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B-1 cell: the precursor of a novel mononuclear phagocyte with immuno-regulatory properties

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ABSTRACT

Characterization of the origin, properties, functions and fate of cells is a fundamental task for the understanding physiological and pathological phenomena. Despite the bulk of knowledge concerning the diverse characteristics mammalian cells, some of them, such as B-1 cells, are still poorly understood. Here we report the results obtained our laboratory on these cells in the last 10 years. After showing that B-1 cells could be cultured and amplified *in vit* a series of experiments were performed with these cells. They showed that B1 cells reside mostly in the peritone and pleural cavities, migrate to distant inflammatory foci, coalesce to form giant cells and participate in granulom formation, both *in vitro* and *in vivo*. They are also able to present antigens to immunologically responsive cells and a endowed with regulatory properties. Further, we have also shown that these cells facilitate different types of infecti as well as tumor growth and spreading. These data are presently reviewed pointing to a pivotal role that these cells m play in innate and acquired immunity.

Key words: B-1 cells, IL-10, inflammation, infection, neoplasia, BALB/*Xid* mice.

INTRODUCTION

B-1 cells, first characterized by Hayakawa et al. in 1985, express the phenotype $IgD^{low}IgM^{hi}CD23\text{-}CD19^{+}CD11b^{+}$ whilst conventional B cells are identified by the phenotype $IgD^{hi}IgM^{low}CD23^{+}CD19^{+}CD11b^{-}CD5^{-}$. Further, B-1a cells differ from B-1b cells by the expression of CD5 molecules in the former.

In addition to the promiscuous expression of markers for lymphoid and myeloid cell lineages, added with the CD5 T cell marker, these cells have also a peculiar distribution in the body's economy of mice: they are predominantly found in pleural and peritoneal cavities, being few in the spleen and almost absent in lymph nodes (Forster et al. 1991, Marcos et al. 1989, Hayakawa et al. 1985). Contrary to the bone marrow origin c ventional B cells, B-1 are long-lived and auto-re cells (Lalor et al. 1989).

The origin of B-1 cells is still a matter of investigation and two current hypotheses try to el this issue. One, addressed by Herzenberg and (1993) says that B-1 cells do not differentiate fro marrow precursors, but from cells which rearrang Ig genes during fetal and neonatal period of life. on this hypothesis, the authors proposed that d lineages of B lymphocytes, B-1 and conventional phocytes, are descendents of distinct precursor being active in different phases of the immune development. This proposal was supported by

and Hayakawa 1995), whereas cells from the paraortic splancnopleura of 9 days embryo reconstitute the B-1a population (Godin et al. 1993). Finally, grafts of fetal omentum reconstitute the populations of B-1a and B-1b cells (Solvason et al. 1991). Based on these data, the authors concluded that B-1 cells appear in ontogeny before the emergence of conventional B cells, thus supporting the two cell lineage origin of B cells.

Conversely, Haughton and collaborators (1993) proposed that all sub-types of B cells emerge from a common progenitor, and that commitment with a cell lineage is dependent on the influence of antigenic selection (Cong et al. 1991). The authors reported that conventional B cells, cultured in the presence of anti-IgM and IL-6, assume the B-1 phenotype. Based on these observations, the authors concluded that the commitment with B-1 phenotype occurs after IgM expression on the cell surface of not fully differentiated B cells. Sustaining this hypothesis, the authors showed that B cells stimulated with thymus-independent antigens, such as carbohydrate from bacteria, or by auto-antigens such as DNA, can differentiate into B-1 cells.

B-1 CELLS HAVE A PECULIAR MORPHOLOGY

Until recently, the morphology of B-1 cells was not fully characterized. Abrahão et al. (2003), 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 (Fig. 1). 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 bridges of the nuclear membrane, suggesting a lobular organization of the nucleus.

