphoantigens or aminobisphosphonates.

Importantly, poly(I:C) did not costimulate IFN γ production in $\alpha\beta$ T cells. These results indicate that TLR3 signalling is differentially regulated in T cell receptor-stimulated $\gamma\delta$ and $\alpha\beta$ T cells. Taken together, the data support the hypothesis that integrated signals from TLR3 and TCR together induce an early antiviral effector function in $\gamma\delta$ T cells, whereas TLR3-expressing $\alpha\beta$ T cells need further costimulatory signals (e.g. *via* CD80/CD86) provided by activated APC.

This study was supported by DFG SPP 1110 Innate Immunity (Ka 502/8-2).

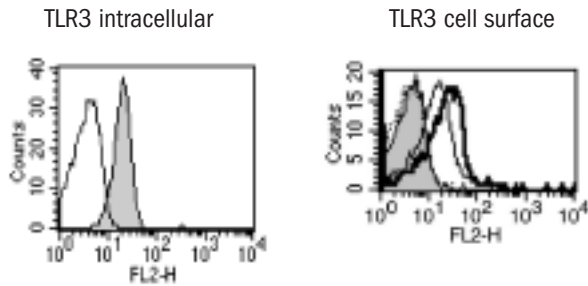


Figure 1. Intracellular and surface expression of TLR3 in $\gamma\delta$ T cells within PBMC. Expression of TLR3 in $\gamma\delta$ T cells was determined intracellularly (left figure) and on the cell surface (right figure) by PE-labeled anti-TLR3 mAb TLR3.7 (Bioscience, Vienna, Austria). Left figure: isotype, thin line; intracellular TLR3, grey histogram; right figure: isotype, dotted line; TLR3-medium, grey histogram; TLR3 after TCR stimulation, thin line; TLR3 after TCR and poly(I:C) stimulation, bold line.

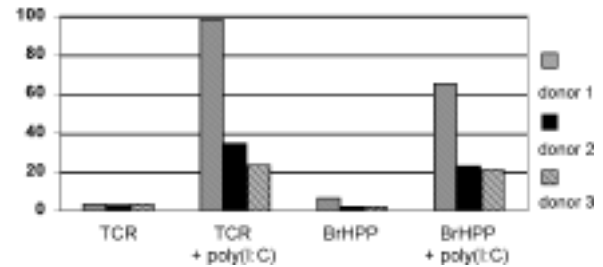


Figure 2. TLR3 ligand poly(I:C) increases IFN- γ production to TCR cross-linking or BrHPP. Highly purified, positively selected $\gamma\delta$ T cells were stimulated through the TCR via immobilized rabbit anti-mouse Ab (ram) or BrHPP with or without poly(I:C). IFN- γ was determined in the supernatant by ELIS. The results of three experiments with different blood donors are shown.

References

- Wesch D, Hinz T, Kabelitz D. Analysis of the TCR V γ repertoire in healthy donors and HIV-1-infected individuals. *Int Immunol* 1998;10:1067-75.
- Altincicek B, J Moll, N Campos, G. Foerster, E Beck, JF Hoeffler, et al. Human $\gamma\delta$ T cells are activated by intermediates of the 2-C-methyl-D-erythritol 4-phosphate pathway of isoprenoid biosynthesis. *J Immunol* 2001;166:3655-8.
- Kunzmann V, Bauer E, Feurle J, Weissinger F, Tony HP, Wilhelm M. Stimulation of $\gamma\delta$ T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. *Blood* 2000;96:384-92.
- Halary F, Peyrat MA, Champagne E, Lopez-Botet M, Moretta A, Moretta L, et al. Control of self-reactive cytotoxic T lymphocytes expressing $\gamma\delta$ T cell receptors by natural killer inhibitory receptors. *Eur Immunol* 1997;27:2812-21.
- Das H, Groh V, Kuijl C, Sugita M, Morita CT, Spies T, et al. MICA engagement by human V γ 2V δ 2 T cells enhances their antigen-dependent effector function. *Immunity* 2001;15:83-93.
- Wesch D, Marischen L, Kabelitz D. Regulation of cytokine production by $\gamma\delta$ T cells. *Curr. Med. Chem.- Anti-Inflammatory & Anti-Allergy Agents* 2005;4:153-60.
- Kabelitz D, Wesch D. Role of $\gamma\delta$ T-lymphocytes in HIV infection. *Eur J Med Res* 2001;6:169-74.
- Dechanet J, Merville P, Lim A, Retiere C, Pitard V, Lafarge X, et al. Implication of $\gamma\delta$ T cells in the human immune response to cytomegalovirus. *J Clin Invest* 1999;103:1437-49.
- Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* 2001;413:732-8.
- Miyagawa F, Tanaka Y, Yamashita S, Minato N. Essential requirement of antigen presentation by monocyte lineage cells for the activation of primary human $\gamma\delta$ T cells by aminobisphosphonate antigen. *J Immunol* 2001;166:5508-14.
- Kunzmann V, Kretzschmar E, Hermann T, Wilhelm M. Polyinosinic-polycytidylic acid-mediated stimulation of human $\gamma\delta$ T cells via CD11c+ dendritic cell-derived type I interferons. *Immunology* 2004;112:369-77.