Published in final edited form as:

Immunol Lett. 2008 October 30; 120(1-2): 79–86. doi:10.1016/j.imlet.2008.07.003.

Participation of Intercellular Adhesion Molecule-2 (CD102) in B lymphopoiesis

Yoshio Yamashita¹, Taku Kouro², Kensuke Miyake³, Kiyoshi Takatsu², Mizuho A. Kido⁴, Teruo Tanaka⁴, Masaaki Goto¹, and Paul W. Kincade⁵

¹Department of Oral and Maxillofacial Surgery, Saga Medical School, Saga, Japan

²Division of Immunology, Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

³Division of Infectious Genetics, Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

⁴Department of Oral Anatomy and Cell Biology, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan

⁵Immunobiology and Cancer Program, Oklahoma Medical Research Foundation, Oklahoma, USA

Summary

The survival and fate of blood cell precursors is dependent on their communication with stromal cells of various types within bone marrow. Monoclonal antibodies have proven to be powerful tools for identifying molecules responsible for such interactions and we now describe one that selectively blocks B lymphopoiesis. The BF/32 antibody inhibited the establishment, but not the maintenance of long term bone marrow cultures capable of lymphocyte production. However, there was no obvious effect on lymphocyte-stromal cell adhesion or responsiveness of pre-B cells to interleukin-7. Furthermore, the reagent had no influence on myeloid precursors or myeloid bone marrow cultures. Injection of adult mice with BF/32 reduced B lineage precursors within bone marrow, but spared mature B cells. Moreover, the reagent did not alter responsiveness of mature B cells to activating stimuli. The 60 kDa protein recognized by this antibody was widely expressed on lymphocytes. Amino terminal protein sequencing and transfection experiments identified it as the murine homologue of ICAM-2 (CD102).

Keywords

ICAM-2 (CD102); B lymphopoiesis; stromal cells; long term bone marrow culture

Introduction

Most growth and differentiation factors are made in limiting quantities by stromal cells within bone marrow and locally presented to hematopoietic precursors of the respective blood cell lineages. Therefore, production of the correct numbers of new cells is highly dependent on close, but transient physical proximity between factor-producing and factor-responsive cells. Of particular interest is how cells committed to a particular blood cell lineage can be

Address correspondence and reprint requests to Dr. Yoshio Yamashita, Department of Oral and Maxillofacial Surgery, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan, Phone number: +81-952-34-2397, Fax number: +81-952-34-2044, e-mail: E-mail: yamashy2@cc.saga-u.ac.jp.

independently formed, spatially organized, nurtured and exported from the marrow. Our previous studies exploited long-term culture models and monoclonal antibodies to learn the molecular basis for this communication, implicating the integrin VLA-4 and its ligand VCAM-1, as well as CD44 and CD9 in the process [1-4]. Additional approaches in this and other laboratories suggested that syndecans, Ly-6 family members, and various other glycoproteins could have important roles in hematopoiesis [5-7]. It is remarkable that this conclusion has not always been supported by the results of gene targeting studies, perhaps because of a high degree of functional redundancy of molecules utilized within bone marrow [8-11]. Indeed, the molecular and functional complexity of this vital organ may yet to be fully appreciated. Here we describe the development of a new monoclonal antibody to ICAM-2 (CD102) and its very discrete influence on B lymphopoiesis.

Methods

Cell lines and animals

The murine IL-7 dependent pre-B cell clones (BC7.7, F10), B cell lymphomas (1A9, BCL₁), and bone marrow stromal cell lines (ST-2, BMS2) were maintained as previously described [4,12]. BALB/c mice and CB17 *scid/scid* mice were obtained from the Animal Facility of Saga Medical School or the Laboratory Animal Resource Facility at OMRF. All experiments reported here were done with female mice at 6-10 wk of age. Wistar rats were purchased from Charles River Japan Inc. (Yokohama, Japan).

MAbs

Wistar rats were immunized six times with the BC7.7 pre-B cell line. Popliteal lymph nodes were removed and fused with SP2/0 myeloma cells (American Type Culture Collection, Manassas, VA). The strategy used for screening is described in the Results. The established antibody, BF/32 was IgG2b/k. Abs were purified from the ascites fluid of CB17 *scid/scid* mice that had been transplanted with the resulting hybridomas using ABx Plus affinity chromatography (J.T.Baker, Phillipsburg, NJ). Control antibody was 14.8 (IgG2b) reactive with CD45R, KY/8.2 (IgG2a) directed against syndecan-4 [12].