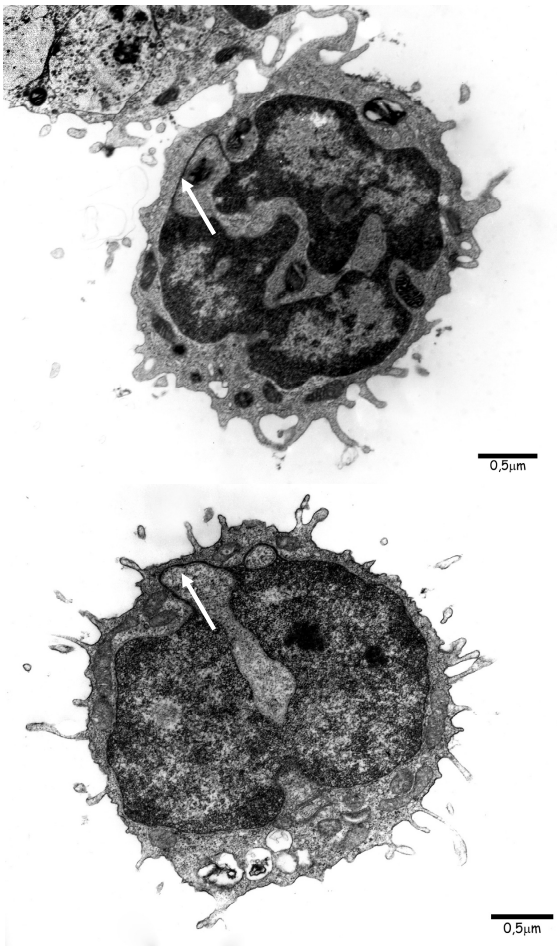

Fig. 1 – Characteristic morphology of B-1b cells. Cells were characterized as B-1 cells using colloidal gold immunocytochemical assays and purified B-1 cells from supernatants of adherent peritoneal cell cultures by a magnetic bead technique. Either B-1a or B-1b cells have a unique morphology which is distinct from that of B-2 cells. The main morphological characteristic of these cells resides in bridges (**arrow**) of the nuclear membrane suggesting a lobular organization of the nucleus.

B-1 CELLS PROLIFERATE *IN VITRO*

B-1 cells represent from 10 to 15% of the total free cell population in the peritoneal cavity of mice. This paucity in cell number has been a limiting factor for the development of experiments directed to the better understand-

them. 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

of mice do not have the ability to adhere to the plastic or glass surface and to differentiate into phagocytic cells.

B-1 CELLS DIFFERENTIATE *IN VITRO* INTO A NOVEL MONONUCLEAR PHAGOCYTE DISTINCT FROM MONOCYTE DERIVED MACROPHAGES

Borrello and Phipps (1995) have cultured B-1a cells *in vitro* and showed that, only 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 that from monocytes. In this direction, Almeida et al. (2001), 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. These cells were named B-1 cell derived mononuclear phagocyte (B-1DMP). Evidences that B-1DMP differentiate from B-1 cells were obtained by the demonstration that B-1 cells express both lymphoid and myeloid transcription factors. Interestingly, B-1DMP cells lose the ability to express lymphoid transcription factors and maintain the expression of myeloid factors. Further, they also lose their ability to express IgM, but maintain the rearrangement of immunoglobulin genes (Popi et al. 2009a).

Although B-1DMP can avidly phagocytose opsonized particles, Popi et al. (2009b) clearly demonstrated that these cells phagocytose higher numbers of *Coxiella burneti in vitro* as compared with bone marrow derived macrophages. Paradoxically, they have also shown that B-1DMP secrete large amounts of NO but kills bacteria in a lesser extent when compared with macrophages.

This phenomenon might result from contamination of the cultures with monocyte-derived macrophages as criticized by colleagues. Experimental results, however, disprove this hypothesis considering that: a) monocyte derived macrophages originate from stem cells in bone marrow of adult vertebrates. In mice, they have two waves of division to become a terminal differentiated cell, the monocyte, which may, under different types of

ity to divide. Our previous report on "macropha
vision *in vitro* and *in vivo* (Mariano and Specto
was a misinterpretation of results, which will b
mented later; b) if mice or total peritoneal cells
radiated before the cells are used to culture B-1
described, they do not grow, although macrophag
spread on the plastic surface. In other words, B-1c
radio-sensitive and macrophages are well known
resistant ones (e Brito et al. 2007); c) B-1 cells
proliferate in culture when cells from *Xid* mice,
mal deprived of B-1 cells, are used and, finally, d
anti-mouse IgM polyclonal antibody is added to t
tures plus mouse fresh serum as a source of c
ment, proliferation of B-1 cells is blocked, ren
only macrophages spread on the plastic surface.

One criticism for the data and for the interpr
that B-1 cells differentiate into a mononuclear pha
is the one of it being an *in vitro* phenomenon th
not occur *in vivo*. Evidences from our laboratory
against these arguments considering that: a) B-1 c
grate from the peritoneal cavity to a site of non-s
inflammation where they become morphological
ilar to monocyte derived macrophages (Almeid
2001); other reports in the literature confirm these
vations (Borrello and Phipps 1996); b) our interpr
that foreign body giant cells resulted from the fu
macrophages *in vivo* (Mariano and Spector 19
proved to be a mistaken interpretation since Bo
al. (2005), in our laboratory, have clearly demon
that B-1 cells are essential for giant cell formatio

These data not only demonstrate a relevant
ipation of B-1b cells in the kinetics of the infl
tory process, but imposes a revisit of the identif
and function of mononuclear cells in inflammat
sions. Also, the participation of B-1 cells on
obtained from experiments with "pure" cultures
herent peritoneal cells must also be re-evaluated
ering that these cells are high producers of IL-10
et al. 1997). This issue only will be solved wh
cific markers for B-1 cells in tissues become avai

Taken together, these data strongly points
existence of a B-1 cell derived from mononucle

lar element of the immune system but a precursor cell which, under different physiological and pathological conditions, is induced to differentiate into a mononuclear phagocyte with functions not fully understood as yet (Popi et al. 2008).

