The Journal of Immunology

RESEARCH ARTICLE | OCTOBER 15 2006

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J Immunol (2006) 177 (8): 5290–5295.
https://doi.org/10.4049/jimmunol.177.8.5290

CXCR5 Identifies a Subset of Vγ9Vδ2 T Cells which Secrete IL-4 and IL-10 and Help B Cells for Antibody Production[1]

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Vγ9Vδ2 T lymphocytes recognize nonpeptidic Ags and mount effector functions in cellular immune responses against microorganisms and tumors, but little is known about their role in Ab-mediated immune responses. We show here that expression of CXCR5 identifies a unique subset of Vγ9Vδ2 T cells which express the costimulatory molecules ICOS and CD40L, secrete IL-2, IL-4, and IL-10 and help B cells for Ab production. These properties portray CXCR5$^+$Vγ9Vδ2 T cells as a distinct memory T cell subset with B cell helper function. *The Journal of Immunology,* **2006, 177: 5290–5295.**

A minor T cell population, γδ T cells have a unique pattern of Ag recognition. In humans, the vast majority of circulating γδ T cells express a TCR comprised of Vγ9 and Vδ2 chains which directly recognize nonpeptide ligands without presentation by MHC molecules (1). The nonpeptide ligands, referred to as phosphoantigens, comprise isoprenoid pathway metabolites derived from mycobacteria (2–5) and tumor cells (6). Accordingly, pharmacological agents promoting accumulation of such metabolites, such as aminobisphosphonates (7), sensitize cells to Vγ9Vδ2 T cell recognition. Moreover, it has been recently found that ATPase expressed on tumor cell surface promotes tumor recognition by Vγ9Vδ2 T cells (8).

Vγ9Vδ2 T lymphocytes are heterogeneous and comprise distinct populations that can be distinguished on the basis of surface markers expression, effector functions, and migratory properties: naive (CD45RA$^+$CD27$^+$) and central memory (T_{CM}[3]; CD45RA$^-$CD27$^+$) cells home to secondary lymphoid organs and lack immediate effector functions, while effector memory (T_{EM}; CD45RA$^-$CD27$^-$) and terminally differentiated (CD45RA$^+$CD27$^-$) cells home to sites of inflammation where they display immediate effector functions such as cytokine production and cytotoxicity, respectively (9). Based on their effector properties, Vγ9Vδ2 T lymphocytes are supposed to play an important role in cellular immune responses against intracellular microorganisms and tumors (10). However, whether Vγ9Vδ2 T lymphocytes also participate in Ab-mediated immune responses remains unclear. Earlier pioneering studies in αβ T cell-deficient mice demonstrated a nonredundant role for γδ T cells in the generation of antimicrobial Abs (11, 12) and autoantibodies (13–15), but as γδ T cell-deficient mice did not show marked defects in IgM and IgG production, γδ T cells may have a modulatory, rather than a primary function in the control of humoral immunity. Ab production was also increased in in vitro cultures of human γδ T cells with B cells (16, 17), but the amount of secreted Ab was low and the mechanisms underlying the observed B cell help were not examined. A more recent study (18) has shown that human γδ T cells are found in the follicles of secondary lymphoid organs, express costimulatory molecules after TCR triggering, and provide B cell help in vitro, but the intrafollicular γδ T cell subset responsible for such an activity was not identified.

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

Materials and Methods

Subjects

PBMC were obtained from the heparinized blood of 15 healthy volunteers (9 males, 6 females, age range 20–23 years). PBMC and tonsils were obtained from seven individuals undergoing tonsillectomy (four males, three females, age range 9–14 years). All individuals gave informed consent to participate to this study.

FACS staining and sorting

PBMC were isolated from heparinized blood or tonsils by Ficoll-Hypaque (Pharmacia Biotech). The following conjugated Abs were used in different combinations: anti-Vδ2 (Coulter), anti-Vγ9 (Coulter), anti-CD27 (BD Pharmingen), anti-CD45RA (Coulter), anti-CD45RO (Coulter), anti-CD3 (Sigma-Aldrich), 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, Czech Republic), anti-CCR5 (BD Pharmingen), anti-CXCR3 (BD Pharmingen), anti-ICOS, anti-CD40L (BD Pharmingen), and anti-CXCR5 (R&D Systems). Data were acquired on a FACSCalibur or a FACSCanto instruments (BD Biosciences) and analyzed using CellQuest software (BD Immunocytometry Systems) or FlowJo (Tree Star). Tonsillar B cells were isolated by use of CD19 microbeads (Miltenyi Biotec), according to the manufacturer's instruction. For isolation of peripheral blood naive B cells, the procedure described in Ref. 19

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Received for publication May 9, 2006. Accepted for publication July 28, 2006.