Immunofluorescence Analysis

Cells in suspension were incubated for 20 min on ice with mAbs. After washing, FITC-labeled mouse anti-rat κ (MAR18.5) mAb (ATCC) was added for an additional 20-min incubation. Propidium iodide was used to exclude dead cells. For dual staining, cells were pre-incubated for 20 min at on ice with supernatant from the anti-FcR mAb 2.4G2 (ATCC), and 10 % normal rat serum and then washed. Labeled cells were then analyzed on a FACScan® (Becton Dickinson Co.). For the analysis of the B progenitor cells in bone marrow (BM), BM cells were stained with 1) APC-conjugated anti-CD19, PE-conjugated anti-CD45R(Phamingen, San Diego, CA), and FITC-conjugated anti-CD24(Phamingen) for Fraction A subset, 2) APC-conjugated anti-CD19, PE-conjugated anti-BP-1(Phamingen), and FITC-conjugated anti-CD43(Phamingen), for Fraction B and C subsets, 3) APC-conjugated anti-CD45R, PE-conjugated anti-CD43, FITC-conjugated anti-IgM(Zymed, San Francisco, CA) for Fraction D ~ F subsets. Cells were then stained with biotinylated BF/32 and PerCP-conjugated streptavidin.

Long-term BM cultures (LTBMCs)

Long-term BM culture was carried out as described previously [13-15]. Whole BM cells were cultured under lymphoid-permissive or myeloid-permissive conditions. In each LTBM system, the cultures were fed by weekly medium replacements. The lineage identify of non-adherent cells was confirmed using fluorescently labeled antibodies specific to CD19 or

CD45R for lymphoid-permissive cultures and Gr-1 for myeloid-permissive cultures (data not shown).

In vivo treatment

BALB/c mice were given an intra-peritoneal injection of BF/32 or a class matched control mAb every 3 days. On day 7, mice were sacrificed and cell suspensions were prepared from spleen, thymus, and bone marrow for phenotypic and functional studies. Viable cell numbers were enumerated by trypan blue exclusion after lysis of red blood cells.

Cell surface biotinylation and immunoprecipitation

Cells (5×10^7 /ml) were washed twice with HBSS, and suspended in saline containing 100 mM Hepes (pH 8.0). Sulfosuccinimidobiotin (Pierce Chemical Co., Rockford, IL) was added to cell suspensions at a concentration of 0.5 mg/ml. After a 30-min incubation at room temperature with occasional shaking, cells were washed and lysed in buffer containing 50 mM Tris/HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 50 mM iodoacetamide, 1 mM PMSF, 10 µg/ml soybean trypsin inhibitor, 2 mM MgCl₂, 2 mM CaCl₂, and 0.1% sodium azide. After a 30-min incubation on ice and following centrifugation, the cell lysates were recovered and incubated with antibody-coupled Sepharose 4B for 2h at 4 °C. After washing with lysis buffer, bound proteins were subjected to SDS-PAGE, blotted onto a nitrocellulose membrane, and visualized with avidin-peroxidase (Zymed, San Francisco, CA) followed by an appropriate substrate.

Colony-forming cell assays

Murine bone marrow cell populations were suspended in 1 ml of assay medium as previously described [8,9]. The semisolid agar cloning assay for B lymphocyte precursors was done with 2 ng recombinant mouse IL-7 (upstate Biotechnology, Lake Placid, NY). The granulocyte-macrophage progenitor assay (CFU-c) was done with 25 µl of 10-fold concentrated L cell-conditioned medium as a source of CSF. All cloning assays were performed in 35-mm Petri dishes and incubated 37 °C, 5% CO₂. Colonies were scored on day 6.

