A subset of Vγ9Vδ2 T cells help B cells for antibody production

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Vγ9Vδ2 T cells are a minor T cell population with a unique pattern of antigen recognition. They are heterogeneous and comprise distinct populations that can be distinguished on the basis of surface markers expression, effector functions and migratory properties. Based on their effector properties, Vγ9Vδ2 T lymphocytes are supposed to play an important role in cellular immune responses against intracellular microorganisms and tumors. However, whether or not Vγ9Vδ2 T lymphocytes also participate in antibody-mediated immune responses remains unclear. Earlier studies in mice and humans have shown that γδ T cells help B cells for antibody production, but the subset responsible for this activity was not identified.

We show here that expression of CXCR5 defines an unique subset of peripheral blood T cells Vγ9Vδ2 cells which upon antigen stimulation express the costimulatory molecules ICOS and CD40L, secrete IL-4 and IL-10 and provide potent B-cell help for antibody production in vitro.

Materials and Methods

FACS staining and sorting

PBMC were isolated from heparinized blood or inflammed tonsils by Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden). The following conjugated antibodies were used in different combinations: anti-Vδ2 (Coulter, Miami, FL), anti-Vγ9 (Coulter), anti-CD27 (BD Pharmingen, San Diego, CA), anti-CD45RA (Coulter), anti-CD45RO (Coulter), anti-CD3 (Sigma, St. Louis, MO), anti-CD25 (BD Pharmingen), anti-CD62L (BD Pharmingen), anti-CCR7 (a gift of Dr. M. Lipp, Max-Delbruch-Center for Molecular Medicine, Berlin, Germany), anti-HLA DR monomorphic (a gift of Prof. V. Horejsi, Institute of Molecular Genetics, Academy of Science of the Czech Republic, Prague), anti-CCR5 (BD Pharmingen), anti-CD40L (BD Pharmingen), anti-ICOS (a generous gift of Dr. R.A. Kroccek), anti-CD40L (BD Pharmingen) and anti-CXCR5 (R&D Systems, Minneapolis, MN, USA).

Data were acquired on a FACSCalibur (BD Biosciences) and analysed using CellQuest software (BD Immunocytometry Systems, San José, CA). Cell sorting was performed on a FACS Vantage (BD Biosciences).

Cytokine production

The medium used throughout was complete RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco), 2 mM L-glutamine, 20 nM Hepes and 100 U/ml penicillin/streptomycin. Sorted Vδ2 cell subsets were labelled with CFSE as above described and cultured at 37°C in the presence of 5% CO2, at 10^6 cells in 96-well flat-bottomed plates (0.2 mL/well), with different concentrations of bromohydrinpyrophosphate (BrHPP, a gift of Dr. J.J. Fournié) in the presence of irradiated (5,000 Rads from a caesium source) allogeneic dendritic cells. IFN-γ, TNF-α, IL-4 and IL-10 levels in the 24-hr culture supernatants were assessed by two-mAbs sandwich ELISA.
assay following manufacturer's recommendations (R&D Systems).

The chemotactic ability of CXCR5+Vγ9Vδ2 cells was assayed using a double-chamber system with 3-μm pores (Transwell Costar, Cambridge, MA), according to literature. Briefly, 10^5 sorted CXCR5+Vγ9Vδ2 cells, were added to the upper chamber and CXCL13 (recombinant human CXCL13, BCA-1, R&D Systems, Minneapolis, MN, 3 μM final concentration) to the lower chamber and incubated at 37°C for 2 hr in a 5% CO₂ humidified incubator. Assays were performed in triplicate. Afterwards, the membrane was removed, washed on the upper side with PBS, fixed, and stained. Migrated cells were counted microscopically at 1,000 magnification in five randomly selected fields per well. Percentage migration was calculated by measuring the counts recovered from the lower chamber and comparing them to the total input counts; results represent the mean ± SD of three independent experiments.

**Antibody production in vitro**

Vγ9Vδ2 T cell help in antibody production was studied as follows. Different subsets of peripheral blood Vγ9Vδ2 T cells were sorted by FACS and co-cultured with sorted tonsillar B cells in 96-well plates at 10^5 cells/well each of T and B cells in the presence or absence of BrHPP for 10 days. IgM, IgG, and IgA levels were assayed using a double-chamber system with 3-μm pores (Transwell Costar, Cambridge, MA), according to manufacturer's recommendations (R&D Systems).

**Results and Discussion**

Expression of the chemokine receptor CXCR5 defines a population of CD4 T helper cells which localizes to B cell follicles and support the production of immunoglobulins.