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[1] This work was supported by grants from the Commission of the European Union, 6FP, contract LSHP-CT-2003-503367, TB-VAC. F.D. was supported by grants from the University of Palermo.

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[3] Abbreviations used in this paper: T_{CM}, central memory T; T_{EM}, effector memory T; BrHPP, bromohydrinpyrophosphate; Th_F, follicular B Th cell.

0022-1767/06/$02.00

was used. Briefly, $CD19^+$ cells were first isolated by the use of CD19 microbeads and were labeled with 25 nM MitoTracker Green FM (Molecular Probes), a substrate for ABCB1, for 25 min at 37° C and washed twice. Following staining for CD27 and surface IgG, naive B cells ($CD19^+CD27^-IgG^-$ $ABCB1^+$) were sorted by FACSVantage (BD Biosciences) with a purity of 99%. Different subsets of Vγ9Vδ2 T cells were sorted similarly by FACSVantage (BD Biosciences).

Cytokine production and chemotaxis assay

The medium used throughout was complete RPMI 1640 medium (Invitrogen Life Technologies) supplemented with 10% heat-inactivated FCS (Invitrogen Life Technologies), 2 mM L-glutamine, 20 nM HEPES and 100 U/ml penicillin/streptomycin. Sorted Vδ2 cell subsets were cultured at 37°C, in the presence of 5% CO_2, at 10^5/ml in 96-well flat-bottom plates (0.2 ml/well), with different concentrations of bromohydrinpyrophosphate (BrHPP) in the presence of irradiated (5000 rad from a cesium source) allogeneic dendritic cells (20). IFN-γ, TNF-α, IL-2, IL-4, and IL-10 levels in the 24-h culture supernatants were assessed by two mAbs sandwich ELISA following manufacturer's recommendations (R&D Systems).

The chemotactic ability of $CXCR5^+V\gamma9V\delta2$ cells was assayed using a double-chamber system with 3-μm pores (Transwell; Costar), according to Ref. 21. Briefly, 10^5 sorted $CXCR5^+$ or $CXCR5^-$ Vγ9Vδ2 cells were added to the upper chamber and CXCL13 (recombinant human CXCL13, BCA-1; R&D Systems, 3 μM final concentration) to the lower chamber and incubated at 37°C for 2 h in a 5% CO_2 humidified incubator. In some experiments, anti-CXCR5 or isotype control mAbs were added to the lower chamber during the test. Assays were performed in triplicate. Afterward, the membrane was removed, washed on the upper side with PBS, fixed, and stained. Migrated cells were counted microscopically at ×1000 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.

Ab production in vitro

Vγ9Vδ2 T cell help in Ab production was studied as follows. Different subsets of peripheral blood Vγ9Vδ2 T cells were sorted by FACS and cocultured with sorted tonsillar B cells or peripheral blood-derived naive 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 in the culture supernatants were determined by ELISA. When naive B cells were used, $F(ab')_2$ of goat anti-human Ig (Jackson ImmunoResearch Laboratories) was added to cultures as a BCR trigger, at the final concentration of 2 μg/ml. Anti-CD40L mAb and ICOS ligand fused to the Fc portion of mouse IgG2a (ICOSL-muIg; both reagents from Alexis through Vinci-Biochem, Firenze, Italy), neutralizing anti-human IL-4 and IL-10 (BD Biosciences) and isotype matched control mAbs, were added at the beginning of cultures at the final concentration of 15 μg/ml. As a control of the ICOSL-muIg, we used mouse IL-7 fused to the Fc portion of mouse IgG2a (a gift from Prof. G. Stassi, Department of Surgical and Oncological Science, University of Palermo, Palermo, Italy).

Statistics

The double-tailed Student *t* test was used to analyze the significance of differences between groups.

Results

Expression of CXCR5 on human Vγ9Vδ2 T cells

Expression of the chemokine receptor CXCR5 defines a population of CD4 Th cells which support the production of Igs (21, 22). CXCR5 expression has been also detected on human Vγ9Vδ2 T cells by some authors (23), but not by others (18).

