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Immunology Letters 98 (2005) 232–244
Immunology Letters
www.elsevier.com/locate/

B-1a cell origin of the murine B lymphoma line BCL_1 characterized by surface markers and bacterial reactivity of its surface IgM

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Received 24 September 2004; received in revised form 19 November 2004; accepted 20 November 2004
Available online 15 December 2004

Abstracts

B cells are divided into two categories: conventional or B-2B cells and B-1B cells, the latter of which are distinguished by their different ontogeny. B cell lymphoma 1 (BCL_1), the first-reported case of a spontaneously developed mouse B-lymphoma, expresses CD5, surface IgM, Mac-1, CD43 and low level of B220, and is likely to have B-1a cell origin. However, antigens recognized by IgM produced by the BCL_1 cells (BCL_1-IgM) have not been identified. Here, we demonstrate that BCL_1-IgM reacts with *Escherichia coli* (*E. coli*). Our initial finding that several recombinant proteins expressed in *E. coli* bound to BCL_1-B20 prompted us to examine the possibility that BCL1 cells may bind *E. coli*. Indeed, BCL_1 cells bound fluorescein-labeled *E. coli*. To elucidate the structure on the BCL_1 cells responsible for *E. coli*-binding, we produced a monoclonal antibody capable of inhibiting BCL^1 binding to *E. coli*. The antibody recognizes an idiotypic epitope on the BCL_1-IgM. Moreover, polyclonal antibody against IgM and secreted BCL_1-IgM purified from the supernatants inhibited BCL_1 binding to *E. coli*. Finally, transfection of non-lymphoid cells with cDNA of heavy and light chains of BCL_1-IgM conferred the cells ability to bind *E. coli*. These results clearly indicate that BCL_1-IgM bind *E. coli* and suggest that BCL_1 lymphoma is a typical B-1 cell-derived lymphoma, characterized not only by the surface phenotype, but also by the reactivity of its IgM with commensal bacteria.

Keywords: B cell lymphoma; Bacterial reactivity; B-1 cells; Somatic mutation

1. Introduction

BCL_1 lymphoma is the first reported spontaneous B cell tumor line that arose in a 2-year-old BALB/c mouse [1–5]. BCL_1 cells express CD5, IL-5R, and IgM on their cell surface and secrete IgM antibody, suggesting that BCL_1 was originated from B-1 cells. Nevertheless, antigens recognized by the antibody produced by BCL_1 have been unknown.

B-1 cells differ from conventional B cells (B-2 cells) in that they are generated predominantly during fetal and neonatal development [6–8]. B-1 cells can be distinguished from the conventional B cells by their surface phenotype: IgM^+, Mac-1^+, and $CD43^+$ and $B220^{low}$ [9,10]. B-1 cells are further classified into B-1a and B-1b cells, the former of which are characterized by their expression of CD5. Although functions of B-1 cells have not been addressed clearly, B-1 cells contribute to the production of most of serum immunoglobulin and natural antibodies, which play a major role in the primary line of defense against infections [11].

In the present study, we found that BCL_1 cells bound *E. coli*. To characterize the structure on BCL_1 cells responsible for binding the bacteria, we produced a monoclonal antibody capable of inhibiting BCL_1 cell binding to bacteria. Sequential immuno-precipitation experiments showed that the antibody recognizes an idiotypic epitope of surface IgM expressed on BCL_1. These results suggest that the IgM antibody

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doi:10.1016/j.imlet.2004.11.019

produced by BCL_1 cells recognizes bacterial antigens expressed by *E. coli*.

2. Materials and methods

2.1. Animals

C57BL/6J mice were purchased from Nippon CLEA (Tokyo, Japan). BALB/c mice were from Nippon SLC (Shizuoka, Japan). A Lewis rat was from Seac Yoshitomi (Yoshitomi, Fukuoka, Japan). All the animals were kept in our own animal facility under specific pathogen-free condition. 6- to 15-week-old female mice were used for experiments throughout the present study.

2.2. Cells and culture condition

A20 cells (BALB/c-derived B lymphoma), BCL_1-B20 cells (BALB/c-derived B lymphoma) [12] and Y3-Ag 1.2.3 cells were obtained from Cell Resource Center for Biochemical Research (Tohoku University, Miyagi, Japan). BCL_1-5B1b cells and BCL_1-CW13.20 cells [13] were obtained from Japanese Collection of Research Bioresources (Tokyo, Japan) and American Type Culture Collection (Rockville, MD), respectively. CH27 cells (B10.H2aH-4bp/Wts-derived B lymphoma) were kindly provided by Dr. K. Nakamura (University of Tokyo, Tokyo, Japan). All these cell lines were maintained in RPMI-1640 medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated FCS, 25 mM HEPES, 100 μg/ml penicillin, 100 U/ml streptomycin, 2 mM glutamine and 50 μM 2-mercaptoethanol. For induction of IgM secretion, BCL_1 cells were cultured in the same medium except containing 10% (v/v) culture supernatants from a mouse IL-5-transfected X63 cell line [14], a generous gift from Dr. Hajime Karasuyama, Tokyo Medical and Dental University, Tokyo, Japan.

