

CD11b-mediated migratory property of peripheral blood B cells

Kazushige Kawai, MD,^a Nelson H. Tsuno, MD,^{a,b} Mika Matsuhashi,^b Joji Kitayama, MD,^a Takuya Osada, MD,^a Jun Yamada, MD,^a Takeshi Tsuchiya, MD,^a Satomi Yoneyama, MD,^a Toshiaki Watanabe, MD,^a Koki Takahashi, MD,^b and Hirokazu Nagawa, MD^a

Tokyo, Japan

Background: CD11b belongs to the integrin family and is expressed on neutrophils, monocytes, natural killer cells, and a subset of lymphocytes. Although CD11b expressed on neutrophils and monocytes has been extensively investigated and has been reported to play an important role in the migration of these subsets of leukocytes, the function of CD11b expressed on a subset of B cells has not yet been clarified. **Objective:** To elucidate the functional activity of CD11b expressed on B cells, we characterized the CD11b-expressing cells among the B-cell population and investigated their migratory ability.

Methods: Isolated peripheral blood CD19⁺ B cells were analyzed by flow cytometry. The migratory ability of B cells was evaluated by the transwell assay, and the contribution of CD11b to this ability was investigated by using an anti-CD11b blocking mAb.

Results: The majority of CD27[−]IgD⁺ naive B cells were CD11b[−], whereas most CD27⁺ memory cells were CD11b⁺. Among the CD27⁺ memory cells, expression of CD11b was stronger on the IgD[−] cells than on the IgD⁺ cells. In the transwell assay, the migrating cells were predominantly CD27⁺IgD[−] cells, most of which expressed CD11b. The addition of an anti-CD11b blocking mAb resulted in the significant reduction of the number of migrating B cells. **Conclusion:** Memory B cells express CD11b and, in contrast with naive B cells, have high migratory ability. CD11b plays an essential role in the homing process of memory cells. (J Allergy Clin Immunol 2005;116:192-7.)

Key words: B cell, peripheral blood, CD11b, migration

CD11b is a member of α -chain integrin and forms a complex with β 2-integrin as Mac-1. CD11b is expressed on neutrophils, monocytes, natural killer cells, and a subset of lymphocytes.¹ CD11b expressed on neutrophils and monocytes has been extensively investigated and

Abbreviations used

BLC: B-lymphocyte chemoattractant
cB: Circulating B cell
CXCR: C-X-C chemokine receptor
FITC: Fluorescein isothiocyanate
PE: Phycoerythrin
PE-Cy5: Phycoerythrin–cyanin 5
SDF-1 α : Stromal cell–derived factor 1 α

reported to play a central role in the migration of leukocytes from peripheral blood to sites of inflammation during the process of host defense.^{1,2} CD11b contributes to firm leukocyte adhesion, not only to the endothelium via intercellular adhesion molecule 1, but also to the underlying subendothelium and interstitial extracellular matrix by binding diverse kinds of ligands, such as fibronectin, collagens, and laminins.³⁻⁷ Their role in the migration of lymphocytes has been less clarified, but in a previous report, we demonstrated that CD11b expressed on CD8⁺ T cells also plays an essential role in cell migration.⁸ Although some peripheral blood circulating B cells also express CD11b,^{9,10} their role in the migration of B cells and the characteristics of this CD11b-expressing subset have not been fully investigated.

The human peripheral blood circulating B cells are classified into 3 subpopulations on the basis of the expressions of CD27 and IgD: CD27[−]IgD⁺ naive B cells (circulating B cell [cB]–1), CD27⁺IgD⁺ memory B cells (cB2), and CD27⁺IgD[−] memory B cells (cB3).¹¹⁻¹⁴ Between these 2 memory B cell subsets, cB3 cells are the classic memory B cells, which have switched from the initial expression of IgM to that of other immunoglobulin subclasses, resulting in surface expression of IgG, IgA, or IgE. On the other hand, although cB2 carries somatic hypermutation, indicating that this population consists of memory cells, it has been reported to express IgM, and has characteristics and gene expression distinct from the ordinary cB3.^{13,14} Weller et al¹⁴ have reported that these cells are circulating splenic marginal zone B cells.