CXCR5 expression was studied on peripheral blood Vγ9Vδ2 T cells. In a population of healthy donors ($n = 15$, age range 20–23 years), ~15% of total Vδ2 T cells are $CXCR5^+$ (14.6 ± 6.2, range 5.3–46.8, see Fig. 1). In a cohort of seven young patients (age range 9–14 years) undergoing tonsillectomy the percentage of peripheral blood $CXCR5^+V\delta2$ T cells was not significantly different from in adults (15.8 ± 7.3, range 4.8–39.5, data not shown). However, $CXCR5^+V\gamma9V\delta2$ T cells were highly enriched in inflamed tonsils where they account for about half the size of the Vγ9Vδ2 T cell population (Fig. 1).

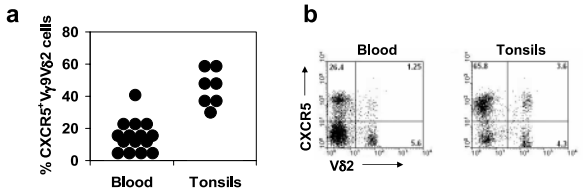

FIGURE 1. Distribution and phenotype of $CXCR5^+V\gamma9V\delta2$ T cells in peripheral blood and tonsils. *a*, Analysis of the relative proportion of $CXCR5^+V\gamma9V\delta2$ T cells in peripheral blood and tonsils. *b*, FACS analysis of peripheral blood and tonsils mononuclear cells stained with different Abs.

We have previously reported (9) that the expression of CD45RA and CD27 Ags defines four subsets of human Vγ9Vδ2 T cells with distinctive functional properties and compartmentalization routes. FACS analysis demonstrates that the vast majority of peripheral blood $CXCR5^+$ Vγ9Vδ2 cells do not express CD45RA, but express CD27, CD45RO, CCR7, and CD62L (Table I), suggesting that most of them have a T_{CM} phenotype. 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 T_{CM} phenotype like their peripheral blood counterpart (see Table I), but most of them express several activation markers and costimulatory molecules (CD25, HLA-DR, CD40L and ICOS, see Table I), 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 (24) is found on the vast majority of peripheral blood $CXCR5^+$ Vγ9Vδ2 T cells (Table I) but is markedly reduced on tonsillar $CXCR5^+V\gamma9V\delta2$ T cells (Table I), suggesting the possibility of a ligand-induced CCR7 down-modulation. Similarly, CD62L expression is reduced on tonsillar $CXCR5^+V\gamma9V\delta2$ T cells, implying that the majority of tonsillar $CXCR5^+V\gamma9V\delta2$ T cells have recently immigrated from circulation. The chemokine receptors CXCR3 and CCR5 were weakly expressed on tonsillar $CXCR5^+V\gamma9V\delta2$ T cells, but were not detected on peripheral blood $CXCR5^+V\gamma9V\delta2$ T cells (Table I).

To assess whether costimulatory molecules expression of tonsil $CXCR5^+V\gamma9V\delta2$ T cells was due to Ag-dependent activation, we sorted $CXCR5^+$ and $CXCR5^-$ Vγ9Vδ2 T_{CM} and Vγ9Vδ2 T_{EM} cells from peripheral blood and cultured in vitro with BrHPP and irradiated dendritic cells. None of the three subsets stained positive

Table I. *Surface markers expression on $CXCR5^+V\gamma9V\delta2^+$ T cells in peripheral blood and inflamed tonsils*[a]

	Peripheral Blood ($n = 15$)	Tonsils ($n = 6$)
CD45RA	1 ± 2 (4)	2 ± 3 (5)
CD45R0	98 ± 7 (85)	95 ± 9 (38)
CD27	72 ± 5 (67)	79 ± 7 (51)
CD25	1 ± 0.4 (2)	65 ± 7 (44)
HLA-DR	0.5 ± 0.1 (4)	58 ± 6 (34)
CD40L	2.4 ± 0.4 (4)	78 ± 9 (84)
ICOS	1.5 ± 0.2 (3)	98 ± 6 (115)
CD62L	95 ± 4 (73)	33 ± 2 (30)
CCR7	97 ± 3.9 (86)	28 ± 5 (25)
CCR5	1 ± 0.1 (5)	15 ± 2 (18)
CXCR3	2.1 ± 0.6 (7)	9.5 ± 3.1 (11)

[a] Peripheral blood and tonsil mononuclear cells were stained with mAbs to Vδ2, CXCR5, and other cell surface markers, and were analysed by FACS. Values indicate the percentage ± SE of $CXCR5^+V\delta2^+$ cells expressing the indicated cell surface markers. Values in parentheses indicate the mean fluorescence intensity mean value.