Cell adhesion assay

BC7.7 pre-B cell line was radiolabeled by incubating 2×10^7 cells/ml in complete medium with 100 µCi of Na₂⁵¹CrO₄ for 1 h at 37°C and washed three times in complete medium. The ST-2 stromal cell clone was plated in 24-well plates (Corning Glass Works, Corning, NY) at 3×10^4 cells/well and allowed to grow overnight before the adhesion assay. The labeled cells (2×10^5 /well) were added to the stromal cell layer and incubated 30 min at 37°C. The unbound cells were removed by three cycles of washing in prewarmed complete medium. Bound cells were lysed with 0.1 N NaOH, 1% Triton X-100, and the ⁵¹Cr was counted with a gamma counter (Hewlett Packard Co., Palo Alto, CA). Percentages of bound cells were determined by the formula: percent bound = $100 \times [(\text{cpm from bound cells})/(\text{input cell associated cpm} - \text{spontaneously released cpm})]$. Antibodies were added at the same time as the labeled cells.

Micro-sequencing of amino-terminal amino acids

Purified BF/32 mAb (25 mg) was coupled to N-Hydroxy-succinidyl-activated HiTrap column (2.5 ml) according to the manufacturer's instructions (Pharmacia P-L Biochemicals Inc., Milwaukee, WI). Cell lysates were prepared from BCL₁ cells (3×10^{10} cells/ 300 ml). Lysis buffer consisted of 50 mM Tris/HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 50 mM iodoacetamide, 1 mM PMSF, 10 µg/ml soybean trypsin inhibitor (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 2 mM MgCl₂, 2 mM CaCl₂, and 0.1% sodium azide. The cell lysate was applied onto the BF/32 mAb-coupled column. The column was washed with buffer containing 20 mM Tris/HCl (pH 7.5), 150 mM NaCl, 0.1% Triton X-100, 150 mM NaCl, and 0.02% sodium azide. Bound proteins were eluted with buffer containing 50 mM glycine/HCl

(pH 2.6) and 0.1% Triton X-100. Each fraction was neutralized immediately with 1M Tris/HCl (pH 8.0) and subjected to SDS-PAGE analysis. Peak fractions were combined, dialyzed against 0.1% Triton X-100 and 10 mM NaCl, and lyophilized. Purified proteins were resolved with SDS-PAGE, blotted onto an Immobilon P membrane (Nihon Milipore, Tokyo, Japan), and visualized with Coomassie brilliant blue R-250 (Nippon Bio-Rad Laboratories, Kanagawa, Japan). The target proteins were excised and loaded onto a protein sequencer (Applied Biosystems, Foster City, CA). Homology was searched within databases of the National Center for Biotechnology Information (NCBI; Bethesda, MD) using a blast algorithm.

cDNA and Transfection

ICAM-2 cDNA full length sequences were amplified by PCR from template DNA prepared BCL₁ cDNA library. To perform PCR, sense and antisense primers, 5'-CTCGAGGAGATGTCTTCTTTTGC and 5'-GCGGCCGCGCAACGTGGGCTCA were designed. PCR products prepared with these oligonucleotides were subcloned into pEF-BOS expression vector (a kind gift from Dr. S.Nagata.). 2 µg plasmid was transfected into 293T cells using a calcium phosphate method. After 2 days of culture, 293T cells were detached with PBS containing 0.2 mM EDTA and stained with the BF/32 or control mAbs.

Results

Establishment of a New mAb, BF/32

A simple strategy for preparing useful monoclonal antibodies has yielded repeated success; whole cells are used as immunogens and the screening involves selection of clones with potential blocking activity [1-4]. In this case, the stromal cell/IL-7 dependent BC7.7 pre-B cell clone was used to immunize Wistar rats and hybridomas were prepared with popliteal lymph cells after the sixth injection. The general objective was to identify molecules expressed on B cell precursors that might mediate interactions with stromal cells. Two hybridoma supernates displayed lymphocyte reactivity and one of them initially attracted attention because of its ability to inhibit the mild aggregation that is typical for the BC7.7 cells. The resulting clone, BF/32 is the subject of this report.

