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Cytokine Control of Memory B Cell Homing Machinery

Meenakshi P. Roy,2 Chang H. Kim, and Eugene C. Butcher 3

The germinal center (GC) is a pivotal site for the development of B cell memory. Whereas GC B cells do not chemotax to most chemokines and do not express the adhesion receptors L-selectin, α4β7, and cutaneous lymphocyte Ag (CLA), memory B cells respond to various chemotactic signals and express adhesion receptors. In this study, we show that CD40 ligand, IL-2, and IL-10 together drive this transition of GC B cells to memory phenotype in vitro, up-regulating memory B cell markers, chemotactic responses to CXCL12, CXCL13, and CCL19, and expression of adhesion receptors L-selectin, α4β7, and CLA. Moreover, addition of IL-4 modulates this transition, preventing chemotactic responses to CXCL12 and CXCL13 (but not to CCL19), and inhibiting the re-expression of L-selectin, but not of CLA or α4β7. CCR7 expression, responsiveness to CCL19, and L-selectin/α4β7 phenotype are coordinately regulated. Thus, IL-2/IL-10 and IL-4 play important and distinctive roles in developing the migratory capacities of memory B cells. The Journal of Immunology, 2002, 169: 1676–1682.

The germinal center (GC) is a pivotal site for the development of memory B cells (1). During the immune response to an Ag, mature B cells are activated by cognate interaction with activated Th cells. This takes place near the boundary between the T cell zone and the B cell follicles (2–4). Subsequently, these activated B cells form the GC. The developing B cells undergo a process of clonal selection and affinity maturation in the GC (5–8) before exiting as memory B cells (1, 9). Indeed, disruption of GC blocks the formation of B cell memory (10–13).

While they are in the GC, B cells lose their ability to migrate. The majority of GC B cells are L-selectin(14), and they are unable to bind to lymph node or Peyer’s patch high endothelial venules or to migrate from blood to lymph nodes or Peyer’s patches in vivo (15, 16). In addition, human GC B cells do not respond to CXC ligand (CXCL)12 in vitro (17). Memory B cells, in contrast, respond well to CXCL12 (17).

Naïve and memory lymphocytes differ fundamentally in the pathways they take through tissues. Naïve lymphocytes that have not encountered Ag are exported from primary lymphoid organs and show relatively homogenous recirculation through secondary lymphoid tissues (18, 19). In contrast, memory/effector lymphocytes generated in secondary lymphoid tissues in response to Ag are composed of distinct subsets with differential tissue-specific homing. This observed difference between the homing behavior of naïve and memory lymphocytes suggests that a shift in migratory phenotype takes place during the naïve to memory transition. In the case of B lymphocytes, this naïve to memory transition takes place in the GC.

In this study, we show that combinations of cytokines, including IL-2, IL-4, and IL-10, and costimulatory signals (CD40 ligand (CD40L)) act in concert to up-regulate the migration of GC B cells, and induce development of distinct memory-like B cell populations with different pathways of recirculation.

Materials and Methods

Cytokines, chemokines, and Abs

The following chemokines and cytokines were purchased from R&D Systems (Minneapolis, MN): IL-2, IL-10, and recombinant human B cell-attracting chemokine-1 (BCA-1; CXCL13). The following cytokines and chemokines were purchased from PeproTech (Rocky Hill, NJ): IL-4, human stromal cell-derived factor (SDF-1; CXCL12), and human CCL19 (EBV-induced molecule 1 ligand chemokine, also known as EB11-ligand chemokine (ELC) or macrophage-inflammatory protein-3β).

The following Abs were purchased from BD Pharmingen (San Diego, CA): FITC- and allophycocyanin-conjugated anti-CD19 (HIB19), unconjugated and FITC-conjugated anti-IgD (IgD-2), and anti-CD44 mAbs before depletion with Dynabeads coated with an Fc-specific human Ab against CD16, and CD56. Cells that bound the Abs were then depleted by two rounds of incubation with Dynabeads coated with a mixture of mouse Abs for CD2, CD3, CD7, CD14, CD16, and CD56. Cells that bound the Abs were then depleted by two rounds of incubation with Dynabeads coated with an Fc-specific human Ab against mouse IgG. This procedure led to >99% pure CD19+ B cells.

