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FURTHER INSIGHTS INTO B-1 CELL BIOLOGY

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Abstract The term B-1 cell was originally proposed to describe a subtype of B lymphocytes, which differs from B conventional cells by anatomical localization, developmental origin, surface markers expression, antibody repertoire and growth properties. B-1 cells express high levels of surface IgM, low levels of B220 (CD45R) and IgD, but not CD23, whereas conventional B-2 cells express high levels of B220 and IgD, CD23 and low levels of IgM. Besides, typical B-1 cells residing in peritoneal cavity also express low levels of Mac-1 (CD11b). Further, B-1 cells are sub classified in B-1a cells, which express CD5, and their phenotypic $CD5^-$ "twins", B-1b cells. Our laboratory has demonstrated that B-1b cells proliferate in cultures of adherent mouse peritoneal cells and differentiate into a mononuclear phagocyte, provisionally named "lymphophage". Yet, that these cells migrate from the peritoneal cavity to a non specific inflammatory lesion. From these observations the origin, differentiation and function of these cells in normal and pathological conditions have been intensively investigated in our laboratory. The morphology of B-1 cells, its participation in giant cell formation and granuloma development *in vitro*, and facilitation of murine melanoma growth will be presented and discussed.

Key words: B-1 cells, phagocytosis

Resumen ***Nuevos aspectos de la biología de las células B-1***. El término célula B-1 fue inicialmente propuesto para describir un subtipo de linfocito B que difiere de los B convencionales por su localización anatómica, su desarrollo, sus receptores de membrana, su repertorio de anticuerpos y sus propiedades de crecimiento. Las células B-1 expresan altos niveles de IgM, bajos niveles de B220 (CD45R) y IgD, pero no CD23, mientras que las células B-2 convencionales expresan altos niveles de B220 y IgD, CD23 y bajos niveles de IgM. Además, las B-1 típicas residen en la cavidad peritoneal y expresan bajos niveles de Mac-1 (CD11b). Más aún, las células B-1 han sido subclasificadas en B-1a que expresan CD5 y en B-1b su fenotipo "mellizo" CD5 negativo. En nuestro laboratorio, hemos demostrado que las células B-1b proliferan en cultivos de células peritoneales adherentes de ratón y se diferencian en una célula mononuclear fagocítica, provisionalmente designada "*lymphophage"* y que estas células al migrar desde la cavidad peritoneal forman una lesión no-inflamatoria. Hemos estudiado estas células *in vitro*, en cuanto a su morfología, su participación en la formación de células gigantes y su desarrollo granulomatoso. También hemos observado que facilitan el crecimiento de un melanoma murino.

Palabras clave: células B-1, fagocitosis

In 1991, B-1/ B-2 lymphocytes were adopted to designate two distinct populations of B cells[1]. B-1 cells were described in the beginning of the eighties when CD5, a T cell marker, was detected in B cells from Chronic Lymphatic Leukemia (B-CLL) in mice[2, 3]. CD5 (Ly-1) was detected in 95% of B-CLL cells and in 3 to 5% of normal lymphocytes from the spleen and tonsils[3]. Ly-1 (CD5) was characterized in a subpopulation of splenic B cells[4] and in 1986, a population of $CD5^+$ B cells was found in the peritoneal cavity of normal mice[4, 5].

In the mouse, B-1 cells constitute a smaller population as compared with that of B-2 cells and are homed predominantly in pleural and peritoneal cavities[6]. Differing from conventional B cells (B-2), B-1 cells are self renewed and apparently not dependent of progenitor cells and proliferative stimuli. This characteristic is due to the constitutive expression of STAT3 (*Signal Transduction and Activator of Transcription*), a cytoplasmatic protein that, after phosphorilation, is translocated to the nucleus after receptor stimulation[7]. This characteristic has been associated with neoplastic transformation of B-1 cells[8].

The surface phenotype of B-1 cells is characterized by the expression of IgM^{hi}, IgD^{lo}, $B220^{lo}$, CD43+, CD11b e CD5 (B-1a). B-1b cells do not express CD5. Based on these characteristics, Plytycz and Seljelid[9] postulated that B-1 cells constitute a promiscuous lineage since they question the paradigm of lineage commitment.

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B-1 cells proliferate *in vitro*

B-1 cells compose from 10 to 15% of the total free cell population in the peritoneal cavity of mice. This paucity has been a limiting factor for the development of experiments directed to the better understanding of these peculiar cell sub sets. Borrello and Phipps[10] developed a model in which B-1a cells purified from the spleen, can be maintained in culture when co-cultivated with fibroblasts.

More recently, we have developed a model to culture B-1 cells *in vitro* which allows the production of larger amounts of these cells. Briefly, total mouse peritoneal cells are cultured for about 1 h on glass or plastic dishes. Non adherent cells are washed out and culture medium plus 10% fetal calf serum added to the cultures which are maintained up to seven days without changing the medium. Under these conditions B-1 cells proliferate[11]. Thies et al. (*in preparation*) have shown in our laboratory that B-1a cells present in the peritoneal cavity of mice do not have the ability to adhere to the plastic or glass surface and to differentiate into phagocytic cell.

