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Potentiation of antigen-stimulated Vγ9Vδ2 T cell cytokine production by immature dendritic cells and reciprocal effect on dendritic cell maturation

Maillet S
Devilder MC
Bouyge-Moreau I
Bonneville M
Scotet E

INSERM, Nantes, France

Correspondence:
E. Scotet, INSERM, 9
Quai Moncousu, Nantes, France.
Phone: +33 240084748
Fax: +33 240356697
E-mail:
emmanuel.scotet@nantes.inserm.fr

Vγ9Vδ2 cells, a major peripheral blood γδ subset representing up to 5 % of the PBL pool in adults 1, have been referred to as innate-like T cells owing to : (i) their ability to recognize conserved antigens (Ag) expressed by a broad range of infected, stressed and/or transformed cells, (ii) their preactivated status resulting from an early (post natal) acquisition of memory markers, (iii) their high frequency in particular tissue locations.[1-3] Two kinds of Ag selectively stimulating Vγ9Vδ2 T cells have been identified: (i) small phosphorylated compounds referred to as phosphoantigen (phosphoAg)[4,5,] which are produced through the isoprenoid biosynthetic pathway of mammalian (such as isopentenyl pyrophosphate (IPP)[6] and non-mammalian cells (such as 4-hydroxy-3-dimethylallyl pyrophosphate) 7 and (ii) complexes comprising ATP synthase subunits, which are found on the surface of some tumor cells.[8] Pharmacological agents acting upstream (like statins) or downstream (like aminobisphosphonates (ABP)) IPP biosynthesis have recently been shown to respectively, inhibit or enhance target cell lysis by Vγ9Vδ2 T cells.[9,10]

Whether or not optimal activation of Vγ9Vδ2 cells requires additional factors provided eg by professional APC remains unclear. Owing to their memory status, these γδ cells are classically considered as dendritic cell (DC)-independent, and accordingly can efficiently expand *in vitro* in response to phosphoantigen and IL-2 in the absence of any adherent cells. However optimal activation of Vγ9Vδ2 T cells by ABP clearly requires the presence of myelomonocytic or transformed cells.[11,12] Several indirect observations suggest that DC can enhance *in vivo* activation and proliferation of other innate-like[13,14] or conventional memory T cell subsets.[15].

Due their potent lytic and bactericidal activities, Vγ9Vδ2 T cells can directly contribute to elimination of infected or tumor cells.[16-19] They may also enhance NK and conventional T cell responses through release of proinflammatory cytokines (such as IFN-γ and TNF-α) and DC priming.[20] Several studies have reported *in vitro* maturation of DC upon coculture with phosphoAg- or ABP -stimulated Vγ9Vδ2 cells.[12,21] However in neither case the outcome of Ag-expressing DC have been precisely studied in these *in vitro* systems. In particular the possibility that DC maturation could exclusively result from a *bystander* process,[22] which would involve DC not interacting with Vγ9Vδ2 T cells but exposed to inflammatory factors released by the latter cells upon interaction and subsequent killing of Ag-expressing DC, could not be ruled out. Besides such experiments have not been performed in the context of infection by Vγ9Vδ2-stimulating pathogens (mycobacteria, ...).

iDC potentiate cytokine responses of phosphoantigen-activated Vγ9Vδ2 T cells

We are studying the APC requirements for optimal *in vitro* activation of Vγ9Vδ2 T cells and the consequences of Vγ9Vδ2 T cell activation on the phenotypic and functional status of Vγ9Vδ2 Ag-expressing APC. Monocyte-derived DC (IL-4/GM-CSF) are cocultured for few hours together with Vγ9Vδ2 T cells (established clones or ex vivo polyclonal populations) in the presence of grading doses of synthethic phosphoAg (BrHPP). Intracellular cytokine levels in T cells are subsequently analyzed by flow cytometry. We show that immature DC (iDC), and to a lesser extent mature DC (mDC), potentiate Th1 and Th2 cytokine (TNF-α, IFN-γ and IL-4), but not cytolytic or proliferative responses, of established Vγ9Vδ2 T cell clones and ex vivo memory Vγ9Vδ2 PBL stimulated by synthetic agonists (Figure 1). Similar results are obtained using when iDC and mDC are pre-incubated with ABP (pamidronate). This suggests the existence of specific costimulatory sig-

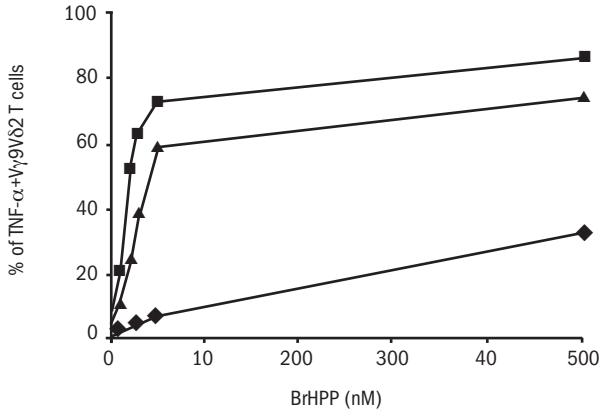

Figure 1. Immature DC potentiate TNF-α production by phosphoantigen-activated human Vγ9Vδ2 T cells. Vγ9Vδ2 T cells (clone G42) were activated for 5 hrs by grading doses of BrHPP in the presence of either iDC (■), mDC (▲) or irrelevant T cells (◆) at a 1/1 T/DC ratio. Intracellular TNF-α was detected in Vδ2⁺ cells by flow cytometry.

nals provided by iDC and/or inhibitory signals provided by mDC which are under investigation.

Activated-Vγ9Vδ2 T cells induce full maturation of iDC

The ability of iDC to potentiate Vγ9Vδ2 production of cytokines required for their own maturation suggested that Vγ9Vδ2 T cells, despite their strong lytic activity, could promote efficient iDC licensing without killing at suboptimal Ag doses. Accordingly Vγ9Vδ2 cells induced accelerated maturation of Ag-expressing iDC (pamidronate-treated iDC) but not *bystander* DC, even within mixed cell populations comprising both Ag-expressing and nonexpressing iDC. Furthermore Vγ9Vδ2 cells induced full differentiation into IL-12-producing cells of iDC infected by Vγ9Vδ2-stimulating mycobacteria that were otherwise unable to induce complete DC maturation. The ability of iDC to efficiently trigger Vγ9Vδ2 production of cytokines required for their own maturation would suggest direct implication of this T cell subset in DC priming. This is in line with previous reports demonstrating an efficient *in vitro* iDC maturation mediated by Ag-stimulated Vγ9Vδ2 CTL. [12,21,23]

Conclusions

In conclusion, we demonstrate an unexpected role played by iDC in activation of a major γδ T cell subset with memory features. The fact that iDC selectively potentiated cytokine but not cytolytic or proliferative responses of Ag-stimulated γδ T cells could be related to the previously demonstrated ability of Vγ9Vδ2 T cells to induce iDC maturation and accordingly, such functional property is presently documented in the context of a natural infection.

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