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B Lymphocyte-Derived IL-16 Attracts Dendritic Cells and Th Cells

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Interaction of B lymphocytes with Th cells is a fundamental step in the establishment of humoral immunity, and recent evidence suggests that direct interaction between B lymphocytes and dendritic cells (DCs) is also an important prerequisite. Factors involved in the selective recruitment of Th cells and DCs by B lymphocytes are insufficiently defined. We set out to delineate the role of IL-16, the soluble ligand of CD4, which is expressed on Th cells and DCs. B lymphocytes express IL-16 mRNA and synthesize bioactive IL-16 protein, and IL-16 is expressed in lymph node follicles in situ. B lymphocyte supernatant efficiently induces migration of CD4+ Th cells, monocyte-derived DCs, and circulating blood DCs in nitrocellulose filter-based assays. Neutralization of IL-16 bioactivity strongly inhibits this migratory response, suggesting that IL-16 might be a major chemotactic factor derived from B cells. The present data further support the idea that IL-16 might have a role in the initiation of cellular as well as humoral immunity by mediating the cellular cross-talk among T lymphocytes, B cells, and DCs, leading to recruitment of these cell types at common anatomical sites. The Journal of Immunology, 2000, 165: 2474–2480.

Interleukin-16 is a polypeptide cytokine that was originally described as a T cell-specific chemoattractant factor (1). It is synthesized by CD4+ and CD8+ T cells and is released in response to Ag, mitogen, histamine, and serotonin (1); furthermore, IL-16 is produced by eosinophils, mast cells, airway epithelial cells of asthmatics, keratinocytes, and monocyte-derived dendritic cells (DCs) (1, 2). Caspase-3 cleaves pro-IL-16, and the 20-kDa IL-16 released is biologically active as a multimer (3, 4). As the natural soluble ligand for CD4, it has been shown to induce chemotaxis in CD4+ T cells as well as in monocytes, eosinophils, and monocyte-derived DCs (1, 2). Besides its chemotactic properties, IL-16 induces the expression of IL-2R (CD25) and activates CD4+ T cells synergistically with IL-2 and IL-15 (5, 6). In contrast, preincubation of CD4+ T cells with IL-16 reduces the proliferative response to CD3/TCR ligation, probably via steric interaction with CD4-MHC binding (7).

Foreign Ag is captured by DCs, processed to antigenic peptides, and presented to CD4+ and CD8+ T lymphocytes in the grooves of MHC II and MHC I molecules, respectively. Subsequently, CD4+ Th cells migrate to the edge of the B cell-rich follicles in lymphoid organs, recognize Ag presented in the groove of MHC II on B lymphocytes by virtue of their TCR, and provide several costimu-

latory signals to B cells, resulting in their activation, proliferation, and ultimately in Ab formation (8). Much is known about T cell trafficking in lymphoid tissue, but few reports have focused on factors directing the chemotraction of T lymphocytes toward B lymphocytes and vice versa (9). Recent reports suggest an important role of direct B cell/DC interaction in B cell proliferation and isotype switching (10–16). Because chemotraction of CD4+ cells is a fundamental property of IL-16, we investigated whether this cytokine is derived from B cells and whether it might have a role in the attraction of CD4+ Th lymphocytes and CD4+ DCs.

