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Determinants of Human B Cell Migration Across Brain Endothelial Cells

Andrea Alter,† Martin Duddy,‡ Sherry Hebert,＊ Katarzyna Biernacki,＊ Alexandre Prat,＊ Jack P. Antel,＊ Voon Wee Yong, † Robert K. Nuttall,‡ Caroline J. Pennington,‡ Dylan R. Edwards,‡ and Amit Bar-Or,*

Circulating B cells enter the CNS as part of normal immune surveillance and in pathologic states, including the common and disabling illness multiple sclerosis. However, little is known about the molecular mechanisms that mediate human B cell interaction with the specialized brain endothelial cells comprising the blood-brain barrier (BBB). We studied the molecular mechanisms that regulate the migration of normal human B cells purified ex vivo, across human adult brain-derived endothelial cells (HBECs). We found that B cells migrated across HBECs more efficiently than T cells from the same individuals. B cell migration was significantly inhibited by blocking Abs to the adhesion molecules ICAM-1 and VLA-4, but not VCAM-1, similar to the results previously reported for T cells. Blockade of the chemokines monocyte chemoattractant protein-1 and IL-8, but not RANTES or IFN-γ-inducible protein-10, significantly inhibited B cell migration, and these results were correlated with the chemokine receptor expression of B cells measured by flow cytometry and by RNase protection assay. Tissue inhibitor of metalloproteinase-1, a natural inhibitor of matrix metalloproteinases, significantly decreased B cell migration across the HBECs. A comprehensive RT-PCR comparative analysis of all known matrix metalloproteinases and tissue inhibitors of metalloproteinases in human B and T cells revealed distinct profiles of expression of these molecules in the different cell subsets. Our results provide insights into the molecular mechanisms that underlie human B cell migration across the BBB. Furthermore, they identify potential common, and unique, therapeutic targets for limiting CNS B cell infiltration and predict how therapies currently developed to target T cell migration, such as anti-VLA-4 Abs, may impact on B cell trafficking. The Journal of Immunology, 2003, 170: 4497–4505.

Regulation of immune cell trafficking across endothelial barriers is central to physiologic immune surveillance and to the capacity of the immune system to mount appropriate immune responses in specific organ compartments. In turn, dysregulated trafficking has been implicated in pathologic states, including target-directed autoimmune diseases, such as multiple sclerosis (MS)1 (reviewed in Ref. 1). A pathologic hallmark of MS, which represents the major cause of neurological disability in young adults in North America, is the infiltration of immune cells across the endothelium of the blood-brain barrier (BBB) and into the CNS, at sites of myelin and axonal injury (2–4). Limiting the migration of immune cells into the CNS may therefore represent an important therapeutic strategy in MS and is predicated upon elucidating the molecular determinants of cell trafficking across the BBB.

Although research has largely focused on the contribution of infiltrating T cells, emerging animal and human studies have underscored the potential roles of B cells in the MS disease process (reviewed in Ref. 5). In the commonly used animal model of MS, experimental autoimmune encephalomyelitis (EAE), and in MS brains, Abs bound to their specific myelin targets have been identified within macrophages at sites of actively demyelinating lesions (6, 7). Also in EAE, B cells may be required for disease induction to whole protein Ags, consistent with the B cells’ unique ability (relative to T cells) to recognize antigenic conformation (8). Several recent studies in myelin oligodendrocyte glycoprotein-induced EAE point to a central role of B cell responses in this model (9, 10). Although T cells appear to be required for the initiation of inflammation, in the absence of B cells, inflammation occurs without demyelination and without neurological dysfunction. Thus, B cells represent an important effector limb of the myelin oligodendrocyte glycoprotein EAE process. Also of interest are recent studies identifying a role for B cell responses in regulating CNS inflammation (11).

In recent studies identifying distinct patterns of tissue injury within MS lesions, the most prevalent pattern was notable for a relative paucity of T cell infiltration, but the presence of a substantial deposition of Ig (Ab) and complement (12–14). Collectively, these findings implicate B cells and their responses in the immune pathology of MS. However, other studies have suggested that certain Ab responses may be beneficial by promoting remyelination (15), and B cells have further been shown to produce nerve growth factors that could support regeneration within the
Given the multiplicity of roles that B cells may play in CNS inflammation, it becomes important to understand the factors that regulate the trafficking of these cells into this compartment. However, little is known about the determinants of human B cell migration across endothelial barriers in general, and how circulating human B cells interact with the specialized endothelial cells of the BBB is essentially unexplored. Several principles of human immune cell migration across endothelial barriers have largely emerged from studies of T cells (reviewed in Ref. 17). Although it is reasonable to speculate that human B cell migration involves similar types of molecular interactions, given the complex anatomy of the immune system and the intricacies of immune regulation, it is unlikely that B and T cells share identical mechanisms of trafficking. Identifying differences between these cell types may provide important insights into both normal and aberrant immune responses, and could guide the development of safer and more refined therapies for a range of inflammatory conditions.