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 B-1DMP will certainly open new avenues for the understanding of lymphoid and myeloid cells physiopathology.

B-1 CELLS MIGRATE FROM THE PERITONEAL CAVITY TO A NON-SPECIFIC INFLAMMATORY LESION

The demonstration that B-1DMP has a high phagocytic ability the demonstration [and] that they might migrate from the peritoneal and pleural cavities to distant inflammatory lesions was mandatory. Aramaki et al. (1998) have shown that B-1 cells migrate to periodontal lesions. Almeida et al. (2001) labeled B-1 cells in culture and transferred these labeled cells to the peritoneal cavity of syngeneic naïve mice. Concomitantly, they implanted round glass coverslips into the subcutaneous tissue of the animals. Four days later, coverslips were removed and histo-autoradiograms prepared. Results showed that about 70% of the cells which adhere 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 the B-1DMP cells being phagocitic, suggested that the participation of B-1DMP in inflammation should be further investigated. In this line, Bogsan et al. (2005), in our laboratory, have clearly demonstrated that B-1 cells are pivotal in foreign body inflammatory giant cell formation. Further, Vigna et al. (2006) have shown that B-1DMP cells are necessary for granuloma formation *in vitro*.

B-1 CELLS ARE ANTIGEN PRESENTING CELLS AND ARE

tion. This observation suggests the possibility of these cells interacting with acquired immunity. Indeed, Alugupalli et al. (2003) have shown that B-1 cells have immunological memory. In our laboratory, De Lorenzo et al. (2007) 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 cells reconstituted animals which were immunized with OVA and showed that foot-pad challenge with OVA was significantly diminished in animals that received cells from previously OVA immunized animals. Also in our laboratory, De-Gennaro et al. (2009), have shown that B-1 cells participate in the mechanisms of oral tolerance. These data strongly suggest not only that B-1 cells are involved with acquired immunity but also that they might be considered as a type of Breg cell.

PARTICIPATION OF B-1 CELLS IN MODELS OF INFECTIOUS DISEASE

As B-1 cells secrete large amounts of auto-reactive IgM, it is assumed by the literature that these cells participate in the mechanisms which govern innate immunity. Nevertheless, experimental investigations question this simplistic interpretation and open the possibility that these cells might have a more complex participation in immunity. For instance, Minoprio et al. (1993) have shown that *Xid* mice, being animals deprived of B-1 cells, are more effective to cope with *T. cruzi* infection than with wild type controls. Moreover, Popi et al. (2008) clearly demonstrated that *Xid* mice, when infected intra-tracheally with *P. brasiliensis*, have a longer survival than wild type counterparts. Reconstitutions of these animals with B-1 cells turn the animals as susceptible to fungus infection as controls do. These data clearly establish a paradoxical phenomenon. Instead of participating in the mechanisms of protection against infectious agents, B-1 cells usually facilitate parasite infection. Similarly, as will be further commented, these cells also facilitate murine melanoma growth and spreading.

100-fold smaller than that observed in wild type mice, which is assumed by the authors as a transient resistance (Junqueira-Kipnis et al. 2005). In our laboratory, a different model has been used (BALBmice Vs. BCG) and, despite not having counted CFUs as yet, if that mentioned difference is valid, it is not corresponded by a clear morphological distinction among observed lesions. Several studies showed the variable behavior of *Xid* mice during different infections: while resistant to *T. cruzi* and *P. brasiliensis*, they are susceptible to *Borrelia hermsii* (Alugupalli et al. 2003), *Strongyloides stercoralis* (Herbert et al. 2002), *Brugia spp* (Paciorkowski et al. 2000) and *Schistosoma mansoni* (Gaubert et al. 1999). Our results allow to state that B-1 cells have a role in the establishment of pulmonary lesions due to BCG, which favors inflammation, as clearly shown by experiments made in irradiated animals. At the 24th day, the B-1a subpopulation seems to provide the main contribution to the infection, but both B-1a and B-1b are equally important for the establishment of inflammation after 38 days. Such cooperation between B-1a and B-1b was recently demonstrated with infections produced by *Streptococcus pneumoniae* (Haas et al. 2005) in which B-1a cells produce antibodies that limit infection, while B-1b cells generate a response that is sufficient to avoid death.