In the current study, we clearly demonstrated that CD27⁺ memory B cells expressed high levels of CD11b, whereas naive B cells expressed low levels. Furthermore, we demonstrated that the expression of CD11b also plays a pivotal role in the migratory properties of B cells.

From ^athe Department of Surgical Oncology and ^bthe Department of Transfusion Medicine, Faculty of Medicine, University of Tokyo.

Supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Ministry of Health, Labor and Welfare of Japan.

Received for publication January 17, 2005; revised March 16, 2005; accepted for publication March 17, 2005.

Available online May 2, 2005.

Reprint requests: Kazushige Kawai, MD, Department of Surgical Oncology, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.

E-mail: kz-kawai@mvd.biglobe.ne.jp.

0091-6749/\$30.00

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doi:10.1016/j.jaci.2005.03.021

METHODS

Reagents and antibodies

The following antibodies coupled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), or tandem dye PE-cyanin 5 (PE-Cy5) were used for flow cytometry: FITC-anti-CD19, FITC-anti-CD27, FITC-anti-IgD, FITC-anti-IgM, FITC-anti-CD5, FITC-anti-CD25, PE-anti-CD11b, and PE-Cy5-anti-CD11b mAbs were obtained from BD Pharmingen (San Diego, Calif), and FITC-anti-C-X-C chemokine receptor (CXCR) 4 and FITC-anti-CXCR5 were obtained from R&D Systems (Minneapolis, Minn). Purified anti-CD11b blocking antibody (preservative-free, mouse IgG1, clone ICRF44) and purified anti-CD19 mAb (preservative-free, mouse IgG1, clone HIB19) were purchased from BD Pharmingen, and the chemokines stromal cell-derived factor 1 α (SDF-1 α) and B-lymphocyte chemoattractant (BLC) were from R&D Systems.

Isolation of peripheral blood CD19⁺ B cells

CD19⁺ B cells were obtained from venous blood drawn from normal healthy volunteers, as described previously,¹⁵ after informed consent. Briefly, PBMCs were isolated by centrifugation on a Ficoll-Paque density gradient (Amersham Pharmacia, Uppsala, Sweden). CD19⁺ B cells were obtained by using a magnetic cell separation system (Miltenyi Biotec, Bergish Gladbach, Germany). For this purpose, PBMCs were incubated with microbeads-coupled anti-CD19 mAb, and the magnetically labeled cells were obtained by positive selection. The purity of the CD19⁺ B cells used in the experiments was more than 97%, as confirmed by flow cytometry (data not shown). All of the following experiments were repeated by using samples from at least 3 different donors, and the representative one was presented.

Detection of cell surface molecules by flow cytometry

Isolated PBMCs or B cells were washed twice with PBS and used for the analysis of cell surface molecules. For 2-color staining, cells were stained with FITC-conjugated and PE-conjugated mAbs. For 3-color staining, cells were incubated with FITC-conjugated, PE-conjugated, and Fc receptor blocking mAbs (Miltenyi Biotec), followed by staining with PE-Cy5-conjugated mAb. Fc receptor blocking mAb was used because PE-Cy5 has an affinity for the Fc receptor expressed on B cells. Data were collected in a FACS Calibur (Becton Dickinson, Mountain View, Calif) and analyzed by using the CellQuest software (Becton Dickinson).