Successful migration of immune cells across endothelial barriers is generally thought to be mediated by the coordinated binding of adhesion molecules and chemokine/chemokine receptor interactions, as well as the actions of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) (reviewed in Ref. 18). Studies of high endothelial venules and HUVECs have contributed to our understanding that the initial tethering of immune cells is mediated by the selectin family of adhesion molecules (19–22). This is followed by rolling of the immune cell, mediated in part by the interaction of VLA-4 on the immune cell surface, with VCAM-1 on the endothelial cell (23). VLA-4 can also bind directly to fibronectin, a component of the basement membrane, through interaction with connecting segment (CS)-1 (24). Another adhesion interaction can be mediated by LFA-1, expressed on all leukocytes, binding with ICAM-1, which is expressed on endothelial cells, as well as on other immune cells (25). Selective and highly regulated expression of chemokines and chemokine receptors contributes to immune cell migration toward chemical gradients (26–29). Chemokine interactions at the stage of initial attraction and tethering can also regulate the activity of downstream integrins (30). Finally, the ability of immune cells to release a range of lytic enzymes such as MMPs further defines the ability of cells to penetrate across endothelial barriers and into the tissue parenchyma (reviewed in Refs. 31 and 32).

It is now established that CNS-derived endothelial cells differ biologically from other endothelial cells (such as high endothelial venules and HUVECs) with regard to gene expression profiles (33) and production of adhesion molecules, chemokines, and cytokines (34). In contrast to non-CNS barriers that constitutively express high levels of both the adhesion molecules VCAM-1 and ICAM-1, we and others have shown that human brain-derived endothelial cells (HBECs) (4) have reduced levels of expression of these adhesion molecules under basal culture conditions and represent a less permissive barrier to cell migration than HUVECs (34, 35). With regard to chemokine secretion, we recently showed that HBECs constitutively secrete the chemokines monocyte chemotactrant protein-1 (MCP-1) and IL-8 (36), but not RANTES or IFN-γ-inducible protein-10 (IP-10). We further found that secretion of RANTES and IP-10 from HBECs can be induced under inflammatory conditions. It is based on their unique biology that HBECs contribute to the ability of the BBB to regulate and restrict the passage of cells and soluble factors from the periphery to the CNS. Studies related to immune cell trafficking into the CNS compartment should therefore strive to use brain-derived endothelial cells, such as HBECs.

The limited studies to date on immune cell migration across HBECs have focused on T cells (reviewed in Ref. 17), and the mechanisms underlying B cell migration into the CNS have not been examined. Circulating human B cells are known to constitutively express the adhesion molecules VLA-4 and LFA-1 (37). The counterreceptors for these (VCAM-1 and ICAM-1, respectively) have been found up-regulated on BBB endothelial cells in MS lesions, at sites of B cell infiltration (38). Little is known about B cell chemoattraction at the level of the BBB. Likewise, although MMPs are known to be involved in CNS infiltration by immune cells (reviewed in Refs. 31 and 32), a comprehensive analysis of human B cell expression of MMPs is lacking, and their role in B cell migration across brain endothelial cells has not been examined.

The purpose of the current study was to evaluate the roles of adhesion molecules, chemokine/chemokine receptor interactions, and MMPs in the migration of circulating human B cells across the BBB. We analyzed the expression of these molecules in ex vivo human B cells and used a recently established Boyden chamber model of the BBB (39) to define the molecular mechanisms that mediate migration of these B cells across HBECs. We demonstrate that human B cells migrate across the brain endothelium more rapidly than autologous T cells. We also show that B cell migration is significantly and selectively inhibited by blocking Abs against the adhesion molecules VLA-4 and ICAM-1 (but not VCAM-1), the chemokines MCP-1 and IL-8 (but not IP-10 or RANTES), and TIMP-1, a natural inhibitor of many of the MMP family members (40). A comprehensive RT-PCR study of all known human MMPs and TIMPs identifies distinct profiles of expression in B cells, compared with T cells.