Selective inhibition of B lymphopoiesis in culture

Addition of purified monoclonal antibodies to cultures represents the next step in our screening process. BF/32 completely inhibited the formation of lymphocytes when present at the initiation of Whitlock-Witte type bone marrow cultures (Fig. 1). The adherent stromal layers of antibody-containing cultures appeared to contain the usual complex mixture of cells and no morphological changes were obvious. Moreover, the antibody did not disrupt on-going lymphocyte production when added to Whitlock-Witte cultures that were established 4-6 weeks previously (Fig. 2). The inhibitory effect of BF/32 was selective for B lymphopoiesis; we recorded only a slight delay in production of myeloid cells when the antibody was continuously present in Dexter type long term bone marrow cultures (data not shown).

There are many potential targets for the BF/32 antibody in primary bone marrow cultures and we investigated the ability of the reagent to directly inhibit pre-B cell growth. Purified BF/32 antibody did not inhibit the IL-7 dependent expansion of BC7.7 cells. Moreover, it had no influence on clonal proliferation of B lineage cells in IL-7 stimulated semisolid agar cultures or myeloid progenitors in CSF containing cultures (Table 1). Flow cytometry revealed that the BF/32 antibody did not stain either of two cloned stromal cell lines (BMS2 and ST-2). Additionally, we found that the reagent recognized most mature lymphocytes but had no influence on the responsiveness of mature B cells to activating signals delivered by LPS, or antibodies directed to sIgM, CD38, CD40, CD72, or CD180/RP105 (Y.Yamashita, unpublished data).

As noted above, BF/32 did not disrupt previously established bone marrow cultures. However, we more rigorously tested the possibility that it might influence adhesive interactions between lymphocyte precursors and stromal cells. The BC7.7 pre-B cell clone that was used for immunization readily adheres to the ST-2 stromal cell clone, but this recognition was unaffected by BF/32. Rather, the adhesion was almost totally inhibited by the integrin VLA-4 specific PS/2 antibody (Fig. 3).

All of these observations suggest that BF/32 is highly specific with respect to its ability to influence early events in establishment of long term bone marrow cultures. It is possible that it directly delivers a negative signal for survival and/or differentiation to hematopoietic cells. This would presumably be a very early precursor required to initiate, but not maintain continued expansion in Whitlock-Witte cultures. As another possibility, the reagent could interfere with communication between lympho-hematopoietic cells and the extracellular matrix. This would represent a step that is uniquely required at the initiation of bone marrow cultures, when a complex cellular environment is assembled. The dramatic and discrete inhibitory effect of BF/32 *in vitro* led to identification of the protein it recognizes.

BF/32 recognizes the murine homologue of ICAM-2 (CD102)

Almost all thymocytes (greater than 95%) were stained by the BF/32 mAb and the antigen was similarly widely represented on lymphocytes in marrow, spleen and lymph nodes (Fig. 4). Four-color flow cytometry revealed that all subset of bone marrow B lymphocyte lineage cells (Fraction A ~F) equally expressed the antigen (Fig. 5). Furthermore, a series of established lymphoid cell lines were tested and most were recognized to similar extents. Immunoprecipitation analysis was then performed using the BC7.7 cell line. This revealed that BF/32 recognizes a single species of approximately 60 kDa and the apparent size was unchanged by reduction (Fig. 6).

The BCL₁ cell line was then used for large scale immunoprecipitation and protein sequencing. A total of 16 amino terminal residues were identified and this represented an exact match with the ICAM-2 (CD102) protein sequence present in the NCBI database (Fig. 7). The known distribution and size of ICAM-2 are consistent with our findings, and staining of transiently transfected 293T cells confirmed the specificity of the BF/32 antibody (Fig.8).

The BF/32 mAb selectively inhibits B lymphopoiesis in vivo

Gene targeting studies have not identified a role for ICAM-2 in lympho-hematopoiesis and we therefore conducted experiments with intact mice. A one week course of intraperitoneal injections of BF/32 or isotype matched control mAb had no effect on numbers of total nucleated cells that could be recovered from femurs. However, there was a very marked and selective depletion of newly formed B cells identified by the high density display of sIgM and CD45R in mice that received BF/32 (Fig. 9). The same was true of CD45R/B220⁺ CD43⁻ precursors. Total numbers of CD45R/B220⁺ sIgM⁻ only slightly declined, suggesting cells near the end of the pathway were preferentially suppressed. This pattern of inhibition was reproducible in three experiments and selective for immature B cells. We recorded no changes in numbers or proportions of splenic lymphocytes (Fig.10a) and neither thymuses (Fig.10b) or lymph nodes appeared to be affected.

