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

Arthur Kaser,* Stefan Dunzendorfer,† Felix A. Offner,‡ Othmar Ludwiczek,* Barbara Enrich,*, Robert O. Koch,* William W. Cruikshank.§ Christian J. Wiedermann,† and Herbert Tilg²*

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 chemotactic 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-2Rα (CD25) and activated CD4⁺ T cells synergistically with IL-2 and IL-15 (5, 6). In contrast, precubination 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 groves 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 grove of MHC II on B lymphocytes by virtue of their TCR, and provide several costimulatory 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 1% 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), CD14 (M5E2), CD19 (B43) and CD56 (B159), CD69 (FN50), and IL-16 (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 intensely 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-IgM-coated microbeads (5 µg/ml; Irvine Scientific, Santa Ana, CA) (17).

Purified CD4+ T lymphocytes were obtained by depletion of monocytes by plastic adherence, 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+ Th 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 (2). In brief, PBMC were resuspended in CM, and 5 × 10^7 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 × 10^7 U/ml IL-4 and 1 × 10^7 U/ml GM-CSF. On day 2, 5 ml of CM supplemented with IL-4 and GM-CSF (both 1 × 10^7 U/ml), and on day 5, 5 ml of CM supplemented with 2 × 10^7 U/ml IL-4 and 2 × 10^7 U/ml GM-CSF were added. On day 6 of culture, DCs were 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 monocyte cells by means of immunomagnetic beads directed against CD3, CD11b, and CD16 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, CD14, CD19, CD56, and HLA-DR.

Northern analysis

Total RNA was prepared from 1 × 10^7 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 spanning nucleotides 930-1839 (GenBank M90391). The probe was radioactively labeled with [32P]dCTP using 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 PMSE, 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 from 200 to 350 mA for 90-150 min (Schleicher & Schuell, Vienna, Austria) 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 1/1500 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 80% 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.

Cytotoxicometric analysis of B lymphocytes

PBMC (1 × 10^6) were washed in PBS/5% 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/100 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 FACSscan (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 1/50 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 networks along gradients of soluble attractants were measured using a 48-well microchemotaxis chamber (Neuroprobe, Bethesda, MD) in which a 5-µm (for lymphocytes) or an 8-µm (for DCs) pore size filter (Sartorius, Gottingen, Germany) separates the upper and lower chamber. Thirty-microliter aliquots of chemotaxtractant 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 × 10^6 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+ and CD8+ T lymphocytes) or 4 h (DCs) at 37°C in humidified atmosphere (5% CO₂). After this incubation time, the noncellular filters were dehydrated, fixed, and stained with hematoxylin-eosin-X, and migration depth of the leading edge was measured by microscope 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
ratio between the distances of directed and undirected migration of DCs and T lymphocytes into the nitrocellulose filters.

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).

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 cytofluorometry 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 significantly increased 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 × 10^6/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 demonstrating 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 identified by CD20 staining (Fig. 4A). IL-16 staining was most pronounced 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 supernatant toward CD4+ Th lymphocytes

Supernatants of unstimulated B lymphocytes (1 × 10^6/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 supernatant 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 supernatant produced significant CD4+ T cell migration (CI, 1.295 ± 0.093; Fig. 5). To evaluate the relative contribution of
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). The inhibitory activity of the mAb was specific for IL-16, because migration toward recombinant MCP-3 was not inhibited.

To evaluate the influence of cellular activation on the chemotactic activity of B cell supernatant, we tested supernatant from anti-CD40/IL-4-stimulated B cells. This supernatant resulted in substantial directed migration of CD4+ T lymphocytes, as demonstrated in Table II. Addition of neutralizing anti-IL-16 mAb resulted in a 62% inhibition of migration (Table II). Similar results were obtained with supernatant from B lymphocytes stimulated with anti-IgM-coated microbeads (data not shown). To prove the activation state of B lymphocytes, we analyzed CD69 expression on anti-CD40/IL-4 treated B cells (data not shown).

**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.

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.

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**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.
B cell supernatant is chemotactic for CD8\(^+\) T cells independently of IL-16

To provide further evidence of the specificity of IL-16 we tested B cell supernatant for its chemotactic properties for CD8\(^+\) T lymphocytes (e.g., a CD4\(^-\) cell type). As shown in Table II, supernatant derived from unstimulated B cells as well as from anti-CD40/IL-4-stimulated B cells was chemotactic for CD8\(^+\) T lymphocytes. However, addition of neutralizing IL-16 mAb did not inhibit the migratory response (Table II).

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. Intracellular 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 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

<table>
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<th>Migrating Cell Type</th>
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<th>% Inhibition</th>
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<td>CD4(^+) T cells</td>
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<td>CD8(^+) T cells</td>
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<td>1.673 ± 0.062</td>
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* Purified monocyte-derived DCs, CD4\(^+\) T, 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.
and B lymphocytes leave their respective compartments, migrate with DCs in the T cell areas of lymphoid organs upon encountering Ag (26–28). At this time, B cells are still randomly distributed and provide evidence that IL-16 derived from B cells might be a major regulator of other chemokines (17, 23–25). Together, these data cytokine derived from B cells despite the activation-induced up-regulation of other chemokines (27). In the primary immune response, T cells form small clusters 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 61% for monocyte-derived DCs. This provides strong evidence that IL-16 remains the predominant chemotactic cytokine derived from B cells despite the activation-induced up-regulation of other chemokines (17, 23–25). Together, these data provide evidence that IL-16 derived from B cells might be a major factor in the cellular interactions required for the initiation of humoral immune responses.

In the primary immune response, T cells form small clusters with DCs in the T cell areas of lymphoid organs upon encountering of Ag (26–28). At this time, B cells are still randomly distributed throughout the follicles (26). B cells responding to Ag undergo arrest in the outer T cell zone and proliferate in response to ligation of a critical number of B cell Ag receptors (29). In a next step, T and B lymphocytes leave their respective compartments, migrate to the edge of the B cell-rich follicles and establish cognate T-B cell interactions (26), leading to the formation of GCs (29–31). Therefore, maturation of the B cell response depends on the availability of primed T cells and the ability of Ag-specific B cells to selectively recruit them. Recent evidence suggests that direct B cell-DC interactions are also a prerequisite for establishing humoral immunity (10–16, 32, 33). However, the accurate sequence of events in DC-B cell-T cell cooperation in vivo has yet to be established. This includes another distinct cell population implicated in B cell maturation, namely follicular DCs (34–37). The presence of GC DCs within primary follicles and the fact that they display a phenotype similar to CD4+CD11c+ blood DCs (20) suggest that DCs originating from blood colonize primary follicles. In line with this model are recent data showing the progressive localization of DCs to B cell follicles during a primary immune response (32). However, in that study migrating DCs where characterized as CD4+ by immunohistochemistry, but this could be due to the low sensitivity of this method compared with that of cytofluorometric analysis. The elucidation of factors involved in the migratory pathway of these cell populations is of critical interest (38). Our in vitro migration data are further corroborated by demonstrating IL-16 expression in B cells in lymph follicles by immunohistochemistry, suggesting an important role in vivo.

Although it has been established that Th/B cell interaction is required for the efficient elucidation of a humoral immune response, few reports have focused on the discovery of factors derived from B cells mediating the selective attraction of T cells (9). Schaniel et al. reported a novel chemokine, ABCD-1, derived from 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.

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