Materials and Methods

Cell isolation

Venous blood from normal adult volunteers was collected into EDTA-containing tubes, in accordance with the guidelines of the McGill Ethical Review Board (Montreal, Canada). PBMCs were isolated by Ficoll density centrifugation (Pharmacia Biotech, Uppsala, Sweden). CD19+ B cells were purified using the MACS system (Miltenyi Biotec, Auburn, CA), following the manufacturer’s protocol without modification. When required, T cells were subsequently isolated from the CD19-negative fraction using CD3 magnetic beads. Isolated cells were washed twice in medium to remove residual EDTA. Unless otherwise defined, the medium used was RPMI 1640 supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 µg/ml), and 2 mM L-glutamate (all from Sigma-Aldrich, St. Louis, MO). Cell viability was assessed by trypan blue exclusion and was invariably >98%. The efficacy of MACS purification was assessed by flow cytometry using triple staining (anti-CD3 FITC, anti-CD19 CyChrome (both from BD PharMingen, Mississauga, Ontario, Canada), and anti-CD14 PE (DAKO, Glostrup, Denmark)). The purity of B and T cell preparations was routinely ≥97%.

Measurements of B cell adhesion molecule and chemokine receptor expression

B cells were stained with anti-LFA-1 FITC or anti-VA-4 PE (BD PharMingen), and expression levels were examined by immunocytochemistry, and by flow cytometry using a BD FACScan. Cytometry data was analyzed using CellQuest software. For immunofluorescent microscopy studies, B cells were fixed (4% paraformaldehyde), blocked (HBSS supplemented with 1 mM HEPES, 2% horse serum, and 10% goat serum, all from Invitrogen Canada, Burlington, Ontario), and stained with anti-VA-4 Ab or isotype control (R&D Systems, Minneapolis, MN), followed by a cytochrome-3-conjugated polyclonal goat anti-mouse IgG and IgM Ab (Jackson ImmunoResearch, West Grove, PA). Stained cells were viewed with a Reichert Polyvar2 Leica fluorescence microscope and photographed on Kodak ektachrome P1600 film.

B cell chemokine receptor gene expression was analyzed by RNase protection assay (RPA). Ex vivo B cells were lysed in TRIZol (Invitrogen Canada), and total RNA was extracted. RPA using Riboquant hCR5 and hCR6 primers (BD PharMingen) was performed following the multiprobe RPA system protocol without modification. Gels were exposed to a phosphor imager and scanned. Results were analyzed by ImageQuant software. Flow cytometry was used to confirm protein expression of CXCR1 and...
CXC CR2. For this purpose, whole blood was stained using the BD PharMingen Whole Blood Staining Protocol without modification, and with fluorescent Abs directed against CXC R1 and CXC R2 (BD PharMingen).

**Migration assays**

Migration assays were conducted in Boyden chambers, as previously described (39). Briefly, each chamber (3-μm-pore-size membrane), precoated with fibronectin (BD PharMingen), was inserted into a well of a 24-well plate to create a two-compartment migration system. The membranes were overlaid with 2.5 × 10⁴ HBECs in a total volume of 500 μl endothelial cell medium (39). The HBECs were derived from temporal lobe specimens resected from young adults undergoing surgical treatment for intractable epilepsy. We have previously reported on the purity of these cultures that is consistently >98% (41). Migration assays were conducted 3 days later, at which time the HBECs formed a confluent layer over the fibronectin. On the day of migration, the endothelial cell medium was aspirated, and the chambers, containing the confluent HBEC/fibronectin layer, were transferred to new wells containing 500 μl of fresh medium. A total of 5 × 10⁵ ex vivo B cells was added to the top compartment in a total volume of 500 μl of medium (detailed above) and incubated at 37°C/5% CO₂. For comparative migration experiments, parallel wells were set up with 5 × 10⁵ ex vivo T cells. After 24 h, 50 μl of 0.5 mol/L EDTA was added to the bottom compartment to mobilize cells, and the plates were placed on a flatbed shaker for 15 min. Cells were collected from the bottom compartment, centrifuged, resuspended, and counted with a hemocytometer. For blower experiments, monoclonal anti-human ICAM-1, VCAM-1, MCP-1, IL-8, IP-10, and RANTES Abs (15 μg/ml; R&D Systems) were added to the top compartment of the Boyden chambers 1 h before migration. Mouse anti-human VLA-4 mAb (15 μg/ml; R&D Systems) and TIMP-1 (0.03 μg/ml; R&D Systems) were added to the top compartment of the Boyden chamber at the time of migration. Monoclonal mouse IgG1 (15 μg/ml; R&D System) was used as a control for all blocking experiments. Migration assays performed on HBECs preincubated with inflammatory cytokines (TNF-α, IFN-γ, or both) were conducted, as previously described (35).