Discussion

Blood cell formation is highly dependent on complex interaction between stromal and hematopoietic cells, and it is important to identify cell surface molecules that mediate that communication. The term “cell adhesion molecules” is commonly used to describe ones that mediate physical attachment, but most of them have additional roles, such as the transmission

of information. Although the fate of hematopoietic cells is controlled by the net influence of many signals, adhesion molecules are required for retention of maturing hematopoietic precursors in bone marrow, where they closely interact with and are dependent on stromal cells. These not only facilitate communication between cells in bone marrow, but between lympho-hematopoietic cells and a rich extracellular matrix. Cell adhesion and matrix molecules probably control movement of precursors and access to regulatory molecules as well as providing signals themselves.

In previous studies, we developed antibodies that blocked physical and functional interactions between pre-B and stromal cells [1-5]. Although these molecules are undoubtedly important, they are unlikely to account all of the associations between B lymphocyte progenitor cells and stromal cells.

We have now established a new mAb, BF/32 that inhibits B cell production and show that it is specific for murine ICAM-2 (CD102). Our results indicate that ICAM-2 may have a previously unsuspected role in B lymphopoiesis.

ICAM-2 has been previously well studied and, like the structurally related ICAM-1 (CD54) and ICAM-3 (CD50) proteins is a known ligand for the β_2 family of leukocyte integrins [16-19]. These include CD11a/CD18 (LFA-1) [16,18], CD11b/CD18 (Mac-1) [20,21], and the dendritic cell-specific, ICAM-grabbing non-integrin (DC-SIGN) protein [22]. We have found by flow cytometric analysis that most lymphocytes present ICAM-2. Especially, ICAM-2 is uniformly present on all subsets of B lymphocyte lineage cells in bone marrow cell suspensions. High level constitutive expression on vascular endothelial cells and less responsiveness than ICAM-1 to inflammatory stimuli are also characteristics of ICAM-2 [16,18,20,21]. Expression is more restricted than ICAM-1 and, unlike ICAM-1, there is little or no induction of ICAM-2 on lymphocytes and endothelial cells by inflammatory mediators. So, ICAM-2 may be important for leukocyte trafficking in uninflamed tissues, as in lymphocyte re-circulation, whereas induction of ICAM-1 on endothelium and other cells by inflammatory cytokines may increase cell-cell interactions and leukocyte extravasation at inflammatory sites. ICAM-1, but not ICAM-2, was required for the migration of T effector cells into the inflamed skin. ICAM-1 is unique in supporting migration into inflamed sites and trapping within the lung [23]. On the other hand, recombinant ICAM-2 can co-stimulate T cells in vitro, suggesting a role in T cell activation. Although ICAM-2 mediates neutrophil transmigration, ICAM-2 appears capable of mediating CD31/PECAM-1-independent leukocyte transmigration [24]. ICAM-2 may also regulate angiogenesis via several mechanisms including survival, cell migration, and Rac activation [25]. Therefore, ICAM-1 and ICAM-2 have selective roles and are products of distinct and homologous genes.

The first N-linked glycan on ICAM-2 contacts an exposed tryptophan residue, defining a conserved glycan-W motif critical for the conformation of the integrin binding domain. The absence of this motif in human ICAM-1 exposes regions used in receptor dimerization and rhinovirus recognition. N-linked glycosylation regulates both conformation and immune related functions of ICAM receptors [26]. Weber investigated structural and functional differences between ICAM-2 from platelets and that from endothelial cells [22]. Sialylation of ICAM-2 on platelets impairs adhesion of leukocytes via LFA-1 and DC-SIGN.

ICAM-2-deficient mice have recently been described by Gutierrez-Ramos and colleagues [27]. The mice appeared normal with respect to size, general health, fertility, and longevity. However, there was prolonged accumulation of eosinophils in lung interstitium concomitant with delayed increases in eosinophil numbers in the airway lumen during the development of allergic lung inflammation. These findings demonstrate that ICAM-2 plays a critical role in eosinophil trafficking and localization within the lung tissue during lung allergic inflammation.