Materials and Methods

Media, reagents, and Abs

The culture medium (CM) used in this study was RPMI 1640 (Schoeller Pharma, Vienna, Austria) supplemented with 10% heat-inactivated (30 min, 56°C) FCS (Life Technologies, Vienna, Austria). 100 U/ml penicillin G, and 100 μg/ml streptomycin (Schoeller Pharma). Recombinant human (rhu) IL-4 and rhuIL-10 were supplied by Schering-Plough Research Institute (Kenilworth, NJ), and rhuIL-6 was purchased from PeproTech (London, U.K.). Recombinant human IL-16 was obtained from PharMingen (Hamburg, Germany), as were mAbs directed against CD3 (HIT3a), CD4 (RPA-T4), CD8 (M5E2), CD19 (B43) and CD56 (B159), and CD16 (14.1). Biotinylated polyclonal Abs (pAbs) against IL-16 were purchased from R&D Systems (Minneapolis, MN; for Western analysis and ELISA) and PharMingen (for intracellular FACS staining). Anti-CD20 mAb was purchased from Dako (Carpinteria, CA). Mouse IgG1 and mouse IgG2a were obtained from Sigma (Vienna, Austria). Biotinylated rabbit IgG was obtained from Pierce (Biotrade, Vienna, Austria). FITC-anti-mouse IgG, human IgG, rabbit IgG, and saponin were purchased from Sigma, and streptavidin-PE was obtained from Becton Dickinson (Vienna, Austria). Recombinant human monocyte chemoattractant protein-3 (MCP-3) and rhuRANTES were obtained from R&D Systems. PMA and LPS (Escherichia coli 055:B5) were purchased from Sigma. Ionomycin and Staphylococcus aureus Cowan I strain (SACS) were obtained from Calbiochem (Biotrade).

Cell culture

PBMC were obtained by density gradient centrifugation (Histopaque 1077, Sigma) of buffy coats obtained from the local blood bank. PBMC were washed and incubated for 45 min at 4°C with an appropriate amount of magnetic beads coated with anti-CD19 (Dynabeads, Dynal, Oslo, Norway). After positive selection of CD19+ cells, Dynabeads were detached by addition of Detach-A-Bead and further incubation for 45 min. Subsequently,
pure B cells (>99%, as determined by cytofluorometry) were additionally washed and cultured at a density of 1 × 10^6 (for ELISA experiments) or 1 × 10^7 (for Northern and Western experiments)/ml in CM for the indicated period of time.

For the generation of B cell supernatant for chemotaxis assays, 1 × 10^6 purified B cells/ml were cultured in RPMI 1640/0.5% BSA as follows: 1) for 24 h without addition of activating agents; 2) for 12 h on anti-CD40 mAb-coated (2 μg/ml, overnight) 48-well plates and addition of IL-4 (1000 U/ml), subsequent intense washing, and further culture for 24 h without activating agents; and 3) for 24 h with addition of anti-lgM-coated microbeads (5 μg/ml; Irvine Scientific, Santa Ana, CA) (17).

Purified CD4^+ T lymphocytes were obtained by depletion of monocytes by plastic adhesion, followed by incubation with anti-CD14, anti-CD16, anti-CD19, and anti-CD56 mAbs. Ab-binding cells were removed by means of anti-mouse Ig-coated magnetic beads (Dynabeads M-450). In the next step, CD8^+ T lymphocytes were depleted by CD8^-coated Dynabeads as recommended by the manufacturer. The resulting CD4^+ T cell population had a purity of >95% as determined by cytofluorometry.

CD8^+ T lymphocytes were obtained as described above for CD19^+ B cells, with anti-CD8^-coated magnetic beads instead of anti-CD19 beads. Purity was >99% as determined by FACs analysis.

Monocyte-derived DCs were generated from PBMC as previously described (18, 19). In brief, PBMC were resuspended in CM, and 5 × 10^5 PBMCs were allowed to adhere in 75-cm² cell culture flasks for 45 min in a 37°C humidified 5% CO₂ atmosphere. Nonadherent cells were removed, and adherent cells were cultured in 10 ml of CM supplemented with 1 × 10^5 U/ml IL-4 and 1 × 10^5 U/ml GM-CSF. On day 2, 5 ml of CM supplemented with IL-4 and GM-CSF (both 1 × 10^5 U/ml), and on day 5, 5 ml of CM supplemented with 2 × 10^5 U/ml IL-4 and 2 × 10^5 U/ml GM-CSF were added. On day 6 of culture, DSF was harvested and purified by magnetic cell separation, employing magnetic beads as previously described (2). The resulting DC population had a purity of >97% as determined by cytofluorometry and CD1a, CD3, CD14, CD19, CD56, and CD40 staining. Following this procedure, DCs were subjected to chemotaxis assay.