**RT-PCR measurements of MMP and TIMP expression in immune cells**

RT-PCR and real-time PCR were used to measure the expression of 21 MMPs and all 4 known TIMPs, in ex vivo purified human B and T cells. Cell subsets were all purified, and total RNA was extracted, as above. The RT-PCR protocol and primers used were recently described (42). Briefly, 1 μg of total RNA was reverse transcribed using 2 μg random hexamers (Amersham Pharmacia Biotech, Buckinghamshire, U.K.) and Superscript II reverse transcriptase (Invitrogen, Paisley, U.K.). Fluorogenic probes for all human MMP and TIMP genes were designed using Primer Express 1.0 software (PE Applied Biosystems, Warrington, U.K.) and synthesized by Applied Biosystems (Foster City, CA). The 18S ribosomal RNA gene was used as an endogenous control; these primers and probes were purchased from Applied Biosystems. PCR were performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems), utilizing the manufacturer’s protocol. Each reaction was performed in 25 μl and contained the equivalent of 5 ng of reverse-transcribed RNA (1 ng RNA for the 18S analyses), 50% TaqMan 2× PCR Master Mix (Applied Biosystems), 100 nM each of the forward and reverse primer, and 200 nM of probe. Conditions for the PCR were 2 min at 50°C, 10 min at 95°C, and then 40 cycles, each consisting of 15 s at 95°C, and 1 min at 60°C. To determine the expression of mRNA levels within the samples, standard curves for the PCR were prepared by using the CDNA from one sample and making 2-fold serial dilutions covering the range equivalent to 20–0.625 ng of RNA (for 18S analyses, the range was from 4 to 0.125 ng). Standard curves for Cₜ (cycle threshold; the cycle at which amplification entered the exponential phase) vs input RNA were prepared, and relative levels of mRNA in each sample were determined. For samples with a Cₜ greater than 35, RNA levels were considered too low to be analyzed.

**Statistics**

All migration assays were performed in duplicate, with at least six independent replicate experiments. Data from comparative migration assays and blocking assays were analyzed using the Student’s paired t test. RT-PCR data were analyzed using the Wilcoxon-sum nonparametric test for paired data. Value of p < 0.05 was considered the threshold for defining statistical significance.

**Results**

**Human B cells migrate across HBECs more efficiently than T cells**

Most studies of immune cell migration across endothelial layers have focused on T cells, and there are essentially no reports on human B cell migration, nor on comparative studies to evaluate the relative migratory efficiency of different immune cell subsets. To address this, we freshly isolated human B and T cells from the circulation of normal donors and tested, in parallel, their capacity to migrate across HBECs. Fig. 1a, depicting flow cytometry images, shows that the purities of isolated B cells (upper row, CD19+/CD3-/CD14-) and T cells (lower row, CD3+/CD19-/ CD14-) were at least 97%. When these populations were assayed in parallel, B cells were found to migrate across the HBEC layer more efficiently than T cells (Fig. 1b; p = 0.008, n = 6).

In previous studies, we have shown that the addition of inflammatory cytokines (e.g., TNF-α) to the HBECs up-regulates their expression of costimulatory molecules and MHC class II and increases the subsequent permeability of the HBECs to soluble molecules (e.g., albumin), but does not increase the rate of transmigration of T cells (36, 41). To examine whether B cells behave differently in this regard, we compared the migration of ex vivo isolated human B and T cells across HBECs that were pretreated with either TNF-α, IFN-γ, or both, using concentrations known to activate the HBECs (41). We extended our previous observations and found that preincubation of HBECs with either or both inflammatory cytokines, at concentrations that increase HBEC permeability to soluble factors, does not significantly change either T or B cell migration (Table I). In subsequent experiments, we focused on the molecular interactions that mediate human B cell migration across brain endothelial cells (summarized in Table II).

**FIGURE 1.** Comparative migration of leukocyte subsets. a, Purity of isolated B and T cells as determined by FACS analysis. B and T cells isolated by MACS demonstrate greater than 97% purity (CD19 or CD3 positive, respectively). b, B cells demonstrate increased migration as compared with T cells. B and T cells were purified ex vivo from normal individuals and migrated in parallel across the HBECs. Histograms represent the average of six independent comparisons from six normal individuals. Error bars represent SEM (n = 6, p = 0.008).
Table I. B and T cell migration across HBECs pretreated with inflammatory cytokines

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>T Cells Migrated % (SD)</th>
<th>B Cells Migrated % (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cytokine</td>
<td>2.9 (0.6)</td>
<td>5.4 (1.1)*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3.3 (0.9)*</td>
<td>5.6 (1.3)*</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>3.3 (1.1)*</td>
<td>5.9 (1.2)*</td>
</tr>
<tr>
<td>TNF-α + IFN-γ</td>
<td>3.5 (1.4)*</td>
<td>5.6 (1.0)*</td>
</tr>
</tbody>
</table>

*With no added cytokine, B cells migrate more efficiently than T cells (n = 6; p = 0.036).