So far, no abnormalities were reported in total leukocyte and red blood cell counts or leukocyte subset numbers in ICAM-2 deficient mice [27]. Partial defects in B lymphopoiesis in bone marrow might have escaped notice or there could have been compensation by adhesive ligands with overlapping functions. As another possibility, ICAM-2 may not be essential for B cell formation, and ligation with our BF/32 mAb could have delivered an unphysiologic signal to late-stage progenitors. Regardless, the findings indicate that ICAM-2 is functional on a discrete subset of bone marrow cells. It will now be important to learn if and how ICAM-2 facilitates intercellular communication between B lymphocytes and surrounding cells.

Acknowledgements

This work has been supported by grant from the Ministry of Education, Science, and Culture of Japan (Y.Y) and by grants AI20069 from the National Institutes of Health (P.W.K). P.W.K. holds the William H. and Rita Bell Endowed Chair in Biomedical Research.

Reference

- [1]. Miyake K, Weissman IL, Greenberger JS, Kincade PW. Evidence for a role of the integrin VLA-4 in lympho-hemopoiesis. *J Exp Med* 1991;173:599–607. [PubMed: 1997648]
- [2]. Miyake K, Medina K, Ishihara K, Kimoto M, Auerbach R, Kincade PW. VCAM-like adhesion molecule on murine bone marrow stromal cells mediates binding of lymphocyte precursors in culture. *J Cell Biol* 1991;114:557–565. [PubMed: 1713592]
- [3]. Miyake K, Underhill CB, Lesley J, Kincade PW. Hyaluronate can function as a cell adhesion molecule and CD44 participates in hyaluronate recognition. *J Exp Med* 1990;172:69–75. [PubMed: 2193100]
- [4]. Oritani K, Wu X, Medina K, Hudson J, Miyake K, Gimble JM, et al. Antibody ligation of CD9 modifies the production of myeloid cells in long-term cultures. *Blood* 1996;87:2252–226. [PubMed: 8630385]
- [5]. Izon DJ, Oritani K, Hamel M, Calvo CR, Boyd RL, Kincade PW, et al. Identification and functional analysis of Ly-6A/E as a thymic and bone marrow stromal antigen. *J Immunol* 1996;156:2391–2399. [PubMed: 8786296]
- [6]. Oritani K, Kanakura Y, Aoyama K, Yokota T, Copeland NG, Gilbert DJ, et al. Matrix glycoprotein SC1/ECM2 augments B lymphopoiesis. *Blood* 1997;90:3403–3413.
- [7]. Oritani K, Kincade PW. Identification of stromal cell products that interact with Pre-B cells. *J Cell Biol* 1996;134:771–782. [PubMed: 8707854]
- [8]. Medina KL, Smithson G, Kincade PW. Suppression of B lymphopoiesis during normal pregnancy. *J Exp Med* 1993;178:1507–1515. [PubMed: 8228804]
- [9]. Borghesi LA, Yamashita Y, Kincade PW. Heparan sulfate proteoglycans mediate Interleukin-7-dependent B lymphopoiesis. *Blood* 1999;93:140–148. [PubMed: 9864155]
- [10]. Tudor KRS, Payne KJ, Yamashita Y, Kincade PW. Functional assessment of precursors from murine bone marrow suggests a sequence of early B lineage differentiation events. *Immunity* 2000;12:335–345. [PubMed: 10755620]
- [11]. Kincade PW, Lee G, Watanabe T, Sun L, Scheid MP. Antigens displayed on murine B lymphocyte precursors. *J Immunol* 1981;127:2262–2268.
- [12]. Yamashita Y, Oritani K, Miyoshi EK, Wall R, Bernfield M, Kincade PW. Syndecan-4 is expressed by B lineage lymphocytes and can transmit a signal for formation of dendritic processes. *J Immunol* 1999;162:5940–5948. [PubMed: 10229831]
- [13]. Whitlock CA, Witte O. Long term culture of B lymphocytes and their precursors from murine bone marrow. *Proc Natl Acad Sci USA* 1982;79:3608–3612. [PubMed: 6980414]
- [14]. Dexter TM, Allen TD, Lajtha LG. Conditions controlling the proliferation of haemopoietic stem cells in vitro. *J Cell Physiol* 1977;91:335–344. [PubMed: 301143]
- [15]. Kincade PW. Experimental models for understanding B lymphocyte formation. *Adv Immunol* 1987;41:181–267. [PubMed: 3318292]
- [16]. Staunton DE, Dustin ML, Springer TA. Functional cloning of ICAM-2, a cell adhesion ligand for LFA-1 homologous to ICAM-1. *Nature* 1989;339:61–64. [PubMed: 2497351]