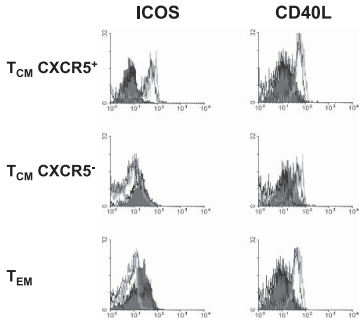

FIGURE 2. Activation-dependent expression of costimulatory molecules in CXCR5$^+$ and CXCR5$^-$Vγ9Vδ2 T cell subsets. FACS analysis of CD40L and ICOS expression on different subsets of peripheral blood Vγ9Vδ2 T cells before (black line) or 48 h (blue line) and 72 h (green line) after stimulation with BrHPP. Control mAb stainings are shown in red filled histograms.

for ICOS and CD40L either in the absence or 24 h after Ag stimulation in vitro (data not shown). However, after 48 and 72 h of Ag stimulation, CXCR5$^+$ and, although at a lower extent CXCR5$^-$ Vγ9Vδ2 T_{CM} and Vγ9Vδ2 T_{EM} cells expressed CD40L, while ICOS expression was detected only on CXCR5$^+$ Vγ9Vδ2 T_{CM} cells (Fig. 2).

Migratory properties and cytokine production of CXCR5$^+$Vγ9Vδ2 T cells

Freshly isolated tonsillar CXCR5$^+$, but not CXCR5$^-$, Vγ9Vδ2 T cells migrated readily in response to CXCL13/BCA-1 (Fig. 3*a*). 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 (Fig. 3*a*). Of note, migration of tonsillar CXCR5$^+$Vγ9Vδ2 T cells in response to CXCL13/BCA-1 was consistently inhibited by anti-CXCR5 mAb (Fig. 3*b*).

We studied the pattern of cytokine production in the CXCR5$^+$ Vγ9Vδ2 T cell subsets after a 24 h stimulation period with BrHPP and irradiated dendritic cells in vitro. To this end, CXCR5$^+$Vγ9Vδ2 T cell were separated into CD27$^+$ and CD27$^-$ cells and their pattern of cytokine production was compared with that of CXCR5$^-$CD27$^-$ (T_{EM}) and CXCR5$^-$CD27$^+$ (T_{CM}) Vγ9Vδ2 T cells. As shown in Table II, CXCR5$^-$CD27$^-$ Vγ9Vδ2 T_{EM} cells produced IFN-γ and TNF-α, but neither IL-4 nor IL-10, thus confirming our previous results (9). CXCR5$^-$CD27$^+$Vγ9Vδ2 T_{CM} cells also produced IFN-γ and TNF-α, but the amounts of cytokines were significantly lower than those produced by Vγ9Vδ2 T_{EM} cells. Ag-stimulated CXCR5$^+$CD27$^+$Vγ9Vδ2 T cells had a different cytokine profile: while producing IFN-γ and TNF-α at a similar extent to CXCR5$^-$CD27$^+$Vγ9Vδ2 T_{CM} cells, they produced significantly higher levels of IL-2, IL-4, and IL-10, when compared with other subsets of Vγ9Vδ2 cells. This cytokine profile was unique to the CXCR5$^-$CD27$^+$ subset, as Ag-stimulated CXCR5$^+$CD27$^-$Vγ9Vδ2 T cells produced IFN-γ and TNF-α, but not IL-2, IL-4, and IL-10.

CXCR5$^+$Vγ9Vδ2 T cells help B cells for Ab production

As CXCR5$^+$Vγ9Vδ2 T cells express costimulatory molecules, produce IL-4 and IL-10, we tested whether or not these cells were able to support B cells to secrete Igs. Peripheral blood CXCR5$^+$ and CXCR5$^-$ Vγ9Vδ2 T cells were further sorted into CD27$^+$ and CD27$^-$ populations and the four T cell subsets were cultured with CD19$^+$ B cells isolated from the tonsil of the same donor, in the presence or absence of BrHPP. Fig. 4 shows one typical experiment of five. B cells produced comparable very low amounts of IgA, IgG and IgM when cultured for 10 days without Vγ9Vδ2 T cells or with the CXCR5$^-$ fractions (i.e., CXCR5$^-$CD27$^+$Vγ9Vδ2 T_{CM} and CXCR5$^-$CD27$^-$Vγ9Vδ2 T_{EM} cells). Similarly, very low IgA, IgG, and IgM levels were detected in cocultures of B cells and CXCR5$^+$CD27$^-$Vγ9Vδ2 T cells. In contrast, coculture of B cells with CXCR5$^+$CD27$^+$ Vγ9Vδ2 T cells 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.