**B cell migration is mediated by VLA-4 interacting with fibronectin, but not with VCAM-1 on the HBECs**

Based on its established role in T cell migration (43), we examined whether VLA-4 mediated B cell migration across HBECs. The surface expression of VLA-4 on ex vivo B cells was confirmed by flow cytometry (Fig. 2a) and by immunofluorescence microscopy (Fig. 2b). When anti-VLA-4 mAb was added to the B cells during the assay, migration was significantly reduced (Fig. 2c; mean inhibition = 68%; p = 0.026, n = 7). Although it has been proposed that, in MS, T cell adhesion to the endothelium of the BBB is mediated in part through VLA-4/VCAM-1 interactions, we found that the addition of anti-VCAM-1 Abs did not inhibit B cell migration in our model (data not shown). We postulated that VLA-4 expressed on the B cells was interacting with its alternate ligand, the CS-1 fragment of fibronectin. To test this, we migrated B cells in the Boyden chamber across fibronectin alone (in the absence of the HBEC layer), and confirmed that VLA-4 blockade significantly inhibited B cell migration across fibronectin alone (Fig. 2d; p = 0.004). We also observed that B cells migrated more efficiently across fibronectin alone, than across the combination of HBECs and fibronectin. This is in keeping with prior reports of T cell migration in the same system (39), and confirms that the HBEC layer does pose a physical barrier to B cell migration.

LFA-1/ICAM-1 interactions are also known to be involved in T cell adhesion (25). We confirmed the presence of LFA-1 on B cells (Fig. 3a) and found that blocking ICAM-1 significantly reduced B cell migration across HBECs, although the effect was less marked than that seen with VLA-4 (Fig. 3b; p = 0.038, n = 8).

**MCP-1/CCR2 and IL-8/CXCR1 and CXCR2 interactions are implicated in the migration of human B cells across HBECs**

To study the potential of B cells to respond to HBEC-derived chemokines, we first used RPA to examine the chemokine receptor mRNA expression of ex vivo B cells. We demonstrated that B cells express CCR2a+b (Fig. 4a), the gene products for the chemokine receptors for MCP-1. The demonstration of functional expression of the CCR2 chemokine receptor on normal human B cells has been previously reported (44). HBECs are known to constitutively secrete MCP-1, as well as IL-8, in the resting state (36). Consistent with this, blocking of MCP-1 reduced migration modestly, but significantly, by a mean of 22% (Fig. 4b; p = 0.002, n = 6). Although, as shown in Fig. 4c, we were unable to detect mRNA for the known receptors for IL-8, CXCR1 (IL-8Rα), or CXCR2 (IL-8Rβ), blockade of IL-8 also inhibited B cell migration by a mean of 24% (Fig. 4d; p = 0.015, n = 6). We proceeded to identify the surface protein expression of both IL-8Rs on our ex vivo isolated B cells, shown in Fig. 4e. Our findings confirm prior reports of functional CXCR1 and CXCR2 expression on normal human B cells (45, 46). In contrast to the roles identified for MCP-1 and

Table II. Summary of blocking experiments

<table>
<thead>
<tr>
<th>Blocking</th>
<th>% Inhibition*</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>VLA-4</td>
<td>68</td>
<td>0.026</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>27</td>
<td>0.038</td>
</tr>
<tr>
<td>MCP-1</td>
<td>22</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-8</td>
<td>24</td>
<td>0.015</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>33</td>
<td>0.012</td>
</tr>
</tbody>
</table>

*Percent inhibition is calculated relative to migration with IgG control.
Contrary to previous views on the immune privileged status of the CNS, it is now appreciated that a low level of circulating immune cells, including B cells, migrates across the BBB in the normal state (47–49). The role of B cells in this physiologic immune surveillance is not fully understood, but it has been shown that, in the presence of intracerebral foreign Ag, specific B cells and plasma cells accumulate in the brain parenchyma and that their effector Abs participate in Ag clearance (50). Such findings suggest that the Ag-seeking behavior of B cells mimics that of T cells, as noted by Hickey (49) in his recent review of normal CNS immune surveillance. In addition to their role in CNS homeostasis, infiltrating B cells are increasingly implicated in CNS inflammatory states, such as MS, in which recent studies suggest that B cell responses may have the dual potential to contribute to CNS injury and/or protection (51–53). These observations underscore the importance of elucidating the mechanisms that regulate the migration of B cells across the specialized endothelial cells of the BBB.