Migration assay

The cell migration assay was conducted by using transwell migration chambers (5- μ m pore size; Corning, Tokyo, Japan). Isolated CD19⁺ B cells were suspended in 0.2% BSA/RPMI, and 2×10^5 cells were loaded to the upper chamber. The lower chamber contained the same medium without or with SDF-1 α (100 ng/mL) or BLC (500 ng/mL) as the chemoattractant. In some experiments, anti-CD11b mAb (clone ICRF44, 20 μ g/mL) or anti-CD19 mAb (clone HIB19, 20 μ g/mL) was added in the upper chamber to evaluate the effect of the blocking antibody. Cells were allowed to migrate to the lower chamber for 4 hours. After the upper chamber was removed, EDTA was added to the lower chamber to give the final concentration of 0.02%, and cells remaining in the lower chambers were recovered. The number of migrating cells was evaluated by counting cells for 60 seconds in the FACS Calibur. Alternatively, to evaluate the percentage of CD11b⁺ cells or that of cB1, cB2, and cB3, migrating cells were stained with FITC-conjugated or PE-conjugated mAbs, as described, and analyzed in the FACS Calibur.

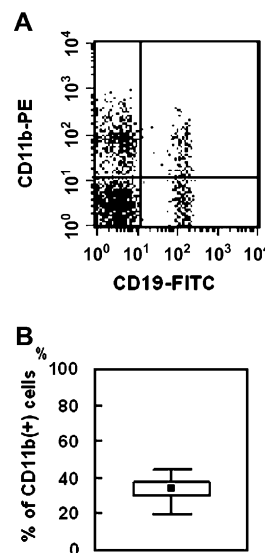


FIG 1. A subset of peripheral blood B cells expresses CD11b. **A**, Flow-cytometric analysis of PBMC. X-axis represents CD19, and Y-axis, the CD11b expression. The representative dot-plot from 12 independent samples is presented. **B**, The percentage of CD11b⁺ B cells.

Statistical analysis

The unpaired Student *t* test was used to determine statistical significance. Differences at *P* < .05 were considered statistically significant.

RESULTS

CD11b⁺ B-cell subpopulations

We studied the populations of circulating human B cells by using double-color immunofluorescence staining. Approximately $\frac{1}{3}$ of peripheral blood B cells were found to be CD11b⁺ by CD19/CD11b staining, as shown in Fig 1, A. Then we purified CD19⁺ B cells from PBMCs of 12 healthy volunteers and determined the proportion of CD11b cells among them (Fig 1, B). The age of donors ranged from 26 to 58 years. The percentage of CD11b⁺ cells among the B-cell population ranged from 19.5% to 44.1%, with a median of 34.2%.

Cell surface molecules

To clarify the differences between CD11b⁺ and CD11b⁻ B-cell subpopulations, the expression of cell surface molecules was analyzed (Fig 2). As shown in Fig 2, the majority of CD11b⁻ peripheral blood B cells were CD27⁻ and IgD⁺ (91.0% and 91.3%, respectively). Among the CD11b⁺ cells, 44.1% were CD27⁺, and 49.6% were IgD⁺, both showing a 2-peaked expression pattern. These data suggested that CD11b⁻ cells were mainly cB1 naive B cells, and CD11b⁺ cells consisted of cB1, cB2, and cB3 subsets. According to the other cell surface molecules, a large portion of CD11b⁻ B cells were IgM⁺ (66.2%), whereas the IgM expression on CD11b⁺