Purification of circulating blood DCs was essentially performed as previously described (20). In brief, PBMC were depleted of T cells, NK cells, and monocytes by means of immunomagnetic beads directed against CD3, CD11b, and CD14 following the manufacturer’s instructions (Miltenyi Biotec, Bergisch Gladbach, Germany). Subsequently, cells were further depleted of B cells by CD19 beads (Miltenyi Biotec). The resulting cell population was subjected to chemotaxis assay. A purity of >95% was ascertained by cytofluorometry and staining for CD3, CD4, CD14, CD19, CD56, and HLA-DR.

Northern analysis

Total RNA was prepared from 1 × 10^6 purified B cells either immediately after positive selection from PBMC or alternatively from B lymphocytes cultured with the indicated agents for 5 h. Ten micrograms of total RNA per lane was gel-electrophoresed and blotted onto nylon membranes as recently described (21). The IL-16 probe was a 909-bp PstI fragment span-ning the 5′-untranslated region (GenBank M90391). The probe was randomly labeled with [32P]dCTP employing the random primed labeling method according to the manufacturer’s (Roche, Vienna, Austria) instructions and hybridized as previously described (21). Control hybridizations were performed with rat cDNA of the housekeeping gene GAPDH to ensure equal loading of RNA.

Western analysis

Cell extracts of 1 × 10^7 purified B cells were prepared by lysis in RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris-HCl (pH 8.0), 0.2 mM PMSF, 1 μg/ml pepstatin, and 0.5 μg/ml leupeptin) for 30 min at 4°C and subsequent centrifugation for 5 min at 16,000 × g. Protein content was estimated by a commercial Bradford assay (Bio-Rad, Vienna, Austria) with BSA standards, and 60 μg of cell lysates were electrophoresed per lane. Proteins were transferred under constant cooling in 25 mM Tris, 192 mM glycine, and 10% (v/v) methanol (Schleicher & Schuell, Dassel, Germany) for 60 min at a 100-V constant voltage. After protein transfer, the polyvinylidene difluoride membrane was incubated with 10% skim milk powder, 0.5% (w/v) Triton X-100, and 0.1% Tween 20 in 20 mM Tris-HCl (pH 7.4) and 150 mM NaCl, blots were incubated with affinity-purified alkaline phosphatase-conjugated goat anti-mouse IgG at a 1:150 dilution for 60 min at 22°C. After washing several times with the above-described buffer, the blots were developed in 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM MgCl₂, 0.033% nitro blue tetrazolium, and 0.017% 5-bromo-4-chloro-3-indolyl phosphate.

ELISA for pro-IL-16 and cleaved IL-16

Rabbit pAb generated to recombinant pro-IL-16 was used to establish an affinity column on cyanogen bromide-coupled Sepharose 4B beads. Supernatants were incubated on the column for 1 h at room temperature before elution with PBS. Cleaved, mature IL-16 would be eluted, whereas pro-IL-16 (cleaved and uncleaved) would remain on the column. ELISA readings were taken before and after column chromatography. The efficiency of the column was checked by applying T cell lysates, and for these samples 90%–95% of the total IL-16 was pro-IL-16. A sandwich ELISA for IL-16 was performed according to standard protocols with immobilized anti-IL-16 14.1 mAb (3 μg/ml in carbonate buffer) and biotinylated IL-16 pAb (200 ng/ml; R&D Systems) as detection Ab. The secondary reagent, streptavidin-peroxidase (Roche), was used according to the manufacturer’s instructions. Tetramethylbenzidine (BM Blue POD substrate, Roche) was used as substrate and measured at 450 nm in an ELISA reader. The standard curve was prepared with rhuIL-16 in a concentration range from 15.63 to 2000 pg/ml.