Of note, CXCR5$^+$Vγ9Vδ2 T cells from peripheral blood did not cause significant increase of Ab production in cocultures with B cells conducted in the absence of BrHPP, but total Vγ9Vδ2 T cells or CXCR5$^+$Vγ9Vδ2 T cells from the tonsils were able to induce significant production of Igs even in the absence of Ag, indicating that they are well equipped for providing B cell help (Fig. 5). The B cell helper activity of CXCR5$^+$Vγ9Vδ2 T_{CM} cells was strictly dependent on their provision of both costimulatory molecules and cytokines, as blocking of CD40L or ICOS caused a drastic reduction of both Ab and similar inhibition was obtained by addition of Abs to IL-10 and IL-4 (Fig. 6), even if the latter seems to be dispensable for IgA production.

Because the source of B cells used in these assays is the inflamed tonsil it would seem likely that CXCR5$^+$Vγ9Vδ2 T cells act by amplifying or enhancing Ab secretion by preformed plasma

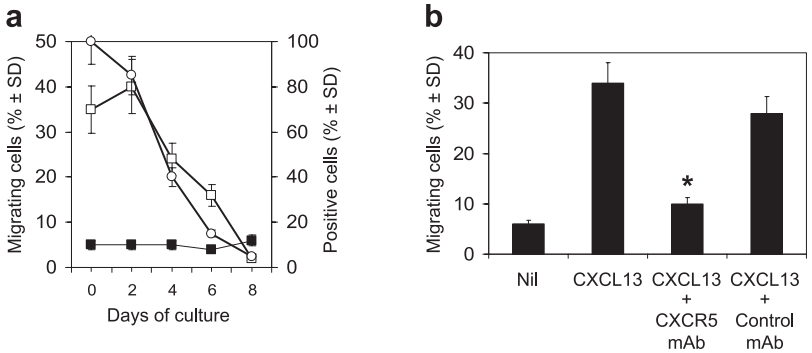

FIGURE 3. Migration to CXCL13/BCA-1 and CXCR5 expression during culture of tonsillar CXCR5$^+$Vγ9Vδ2 T cells. In *a*, freshly isolated tonsillar CXCR5$^+$ (□) or CXCR5$^-$ (■) Vγ9Vδ2 T cells were cultured with BrHPP for up to 8 days and examined for in vitro migration to CXCL13/BCA-1 (3 μM, final concentration) and CXCR5 expression (○). In *b*, migration of tonsillar CXCR5$^+$Vγ9Vδ2 T cells to CXCL13/BCA-1 was conducted in the presence of anti-CXCR5 or isotype-matched control mAbs (15 μg/ml, final concentration). Data are representative of three independent experiments. *, $p < 0.05$ when compared with groups consisting of CXCR5$^+$Vγ9Vδ2 T migrating to CXCL13 in the absence of any Ab or in the presence of isotype-matched control mAb.

Table II. *Cytokine production by CXCR5$^+$ and CXCR5$^-$Vγ9Vδ2 T cells*[a]

	Vγ9Vδ2 T Cell Subset			
	CXCR5$^+$CD27$^+$	CXCR5$^+$CD27$^-$	CXCR5$^-$CD27$^+$	CXCR5$^-$CD27$^-$
IFN-γ	100 ± 20	110 ± 20	250 ± 60	1400 ± 320[b]
TNF-α	150 ± 40	160 ± 15	200 ± 55	2300 ± 450[b]
IL-2	550 ± 65[c]	10 ± 5	40 ± 10	20 ± 10
IL-4	600 ± 25[c]	10 ± 10	10 ± 5	50 ± 5
IL-10	240 ± 30[c]	10 ± 5	10 ± 5	10 ± 5