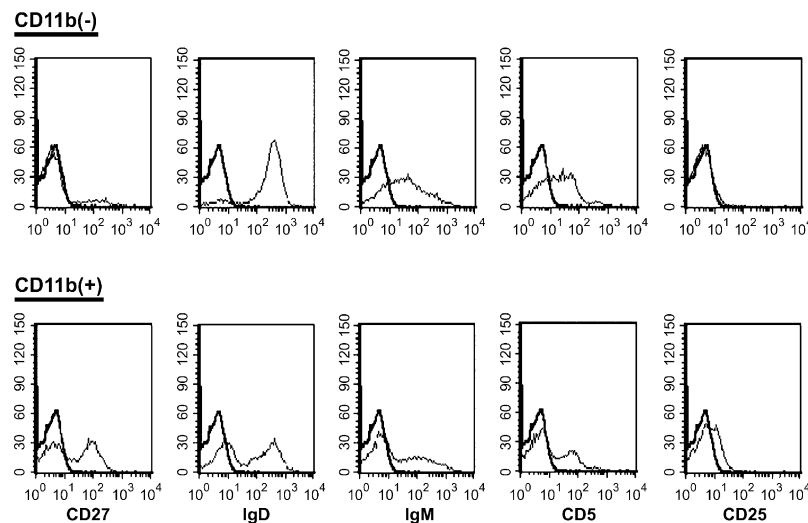


FIG 2. Expression of cell surface molecules. Purified B cells were stained with FITC-mAbs and PE-CD11b mAb, and the expression of cell surface molecules was investigated on the CD11b⁻ (upper) and CD11b⁺ (lower) populations. In each figure, the **bold line** represents the negative control, and the **solid line**, the expression of the respective cell surface markers.

cells was bimodal, with 60.2% IgM⁻. Similarly, CD5 expression was found in 45.8% of CD11b⁻ B cells but in only 27.4% of CD11b⁺ B cells. The CD25 expression level was higher on CD11b⁺ B cells than on CD11b⁻ cells (11.7 vs 6.0, mean fluorescence intensity).

Expression of CD11b on memory and naive B cells

The expression of CD11b on the 3 peripheral blood B-cell subpopulations—CD27⁻ IgD⁺ naive cB1, CD27⁺ IgD⁺ memory cB2, and CD27⁺ IgD⁻ memory cB3—was investigated by 3-color staining (Fig 3). As shown in Fig 3, A, the majority of CD11b⁻ cells were cB1 (91.6%), whereas CD11b⁺ cells contained all cB1, cB2, and cB3 subsets, consistent with the result of the 2-color staining. We then evaluated CD11b expression on cB1, cB2, and cB3 subsets. The majority of cB1 were CD11b⁻ (77.4%), whereas large part of cB2 and cB3 memory cells were CD11b⁺ (71.5% and 81.3%, respectively; Fig 3, B). Furthermore, the CD11b expression level on cB3 was significantly higher than that on cB2.

Migratory properties of peripheral blood B cells

Then, to investigate the functional activity of CD11b expressed on B cells, the migratory property of B cells was examined. As shown in Fig 4, A, although only 28.3% of the purified peripheral blood B cells were CD11b⁺, migrating cells were predominantly CD11b⁺, regardless of the presence or absence of chemoattractants. Furthermore, as determined by CD27/IgD double-staining, CD27⁺ memory cells were significantly enriched in the migrating cells population (Fig 4, B). Especially, regardless of the experimental conditions, the cB3 subset occupied more than 40% of migrating cells, whereas the

percentage of the cB3 subset before migration was no more than 11.1%. As shown in Fig 5, both SDF-1 α and BLC significantly increased the number of migrating cells, and addition of the anti-CD11b blocking mAb markedly inhibited the migration of B cells. Interestingly, both CD11b⁻ and CD11b⁺ B cells expressed CXCR4 and CXCR5, the receptors for SDF-1 α and BLC, respectively. The expression levels of these receptors, however, did not differ between the 2 subsets (Fig 6). These data strongly suggest that the expression of CD11b, but not the chemokine receptors, plays an important role in the migratory ability of memory B cells.

DISCUSSION

CD11b is a member of the integrin family of adhesion molecules and is expressed on a variety of leukocytes, including granulocytes, monocytes, and a subset of lymphocytes. CD11b expressed on neutrophils and monocytes has been extensively investigated, and its important role in the migration of leukocytes from blood stream to the site of inflammation has been confirmed.³⁻⁷ The role of CD11b expressed on lymphocytes was poorly investigated, but in a recent report, we could demonstrate that CD11b expressed on a subset of CD8⁺ cytotoxic T cells is also essential for the migration of this T-cell subset.⁸ On the other hand, the functional activity of CD11b expressed on B cells remains to be investigated. The life cycle of B cells has been more intensively investigated in mouse or rat settings than in human beings. The B-1 cell is a subset of murine B cells and expresses CD11b.¹⁶⁻¹⁸ They primarily reside in the peritoneal cavity and, by producing IgM, are known to play an important role in host defense. However, the B-cell system of human beings is known to be distinct from that of mouse or rat. Although some

