自己免疫疾患に關与する Ly-1 B 細胞の分化***

The LY-1 B Cell Lineage

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T 細胞特有のマーカーと考えられていた Ly-1 分子が, ある種の B 細胞に表現されていることを明らかにした. この Ly-1 B 細胞の表面免疫グロブリンリセプターの質的・量的検索を FACS を用いた二重染色法を駆使して行った. さらに, この Ly-1 B 細胞の分化経路を検索し, 通常の B 細胞とは異なることを裏づける結果を得た.

索引用語: Ly-1, B cell, FACS, two color analysis, PEC

Key words: Ly-1, B 細胞, FACS, 二重染色法, PEC

INTRODUCTION

The murine Ly-1 lymphocyte surface glycoprotein was defined initially with conventional antisera in cytotoxic assays. As such, it appeared to be expressed exclusively on helper T cells. Later, however, Fluorescence Activated Cell Sorter (FACS) analyses and sorting studies with monoclonal antibody reagents showed that all T cells express Ly-1, regardless of functional subclass¹⁾ Furthermore, these studies^{2,3)} showed that Ly-1 is expressed on several murine B cell tumors and introduced evidence suggesting that this glycoprotein may also be expressed on a small proportion of normal murine splenic B cells^{4,5)}.

Similar studies with human lymphocytes demonstrated the homologous (Leu-1) cell surface antigen on all normal T cells⁶⁾, on some B

cell tumors (particularly chronic lymphocytic leukemias)⁷⁾ and, as in the mouse, on a small proportion of apparently normal B cells⁸⁾. Thus, a series of earlier findings foreshadowed contemporary evidence demonstrating Ly-1 and Leu-1, respectively, on subsets of murine and human B cells and showing further that Ly-1 marks functionally distinct B cells that play a major role in autoimmunity in the mouse.

Here we summarize the physical and functional characteristics that distinguish Ly-1 B cells from the majority of splenic and lymph node B cells. We focus on data from cell transfer and antibody treatment studies, which locate Ly-1 B cells in a separate developmental lineage that branches off from the lymphocyte developmental lineage during prenatal or early neonatal life. We then consider various genetic defects that influence autoantibody production and Ly-1 B representation and, finally, we discuss potential homologies between Ly-1 B and B cells in other species and the possible role(s) that Ly-1 B cells may play as producers of

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anti-idiotypic and other autoantibodies found in normal animals. Thus we attempt to develop a fairly comprehensive view of the Ly-1 B lineage and thereby to create an overall context within which our studies and those of others presented in this volume can be understood.

METHODOLOGY

By and large, the serological and cell transfer studies that we have conducted rely on commonly used methods that have been perfected over the last 20 years in a variety of laboratories. The FACS studies, however, utilize certain unique methods and instrumentation developed quite recently in our laboratory, partly in response to needs generated by the B cell studies discussed here. In particular, we have relied heavily on the advanced capabilities provided by our six-parameter dual-laser FACS instrument⁹⁾ which is coupled to a VAX computer running FACS-DESK software written (in our laboratory) to enable rapid and efficient multiparameter FACS data collection, storage and processing¹⁰⁾. The availability of this instrumentation and software allows us to routinely analyze and sort doubly- or triply-stained cells from large numbers of samples and to reliably detect minor subpopulations, even those containing only 0.5 to 1 % of total live cells in the sample.

The FACS-DESK software provides novel types of contour maps that significantly improved the display of correlated multiparameter immunofluorescence staining data¹⁰⁾. In particular, we make intensive use of probability contour maps, which tend to emphasize subpopulations in proportion to their representation in the overall population. These contour maps, used to display virtually all correlated immunofluorescence data in our publications,

tend to be more visually reliable than the maps (generated with commercial software) in standard use, in which the number of contours drawn around a peak is proportional to its height rather than to the number of cells it encompasses.

The smoothing method FACS-DESK uses when drawing the contour maps shown in our studies also improves the reliability and utility of the data display¹⁰⁾. In essence, use of this method minimizes the influence of statistical variation on the location of contours so that contour lines tend to have fewer random jagged edges. This substantially reduces distracting visual "noise" in the display and thus greatly facilitates the recognition and comparison of contour patterns that delineate subpopulations in the various analyses. The recognition and functional characterization of the minute Ly-1 B subpopulation in normal spleen (see Figure 3) attests to the validity of the smoothing and contouring algorithm used in these studies.

Finally, the recent development of new, highly efficient fluorochromes that have suitable excitation and emission spectra for multiparameter analyses and can readily be coupled to monoclonal antibodies opened the way to effective three-color and four-color immunofluorescence studies^{11,12)}. The availability of these plant-derived (phycobiliprotein) fluorochromes¹³⁾ and a multiparameter FACS system on which they could be utilized proved extremely important for our studies because it enabled the direct detection of cell surface phenotypes that could only be inferred from two-color analyses¹²⁾.