B-1 CELLS INFLUENCE GROWTH AND SPREADING OF MURINE MELANOMA *IN VIVO*

Our laboratory has generated three different lineages of murine melanoma: on the one hand, by *in vivo* selection and by cloning B16 mouse melanoma cells; two sublines with different behavior (high and low metastatic cells) were produced, thus creating a quantitative model for measuring metastatic capacity (Staquicini et al. 2003). On the other, starting from a non-tumorigenic melanocyte cell line (melan-a) (Bennett et al. 1987), Jasiulionis and her group, along epigenetic studies, by sequential stressing procedures of adhesion and deadhesion, were able to lead these melanocytes to malignant cell lines that, in the end, became tumorigenic and metastatic. Since the intermediary lines were also kept and available, it is now

was possible to investigate the participation of B
in the whole process, thus reaching interesting re

Absence of B-1 cells, as happens in *Xid* r
well as in animals whose peritoneal and pleura
ties were previously irradiated, is sufficient to p
arrest B16 tumor growth and inhibit formation o
static pulmonary nodules. This phenomenon is
pendent on IL-10, a cytokine produced in large a
by these cells, and can be completely reverted by
constitution of the B-1 population with syngene
(Staquicini et al. 2008). The minor participation
10 in this process was confirmed by using kn
mice (Pérez et al. 2008), in which all results we
similar to those seen in wild type animals. It is
dent, however, on cell-to-cell interaction and not
uble factors, since no differences in behavior w
served after co-cultivation with unattached cells
swell plates. When co-cultivated, both B-1 and
noma cells form tightly attached clusters, most pr
dependent on adhesion molecules present on the
of both cells. Also, we have shown that B-1 c
by increasing the metastatic capacity of melanon
by up-regulating their amount and phosphorylati
els of the constitutionally phosphorylated extrac
signal-regulated kinase (ERK) (Pérez et al. 2008
high malignancy of the resulting cells is probab
to differential expression of surface molecules
be disclosed.

The transformation of non-tumorigenic mela
cells into a very malignant subline by the adhes
adhesion procedure represents a patient and tim
suming method. Similar results, however, can
tained by simply cocultivating melan-a in the pr
of B-1 cells for 48 to 72 hs. These melanocyti
even after the riddance of B-1 cells, become tumo
and metastatic such as those coming from the seq
stress experiments. Using phage display metho
we tried to uncover surface molecules that coul
differential expression and be potentially respons
such malignant behavior. As yet, since experime
still ongoing, preliminary results have shown an
mal expression of some proteoglycans, mainly P

metastatic phenotype of these cells. On the other hand, we have already provided enough evidence of B-1 cells influencing the out coming of murine melanoma.

FINAL COMMENTS

Little is known on the physiology of B-1 cells, as are their functions and insertion on the immune system as a whole. This is most probably due to their paucity and localization and to the difficulties of their cultivation until recently. Literature is scarce and the number of research groups dealing with these issues is very small. These circumstances made the contributions on this knowledge provided by our group already recognized by the community of investigators involved with B cells. We described how to cultivate and amplify the number of cells, showed their participation on different inflammatory processes, as well as their influence on the outcome of some tumor disorders. We also added information on migration, participation on granuloma formation and interactions with other cells, either of the immune system or from different origins. The not fully differentiated condition of these cells, which allow them, under some special situations, to perform myeloid functions, as yet confined to macrophages, must not be neglected.

Thus, our knowledge on the participation of B-1 cells in physiology and pathology has become primordial. Nevertheless, evidence that these cells are endowed with tolerogenic properties, among others, is an exciting clue to explain unsolved problems in immunopathology. One might speculate that the absence of B-1 cells in *Xid* mice renders these animals more resistant to infection and tumor growth due to the release of T cell specific functions by unknown regulatory mechanisms imposed by B-1 cells. Once definitely proved, these cells might become a population to be regarded as one of the most important lineages composing the immune system.

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ensão dos fenômenos fisiológicos e patológicos. Apesar da grande quantidade de conhecimentos relativos às diversas características das células de mamíferos, muitas delas, inclusive células B-1, são pouco entendidas. Depois de mostrar que as células B-1 podem ser cultivadas e amplificadas *in vitro*, uma série de experimentos visando esclarecer as propriedades dessas células puderam ser feitos em nosso laboratório nos últimos 10 anos. Assim, pudemos demonstrar que células B-1 residem principalmente nas cavidades peritoneal e pleural do camundongo, migram para focos inflamatórios distantes, coalescem para formar células gigantes e participam na formação de granulomas, tanto *in vitro* como *in vivo*. São também capazes de apresentar antígenos a células responsivas e são dotadas de propriedades imuno-regulatórias. Mostramos ainda que estas células favorecem diferentes tipos de infecções bem como o crescimento e metastatização tumoral. Esses resultados sugerem que células B-1 devem exercer papel central na imunidade como um todo.

Palavras-chave: células B-1, IL-10, inflamação, infecção, neoplasia, camundongos BALB/*Xid*.

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