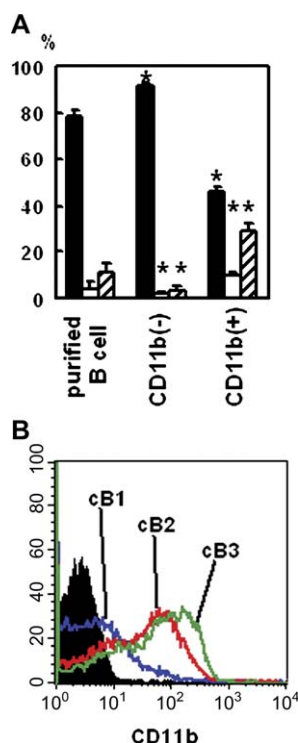


FIG 3. CD11b expression of cB1, cB2, and cB3 B-cell subpopulations. **A**, Purified B cells were stained with FITC-anti-CD27, PE-anti-IgD, and PE-Cy5-anti-CD11b mAbs. The percentage of cB1 (filled bar), cB2 (open bar), and cB3 (shaded bar) subpopulations among purified B cells (left), CD11b⁻ B cells (middle), and CD11b⁺ B cells (right) is shown. Data are presented as means $\pm$ SDs from 3 independent experiments using samples from the same donor. *Statistically significant difference from the result of total purified B cells. **B**, The CD11b expressions of cB1 (blue line), cB2 (red line), and cB3 (green line) subpopulations are presented. Filled black line represents the negative control.

reports document the expression of CD11b in a subset of human peripheral blood B cells,^{9,10} the functional activities of CD11b⁺ B cells remain to be investigated.

Initially, we confirmed the expression of CD11b on peripheral blood B cells to be found in approximately 30% of the cells. Then, we evaluated the differences between the CD11b⁺ and CD11b⁻ B cells concerning the expression of cell surface molecules. Human adult circulating B cells are classified into 3 subpopulations on the basis of the expressions of CD27 and IgD: cB1, cB2, and cB3 subsets. cB1 consists of naive B cells, and cB2 and cB3 of memory B cells. Among the CD11b⁻ cells, the majority were IgD⁺CD27⁺IgM⁺. They were negative for CD25, and approximately half of them were CD5⁺. These data suggest that the CD11b⁻ population mainly consisted of cB1 cells, and this was confirmed by IgD/CD27/CD11b 3-color staining. In contrast, CD11b⁺ cells were more heterogeneous, because the expressions of IgD, CD27, IgM, and CD5 were all bimodal, with positive and negative subpopulations. The 3-color staining analysis revealed that all of the cB1, cB2, and cB3 subsets were present in the CD11b⁺ population. Memory B cells were reported to have higher migratory ability than naive

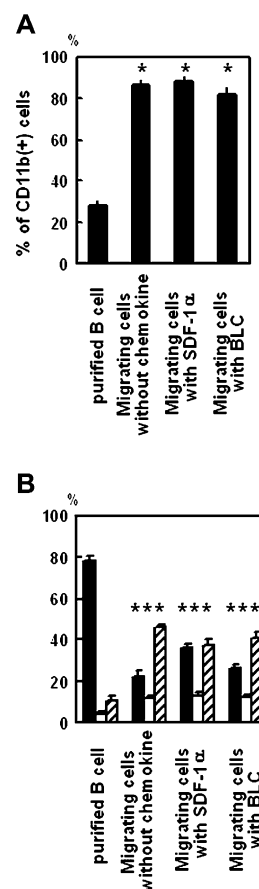


FIG 4. Migration assay. **A**, Purified B cells were allowed to migrate in a transwell assay system. Purified B cells as well as migrated cells were stained with anti-CD11b mAb, and the percentage of CD11b⁺ cells was evaluated by flow cytometry. **B**, Alternatively, cells were stained with anti-CD27 and anti-IgD mAbs, and the percentage of cB1 (filled bar), cB2 (open bar), and cB3 (shaded bar) subpopulations was evaluated. Data are presented as means $\pm$ SDs from triplicate experiments. *Statistically significant difference from the result of purified B cells.