B-1 cells have a peculiar morphology

Until recently, the morphology of B-1 cells was not fully characterized. Abrahão et al[12] in our laboratory identified B-1 cells using colloidal gold immunocytochemical assays and purified B-1 cells from supernatants of adherent peritoneal cell cultures by a magnetic bead technique. The findings led the authors to demonstrate that, in mice, either B-1a or B-1b cells have a unique morphology distinct from that of B-2 cells. The main morphological characteristic of these cells resides in re-entrances of the nuclear membrane suggesting a lobular organization of the nucleous.

B-1 cells differentiate *in vitro*, into a novel mononuclear phagocyte distinct from monocyte derived macrophages

Borrello and Phipps[10] have cultured B-1a cells *in vitro* and showed that when co-cultured with fibroblasts they differentiate into a phagocytic cell similar to macrophages. Based on these observations, the authors claim that macrophages might have a distinct origin other than from monocytes.

Recently, Almeida et al[11] in our laboratory have shown that the transference of B-1b cells obtained from cultures as above described to a fresh culture medium, induces these cells to differentiate into a bipolar mononuclear cell with a high capacity to phagocytose particles via Fc and mannose receptors. Herzenberg (*personal communication*) has baptized these cells as *lymphophages*.

Evidences that lymphophages differentiate from B-1 cells were obtained by the demonstration that B-1 cells express both lymphoid and myeloid transcription factors. Interestingly, lymphophages lose the ability to express lymphoid transcription factors and maintain the expression of myeloid factors. Further, lymphophages lose their ability to express IgM but maintain the rearrangement of immunoglobulin genes (Popi et al. *submitted*).

Although lymphophages avidly phagocytose opsonized particles, Popi et al (*submitted*) clearly demonstrated that lymphophages phagocytose higher numbers of *Coxiella burneti in vitro* as compared with bone marrow derived macrophages. Paradoxically, they have also shown that lymphophages secret large amounts of NO but kills bacteria in a lesser extent when compared with macrophages.

B-1 cells migrate from the peritoneal cavity to a non specific inflammatory lesion

The demonstration that lymphophages have a high phagocytic ability, the isclosure demonstration that they might migrate from the peritoneal and pleural cavities to distant inflammatory lesions was mandatory. Fulano have shown that B-1 cells migrate to periodontal lesions. Almeida et al[11] labeled B-1 cells in culture and transferred these labeled cells to the peritoneal cavity of syngenic naive mice. Concomitantly, they implanted round glass cover slips into the subcutaneous tissue of the animals. Four days later, cover slips were removed and histo-autoradiograms prepared. Results showed that about 70% of the cells adherent to the glass had their nuclei labeled, thus demonstrating that these cells have the ability to migrate from the peritoneal cavity to a distant inflammatory lesion.

These observations, added to the fact that lymphophages are phagocytic cells, suggested that the participation of lymphophages in inflammation should be further investigated. In this research line, Bogsan et al[13], in our laboratory, have clearly demonstrated that B-1 cells are pivotal in foreign body inflammatory giant cell formation. Further, Vigna et al[14] have shown that lymphophages participate in granuloma formation *in vitro*.

B-1 cell are antigen presenting cells and participate of acquired immunity

Vigna et al.[14] have shown that, among other cell types, B-1 cells had the property of antigen presentation. This observation suggests the possibility of these cells to interact with acquired immunity. Indeed, Gallupar[15] have shown that B-1 cells have immunological memory. In our laboratory Pizarro De Lourenzo et al (*submitted*) made a very elegant experiment showing that B-1 cells have immunological memory. BALB/c mice were immunized

with ovalbumin (OVA). Peritoneal cells from immunized or non immunized mice were adoptively transferred to the peritoneal cavity of BALBc/Xid mice, characteristically deprived of B-1 cells. Later, these B-1 cell reconstituted animals were immunized with OVA and showed that footpad challenge with OVA was significantly diminished in animals that received cells from previously OVA immunized animals. These data, strongly suggest not only that B-1 cells are involved with acquired immunity but also that they might be considered as a B_{reg} cell.

The implications of these observations in the physiopathology and immunity of B-1 cells remains open for further investigations. The "promiscuous" expression of both myeloid and lymphoid characteristics in a single cell type and the factors which govern B-1 cell differentiation in lymphophages will certainly open new avenues for the understanding of lymphoid and myeloid cells physiopathology.

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Siempre he creído que un investigador auténtico debe experimentar, pensar, leer e intercambiar ideas todos los días. Trabajar en algo que interesa o apasiona es un placer, es una de las felicidades humanas más grandes.

Bernardo A. Houssay (1887-1971)