- [17]. Wu H, Bickford JK, Luther E, Carpenito C, Takei F, Springer TA. Characterization of murine intercellular adhesion molecule-2. *J Immunol* 1996;156:4909–4914. [PubMed: 8648141]
- [18]. de Fougères AR, Stacker SA, Schwarting R, Springer TA. Characterization of ICAM-2 and evidence for a third counter-receptor for LFA-1. *J Exp Med* 1991;174:253–267. [PubMed: 1676048]
- [19]. Sansom D, Borrow J, Solomon E, Trowsdale J. The human ICAM2 gene maps to 17q23-25. *Genomics* 1991;11:462–464. [PubMed: 1769660]
- [20]. Xie J, Li R, Kotovuori P, Vermot-Desroches C, Wijdenes J, Arnaout MA, et al. Intercellular adhesion molecule-2 (CD102) binds to the leukocyte integrin CD11b/CD18 through the A domain. *J Immunol* 1995;155:3619–3628. [PubMed: 7561061]
- [21]. Damle NK, Klussman K, Aruffo A. Intercellular adhesion molecule-2, a second counter-receptor for CD11a/CD18 (leukocyte function-associated antigen-1), provides a costimulatory signal for T-cell receptor-initiated activation of human T cells. *J Immunol* 1992;148:665–671. [PubMed: 1346150]
- [22]. Weber KS, Alon R, Klickstein LB. Sialylation of ICAM-2 on platelets impairs adhesion of leukocytes via LFA-1 and DC-SIGN. *Inflammation* 2004;28:177–188. [PubMed: 15673159]
- [23]. Lehmann JC, Jablonski-Westrich D, Haubold U, Gutierrez-Ramos JC, Springer T, Hamann A. Overlapping and selective roles of endothelial intercellular adhesion molecule-1 (ICAM-1) and ICAM-2 in lymphocyte trafficking. *J Immunol* 2003;171:2588–2593. [PubMed: 12928410]
- [24]. Huang MT, Larbi KY, Scheierrmann C, Woodfin A, Gerwin N, Haskard DO, et al. ICAM-2 mediates neutrophil transmigration in vivo: Evidence for stimulus-specificity and a role in PECAM-1-independent transmigration. *Blood* 2006;107:4721–4727. [PubMed: 16469869]
- [25]. Huang MT, Mason JC, Birdsey GM, Amsellem V, Gerwin N, Haskard DO, et al. Endothelial intercellular adhesion molecule (ICAM)-2 regulates angiogenesis. *Blood* 2005;106:1636–1643. [PubMed: 15920013]
- [26]. Jimenez D, Roda-Navarro P, Springer TA, Casasnovas JM. Contribution of N-linked glycans to the conformation and function of intercellular adhesion molecules (ICAMs). *J Biol Chem* 2005;280:5854–5861. [PubMed: 15545280]
- [27]. Gerwin N, Gonzalo J-A, Lloyd C, Coyle AJ, Reiss Y, Banu N, et al. Prolonged eosinophil accumulation in allergic lung interstitium of ICAM-2-deficient mice results in extended hyperresponsiveness. *Immunity* 1999;10:9–19. [PubMed: 10023766]

Figure 1.

A new mAb, BF/32 blocks lymphopoiesis in LTBM.

Replicate cultures of murine bone marrow were prepared and maintained with a control antibody to CD45R (14.8), BF/32 mAb (10 µg/ml), or medium alone as indicated at the beginning of culture and replenished with each feeding. Numbers of nonadherent cells were collected and counted at weekly intervals. The data are presented as the mean ± SD of quadruplicate samples and are representative of one of three experiments.

Figure 2.