In vivo, the BBB is a dynamic and integrated structure in which brain endothelial cells are intimately associated with other cell types, including pericytes, perivascular macrophages, and astrocytic foot processes (54). In prior work, we found that the addition of astrocyte-conditioned medium to HBECs decreased the permeability of soluble molecules across this barrier, but did not affect cellular migration (55). Although the BBB can never be fully simulated in vitro, the current study is the first to examine the molecular interactions involved in the migration of human B cells across HBECs. Our report on the expression profiles of chemokine receptors and MMPs in ex vivo human B cells is likely to be of relevance to the trafficking of these cells into non-CNS compartments as well.

Since the pivotal work by Hickey et al., which defined the low basal rate of immune cell entry into the CNS as part of normal immune surveillance, no studies have examined the relative migration efficiencies of immune cell subsets across this barrier. Our comparative migration studies show that ex vivo B cells migrate across HBECs more efficiently than T cells. Although it remains to be ascertained whether these in vitro findings indeed reflect a genuine hierarchy for migration of immune cell subsets across the resting BBB in vivo, it is likely that this differential migration reflects distinct expression profiles of adhesion molecules, chemokine receptors, and MMPs in each cell type. Defining the specific molecular interactions involved in the trafficking of selected immune cell subsets has important therapeutic implications. In the case of B cells, such insights may help to develop interventions that selectively target B cell-predominant autoimmune states, or B cell neoplasms. Furthermore, it may be possible to predict the impact of other immune interventions on human B cell trafficking, such as anti-VLA-4 therapy, currently in clinical trials of human autoimmune disease.

In this context, we confirmed the expression of VLA-4 on the surface of circulating human B cells and found that blocking VLA-4 significantly inhibited migration of these B cells across the HBEC/fibronectin barrier. Surprisingly, blocking VCAM-1 (the predicted VLA-4 ligand on HBECs) had no effect on migration. We hypothesized that VLA-4 on B cells was binding to its alternate ligand, the CS-1 fragment of fibronectin, present in our system as a proxy of the basement membrane. This was confirmed with the demonstration that VLA-4 blockade strongly inhibited the migration of B cells across fibronectin, in the absence of the HBEC layer. In contrast to the lack of inhibition seen with VCAM-1 blockade, ICAM-1 blockade significantly inhibited B cell migration across the HBECs, consistent with the demonstrated expression of its ligand, LFA-1, on the surface of ex vivo B cells. LFA-1/ICAM-1 interactions have been previously shown to be involved in T cell migration in the same model (39), and have also been implicated in the pathogenesis of MS (56).

Recent studies in murine systems highlight the roles of specific chemokine/chemokine-receptor interactions in controlling B cell
and CXCR2 is seen expressed on 12.1% of B cells. One of
CXCR1 (solid lines) and granulocytes (dashed lines, as positive controls). Corrected for appropriate isotype controls (lower panels). Corresponding histogram plots (left upper panel). CXCR1 (left upper panel) and CXCR2 (right upper panel). Corresponding histogram plots (lower panels) demonstrate chemokine receptor expression on B cells (solid lines) and granulocytes (dashed lines, as positive controls). Corrected for appropriate isotype controls (<2%), CXCR1 is seen expressed on 6.8% of B cells, and CXCR2 is seen expressed on 12.1% of B cells. One of five independent experiments is shown.