Cytotoxic analysis of B lymphocytes

PBMC (1 × 10^9) were washed in PBS/2% FCS, resuspended in 250 μg/ml hlgG/PBS/2% FCS, and incubated for 20 min at 4°C. After pelleting, PBMC were incubated with anti-CD19 at 10 μg/ml for 30 min at 4°C. After washing in PBS/2% FCS, a 1/40 dilution of FITC-anti-mouse IgG in PBS/2% FCS was incubated for 30 min at 4°C. Subsequently, PBMC were washed twice in PBS and resuspended in 2% formaldehyde/PBS for 10 min, washed once in PBS, and incubated with 0.1% saponin/10% goat serum/250 μg/ml rabbit IgG/PBS for 10 min at room temperature for permeabilization. After pelleting, 10 μg/ml biotinylated IL-16 pAb or the respective biotinylated control Ig in PBS/2% FCS/0.1% saponin was incubated for 30 min at room temperature, followed by washing in PBS/2% FCS/0.1% saponin. Subsequently, PBMC were incubated with a 1/25 dilution of streptavidin-PE in PBS/2% FCS/0.1% saponin for 30 min at room temperature, washed twice in PBS/2% FCS, and immediately analyzed on a FACScan (Becton Dickinson, Mountain View, CA). For analysis of the activation marker CD69, purified B cells were blocked with hlgG as described above, followed by anti-CD69-FTTC (10 μg/ml) or IgG1-FTTC (10 μg/ml), washed, and analyzed. Data analysis was performed with CellQuest software (Becton Dickinson).

IL-16 immunohistochemistry

Tissue sections of human tonsils were acquired from excess tissue obtained during elective surgery. Serial sections were fixed in 2% paraformaldehyde in Tris buffer for 8 min at 4°C. Sections were incubated overnight at 4°C with biotinylated IL-16 pAb (dilution 1/500 in 1% BSA/0.1% saponin in Tris buffer). Blocking of specific binding sites of the pAb was performed with 10 μg/ml rhuIL-16. Subsequently, streptavidin-peroxidase was added at a dilution of 1/800 and incubated for 30 min, and the signal was developed with 3,3′-diaminobenzidine (Sigma). Slides were counterstained with hemalum. Identification of B lymphocytes was performed by anti-CD20 mAb according to standard protocols.

Chemotaxis assay

Migration of cells into cellular matrices along gradients of soluble attractants were measured using a 48-well chemotaxis chamber (Neuroprobe, Bethesda, MD) in which a 5-μm (for lymphocytes) or an 8-μm (for DCs) pore size filter (Sartorius, Göttingen, Germany) separates the upper and lower chamber. Thirty-microliter aliquots of chemotactant solution or control medium (RPMI 1640/0.5% BSA) were added to the lower wells of the chamber, then the filter was carefully layered onto the wells and covered with a silicon gasket and the top plate. Fifty microliters of cell suspension (1 × 10^7 cells/ml) were seeded in the upper chamber. Purified CD4^+ T lymphocytes, CD8^+ T lymphocytes, monocyte-derived DCs, and circulating blood DCs were allowed to migrate toward attractants in the lower chambers for 2 h (CD4^+ /CD8^+ T lymphocytes) or 4 h (DCs) at 37°C in humidified atmosphere (5% CO₂). After this incubation time, the total cell suspensions were washed, fixed, and stained with hematoxylin-eosin. Migration depth of cells into the filter was determined by microscopy measuring the distance (microns) from the surface of the filter to the leading front of cells before any cells had reached the lower surface (leading front assay). Data are expressed as the chemotaxis index (CI), which is the...
equal loading was ascertained by hybridization with the housekeeping
test. A difference with p the CI were compared by Kruskal-Wallis ANOVA and Mann-Whitney U
test. A difference with p < 0.05 was considered significant. Calculation
was performed using the StatView software package (Abacus Concepts,
Berkeley, CA).