2.3. Antibodies (Abs)

Goat anti-mouse IgM (μ chain specific) antibody was purchased from ICN (Costa Mesa, CA). Fluoresceinisothiocyanate (FITC)-conjugated monoclonal rat anti-mouse IgM antibody, biotin-conjugated monoclonal rat anti-mouse IgM antibody, PerCP-conjugated anti-mouse CD5, a purified IgM antibody (6-27.5; anti-mouse H-$2D^p$/H-$2K^p$), FITC-conjugated anti-mouse B220 (CD45R) antibody, and FITC-conjugated anti-mouse CD43 antibody were purchased from BD Biosciences (Sand Diego, CA). SK1, which recognizes a private epitope on BCL_1-B20 and −CW13.20, was produced in this study as described below. Mouse anti-rat κ light chain antibody (MAR18.5) and rat anti-Mac-1 (CD11b) antibody (M1/70) were purified from the culture supernatant of the hybridomas obtained from American Type Culture Collection and were conjugated with FITC in a standard protocol. Secreted BCL_1-IgM were purified from the culture supernatant of the BCL_1 cells using ImmunoAssist MG-PP (Kanto Kagaku, Tokyo, Japan) according to the protocol provided by the manufacturer. Rat anti-idiotypic BCL_1-IgM antibody (Mc106A5) and hamster anti-idiotypic BCL_1-IgM antibody (AT65) were generous gifts from Dr. Alison Tutt, Southampton General Hospital, Southampton, UK.

2.4. Soluble protein tetramers

Soluble mouse Ly49A tetramers were prepared as described previously [15]. Production of soluble mouse CD8α will be published elsewhere (M. Ito, K. Yamamoto and N. Matsumoto, manuscript in preparation). cDNA encoding the extracellular portion of mouse CD8α was amplified by polymerase chain reactions (PCR) using cDNA from concanavalin A-stimulated T cells prepared from a C57BL/6 mouse as a template. Those of mouse NKR-P1C and mouse NKp46 were amplified by PCR using cDNA from IL-2-activated natural killer cells prepared from C57BL/6J mice. Used primers were CD8α sense 5′-AAA*CATATG*AAGCCACAGGCACCCG, CD8α antisense 5′-GCCACGGGGCCGACA-3′, NKR-P1C sense 5′-GAT*CCCGGG*CAAAAACCATCAAGAGAAAAAT-3′, NKR-P1C antisense 5′-CTA*GGATCC*GCAGTCAGGAGTCATTA-3′, NKp46 sense 5′-AAA*CATATG*GAAAAGGAGACTCTCCCG-3′, and NKp46 antisense 5′-AAA*CCCGGG*GAGATTCTGGGTTGTGTGA. The PCR-generated cDNA fragment of NKR-P1C was cloned into the bacterial expression vector pET3cN-bio, which was designed to express a recombinant protein with a N-terminal enzymatic biotinylation signal [15]. The PCR-generated cDNA fragments of CD8α and NKp46 were cloned into the bacterial expression vector pET3cN-bio, which was designed to express a recombinant protein with a C-terminal enzymatic biotinylation signal [16]. The constructs were sequenced to confirm for correct sequence and frame. CD8α and NKR-P1C were refolded by dilution as described [15]. NKp46 was refolded by stepwise removal of guanidine HCl as described [17]. Then these refolded proteins were purified by sequential ion exchange and gel filtration chromatography, and then biotinylated with BirA biotin ligase (Avidity, Denver, CO). Each of the biotinylated proteins used in this study migrated as a single band on standard SDS-PAGE gels under reducing condition. Gel filtration analysis of the biotinylated proteins revealed that Ly49A, CD8α and NKR-P1C were monomers while NKp46 were oligomerized to form high molecular weight complex. The proteins were complexed with R-phycoerythrin (PE)-labeled streptavidin (PE-SA) (BD Biosciences) at a molar ratio of 4:1.

2.5. Flow cytometry

For staining with the soluble protein tetramers, 2×10^5 cells were incubated with 25 μg/ml of the tetramers or control PE-SA for 30 min on ice. The cells were then washed twice with Hank's balanced salt solution (Nissui Pharmaceutical,