[a] Peripheral blood CXCR5$^+$ and CXCR5$^-$Vγ9Vδ2 T cells were separated into CD27$^+$ and CD27$^-$ cells and equal numbers of each of the four subset were cultured with BrHPP. Cytokine levels were assessed by ELISA and data expressed as picrograms per milliliter ± SD. Similar results were obtained in three independent experiments.
[b] A value of $p < 0.005$ and [c] $p < 0.001$ when compared to values in all other groups.

cells. We therefore investigated whether CXCR5$^+$Vγ9Vδ2 T cells can drive naive B cells to become Ab-secreting plasma cells. To this end, peripheral blood CXCR5$^+$Vγ9Vδ2 T cells were cultured with highly purified naive B cells isolated from the peripheral blood of the same donors, in the presence or absence of BrHPP and F(ab′)$_2$ of goat anti-human Ig as a BCR trigger, and Ab production was assessed 10 days later.

As shown in Fig. 7 (one typical experiment of three), CXCR5$^+$Vγ9Vδ2 T cells provided help to naive B cells to produce IgM, IgG, and IgA Abs, thus indicating that this T cell subset is also able to provide help.

Discussion

Our study provides evidence of a direct role for γδ T cells in the control of humoral immune response and allows us to assign this property to a subsets of Vγ9Vδ2 T cells that express CXCR5.

Circulating CXCR5$^+$Vγ9Vδ2 T cells uniformly coexpress CD45R0, CCR7, and CD62L, but do not express either activation (CD25 and HLA-DR) or costimulatory (CD40L and ICOS) molecules. This subset was heterogeneous in terms of CD27 expression, with the majority (70%) being CD27$^+$. Thus, peripheral CXCR5$^+$Vγ9Vδ2 T cells likely represent a subpopulation of T_{CM} cells and, thus, differ from effector memory Vγ9Vδ2 T cells, which are proposed to directly participate in immune responses at inflammatory sites (9). Accordingly, circulating CXCR5$^+$ Vγ9Vδ2 T cells lack chemokine receptors, such as CCR5 and CXCR3, that are typically involved in recruitment of effector memory T cells to inflammatory sites in peripheral tissues. This chemokine receptor profile suggests that CXCR5$^+$Vγ9Vδ2 T cells home preferentially into secondary lymphoid tissues and, therefore, CXCR5$^+$Vγ9Vδ2 T cells from tonsils were selected for further studies.

Practically 50% Vγ9Vδ2 T cells in tonsils are CXCR5$^+$ and the majority express activation markers (CD69 and HLA-DR) and costimulatory molecules (CD40L and ICOS) suggesting their engagement in B cell activation. Drastic reduction in cell surface CCR7 and CD62L expression indicates that the majority of local CXCR5$^+$Vγ9Vδ2 T cells have recently entered the tonsils.

Tonsillar CXCR5$^+$Vγ9Vδ2 T cells readily migrate in response to CXCL13/BCA-1, and the response was consistently inhibited by anti-CXCR5 mAb demonstrating that CXCR5 is functional.

However, both CXCR5 expression and responsiveness to CXCL13/BCA-1 are sensitive to Vγ9Vδ2 T cell activation: in fact, they increased upon phosphoantigen activation, but by day 3 they consistently decrease. The requirements for induction of CXCR5 expression in Vγ9Vδ2 T cells are not defined. Preliminary data indicate that phosphoantigen stimulation in the presence or absence of IL-2 are not sufficient. In mice, CXCR5 expression during primary responses was shown to depend on sequential signaling by CD28 and OX40, suggesting the requirement for APCs (25, 26).

Expression of CXCR5 on human Vγ9Vδ2 T cells is a matter of debate. While Kabelitz and coworkers (23) found this receptor being expressed by a subset of Vγ9Vδ2 T cells, Moser and colleagues (18) did not detect CXCR5 expression on both peripheral blood and tonsillar Vγ9Vδ2 T cells. Moreover, Forster et al. (27) found that in healthy individuals, 2% of peripheral blood γδ T cells, but ~23% of tonsillar γδ T cells express BLR1 (presumably CXCR5) and this percentage consistently increased in HIV-infected individuals. We have no obvious explanation for the