Lymphoid-permissive LTBMcs were established for 4 weeks in without antibodies and then treated weekly in the presence or absence of 10 $\mu\text{g/ml}$ mAbs. The data are presented as the mean $\pm$ SD of quadruplicate samples and are representative of one of three experiments.

Figure 3.

New mAb has no effect on the BC7.7 cell adhesion to stromal cells.

BC7.7 cells were ^{51}Cr labeled, and used in the cell adhesion assay as described in the Materials and Methods. Radiolabeled cells were cultured with or without the indicated antibodies (10 $\mu\text{g}/\text{ml}$). The values are presented as the means $\pm$ SD for triplicate wells.

Figure 4.

Expression of BF/32 antigen in various lymphoid organs of BALB/c mice. Control profiles were stained with second reagent (FITC-labeled mouse anti-rat κ mAb) only and shown as bold line.

Figure 5.

BF/32 antigen expression on BM B progenitor cells. Cells were stained simultaneously either with CD19-APC, CD24-FITC, CD45R-PE, Biotinylated BF/32 and PreCP-streptavidin for Fraction A subset. Cells were stained with CD19-APC, CD43-FITC, BP-1-PE, Biotinylated BF/32 and PreCP-streptavidin for Fraction B, C subsets. Cells were stained with CD45R-APC, IgM-FITC, CD43-PE, Biotinylated BF/32 and PreCP-streptavidin for Fraction D~F subsets. Data for cells falling in each gate were collected and the expression is indicated by the lower histograms.

Figure 6.

The BF/32 mAb recognizes a monomeric molecule with 60 kDa molecular mass.

Cell surface proteins on the BC7.7 pre-B cell line were biotinylated and immunoprecipitated with BF/32-bound beads (lane 2 and 4) or with unconjugated beads (lane 1 and 3). Precipitated proteins were subjected to SDS-PAGE (9 % acrylamide) under reducing conditions (lane 1 and 2) or under nonreducing conditions (lane 3 and 4). Blotted proteins were visualized with HRP-streptavidin and an enhanced chemiluminescence system.

	1		11
BF/32 antigen	:	XEKA FEVYI	X SEXQIV
Murine CD102	:	SGEKA FEVYI	WSEKQIV

Figure 7.

Partial amino acid sequence of the BF/32 Ag and its identity with murine CD102.

The amino acid sequence of the NH₂-terminal of BF/32 Ag was determined as described in the Methods, and is shown in single letter code. The letter X denotes an undefined residue.

Figure 8.

Flow cytometry analysis of 293T cells transfected with murine CD102 cDNA. Transfected 293T cells were labeled with BF/32 or a control antibody, JF/9 (anti-CD9). Control profiles were stained with second reagent (FITC-labeled mouse anti-rat κ mAb) only and shown as bold line.

Figure 9.

Inhibition of B lymphopoiesis in adult mice treated with BF/32 mAb. Adult BALB/c mice were injected intraperitoneally with 1 mg per treatment on days 0, and 4 with either BF/32 or isotype matched control antibody. Bone marrow cells were analyzed on day 7 by flow cytometry as described in the Methods for the expression of CD45R (B220) and CD43 (upper panel). CD43⁺BM cells were resolved into two subsets on the basis of CD45R and IgM expression (lower panel). The percentages of cells within selected quadrants are shown.

<Spleen>

<Thymus>

Figure 10.

Effect of BF/32 mAb on splenic (a) and thymic (b) subpopulations in mice. Mice were treated with BF/32 mAb or control antibodies as in Fig. 7. Splenocytes analyzed for the expression of CD19 and CD3, thymocytes analyzed for the expression CD4 and CD8. The percentages of the cells within selected quadrants are shown.

Table. 1

The BF/32 mAb does not suppress clonal proliferation of lymphoid or myeloid progenitors

mAb	CFU-IL7	CFU-c
Control	181	179
BF/32	154	149

BF/32 mAb or a class matched control mAb, 14.8 were added to CFU-IL-7 and CFU-c colony assays of whole murine marrow cells. The data are presented as mean numbers of colonies per 10^5 cells cultured in semisolid agar with IL-7 (CFU-IL-7) or L cell-conditional medium (CFU-c). Similar results were observed in three independent experiments.