B lymphocyte purification

GC B cells were purified from human tonsils obtained from children undergoing routine tonsillectomy. Tonsillar mononuclear cells were obtained by standard Ficoll-Hypaque gradient method. Subsequently, CD19+ cells were obtained by negative selection using the B cell purification kit from Dynabeads (DynaBiotec, Great Neck, NY). Briefly, tonsillar cells were incubated with a mixture of mouse Abs for CD2, CD3, CD7, CD14, CD16, and CD56. Cells that bound the Abs were then depleted by two rounds of incubation with Dynabeads coated with an Fc-specific human Ab against mouse IgG. This procedure led to >99% pure CD19+ B cells. Purification of GC B cells was done by further incubating cells with anti-IgD and anti-CD44 mAbs before depletion with Dynabeads coated with
anti-mouse IgG (Dynal Biotech). The resulting population was >99% CD19+CD38+CD20+IgD+ GC B cells.

Cell lines
Dr. Y. J. Liu of DNAX (Palo Alto, CA) kindly provided CD40L transfected and untransfected L cells (20). Before each experiment, the levels of CD40L expression were confirmed by FACS analyses using FITC-conjugated anti-CD40L Ab. The low levels of CD40L on the transfectants (increased staining relative to untransfected L cells: 20% with a change in mean fluorescence intensity (MFI) of 8–10 on a log scale) correspond to previously reported physiological values (21).

In vitro generation of memory B cells
Purified GC B cells were differentiated into memory phenotype B cells in vitro, as described (20). In brief, CD40L-transfected L cells (expressing low levels of CD40L) were treated with mitomycin C (50 μg/ml). After extensive washing, the cells were then plated onto flat-bottom 48-well plates, at a density of 2 × 10⁵ cells/well. The cells were allowed to adhere for at least 3 h at 37°C. Purified GC B cells were then added to each well at a density of 2 × 10⁵ cells/well. Cultures were set up in the presence of RPMI 1640 medium supplemented with L-glutamine, 10% heat-inactivated FCS, 100 U/ml penicillin, 100 U/ml streptomycin, and 80 μg/ml gentamicin (Sigma-Aldrich, St. Louis, MO). Cytokines were added at concentrations of 100 ng/ml. B cells were cultured at 37°C in 5% CO₂ for 3 days, followed by 5 days of culture on freshly prepared CD40L transfectants.

Chemotaxis assays
After 8 days of culture, B cells were harvested. Cells were treated with 5 mM EDTA for 10 min at 37°C, to break up clumps. Chemotaxis assays were performed in 5-μm-pore Transwell inserts (Costar, Cambridge, MA), as described by Campbell et al. (22). Chemokines were used at the following concentrations: 5 μg/ml CXCL13, 1 μg/ml CXCL12, and 1 μg/ml CCL19. Chemotaxis proceeded for 3 h at 37°C. Cells were harvested from the wells after removal of the Transwell insert. A known number of beads (Polysciences, Warrington, PA) was added to each sample before analyzing by flow cytometry. Migrated cells were counted by scatter gating on lymphocytes.

Flow cytometry analyses
For GC B cell staining, ficollated tonsillar cells were stained with unconjugated Abs to L-selectin, CD21, CD44, and CD10. Alternatively, FITC-conjugated Abs to CD44 and B7-1 were added. After incubation, cells were washed and incubated with either FITC anti-mouse Ig or FITC anti-rat IgM. After washing, cells were blocked with 10% mouse or 10% rat serum. Subsequently, allophycocyanin-conjugated anti-CD19 and PE-conjugated anti-CD38 were added. For analysis, CD19+, CD38+, and IgD- cells (GC B cells) were gated on.

For memory B cell staining, cells were harvested after 8 days of culture and treated with 5 mM EDTA for 5 min to break up clumps. Cells were then incubated with appropriate unconjugated Abs. After incubation, cells were washed and stained with either FITC-conjugated anti-mouse Ig or FITC-conjugated anti-rat IgM. For analysis, the B cell population was distinguished from the residual L cells by forward and side scatter and CD19 staining. Expression of chemokine receptors was examined using unconjugated mAbs to CXCR7, CXCR4, or CXCR5 (or isotype-matched control mAbs), a biotinylated horse anti-mouse IgG secondary Ab (Vector Laboratories, Burlingame, CA), and streptavidin CyChrome or streptavidin allophtocoycin (BD PharMingen).