Results

B lymphocytes are a source of IL-16

Highly purified B lymphocytes were obtained by positive selection
of CD19+ cells employing magnetic beads. Obtained B cells were
>99% pure as determined by cytfluorometry and staining for
CD3, CD14, CD16, CD19, and CD56 (data not shown). B lymphocytes
did not express the activation Ag CD69 either immediately
after isolation or after 24 h of culture in medium alone (data
not shown), thus showing that the isolation procedure does not
induce cell activation. mRNA analysis revealed constitutive
expression of IL-16 in freshly isolated B cells (Fig. 1). Western
analysis of cell extracts derived from freshly isolated purified B lymphocytes
showed a single band at ~80 kDa, representing premature IL-16, but no band at ~20 kDa as would be expected
for bioactive cleaved IL-16 (Fig. 2), suggesting that cleaved IL-16 is readily secreted, rather than stored, in B lymphocytes. Corresponding
to these data, intracellular FACS analysis showed preformed
IL-16 in the CD19+ subpopulation (i.e., B cells) of freshly isolated
PBMC (Fig. 3). Stimulation of purified B cells for 5 h with various
agents showed that IL-4, IFN-γ, and SACS down-regulate IL-16
mRNA expression. IL-10, IL-6, and anti-CD40 had no effect (Fig.
1). Twenty-four-hour culture of unstimulated purified B cells at a
density of 1×10⁶/ml resulted in IL-16 synthesis of 506 ± 61 pg/ml.
Analysis of IL-16 protein synthesis after culture with various cytokines for various time periods did not show statistically
significant changes compared with unstimulated B cells, thus
providing no correlation between IL-16 mRNA regulation and
IL-16 protein synthesis (Table I). ELISA performed after depletion
of supernatants from pro-IL-16 with anti-pro-IL-16 pAb by affinity
chromatography yielded identical results, showing that all IL-16
detected in the supernatant is bioactive, mature IL-16 (data not
shown). In addition, a dose response for IL-16 chemotaxis was not
altered by the column (data not shown).

B lymphocytes in tonsil lymphatic tissue express IL-16 protein

Immunohistochemistry was performed on serial tissue sections of
tonsils obtained during elective surgery. B lymphocytes were iden-
tified by CD20 staining (Fig. 4A). IL-16 staining was most pro-
nounced in the mantle zone and less intense in the germinal center
(GC; Fig. 4, B and C). Fig. 4D proves specificity of IL-16 staining
by preincubation of the IL-16 mAb with rhuIL-16.

IL-16 is a major constituent of chemotactic activity of B cell
superantigen toward CD4+ Th lymphocytes

Supernatants of unstimulated B lymphocytes (1×10⁶/ml) and IL-
4/anti-CD40-stimulated B cells were tested for chemotactic activity
toward CD4+ Th lymphocytes. Migration data are expressed as the
depth of migration into nitrocellulose filters induced by B cell
superantigen compared with migration induced by medium alone
(CI). Undiluted supernatant from unstimulated B cells resulted in
a CI of 1.615 ± 0.077 (Fig. 5). Even a 1000-fold dilution of this
superantigen produced significant CD4+ T cell migration (CI,
1.295 ± 0.093; Fig. 5). To evaluate the relative contribution of

Statistical analysis

ELISA data and chemotaxis data are expressed as the mean ± SEM. The
significance of ELISA data was determined by Student’s t test for paired
samples using the SPSS software package (SPSS, Chicago, IL). Means of
the CI were compared by Kruskal-Wallis ANOVA and Mann-Whitney U
test. A difference with p < 0.05 was considered significant. Calculation
was performed using the StatView software package (Abacus Concepts,
Berkeley, CA).
IL-16 in chemotactic activity of supernatant from unstimulated B cells, we added neutralizing IL-16 mAb and found that CI was reduced almost to background levels (Fig. 5). However, undiluted B cell supernatant efficiently induced migration of DCs, which could be blocked by anti-IL-16 mAb (CI, 1.099 ± 0.037 with undiluted supernatant). As shown in Fig. 5, the inhibitory effect of the mAb was specific for IL-16, because migration toward recombinant MCP-3 was not inhibited.

Furthermore, we tested supernatant from anti-CD40/IL-4-stimulated B cells as shown in Table II. The relative contribution of IL-16 to the chemotactic response was 61% as determined by neutralization experiments. Similar results were obtained with supernatant from B lymphocytes stimulated with anti-IgM-coated microbeads (data not shown).