B cells,^{19,20} and therefore, if cell surface CD11b expression is crucial for the migration of B cells, similar to neutrophils and monocytes, we hypothesized that the expression levels of CD11b were higher on cB2 and cB3 subsets than on cB1. The 3-color staining analysis revealed that the expression of CD11b was the highest in cB3, followed by cB2 and cB1, in this order, which confirmed our hypothesis.

Afterward, we investigated the contribution of CD11b to the migratory ability of peripheral blood B cells. The migratory activity of B cells clearly correlated with the CD11b expression, because the migrating cells were predominantly CD11b⁺, and significantly enriched with cB3 cells. In addition, blocking of CD11b binding by the specific antibody markedly reduced the number of migrating cells. These data indicated that CD11b⁺ cells had higher migratory ability than CD11b⁻ cells; however, the expression of chemokine receptors did not differ between these subsets. Therefore, the difference in the migratory

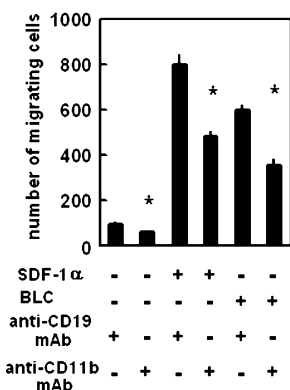


FIG 5. Effect of CD11b blocking with the specific mAb on the migratory activity of B cells. Purified B cells were allowed to migrate in the absence or presence of chemokines in the lower chamber and anti-CD19 or anti-CD11b mAb in the upper chamber. Data are presented as means $\pm$ SDs from triplicate experiments. *Statistically significant difference compared with the negative control.

property between the CD11b⁺ and CD11b⁻ cells was supposed to be dependent not on the expression of chemokine receptors but on CD11b expression. Although the migration was significantly affected by blocking CD11b mAb, the inhibition rate was as high as 40%. In addition, a small portion of CD11b⁻ cells also migrated. These data suggest that molecules other than CD11b are also involved in the migratory property of B cells.

Several other adhesion molecules have been reported to play important roles in B-cell homing. Integrin $\alpha 4\beta 1$, $\alpha 4\beta 7$, and $\alpha E\beta 7$, and cutaneous lymphocyte antigen are expressed on a subset of B cells, and the heterogeneity in the expression of these adhesion molecules is supposed to contribute to the diversity of B-cell trafficking.²¹⁻²⁵ Different lymphoid organs express different ligands for these molecules, ie, vascular cell adhesion molecule 1, mucosal addressin cell adhesion molecule, or E-selectin, and this enables B cells to migrate selectively to the targeted organ.^{26,27} Although the involvement of CD11b in B-cell trafficking has not yet been clarified, here we could clearly demonstrate that CD11b is also pivotal for the migratory ability of B cells. Because the expression of CD11b on each of the cB1, cB2, and cB3 subsets showed a single-peak pattern, it might not affect the destination of trafficking but rather determine the general migratory ability of each subset. Our result suggested that memory cells, especially immunoglobulin class-switched ones, had high migratory property and naive cells had low migratory ability. Naive and memory B cells are known to have different trafficking systems.^{26,28} Naive cells mainly home to secondary lymphoid tissues, such as lymph nodes, Peyer's patches, tonsils, and spleen, and receive positive and negative selections.^{26,29} Because naive B cells express only low levels of CD11b, CD11b may be not essential for this homing process. On the other hand, although memory cells traffic through lymphoid organs as well, they can also access and recirculate