FIGURE 4. The role of chemokine/chemokine receptor interactions in B cell migration across HBECs. a, Chemokine receptor expression in ex vivo B cells from two individuals (1, 2). RPA data for chemokine receptor expression: positive expression of CCR2a + b, CCR2a, and CCR2b; negative expression of CCR1, CCR3, CCR4, and CCR5. GADPH used as control for loading. b, MCP-1 is involved in B cell migration. Each line represents a single experiment on purified B cells isolated from a normal individual and migrated across the combined HBEC + fibronectin barrier, in the presence of either the appropriate control Ab (IgG1) or a mAb against MCP-1 (α-MCP-1). Independent experiments on six different donors were performed. Anti-MCP-1 significantly inhibits migration across fibronectin + HBECs (mean migration IgG1, 7.4%; mean migration anti-ICAM-1, 5.8%; n = 6, p = 0.002). c, Chemokine receptor expression in ex vivo B cells from two individuals (1, 2). RPA data for chemokine receptor expression: positive expression of CXCR4, BLR-1, and BLR-2; negative expression of CXCR1, CXCR2, and CXCR3. GADPH used as control for loading. d, IL-8 is involved in B cell migration. Each line represents a single experiment on purified B cells isolated from a normal individual and migrated across the combined HBEC + fibronectin barrier, in the presence of either the appropriate control Ab (IgG1) or a mAb against IL-8 (α-IL-8). Independent experiments on six different donors were performed. Anti-IL-8 significantly inhibits migration across fibronectin + HBECs (mean migration IgG1, 7.4%; mean migration anti-ICAM-1, 5.6%; n = 6, p = 0.015). e, CXCR1 and CXCR2 expression by ex vivo B cells confirmed by FACS. Whole blood staining using anti-CD19 and anti-CXCR1 or anti-CXCR2 fluorescent Abs was performed on fresh blood samples obtained from normal donors. Representative whole blood analysis from a normal individual is shown. Dot plots (upper panels) demonstrate subpopulation of B cells (CD19+) expressing CXCR1 (left upper panel) and CXCR2 (right upper panel). Corresponding histogram plots (lower panels) demonstrate chemokine receptor expression on B cells (solid lines) and granulocytes (dashed lines, as positive controls). Corrected for appropriate isotype controls (<2%), CXCR1 is seen expressed on 6.8% of B cells, and CXCR2 is seen expressed on 12.1% of B cells. One of five independent experiments is shown.

Table III. Summary of MMP and TIMP profiling of B and T cells by quantitative RT-PCR

<table>
<thead>
<tr>
<th>Gene Expressed</th>
<th>B Cell Expression</th>
<th>T Cell Expression</th>
<th>B-T Cell Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-7</td>
<td>0.22</td>
<td>1.0</td>
<td>p = 0.54</td>
</tr>
<tr>
<td>MMP-8</td>
<td>1.75</td>
<td>1.0</td>
<td>p = 0.31</td>
</tr>
<tr>
<td>MMP-11</td>
<td>11.25</td>
<td>1.0</td>
<td>B &gt; T; p = 0.03</td>
</tr>
<tr>
<td>MMP-14</td>
<td>Detected</td>
<td>nd²</td>
<td></td>
</tr>
<tr>
<td>MMP-15</td>
<td>1.3</td>
<td>1.0</td>
<td>p = 0.06</td>
</tr>
<tr>
<td>MMP-17</td>
<td>39</td>
<td>1.0</td>
<td>B &gt; T; p = 0.03</td>
</tr>
<tr>
<td>MMP-23</td>
<td>0.38</td>
<td>1.0</td>
<td>p = 0.44</td>
</tr>
<tr>
<td>MMP-24</td>
<td>0.33</td>
<td>1.0</td>
<td>p = 0.63</td>
</tr>
<tr>
<td>MMP-25</td>
<td>nd²</td>
<td>Detected³</td>
<td></td>
</tr>
<tr>
<td>MMP-27</td>
<td>0.8</td>
<td>1.0</td>
<td>p = 0.84</td>
</tr>
<tr>
<td>MMP-28</td>
<td>0.02</td>
<td>1.0</td>
<td>B &lt; T; p = 0.03</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>0.19</td>
<td>1.0</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>3.29</td>
<td>1.0</td>
<td>p = 0.09</td>
</tr>
</tbody>
</table>

⁎ Genes tested, but not detected in either B or T cells: MMP-11, -2, -3, -9, -10, -12, -13, -16, -18, -19, -26 and TIMP-3 and -4.
* B cell expression is represented relative to T cell expression.
Detected: samples that had a cycle threshold less than 35 and could be detected, but could not be compared.
nd, Not detected, and refers to samples that had a cycle threshold greater than 35.

previouly shown that unstimulated HBECs constitutively secrete the chemokines MCP-1 and IL-8, but not IP-10 or RANTES (36). To identify candidate interactions for B cells, we studied the chemokine receptor expression of ex vivo human B cells by RPA and the effects of a panel of anti-chemokine Abs on the migration of these B cells across HBECs. In keeping with previous reports (44), trafficking into lymph nodes and Peyer’s patches (57), and between distinct zones within lymphoid organs (58). Little is known, however, about B cell trafficking at the level of the BBB.
we detected B cell mRNA expression of the MCP-1 receptors (CCR2, CCR2a, and CCR2b) and show that blocking MCP-1 significantly inhibited B lymphocyte migration in our system, as predicted by the known basal HBEC secretion of MCP-1 (36). Although blocking IL-8 also significantly inhibited migration, we could not detect mRNA expression of the known IL-8Rs, CXCR1 or CXCR2, in ex vivo B cells. This suggests that IL-8 is interacting with an alternate chemokine receptor, or that the known receptors are present at the protein level, but not actually transcribed in ex vivo B cells, or are expressed below our detection sensitivity. Our findings are consistent with previous reports implicating IL-8 in B lymphocyte chemotraction (46, 59) and serve to extend these reports to a model that incorporates an endothelial barrier. Of interest, blocking IL-8 has been reported to have no effect on the migration of T cells across HBECs (36), suggesting that IL-8 secretion by resting HBECs may preferentially facilitate B lymphocyte migration, perhaps representing a mechanism that accounts for our observation that B cells migrated more efficiently than T cells across this barrier.