Definition of the Ly-1 B lineage

The extent of the differences between Ly-1 B cells and conventional B cells is sufficient in

itself to suggest that the Ly-1 B belong to a separate developmental lineage. The Ly-1 B cells have a unique surface phenotype : in addition to being identifiable because they express Ly-1 , they can be recognized because they express distinctive levels of several standard B cell surface markers, e. g., IgM, IgD, B220. Furthermore, they show a distinctly different localization pattern from conventional B cells in that they are present at quite high frequencies in the peritoneal cavity in normal animals but tend to be rare in spleen and undetectable in lymph nodes^{5,12,14,15,16,17,18,19,20,21,22}.

B cell frequency differences in normal and immunologically defective mice also point to a basic distinction between Ly-1 B and conventional B cells⁵. By and large, individual normal and immunodeficient mouse strains show characteristic, genetically controlled Ly-1 B frequencies that vary independently of other B cell frequencies. In general, immunologically normal mouse strains have roughly the same number of Ly-1 B cells. Immunodeficient strains, in contrast, tend to have either markedly elevated (e. g., NZB and motheaten viable mice) or markedly depressed Ly-1 B frequencies (e. g., CBA/N and other X-id strains)^{5,16,17,21}. Similar frequency abnormalities can be induced in normal mice by treating neonates with monoclonal anti-Ig antibodies. Such treatments result in the selective depletion or selective survival of the Ly-1 B cell population (depending on the specificity of the reagent), sometimes for the life time of the animal (manuscript in preparation).

Ontologically, Ly-1 B cells also present a substantially different picture⁵. They are among the earliest B cells detectable in spleen ; however, their predominance recedes as con-

ventional B cell populations develop and fill the spleen. Thus, by 6 wk of age, they represent less than 2 % of total splenic B cells.

Finally, functional studies distinguish Ly-1 B from conventional B cells. Ly-1 B produce many of the commonly studied IgM autoantibodies in autoimmune mice. Furthermore, recent data suggest that they may be responsible for producing much of the autoantibody¹⁴ and anti-idiotypic antibodies produced by normal animals²³. Conventional B cells, in contrast, tend to participate much less frequently in autoimmune responses and, instead, appear to be concerned principally with producing antibodies to exogenously introduced antigens¹⁴.

This extensive series of differences (discussed in more detail below) provides both the rationale and the tools for cell transfer studies that, demonstrate directly that Ly-1 B cells belong to a distinct developmental lineage¹⁵. In essence, these studies identify the donor-derived B cell populations present in irradiated recipients reconstituted with progenitors from various sources. They show that progenitors for Ly-1 B cells are mainly found in the peritoneum and carry surface Ig, whereas progenitors that reconstitute conventional B cell populations typically lack surface Ig and are found in bone marrow rather than in peritoneum. We review this evidence, which dissociates Ly-1 B progenitors from the traditional B cell progenitors in bone marrow, in the sections that follow.

Ly-1 B cell characteristics

Ly-1 B cells express all of the usual B cell surface markers (in conjunction with Ly-1). Several of these markers, however are expressed at levels that are substantially different from the levels found on most splenic and lymph

Figure 1 Cell surface phenotype of peritoneal Ly-1 B cells (revealed by three-color FACS analyses). Peritoneal cells from 3-month-old BALB/c mice were stained simultaneously with 3 monoclonal antibodies: anti-IgM, 331.12/FITC; anti-Ly-1, 53-7.6/allophycocyanin; anti-B220/6B2, RA 3-6B2/biotin or an anti-IgD allotype antibody, anti-Igh-5a, AMS 15.1/biotin. Biotin-conjugated antibodies were revealed by Texas-Red/avidin. Dead cells were excluded by propidium iodide staining. All analyses were conducted as previously described¹²⁾. The box in each panel delineates the Ly-1 B population.

node B cells (see Fig. 1). IgM, for example, tends to be 5- to 10-fold higher on Ly-1 B cells. IgD, in contrast, tends to be 10- to 20-fold lower. Ia, ThB and certain B220 determinants also show distinct (although less dramatic) level differences; and FACS light scatter levels similarly show small but clear differences (Fig. 2). None of these markers is individually suffi-

cient to resolve Ly-1 B cells definitively; however, when used together in two-color and three-color FACS studies, they clearly delineate the Ly-1 B and other B cell populations for analysis and sorting.

Cytotoxic treatment with monoclonal anti-Ly-1 antibodies (particularly C3PO) have also been used to identify Ly-1 B cells²⁴⁾. In some

Figure 2 Forward angle and wide angle light scatter distinguish Ly-1 B cells. Peritoneal cells from a 3-month-old BALB/c mouse were stained for surface IgM (331.12/FITC) and IgD (AMS 15.1/biotin). The gated contour maps shown in the bottom two panels represent the IgM vs IgD distribution of the cells within the scatter gates depicted by the boxes shown in the upper left hand panel. All Ly-1 B cells in the analyzed peritoneal population are contained within the large lymphocyte population whose IgM vs IgD distribution is shown in the lower left panel. The Ly-1 B cells represent approximately 80% of this population. The remaining cells represent a related Ly-1⁻ population.

studies, this method has been used to deplete Ly-1 B cells from splenic B cell populations in order to find out whether Ly-1 B participate in or influence a particular antibody response^{25,26}. In principle, direct analysis and sorting of Ly-1 B cells would be more satisfactory for these purposes; however, these studies clearly demonstrate that cytotoxic depletion analysis represents a viable alternative when the need for many cells or the lack of FACS facilities mitigates against FACS use.