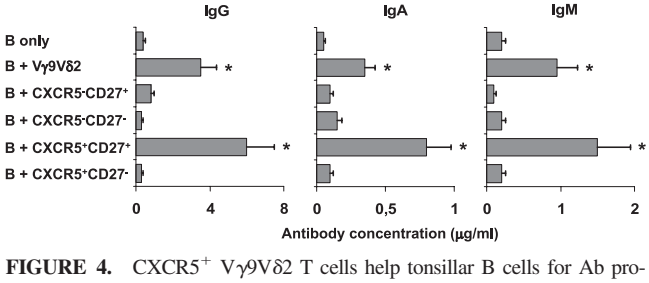

FIGURE 4. CXCR5$^+$ Vγ9Vδ2 T cells help tonsillar B cells for Ab production. Tonsillar B cells were cultured alone or in the presence of equal numbers of the CXCR5$^+$CD27$^+$, CXCR5$^+$CD27$^-$, CXCR5$^-$CD27$^+$, and CXCR5$^-$CD27$^-$ subsets of Vγ9Vδ2 T lymphocytes, in the presence of BrHPP. Ten days later, total IgG, IgA, and IgM levels in culture supernatants were assessed by ELISA. One of five different experiments is shown. *, $p < 0.001$ when compared with the group consisting of B cell cultured with medium.

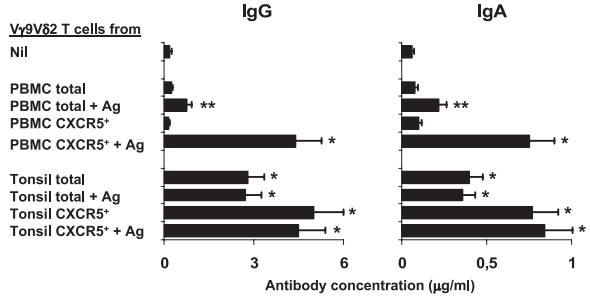

FIGURE 5. Ag requirement for CXCR5$^+$ Vγ9Vδ2 T cell help. Tonsillar B cells were cultured alone (Nil) or with peripheral blood- or tonsillar-derived total Vγ9Vδ2 T lymphocytes or their CXCR5$^+$ subset, in the presence or absence of BrHPP. Ten days later, total IgG and IgA levels in culture supernatants were assessed by ELISA. One of three different experiments is shown. *, $p < 0.001$ and **, $p < 0.01$ when compared with the group consisting of B cell cultured with medium (Nil).

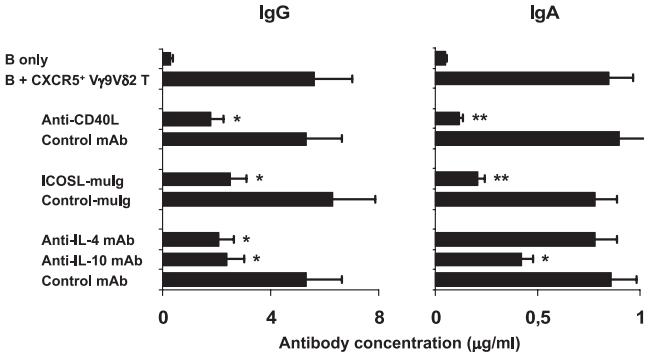

FIGURE 6. CXCR5$^+$ Vγ9Vδ2 T-B cell cooperation requires both costimulatory molecules and cytokines. Cocultures of tonsillar B cells and peripheral blood CXCR5$^+$Vγ9Vδ2 T lymphocytes were conducted as described in the legend to Fig. 4, but in the presence of mAbs to costimulatory molecules or cytokines (see *Materials and Methods*). *, $p < 0.005$ and **, $p < 0.001$ when compared with the group consisting of B cells cultured with CXCR5$^+$Vγ9Vδ2 T lymphocytes and BrHPP.

discrepancy in CXCR5 expression on peripheral blood T cells between these studies but the results report here clearly demonstrate CXCR5 expression by a subset of human Vγ9Vδ2 T cells.

CXCR5 defines a subset of CD4 memory Th cells which are now termed follicular B Th (Th_F) cells (28). Peripheral blood Th_F cells belong to the subset of T_{CM} cells, i.e., they are resting, CCR7$^+$ and nonpolarized (do not produce Th1/Th2-type cytokines upon stimulation). Tonsillar Th_F cells are also nonpolarized but differ from peripheral blood Th_F cells in that they express a range of activation markers and costimulatory molecules.

Th_F cells are highly efficient at providing B cell help and, thus, are expected to be well-equipped with costimulatory molecules and cytokines. In this regard, a significant fraction of tonsillar Th_F cells express CD40L and the majority also express ICOS. However, the cytokine secretion capacity of tonsillar Th_F cells is very limited and only a subpopulation of germinal center-localized Th_F cells has indeed been identified which produces IL-10.