Because CD4+CD11c+ circulating blood DCs have been suggested as the DC population migrating into lymph node follicles subsequently constituting germinal center DCs (20), we tested B cell supernatant for its chemotactic activity on circulating blood DCs. Circulating blood DCs were obtained by depletion of CD3+, CD11b+, CD16+, and CD19+ cells employing immunomagnetic microbeads. The resulting cell population had a purity of >95% as determined by cytofluorometry for lineage markers and HLA-DR (data not shown). Extensive chemotaxis analysis was impossible due to the very low frequency of this cell type in circulating blood. However, undiluted B cell supernatant efficiently induced migration of circulating blood DCs (CI, 1.64 ± 0.08), which could substantially be blocked by adding neutralizing anti-IL-16 mAb (CI, 1.26 ± 0.04; Table III). As shown in Table III, rhuIL-16 induced significant migration of DCs, which could be blocked by anti-IL-16 mAb as well.

### Table I. IL-16 synthesis in B lymphocytes

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mean ± SEM</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium alone</td>
<td>506 ± 61</td>
<td></td>
</tr>
<tr>
<td>PMA 10 ng/ml</td>
<td>552 ± 101</td>
<td>0.674</td>
</tr>
<tr>
<td>PMA/IONOMycin</td>
<td>500 ng/ml</td>
<td>708 ± 144</td>
</tr>
<tr>
<td>LPS 10 µg/ml</td>
<td>593 ± 98</td>
<td>0.480</td>
</tr>
<tr>
<td>SACS 1:5000</td>
<td>638 ± 131</td>
<td>0.481</td>
</tr>
<tr>
<td>IL-4 1000 U/ml</td>
<td>590 ± 147</td>
<td>0.920</td>
</tr>
<tr>
<td>IL-6 100 U/ml</td>
<td>651 ± 147</td>
<td>0.425</td>
</tr>
<tr>
<td>IL-10 100 U/ml</td>
<td>478 ± 117</td>
<td>0.427</td>
</tr>
<tr>
<td>IFN-γ 100 U/ml</td>
<td>533 ± 60</td>
<td>0.779</td>
</tr>
<tr>
<td>Anti-CD40 1 µg/ml</td>
<td>769 ± 231</td>
<td>0.301</td>
</tr>
<tr>
<td>Anti-CD40 + IL-4</td>
<td>711 ± 119</td>
<td>0.148</td>
</tr>
<tr>
<td>Anti-CD40 + IFN-γ</td>
<td>1171 ± 345</td>
<td>0.127</td>
</tr>
<tr>
<td>Anti-CD40 + IL-4</td>
<td>826 ± 164</td>
<td>0.998</td>
</tr>
</tbody>
</table>

a Purified B cells (1 × 10⁶ per ml) were cultured for 24 h with addition of indicated agents. Supernatants were assayed for total IL-16 (pg/ml) by ELISA. Pro-IL-16 was not detected in the same samples, suggesting that all IL-16 detected is cleaved IL-16. Significance was calculated by Student’s t test for paired samples for the comparison of B cells stimulated with indicated agents compared to B cells cultured in medium alone. Various other time points (2, 4, 6, 12, 48, and 72 h) were evaluated, but not statistically significant regulation was observed (n = 7).

**IL-16 is a major constituent of chemotactic activity of B cell supernatant toward DCs**

To gain clues as to whether B cells secrete factors attracting DCs, B cell supernatant was tested for chemotactic activity on highly purified monocyte-derived DCs. Supernatant from unstimulated B cells efficiently attracted monocyte-derived DCs, with a maximum CI of 1.634 ± 0.060 (Fig. 5). Significant migration was even noted at a 100-fold dilution (CI, 1.325 ± 0.058). To identify the role of IL-16, we added neutralizing anti-IL-16 mAb and found that migration was almost completely inhibited (CI, 1.099 ± 0.037 with undiluted supernatant). As shown in Fig. 5, the inhibitory effect of the mAb was specific for IL-16, because migration toward recombinant MCP-3 was not inhibited.