Hemolytic plaque forming cell (PFC) assays that reveal cells producing autoantibodies to mouse erythrocytes treated with the enzyme bromelain (BrMRBC) have proven useful for detecting the presence of Ly-1 B cells, even when there are too few Ly-1 B cells present to reliably detect by FACS analyses. The failure to detect the anti-BrMRBC PFC does not necessarily mean that Ly-1 B are missing; however, in our experience, these PFC provide a reliable indicator for Ly-1 B presence since

Figure 3 Cell surface phenotype of splenic Ly-1 B cells defined by four-color FACS analysis. Spleen cells from a 3-month-old BALB/c were simultaneously stained with 4 reagents : anti-IgM, 331.12/FITC) ; anti-Igh-5a (IgD), AMS-15.1/biotin ; anti-Ly-1, 53-7.6/allophycocyanin ; and anti-B220/6B2, RA3-6B2/phycoerythrin. The “ungated” contour map (lower left) shows the Ly-1 vs IgM distribution for all splenic lymphocytes. The “gated” contour map (lower right) shows the Ly-1 vs IgM profile for the cells that fall within the gates depicted by the shaded box in the B220 vs IgD contour map (upper panel).

1) they are all contained within the Ly-1 B population¹⁴⁾ 2) they are always present at reasonable frequencies in (LPS-stimulated) animals that have normal numbers of Ly-1 B cells; and, 3) they are always either undetectable or reduced in frequency in Ly-1 B-deficient animals (manuscript in preparation).

Ly-1 B location in vivo

Analysis of B cell frequencies in various lymphoid organs demonstrates that Ly-1 B cells localize quite differently from conventional B cells^{5,16)}. In general, Ly-1 B are barely detectable in spleen (Fig. 3) and are almost

never found in lymph nodes (except in animals with advanced autoimmune disease). Furthermore, they represent less than 1 % of the total B cell population in a normal adult mouse. Nevertheless, they frequently comprise more than half the floating and loosely-attached B cells recoverable from the peritoneum (20~40% of total).

This concentration in the peritoneum appears to be due to the operation of a homing mechanism, since injecting irradiated animals intravenously with allotype congenic Ly-1 B cells results in the specific accumulation of donor-derived Ly-1 B cells in the peritoneum

Table 1 Characteristics shared by murine Ly-1 B cells and rat marginal zone B cells

Phenotype	Bright for IgM, low-to-negative for IgD. Slightly larger than bright IgD ⁺ follicular B cells
Organ Location	Present in spleen but not nodes
Ontogeny	Develop early in post-natal life
Precursors	Distinct from precursors for bright IgD ⁺ B cells that populate follicles in spleen and nodes
Antibody	Depleted by treatment of neonates with anti-IgM but persist at normal levels in anti-IgD-treated animals

shortly after injection (Hayakawa, unpublished data). These cells gradually disappear and are no longer detectable a week to 10 days later. Their relationship to the reconstituted peritoneal Ly-1 B populations that become visible 3 to 4 wk after transfer has yet to be determined.

The anatomical location of the small number of Ly-1 B cells found in normal spleen also remains to be determined; however, it is possible that these cells reside in or near the marginal zones that separate the white pulp and red pulp areas of the spleen. We make this suggestion based on the description, by McClennan, Gray, Bazin and co-workers, of two apparently distinct B cell lineages in the rat that exist in anatomically distinct locations in the spleen^{27,28,29}. The cells from one of these putative lineages, located mainly in the splenic marginal zone, appear quite similar to Ly-1 B with respect to Ig surface phenotype and several other properties (see Table I). Thus it is possible that the anatomical location of these cells (in the rat spleen) predicts the anatomical location of the mouse Ly-1 B population, i.e., in the marginal zone of the mouse spleen.

Potential Ly-1 B cell homologs in other species

In addition to the rat B cell population(s) discussed above, there is a human B cell population that appears to be homologous to the murine Ly-1 B lineage⁸. This human population carries the Leu-1 antigen, which is structurally homologous to Ly-1 (S. Huang, manuscript in preparation). It has been associated with the production of a variety of autoantibodies (e. g., cold hemagglutinins, cytoskeletal antibodies, rheumatoid factor)³⁰. Furthermore, it has been shown to be elevated in a proportion of patients with rheumatoid arthritis³¹ (Hardy et al., personal communication; Taniguchi and Okumura, personal communication).

There is also reasonable evidence suggesting that Ly-1 B may be homologous to the predominant B cell population in chickens³².

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