Results
GC B cells fail to migrate to chemokines or undergo desensitization; naive and memory B cells resensitize responses to CCL19 and CXCL13 after short-term incubation in vitro
Tonsillar naive, memory, and GC B cells were distinguished from each other by expression of IgD and CD38: naive cells are IgD+CD38-; memory B cells are IgD-CD38+; GC B cells are IgD-CD38- (33). We examined the chemokine receptor expression and chemotactic responses of these subsets to CCL19/ELC, CXCL12/SDF-1, and CXCL13/BCA (Fig. 1). Unlike freshly isolated naive and memory B cells, GC B cells do not migrate to CXCL12, as has also been shown by Bleul et al. (17). However, freshly isolated tonsillar naive and memory B cells also fail to migrate to CCL19 and CXCL13, despite high levels of expression of CXCR4 and CXCR5 (Fig. 1A). We reasoned that the poor chemotactic ability of freshly isolated tonsillar B cells could be due to desensitization. Indeed, when cultured for 16 h in vitro, naive and memory B cells re-expressed CCR7 (Fig. 1B, right panel) and migrated well to CCL19, CXCL12, and CXCL13 (Fig. 1A). This process of resensitization observed in naive and memory B cells could not be inhibited by cycloheximide, a protein synthesis inhibitor (not shown). In contrast, GC B cells do not migrate to any of these chemokines, even after incubation for 16 h in vitro.

In the presence of CD40L, responses of naive and memory B cells to CXCL13 were further up-regulated (Fig. 1A). However, GC B cells cultured overnight, either in medium alone or in the

**FIGURE 1.** GC B cells fail to migrate to chemokines. A, Freshly isolated tonsillar B cells were either assessed directly or cultured for 16 h at 37°C with or without CD40L. Cells were then added at 1 × 10⁵/well to Transwell inserts placed in wells containing the following chemokines at optimal chemotactic concentrations: CCL19 at 1000 ng/ml; CXCL13 at 5000 ng/ml; CXCL12 at 100 ng/ml. Migrated B cells were stained with PE-conjugated anti-CD38 and FITC-conjugated anti-IgD to distinguish among naive, memory, and GC B cells. Results are expressed as a percentage of total input, and error bars show the range of duplicates. Background migration has been subtracted. The results are representative of three independent experiments. B, Chemokine receptor expression of naive, memory, and GC B cells, freshly isolated or after a 16-h incubation with CD40L.
presence of CD40L as a survival signal, remained unable to respond to these chemokines (Fig. 1A) even though they re-expressed CCR7 (Fig. 1B, right panel).

Examination of CXCR4 and CXCR5 expression on these B cell subsets suggests that receptor expression is necessary, but not sufficient, for chemotaxis (Fig. 1). Thus, freshly isolated B cell subsets expressed high levels of CXCR4 and CXCR5, even though they do not chemotax to their ligands.

**IL-2 and IL-10 synergize to up-regulate chemotactic responses**

Both IL-2 and IL-10 have roles in B cell differentiation. IL-2 induces B cell proliferation (23, 24). IL-10 induces the proliferation and differentiation of activated B cells, and also stimulates the secretion of IgG by these cells (25–27). To determine whether these cytokines could play a role in restoring migratory responses of GC B cells, purified GC B cells were incubated with CD40L, in the presence of IL-2 or IL-10 or both. After 8 days, B cells were harvested and their chemotaxis tested (Fig. 2). Neither IL-2 nor IL-10 alone induced significant chemotaxis in CD40L-stimulated GC B cells. In contrast, GC B cells cultured with CD40L, IL-2, and IL-10 showed greatly increased chemotaxis to CXCL12, CCL19, and CXCL13. Thus, IL-2 and IL-10 synergize to up-regulate chemotaxis in CD40L-stimulated GC B cells. Interestingly, IL-2 and IL-10 also act synergistically to induce the transition of GC B cells (CD38<sup>high</sup>CD20<sup>low</sup>) to a memory-like B cell phenotype (CD38<sup>low</sup>CD20<sup>high</sup>) (20) (reproduced in experiments not shown). Thus, the combination of IL-2, IL-10, and CD40L appears to reproduce a complex developmental program similar to that shown). Thus, the combination of IL-2, IL-10, and CD40L appears to reproduce a complex developmental program similar to that involved in GC to memory cell differentiation in vivo.