CXCR5 expression was studied on peripheral blood and tonsillar Vγ9Vδ2 T cells. Within PBMC, about 15% of total Vγ9Vδ2 T cells are CXCR5+, while CXCR5+Vγ9Vδ2 T cells are highly enriched in inflamed tonsils where they account for about half the size of the Vγ9Vδ2 T cell population. As shown in Table 1, the vast majority of CXCR5+Vγ9Vδ2 cells does not express CD45RA, but express CD27, CD45RO, CCR7 and CD62L, thus identifying them as a subpopulation of T central memory (Tcm) Vγ9Vδ2 cells. Peripheral blood CXCR5+Vγ9Vδ2 T cells do not express the activation markers CD25 and HLA-DR and also lack expression of the costimulatory molecules CD40L and ICOS. In tonsils, CXCR5+Vγ9Vδ2 T cells had a Tcm phenotype like their peripheral blood counterpart, but most of them express several activation markers and costimulatory molecules (CD25, HLA-DR, CD40L and ICOS), suggesting that they are engaged in immune responses occurring in tonsils. Moreover, expression of CCR7, which causes homing to the T cell areas of secondary lymphoid tissues is found on the vast majority of peripheral blood CXCR5+Vγ9Vδ2 T cells but is markedly reduced on tonsillar CXCR5+Vγ9Vδ2 T cells, suggesting the possibility of a ligand-induced CCR7 downregulation. Similarly, CD62L expression is reduced on tonsillar CXCR5+Vγ9Vδ2 T cells, implying that the majority of tonsillar CXCR5+Vγ9Vδ2 T cells have recently immigrated from circulation. The chemokine receptors CXCR3 and CCR5 were weakly expressed on tonsillar CXCR5+Vγ9Vδ2 T cells, but were no detected on peripheral blood CXCR5+Vγ9Vδ2 T cells (Table 1). This finding is in agreement with our previous results demonstrating that CXCR3 and CCR5 are expressed by TEM and TEMRA, but not by TCM Vγ9Vδ2 T cells.

Freshly isolated tonsillar CXCR5+Vγ9Vδ2 T cells migrated readily in response to CXCL13/BCA-1 (Figure 2a). Responsiveness to BCA-1 was enhanced when the cells were cultured in vitro with BrHPP, but by day 3 of culture migration in response to BCA-1 consistently decreased; this effect was paralleled by CXCR5 receptor expression, which uniformly decreased upon in vitro culture with BrHPP (Figure 2a). Of note, migration of tonsillar CXCR5+Vγ9Vδ2 T cells in response to CXCL13/BCA-1 was consistently inhibited by anti-CXCR5 mAb (Figure 1b).

We then studied the pattern of cytokine production in CXCR5+ and CXCR5+Vγ9Vδ2 Tcm and Vγ9Vδ2 Tem cells, after a 24 hrs stimulation period with BrHPP and irradiated dendritic cells in vitro. As shown in Figure 2a, CXCR5+Vγ9Vδ2 Tem produced very few amounts of cytokines, whereas Vγ9Vδ2 Tem cells produced significant amounts of IFN-γ and TNF-α, but neither IL-4 nor IL-10, thus confirming our previous results (1). However, antigen-stimulated CXCR5+Vγ9Vδ2 Tem cells had a different cytokine profile as they produced IL-2, IL-4

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<tr>
<th>Table 1. Surface markers expression on CXCR5+Vγ9Vδ2 T cells in peripheral blood and inflamed tonsils.</th>
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<tr>
<td><strong>Peripheral blood</strong> (n = 15)</td>
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<tr>
<td><strong>Tonsils</strong> (n = 6)</td>
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<tr>
<td>CD45RA (n = 15)</td>
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<td>CD45RO (n = 15)</td>
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<td>CD25 (n = 15)</td>
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<td>CXCR3 (n = 15)</td>
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Peripheral blood and tonsil mononuclear cells were stained with mAbs to Vγ9Vδ2, CXCR5 and other cell surface markers, and were analysed by FACS. Values indicate the percentage±SE of CXCR5+Vγ9Vδ2 cells expressing the indicated cell surface markers. Values in brackets indicate Mean Fluorescence Intensity mean value.
and, to a lower extent, IL-10, but neither IFN-γ nor TNF-α.

As CXCR5+ Vy9Vδ2 T cells express costimulatory molecules, produce IL-4 and IL-10 and localize to B cell follicles, we tested whether or not these cells were able to support B cells to secrete immunoglobulins. Peripheral blood derived CXCR5+ and CXCR5- Vy9Vδ2 Tcm and Vy9Vδ2 Tdm cells were sorted and cultured with CD19+ B cells isolated from the tonsil of the same donor, in the presence or absence of BrHPP. Figure 2b shows one
typical experiment out of five. B cells produced comparable very low amounts of IgA, IgG and IgM when cultured for 10 days without \(\gamma\delta T\) cells or with the CXCR5\(^+\) fractions (i.e. CXCR5\(^+\) \(\gamma\delta T_{CM}\) and \(\gamma\delta T_{EM}\) cells). In contrast, co-culture of B cells with CXCR5\(^-\) \(\gamma\delta T_{CM}\) and BrHPP resulted in an 18-fold increase in the production of IgG, 8-fold increase in the production of IgA and 7-fold increase in the production of IgM.

In conclusion, data reported in this paper allow us to identify a unique subset of peripheral blood \(T_{CM}\) \(\gamma\delta T\) cells defined by expression of CXCR5, which express the costimulatory molecules ICOS and CD40L, secrete IL-2, IL-4 and IL-10, localize to B cell follicles and provide potent B-cell help for antibody production \textit{in vitro}. Thus, CXCR5\(^-\) \(\gamma\delta T_{CM}\) cells may influence humoral immune responses early during microbial infections, before full development of acquired responses mediated by CD4 T cells, which depends on a series of time-consuming steps, including Ag uptake and processing by tissue DCs, their relocation to draining lymph nodes, and T-cell priming and effector cell development.

### References