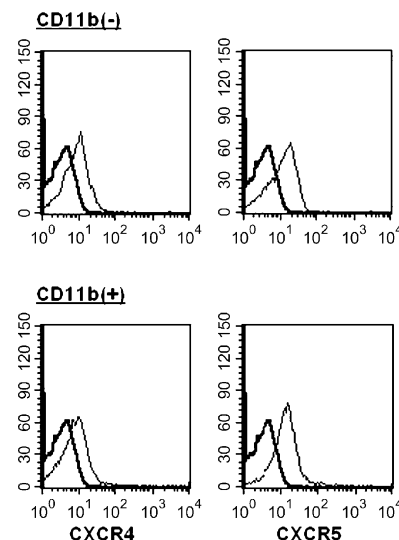


FIG 6. Expression of chemokine receptors. Purified B cells were stained with FITC-mAbs and PE-CD11b mAb, and the expression of chemokine receptors on CD11b⁻ (upper) and CD11b⁺ (lower) subpopulations was evaluated. In each figure, the **bold line** represents negative control, and the **solid line**, the expression of CXCR4 and CXCR5.

through extralymphoid immune effector sites, such as the intestinal lamina propria, pulmonary interstitium, inflamed skin, and joints.^{26,30} High expression of CD11b on memory B cells was supposed to play a central role in this extravasating infiltration of B cells.

Our data show for the first time that CD11b expression plays an important role in the migration of B cells and is highly expressed on memory cells. Further studies will be necessary to determine the precise mechanisms involved in the trafficking of B cells and the regulation by CD11b.

REFERENCES

- Springer TA. Adhesion receptors of the immune system. *Nature* 1990; 346:425-34.
- Nielsen HV, Christensen JP, Andersson EC, Marker O, Thomsen AR. Expression of type 3 complement receptor on activated CD8⁺ T cells facilitates homing to inflammatory sites. *J Immunol* 1994;153:2021-8.
- Thompson HL, Matsushima K. Human polymorphonuclear leucocytes stimulated by tumour necrosis factor- α show increased adherence to extracellular matrix proteins which is mediated via the CD11b/18 complex. *Clin Exp Immunol* 1992;90:280-5.
- Lundgren-Akerlund E, Olofsson AM, Berger E, Arfors KE. CD11b/CD18-dependent polymorphonuclear leucocyte interaction with matrix proteins in adhesion and migration. *Scand J Immunol* 1993;37: 569-74.
- Bohnsack JF, Akiyama SK, Damsky CH, Knape WA, Zimmerman GA. Human neutrophil adherence to laminin in vitro: evidence for a distinct neutrophil integrin receptor for laminin. *J Exp Med* 1990;171:1221-37.
- Ding ZM, Babensee JE, Simon SI, Lu H, Perrard JL, Bullard DC, et al. Relative contribution of LFA-1 and Mac-1 to neutrophil adhesion and migration. *J Immunol* 1999;163:5029-38.
- Smith CW, Marlin SD, Rothlein R, Toman C, Anderson DC. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils in vitro. *J Clin Invest* 1989;83:2008-17.