**FIGURE 4.** IL-16 immunohistochemistry. IL-16 synthesis was studied in serial tissue sections of human tonsil lymphatic tissue obtained routinely during elective surgery. A. Identification of B lymphocytes within lymph follicles by CD20 staining. Magnification, ×200. B. IL-16 reactivity in lymphatic tissue detected by anti-IL-16 pAb. IL-16 staining is most pronounced in the mantle zone and less intense in the GC. Magnification, ×200. C. IL-16 reactivity in the GC. Magnification, ×400. D. Blocking of IL-16 reactivity by preincubation of IL-16 pAb with rhuIL-16. Magnification, ×200.
**Discussion**

Herein we report that human B cells are a source of IL-16. We provide evidence that freshly isolated B cells contain IL-16 mRNA as determined by Northern analysis. Western analysis of cellular extracts derived from freshly isolated B cells revealed an ~80-kDa band, representing pro-IL-16. In accordance with previous reports (2, 3, 22), we suggest that bioactive 20-kDa IL-16 is readily released into supernatant rather than stored in B lymphocytes. Intra-cellular FACS staining of PBMC confirmed that the CD19+ B cell population stains positively for IL-16. Culture of highly purified B cells for various time periods revealed constitutive synthesis and release into supernatant of cleaved, biologically active IL-16. This was not due to cellular activation during the isolation procedure, because B cells did not express the activation Ag CD69 immediately after isolation or after 24 h in culture in medium alone, while addition of PMA/ionomycin efficiently up-regulated CD69 (data not shown). Investigating modulation of IL-16 mRNA expression and protein synthesis by various cytokines, we noted that some mechanisms in regulating IL-16 release (3).

**Table II. Chemotaxis with supernatant derived from activated B cells**

<table>
<thead>
<tr>
<th>Migrating Cell Type</th>
<th>B Cell Stimulation</th>
<th>CI ± SEM</th>
<th>p</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cells</td>
<td>Anti-CD40/IL-4-stimulated plus IL-16 mAb</td>
<td>1.584 ± 0.070</td>
<td>&lt;0.05</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>+ IL-16 mAb</td>
<td>1.222 ± 0.043</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocyte-derived DC</td>
<td>Anti-CD40/IL-4-stimulated plus IL-16 mAb</td>
<td>1.540 ± 0.082</td>
<td>&lt;0.05</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>+ IL-16 mAb</td>
<td>1.212 ± 0.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>Unstimulated</td>
<td>1.280 ± 0.057</td>
<td>NS</td>
<td>No inhibition</td>
</tr>
<tr>
<td></td>
<td>plus IL-16 mAb</td>
<td>1.345 ± 0.040</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>plus anti-IL-16 mAb</td>
<td>1.408 ± 0.063</td>
<td>NS</td>
<td>No inhibition</td>
</tr>
<tr>
<td></td>
<td>rhuRANTES</td>
<td>1.625 ± 1.673</td>
<td>NS</td>
<td>No inhibition</td>
</tr>
<tr>
<td></td>
<td>+ anti-IL-16 mAb</td>
<td>1.673 ± 0.062</td>
<td></td>
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</table>

*p Purified monocyte-derived DCs, CD4+, and CD8+ T lymphocytes were subjected to chemotaxis into nitrocellulose filters toward undiluted supernatant derived from anti-CD40/IL-4-stimulated B lymphocytes as described in Materials and Methods. For CD8+ T lymphocytes, migration toward unstimulated B cell supernatant and rhuRANTES (20 ng/ml) is additionally included. Results are expressed as CI ± SEM, and the effect of IL-16 neutralization by mAbs (10 μg/ml) is expressed as percent inhibition of the migratory response (n = 3). Statistical analysis evaluates the significance of addition of neutralizing anti-IL-16 mAb. A value of p < 0.05 was considered statistically significant. Similar results were obtained with supernatant derived from anti-huIgM-coated microbead-stimulated B cells.
as in the GC. The weaker IL-16 staining in the GC compared with that in the mantle zone might reflect enhanced release of IL-16 from B cells and consecutive depletion of intracellular stores. Although we did not observe up-regulation of the activation marker CD69 on unstimulated B cells cultured for 24 h as described in the previous paragraph, we cannot definitely exclude the possibility that B cells release IL-16 spontaneously only in the in vitro culture system and not in quiescent state in vivo. This might explain the absence of CD4+ T lymphocytes and DCs in the mantle zone, in contrast to the GC, where both cell types are found and probably recruited by IL-16 derived from B lymphocytes.