In contrast to the effects of MCP-1 or IL-8 blockade, we observed no effect on B cell migration when either IP-10 or RANTES was blocked. This is not surprising given that resting HBECs do not express IP-10 or RANTES (36) and, furthermore, we could not detect B cell mRNA expression of CXCXR3 (receptor for IP-10) or CCR1, CCR3, CCR4, and CCR5 (receptors for RANTES). In keeping with previous reports (60–62), we found that ex vivo human B cells expressed CXCXR4 (the receptor for stromal cell-derived factor-1), BLR-1 (receptor for BLC), and BLR-2 (receptor for 6CK/SLC and macrophage-inflammatory protein-3β), which are believed to be involved in B cell homing to secondary lymphoid structures. The potential contribution of these chemokine receptors to B cell interaction with HBECs remains to be elucidated.

Members of the MMP family play important physiological roles in the final steps of immune cell migration by mediating invasion of the subendothelial basement membranes and the extracellular matrix (reviewed in Refs. 18 and 63). In addition, MMP dysregulation has been implicated in the pathogenesis of target-directed autoimmune disease, including MS (reviewed in Refs. 31 and 32). The potential role of MMPs in B cell migration across HBECs has not been ascertained. Indeed, to date, there have been no comprehensive studies of MMP expression in human B cells, nor has it been established whether circulating human B and T cells express identical, or distinct, profiles of MMPs. We first demonstrated a role for MMPs in B cell trafficking across HBECs by showing that TIMP-1, a relatively nonselective inhibitor of MMP, decreases B cell migration, as was previously shown for T cells in the same system (36). We next wished to determine whether B and T cells express distinct profiles of MMPs or TIMPs, and provide the first comprehensive analysis of the expression profiles of all known human MMPs and TIMPs in ex vivo human B and T cells. B cells were found to express higher levels of MMP-11 and MMP-17, whereas T cells expressed significantly higher levels of MMP-28. Interestingly, T cells also expressed higher levels of the MMP inhibitor, TIMP-1, which may serve to restrict the effects of T cell MMPs and may contribute to our demonstration that T cells migrate less efficiently than B cells. Further elucidation of how specific MMPs contribute to B and T cell migration will be possible as more selective MMP-blocking agents become available.

In summary, this study is the first to examine B cell migration across brain endothelial cells. A striking feature, given the focus on T cell trafficking in MS, is that human B cells migrate across the brain endothelium more rapidly than autologous T cells. We find that, similar to prior reports on T cell migration across HBECs (36, 39), B cell migration is significantly and selectively inhibited by blocking Abs against the adhesion molecules VLA-4, an observation that is of particular interest given the promising results of the recently published trial of Antegren (an anti-VLA-4 Ab) in MS (64). We also find significant inhibition of B cell migration by blocking ICAM-1 and the chemokine MCP-1 (but not IP-10 or RANTES). Of interest, IL-8 blockade, which did not inhibit T cell migration in the same system (36), significantly inhibited B cell migration across HBECs. This selective responsiveness of B cells to MCP-1 (known to be secreted from resting HBECs) may explain in part the enhanced capacity of B cells to migrate across the barrier relative to T cells, and may be relevant during the course of normal immune surveillance of the intact and noninflamed BBB. We further demonstrate a role for MMPs in the migration of human B cells across HBECs and provide the first comprehensive analysis of all known MMPs and TIMPs in human B and T cells, demonstrating distinct profiles of expression of these molecules in the two lymphocyte populations. Our findings provide insights into the general regulation of human B cell trafficking, with a focus on their participation in normal immune surveillance, as well as in inflammatory conditions, of the CNS. In addition, our results identify potential therapeutic targets for limiting CNS B cell infiltration.
and predict how current therapies directed at immune cell trafficking may impact on circulating B cells.

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References