8. Kawai K, Tsuno NH, Kitayama J, Okaji Y, Yazawa K, Asakage M, et al. Epigallocatechin gallate attenuates adhesion and migration of CD8+ T cells by binding to CD11b. *J Allergy Clin Immunol* 2004;113:1211-7.
9. Postigo AA, Corbi AL, Sanchez-Madrid F, de Landazuri MO. Regulated expression and function of CD11c/CD18 integrin on human B lymphocytes: relation between attachment to fibrinogen and triggering of proliferation through CD11c/CD18. *J Exp Med* 1991;174:1313-22.
10. Kasaian MT, Ikematsu H, Casali P. Identification and analysis of a novel human surface CD5- B lymphocyte subset producing natural antibodies. *J Immunol* 1992;148:2690-702.
11. Agematsu K, Hokibara S, Nagumo H, Komiyama A. CD27: a memory B-cell marker. *Immunol Today* 2000;21:204-6.
12. Maurer D, Fischer GF, Fae I, Majdic O, Stuhlmeier K, Von Jeney N, et al. IgM and IgG but not cytokine secretion is restricted to the CD27+ B lymphocyte subset. *J Immunol* 1992;148:3700-5.
13. Shi Y, Agematsu K, Ochs HD, Sugane K. Functional analysis of human memory B-cell subpopulations: IgD+CD27+ B cells are crucial in secondary immune response by producing high affinity IgM. *Clin Immunol* 2003;108:128-37.
14. Weller S, Braun MC, Tan BK, Rosenwald A, Cordier C, Conley ME, et al. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. *Blood* 2004;104:3647-54.
15. Kawai K, Tsuno NH, Kitayama J, Okaji Y, Yazawa K, Asakage M, et al. Epigallocatechin gallate, the main component of tea polyphenol, binds to CD4 and interferes with gp120 binding. *J Allergy Clin Immunol* 2003;112:951-7.
16. Arnold LW, Pennell CA, McCray SK, Clarke SH. Development of B-1 cells: segregation of phosphatidyl choline-specific B cells to the B-1 population occurs after immunoglobulin gene expression. *J Exp Med* 1994;179:1585-95.
17. Carsetti R, Rosado MM, Wardmann H. Peripheral development of B cells in mouse and man. *Immunol Rev* 2004;197:179-91.
18. Elphick GF, Wieseler-Frank J, Greenwood BN, Campisi J, Fleshner M. B-1 cell (CD5+/CD11b+) numbers and nIgM levels are elevated in physically active vs. sedentary rats. *J Appl Physiol* 2003;95:199-206.
19. Roy MP, Kim CH, Butcher EC. Cytokine control of memory B cell homing machinery. *J Immunol* 2002;169:1676-82.
20. Ottonello L, Corcione A, Tortolina G, Airoldi I, Albesiano E, Favre A, et al. rC5a directs the in vitro migration of human memory and naive tonsillar B lymphocytes: implications for B cell trafficking in secondary lymphoid tissues. *J Immunol* 1999;162:6510-7.
21. Rott LS, Briskin MJ, Butcher EC. Expression of alpha4beta7 and E-selectin ligand by circulating memory B cells: implications for targeted trafficking to mucosal and systemic sites. *J Leukoc Biol* 2000;68:807-14.
22. Yoshino T, Okano M, Chen HL, Tsuchiyama J, Kondo E, Nishiuchi R, et al. Cutaneous lymphocyte antigen is expressed on memory/effector B cells in the peripheral blood and monocytoid B cells in the lymphoid tissues. *Cell Immunol* 1999;197:39-45.
23. Lo CG, Lu TT, Cyster JG. Integrin-dependence of lymphocyte entry into the splenic white pulp. *J Exp Med* 2003;197:353-61.
24. Lu TT, Cyster JG. Integrin-mediated long-term B cell retention in the splenic marginal zone. *Science* 2002;297:409-12.
25. Csencsits KL, Walters N, Pascual DW. Cutting edge: dichotomy of homing receptor dependence by mucosal effector B cells: alpha(E) versus L-selectin. *J Immunol* 2001;167:2441-5.
26. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. *Science* 1996;272:60-6.
27. Berg EL, McEvoy LM, Berlin C, Bargatze RF, Butcher EC. L-selectin-mediated lymphocyte rolling on MAdCAM-1. *Nature* 1993;366:695-8.
28. Picker LJ. Control of lymphocyte homing. *Curr Opin Immunol* 1994;6:394-406.
29. Butcher EC. The regulation of lymphocyte traffic. *Curr Top Microbiol Immunol* 1986;128:85-122.
30. Mackay CR. Immunological memory. *Adv Immunol* 1993;53:217-65.