To determine factors involved in the selective attraction of Th cells and DCs toward B cells, we tested supernatant derived by 24-h culture of highly purified B lymphocytes for its ability to induce the migration of Th cells and DCs into nitrocellulose filters. This assay has previously been employed in the evaluation of IL-16 as a chemotactic factor for DCs (2). For reasons of comparison, we demonstrated IL-16-induced migration of DCs through polycarbonate filters, showing similar results and confirming the reliability of our method (2). We furthermore provided evidence that the migratory activity of IL-16 toward monocyte-derived DCs is chemotactic rather than chemokinetic (2). Herein we demonstrate that B cell supernatant efficiently attracts CD4+ T lymphocytes, monocyte-derived DCs, and circulating blood DCs. To delineate the role of IL-16 in chemotactic activity of B cell supernatant, we neutralized IL-16 bioactivity by adding anti-IL-16 mAb and found the migratory response almost completely abrogated with supernatant derived from unstimulated B cells. This was true for CD4+ T lymphocytes, monocyte-derived DCs, and circulating blood DCs. To evaluate the influence of cellular activation of B lymphocytes and the consecutive up-regulation of various chemokines, we tested supernatant of anti-CD40/IL-4- and B cell Ag receptor-stimulated B cells as well. Neutralization of IL-16 by mAbs resulted in a decrease in migration of 62% for CD4+ T lymphocytes and DCs to activated murine B lymphocytes and DCs that selectively acts on activated T cells (39). The human orthologue, STCP-1 (MDC), acts specifically on chronically activated Th2 lymphocytes (23, 24). ABCD-2, which acts via CCR4, as does ABCD-1, has also been proposed as a factor attracting T lymphocytes toward activated B cells (25). Macrophage inflammatory protein-1β has been reported to be derived from Ag receptor-triggered B lymphocytes and to partly account for T cell chemotaxis toward activated B cells (17). IL-16 is theoretically a well-suited cytokine to meet the biological requirement of selectively attracting CD4+ Th cells, because it acts via CD4. Regarding the recently discovered direct interaction between DCs and B cells, no factors involved in DC attraction by B cells besides the herein reported IL-16 have been identified to date to our knowledge. We conclude that IL-16 could orchestrate the interaction of Th cells, B cells, and DCs during the induction phase of an immune response.

Table III. Circulating DC chemotaxis

<table>
<thead>
<tr>
<th>Chemoattractant Mean SEM p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>1.000 0.000</td>
</tr>
<tr>
<td>+ mAb (10 µg/ml)</td>
</tr>
<tr>
<td>1.096 0.038</td>
</tr>
<tr>
<td>IL-16 (10 ng/ml)</td>
</tr>
<tr>
<td>1.612 0.079 0.029</td>
</tr>
<tr>
<td>+ mAb (10 µg/ml)</td>
</tr>
<tr>
<td>1.149 0.051</td>
</tr>
<tr>
<td>Supernatant (1:1)</td>
</tr>
<tr>
<td>1.637 0.082 0.029</td>
</tr>
<tr>
<td>+ mAb (10 µg/ml)</td>
</tr>
<tr>
<td>1.256 0.038</td>
</tr>
</tbody>
</table>

*p Circulating blood DCs were purified and subjected to chemotaxis assay as described in Materials and Methods. Migration of DCs into nitrocellulose filters toward thall-16 and B cell supernatant is compared to migration induced by medium and expressed as Ct ± SEM. Statistical analysis evaluates the significance of addition of neutralizing anti-IL-16 mAb. A value of p < 0.05 was considered statistically significant.

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