TLR3 ligand induces a direct costimulatory effect on phosphorylated stimulated human γδ T cells, but not on aminobisphosphonate-stimulated human γδ T cells

δ T cells expressing Vγ9 paired with Vδ2 account for up to 95% of γδ T cells in the peripheral blood. These cells recognize phosphorylated, nonproteinaceous intermediates of the non-mevalonate pathway of the bacterial isoprenoid biosynthesis pathway (phosphoantigens). Additionally, the same subset of Vγ9Vδ2 T cells has been shown to be activated by therapeutically used aminobisphosphonates. Moreover, Vγ9Vδ2 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. γδ T cells activate innate immune cells, facilitate adaptive immune responses by αβ T cells, and play a not precisely defined role during antiviral immunity.

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 γδ and αβ 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 absence of TLR3 ligand polyinosinic-polycytidylic acid (poly[I:C]) (Figure 1). TLR3 binds double-stranded viral RNA and the synthetic analog poly[I:C]. 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 γδ 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-TCRγδ 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 Vγ9Vδ2 T cells in the absence of APC. This fits well with the observation by others that γδ T cells do not directly recognize aminobisphosphonates but rather respond to ligands produced by e.g. monocytes following treatment with aminobisphosphonates.

In line with the studies of Kunzmann and coworkers, we observed that phosphoantigen-activated γδ 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 γδ 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 γδ T cells by microbial phosphoantigens or aminobisphosphonates.

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

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References