**IL-4 selectively suppresses the effects of IL-2 and IL-10**

IL-4 and IL-10 play important roles in B cell function and in the humoral immune response (28–30). The following studies had two aims: first, to compare the role of these cytokines in determining chemokine responsiveness and adhesion receptor phenotype of B cells making the transition from GC to memory phenotype; and second, to assess the respective contributions of CD40L stimulation and of cytokines added.

Purified GC B cells were incubated with CD40L transfectants with or without IL-4. After 8 days, cells were harvested, and their ability to chemotax to CXCL12, CCL19, and CXCL13 was tested (Fig. 3A). Cultured GC B cells with CD40L and IL-4 did not significantly alter their ability to respond to any of the chemokines tested. Although IL-4 did not stimulate GC chemotactic ability, we reasoned that it may modulate the inductive effect of IL-2 and IL-10. Therefore, we incubated GC B cells with all three cytokines and IL-4 selectively suppressed chemotactic responses of GC B cells developed in the presence of CD40L and IL-2 and IL-10. Under these conditions, chemotaxis to CXCL12 and CXCL13 is decreased, without affecting chemotaxis to CCL19 (Fig. 3B and Table I).

Addition of IL-2 and IL-10 increased the intensity of chemokine receptor staining with a concomitant increase in chemotactic response (Figs. 1 and 4 and Table I). As shown in Figs. 1 and 4, CCR7 is expressed at very low levels on both fresh GC B cells (Fig. 1) and GC B cells cultured with CD40L alone for 8 days (Fig. 4), and is up-regulated on the addition of IL-2 and IL-10 (Fig. 4). This is in contrast to CXCR4 and CXCR5, which are expressed at

**FIGURE 2.** IL-2 and IL-10 up-regulate chemotactic responses synergistically. Purified GC B cells were cultured with CD40L transfectants at a density of 2 × 10<sup>5</sup>/well. IL-2 and/or IL-10 were added at concentrations of 100 ng/ml each. After an 8-day incubation, cells were harvested and treated with EDTA to break clumps. Cells were then added at 1 × 10<sup>7</sup>/well to Transwell inserts placed in wells containing the following chemokines: CCL19 at 1000 ng/ml; CXCL13 at 5000 ng/ml; CXCL12 at 100 ng/ml. B cells were migrated for 3 h. Results are expressed as a percentage of total input, and error bars show the range of duplicates. Background migration has been subtracted. The results are representative of 10 independent experiments.

**FIGURE 3.** IL-4 suppresses the effect of IL-2 and IL-10. A, IL-4 does not induce substantial migration. B, IL-4 suppresses migration to CXCL13 and SDF, but not to CCL19. GC B cells were cultured with CD40L and either IL-2 and IL-10 or IL-2, IL-4, and IL-10. Data in both panels represent results from the same experiment. Purified GC B cells were cultured with CD40L transfectants at a density of 2 × 10<sup>5</sup>/well. Cytokines were added as indicated at concentrations of 100 ng/ml. After an 8-day incubation, cells were harvested and treated with EDTA to break clumps. Cells were then added at 1 × 10<sup>7</sup>/well to Transwell inserts placed in wells containing the following chemokines: CCL19 at 1000 ng/ml; CXCL13 at 5000 ng/ml; CXCL12 at 100 ng/ml. B cells were migrated for 3 h. Results are expressed as a percentage of total input, and error bars show the range of duplicates. Background migration has been subtracted. Results are representative of 10 independent experiments. **+, These differences were statistically significant for SDF and BCA, but not for ELC using Student’s t test with a cutoff of p < 0.05.**
high levels on all these cell types (Figs. 1 and 4 and Table I) (although their expression is further up-regulated on cells cocultured with IL-2 and IL-10). Thus, CCR7 is regulated differently from CXCR4 and CXCR5. Importantly, the addition of IL-4 to GC B cells cultured with CD40L, IL-2, and IL-10 suppressed expression of CXCR4 and CXCR5 by >5-fold. This is consistent with the decreased chemotaxis of these cells to CXCL12 and CXCL13 (Fig. 3 and Table I). Similarly, expression of CCR7 was decreased 2- to 3-fold when IL-4 was added to cultures containing IL-2 and IL-10 (Figs. 1 and 4 and Table I). In general, chemokine receptor levels correlated with chemotactic ability only in cultures with added cytokines. Thus, GC B cells cultured with CD40L alone, without added cytokines, expressed both CXCR4 and CXCR5. However, these cells did not respond to either CXCL12 or CXCL13. Relative changes in chemokine receptor levels of B cell cultures with added cytokines are predictive of chemotactic ability of these cells. Thus, a combination of costimulatory signals and cytokines is necessary for reconstituting the migratory ability of memory B cells (summarized in Table I).