Differently than CXCR5$^+$CD4$^+\alpha\beta^+$ memory T cells, that are poor producers of cytokines (21, 22), CXCR5$^+$Vγ9Vδ2 T cells have a Th2-type pattern of cytokine production upon Ag stimulation in vitro, as they secrete IL-2, IL-4, and IL-10. This contrasts, with the cytokine production pattern of the CXCR5$^-$ T_{EM} subsets of Vγ9Vδ2 T cells, which preferentially secrete IFN-γ and TNF-α. Moreover, secretion of IL-4 and IL-10 seems confined to the CD27$^+$CXCR5$^+$ subset of Vγ9Vδ2 T cells. The finding of a population of Vγ9Vδ2 T cells that secretes IL-4 and IL-10 is not new, and expands previous results demonstrating IL-4 production by resting (29–31) and Vγ9Vδ2 T cell clones (32, 33), most of which express CD27 (M. Bonneville and E. Scotet, unpublished observations).

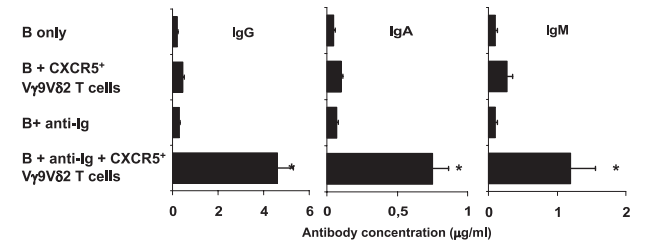

FIGURE 7. CXCR5$^+$ Vγ9Vδ2 T help naive B cells for Ab production. Naive B cells were sorted from the peripheral blood as indicated in *Materials and Methods*, and were cultured alone or with peripheral blood CXCR5$^+$ Vγ9Vδ2 T lymphocytes, in the presence of BrHPP and $F(ab')_2$ of goat anti-human Ig as a BCR trigger (2 μg/ml final concentration). Ten days later, total IgG, IgA, and IgM levels in culture supernatants were assessed by ELISA. One of three different experiments is shown. *, $p < 0.001$ when compared with all other groups.

Production of Th2-type cytokines together with expression of CD40L and ICOS strongly suggests that tonsillar CXCR5$^+$Vγ9Vδ2 T cells are engaged in B cell activation and help for Ab production. Interestingly, and differently than CD4$^+$ follicular T cells, CD40L, and ICOS are induced late during activation and persist longer, a finding that may be important for their function within germinal centers. In fact, ICOS induces production of various cytokines from recently activated T cells and critically participates in T cell-dependent immune responses (34) and CD40-CD40L interaction is an essential step during a T cell-dependent B cell response as it regulates proliferation of B cells, Ig class switching, and Ab production (35). Consequently, when coculturing sorted tonsillar B cells with various subsets of Vγ9Vδ2 T cells, we observed Ig production only within the CXCR5$^+$CD27$^+$ fraction, thus identifying this cell population as the classical helper cells. Moreover, Ig production was consistently inhibited by blocking CD40-CD40L and ICOS-ICOSL interactions, or by neutralization of IL-4 or IL-10. Due to the preactivation status of tonsillar B cells and that exogenous stimuli were not required for B cell help by tonsillar Vγ9Vδ2 T cells, one may argue that CXCR5$^+$Vγ9Vδ2 T cells may only be active on already activated B cells and hence during secondary Ab responses. However, the finding that circulating CXCR5$^+$Vγ9Vδ2 T cells are also able to help circulating naive B cells for Ab production, strongly suggests that they play an important regulatory role in all aspects of humoral immunity.

Contribution of CXCR5$^+$Vγ9Vδ2 T cells to Ab-mediated immune responses may occur 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. Accordingly, Vγ9Vδ2 T cells induce maturation of myeloid dendritic cells (33, 36–38) and, even if for a short period of time upon Ag activation, may function as APCs themselves (39, 40).

Thus, Vγ9Vδ2 T cells may influence the subsequent adaptive immune responses through interaction with peripheral dendritic cells and B cells in reactive secondary lymphoid tissues.

Acknowledgments

We thank Dr. M. Lipp, Prof. V. Horejsi, and Prof. G. Stassi for providing us with reagents.

Disclosures

The authors have no financial conflict of interest.

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