**Table 1. Summary of chemokine receptor expression and chemotaxis data**

<table>
<thead>
<tr>
<th>Chemokine</th>
<th>GC B Cells Cultured With CD40L/IL-2, 10</th>
<th>CD40L/IL-4</th>
<th>CD40L/IL-2, 4, 10</th>
<th>CD40L alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR7 (ELC)</td>
<td>No change</td>
<td>++</td>
<td>++</td>
<td>No change</td>
</tr>
<tr>
<td>CXCR4 (SDF)</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CXCR5 (BCA)</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Chemotaxis to CCL19 (ELC)</td>
<td>No change</td>
<td>++</td>
<td>No change</td>
<td>++</td>
</tr>
<tr>
<td>CXCL12 (SDF)</td>
<td>No change</td>
<td>++</td>
<td>No change</td>
<td>+</td>
</tr>
<tr>
<td>CXCL13 (BCA)</td>
<td>No change</td>
<td>+</td>
<td>No change</td>
<td>+</td>
</tr>
</tbody>
</table>

Data for chemokine receptor expression were obtained by setting the MFI of staining of CCR7 on GC B cells as a standard. Alternatively, chemotaxis of GC B cells to CCL19 (ELC) was used as a standard for chemotaxis data. Results are representative of six independent experiments.

**Discussion**

These studies provide the first direct demonstration that cytokines may differentially control the migratory ability of memory B cells during the GC to memory B cell transition. Moreover, IL-2/IL-10 and IL-4 have distinct and often opposing effects on the migratory ability of memory B cells. The combination of IL-2 and IL-10 up-regulates responses of memory B cells to CCL19 and CXCL13 as well as to CXCL12, and also up-regulates expression of the adhesion receptors L-selectin and α4β7, Thus, IL-2 and IL-10 may play a critical role in multiple aspects of the developmental program of GC to memory B cell differentiation and may be required for GC B cells to regain migratory competence. IL-4, in contrast, inhibits the IL-2/IL-10-induced chemotactic responses to CXCL13.
Subsets were phenotypically similar, except for their differential from GC B cells when they mature. Thus, all memory-like B cell cultured B cells had down-regulated CD38, a marker that is lost memory/activated B cell markers (CD44, B7-1). In addition, all cultures had lost GC markers (CD10) and had up-regulated of cells. After an additional 3–8 days in culture, GC B cells exhibit reduced CD38 and CD20, but no additional expansion added cytokines (data not shown). After 3 days in culture, GC B cells stimulated through CD40L in the presence or absence of cytokines showed decreased apoptosis, irrespective of the cytokines investigated in this study.

GC B cells stimulated through CD40L in the presence or absence of cytokines showed decreased apoptosis, irrespective of added cytokines (data not shown). After 3 days in culture, GC B cells exhibit reduced CD38 and CD20, but no additional expansion of cells. After an additional 3–4 days in culture (6–8 days total), all cultures had lost GC markers (CD10) and had up-regulated memory/activated B cell markers (CD44, B7-1). In addition, all cultured B cells had down-regulated CD38, a marker that is lost from GC B cells when they mature. Thus, all memory-like B cell subsets were phenotypically similar, except for their differential expression of adhesion markers and chemokine receptors (summarized in Tables I and II). Cultures with added IL-2 and IL-10 or IL-2, IL-4, and IL-10 showed 3- to 4-fold expansion in numbers at the end of the culture period (6–8 days). Thus, cultures with IL-2/IL-10 show similar expansion to cultures with IL-2, IL-4, and IL-10 but show disparate ability to chemotax. Cultures with CD40L alone, or CD40L and IL-4, showed 1- or 2-fold increase in numbers, respectively.

The kinetics of GC to memory differentiation observed in vitro (6–8 days) parallels that of in vivo GC formation and memory induction. Previous studies have shown that the initial transition of naïve B cells to GC to memory in vivo takes 6–8 days (35, 36). However, memory generation after primary Ag response is inefficient. The in vitro system used in this study may more closely mimic secondary immune responses, because we are starting with well-established GC. Even in this case, in vivo responses and generation of memory are thought to take 6–10 days after stimulation (35). So the kinetics in vitro is consistent with that in vivo, to the extent that it is known.

![Image](https://www.jimmunol.org/)

**FIGURE 5.** IL-2/IL-10 and IL-4 have distinct and interactive effects on adhesion receptor expression. Purified GC B cells were cultured with CD40L transfectants at a density of 2 × 10^5/well. Cytokines were added as indicated at concentrations of 100 ng/ml. GC B cells were cultured with CD40L and IL-2 and IL-10 (CD40L/IL-2, -10); IL-4 (CD40L/IL-4); or IL-2, IL-4, and IL-10 (CD40L/IL-2, -4, -10); or without added cytokines (CD40L alone). After an 8-day incubation, cells were harvested and stained with anti-L-selectin (DREG-56) and anti-CD21 (B-ly4), followed by a FITC-conjugated secondary Ab. For α_β_ and CLA expression, cells were stained with PE-conjugated anti-α_β_ (Act-1) and FITC-conjugated anti-CLA (clone 452). The dotted lines represent binding of isotype control. Live cells were selected using a forward and side scatter and CD19 staining. Adhesion receptor-staining values are shown as the difference in mean intensity of staining vs isotype control. Results are representative of six different experiments.

### Table II. Summary of adhesion receptor expression

<table>
<thead>
<tr>
<th>Homing</th>
<th>GC B Cells</th>
<th>CD40L/IL-2, 10</th>
<th>CD40L/IL-4</th>
<th>CD40L/IL-2, 4, 10</th>
<th>CD40L alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptors</td>
<td>L-selectin</td>
<td>No change</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>CD21</td>
<td>+++</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>α_β_</td>
<td>−</td>
<td>+</td>
<td>No change</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CLA</td>
<td>No change</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

*Data were obtained by setting the MFI of staining of L-selectin on GC B cells as a standard. Figures were obtained by normalizing MFI of staining against this standard. +, A 2- to 5-fold increase in MFI; ++, a 5- to 20-fold increase in MFI; ++++, a 20- to 100-fold increase in MFI; −, a 2- to 10-fold decrease in MFI; −−, a 10- to 100-fold decrease in MFI; No change, no significant difference over standard (<2-fold change from the standard MFI). Results are representative of six independent experiments.*
Arpin et al. (20) have shown that the memory-like B cells derived in these cultures can proliferate on reculture with CD40L, IL-2, and IL-10. Thus, the low CD38lowCD20low cells generated have some features of memory B cells: phenotype, ability to respond to proliferative signals, low levels of intracytoplasmic and secreted Ig, and ability to chemotax. In contrast, there are indications that they represent a transitional stage in memory development rather than mature “memory” B cells, as exemplified by their blast morphology and the coexpression of adhesion receptors αβ and CLA. Both these features are uncharacteristic of mature memory B cells (34) and indicate that there may be additional signals required for memory development.

Most mature B lymphocytes can recirculate through both the lymph and blood, and are able to enter the lymphatic follicles of both spleen and lymph nodes. Recent studies have described a CD21−L-selectin− subset of B cells that are excluded from the lymphatic recirculation pathway and that migrate preferentially through the peripheral blood and spleen (37–39). Memory-like B cells developed in vitro in the presence of IL-4 resemble this CD21−L-selectin− population described in sheep (37, 38). In general, the L-selectin− phenotype confers increased homing to the spleen. Thus, splenic lymphocyte entry does not require L-selectin (40, 41), and lymphocytes from L-selectin knockout mice show increased homing to the spleen (42, 43). Our results lead to the hypothesis that IL-4 may be involved in the development of this L-selectin−CD21− subset of B cells.

T cells in the human GC are CXCR5+ and CD57+ and have been shown to produce IL-2, IL-4, and IL-10, among other cytokines (44). In fact, IL-10 is more efficiently provided by the CD57+ T cells in the apical light zone of GC than by CD57− T cells outside of GC (44). There are few IL-4-producing cells in lymphoid tissues. Only 1% of GC T cells produce IL-4, compared with 2–4% of circulating memory T cells, and even higher levels are expressed at sites of inflammation (45). T cells outside of GC or follicles are even less efficient in producing IL-4 than GC T cells. These data suggest that the cytokine milieu in and around GC is heterogeneous, but that GC B cells localized in the apical light zone of GC may get more IL-10 signal (in addition to IL-2 and CD40L). Our results suggest that these signals may be required for GC B cells to gain the migratory machinery to exit the GC and recirculate. In addition, IL-4 may become more important at sites of inflammation during Th2-oriented immune responses.

IL-4 may exert its suppressive effect by inhibiting the effect of IL-2. IL-2 promotes the proliferation and differentiation of B cells (23, 46–48). IL-4 has been shown to suppress this IL-2-driven process (24, 49–51). Specifically, IL-4 blocks the up-regulation of IL-2Rs (high and low affinity), induced by IL-2 on normal human B cells (52). This down-regulation of IL-2Rs may be partly due to the accelerated endocytosis of IL-2R p75 by IL-4 (53). Our studies show that IL-2 is essential in up-regulating chemotactic ability of GC B cells. IL-4 may act by blocking the effect of IL-2.

The ability to respond to CCL19 may be important for redistribution within and potentially for exit from lymphoid tissues. CCL19 is expressed in the T cell areas of lymphoid follicles, by dendritic cells, macrophages, and some nonhemopoietic cells. The expression of CXCL13, in contrast, is restricted to stromal cells in the B cell areas of the follicles (reviewed in Ref. 54). Thus, the ability to respond to CCL19 vs to CXCL13 may help determine the movement and/or localization of cells in the T or B cell areas of secondary lymphoid tissues. Addition of IL-4 to cultures containing IL-2 and IL-10 does not suppress the induced response to CCL19. Thus, memory-like B cells developed in the presence of IL-2 and IL-10, with or without IL-4, can respond to CCL19. This implies that these memory B cells may be able to move away from the B cell areas into the T cell areas. These cells may then eventually exit via efferent lymphatics in the T cell areas.

CD40 stimulation of GC B cells is necessary to prevent their apoptosis and promote survival (55). CD40L-deficient mice do not develop GCs (12, 13), and the administration of anti-CD40L Ab can block both GC formation and dissolve established GCs (11). Brandes et al. (56) have shown that short-term stimulation through CD40 can transiently increase the responses of mature B cells to chemokines. Their studies were done with CD19+ B cells, without separation into naive or GC B cells. We describe the effects of low levels of CD40 stimulation on purified GC B cells and further show differential resensitization among naive, memory, and GC B cells. Our studies show that long-term stimulation with low levels of CD40L alone is not sufficient for the development of memory B cells that are able to respond to chemokines. The addition of cytokines as well as the dose of CD40L are important for this process. Under high levels of CD40 stimulation, cells are less responsive to modulation by added cytokines.

B cells developed in vitro in the presence of IL-4 lack receptors that are involved in trafficking to lymph nodes, but express adhesion receptors (αβ, CLA) that may enable homing to extralymphoid and mucosal sites. We conclude that a complex combination of cytokines and costimulatory signals within the GC determines the migratory properties of memory B cells, and that within this complex environment IL-2, IL-10, and CD40L may play essential permissive roles, and IL-4 a regulatory role in the re-expression of migratory competence and specific homing properties by GC B cells (summarized in Tables I and II).

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References


