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Tolerogenic property of B-1b cells in a model of allergic reaction☆

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Abstract

Since B-1 cells were first described, their origin and function remain controversial. Given the ability to produce natural antibodies and large amounts of IL-10, there is a consensus about their role in innate immunity. More recently, however, B-1 cells have been associated to adaptive immunity as well, due to the demonstration of immunological memory and antigen presentation capability. Here we demonstrate that adoptive transfer of pre-sensitized B-1b cells (obtained from OVA-sensitized mice) to naïve B-1 deficient animals, drastically affects the ability of transplanted animals to mount an adaptive response upon immunization with OVA. In contrast to naïve B-1 populated mice, mice transplanted with sensitized B-1 exhibit lower anti-OVA antibody levels, milder footpad swelling in response to OVA subcutaneous injection and reduced granulomatous reaction to OVA-coated beads. Moreover, we show that these pre-sensitized B-1 cells, when acting as APCs, induce poor T cell proliferation *in vitro* when compared with macrophages or B-1 cells obtained from naïve mice. This property may be due in part to insufficient expression of the co-stimulatory molecule CD86, necessary for optimal antigen presentation. In conclusion, our data suggest a novel role for B-1 cells as part of suppressor mechanisms in the immune system.

Keywords: B cells; Tolerance; Supression; Anergy; Allergy

1. Introduction

The observation that malignant cells from patients suffering from chronic lymphatic leukemia express both lymphoid and myeloid surface markers stimulated the search for cells bearing this phenotype physiologically. In 1980s Herzenberg and her group demonstrated that Ly-I cells featured these characteristics and could be found in the mouse peritoneal and pleural cavities, but were rare in the spleen and virtually absent in lymph nodes [1,2]. These findings supported a new classification of B cells. In 1991, the division of B cells into three subpopulations was proposed: conventional B cells (B-2), which evolve to plasma cells, B-1a and B-1b cells [3].

B-1 cells have a peculiar morphology not compared with the morphology of B-2 cells [4]. B-1b cells express CD11b (Mac-1), CD19, CD45 (B220) and IgM surface markers, whereas B-1a cells have added to this repertoire the T cell-related CD5 molecule. The mixed myeloid and lymphoid characteristics of these cells have justified their designation as "promiscuous cells", and may contribute to their remarkable plasticity. For instance, B-1b cells obtained from cultures of mouse peritoneal adherent cells differentiate into a mononuclear phagocyte [5]. In the course of such differentiation, the cells loose expression of lymphoid transcription factors, while maintaining the expression of myeloid transcription factors (personal communication). In addition, they are shown to migrate to inflammatory foci and to participate in giant cell formation [6] and to present antigen [7].

Despite the increasing volume of information about B-1 cells, the role played by them in innate and acquired immune response is not clear. As they are the major producers of self-reactive IgM [8,9], a number of reports have linked them with resistance to infection [10–12], tumor development [13] and autoimmune

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manifestations [14,15]. Paradoxically, Xid mice, which lack B-1 cells due to a mutation in the Bruton's tyrosine kinase [1,16], cope more efficiently with *Trypanosoma cruzi* infection as compared with wild type counterparts [17]. It was also demonstrated that depletion of B-1 cells by selective irradiation turns the animals significantly more resistant to growth and metastatization of melanoma cells. The same phenomenon was observed when Xid mice were inoculated with this neoplastic cell lineage (personal communication). These data point to a down-modulatory effect of B-1 cells in the immune response.

The putative suppressor mechanisms by which B-1 cells facilitate parasite infection, autoimmune manifestations and melanoma malignancy are not understood. In an attempt to link B-1 cells with acquired immunity, Alugupalli et al. [18] demonstrated that B-1 cells have immunological memory, since animals lacking B-1b lymphocytes are susceptible to *Borrelia hermsii* infection but become protected upon adoptive transfer of this cell subtype derived from convalescent mice. Seeking to understand the role that B-1 cells might play in acquired immune response, we adoptively transferred B-1 cells from ovalbumin immunized wild type mice to Xid mice and investigated the subsequent generation of response to the antigen. Results demonstrated that B-1 cells exert a potent tolerogenic property in this model. The significance of this finding for innate and acquired immunity is discussed.

2. Material and methods

2.1. Experimental design

BALB/Xid mice were adoptively transferred with B-1 cells obtained from either näive or OVA-immunized BALB/c mice (immunization with hen egg white as described below), constituting NB-1 (näive B-1) and IB-1 (immune B-1) groups, respectively. Non-transplanted BALB/c and BALB/Xid mice were used as controls. The four groups were immunized with OVA 24 h after adoptive transfer. Fourteen days post-immunization, the animals were submitted to one of the following challenge procedures to assess the magnitude of response to OVA: [1] footpad injection of purified OVA and measurement of swelling as a parameter of hypersensibility response; [2] intrapulmonary injection of OVA-coated beads and assessment of granulomatous reaction by histology 14 days later. Anti-OVA humoral response was also evaluated.

2.1.1. Mice

BALB/c female mice and BALB/Xid mice were obtained from the Animal Facility of the Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, Brazil. The experimental groups of 5–6 animals were 5–7 weeks old and maintained in controlled light and temperature receiving water and food *ad libitum*. This study was approved by Research Ethical Committee from Federal University of São Paulo, Brazil.

2.1.2. B-1b cell purification and transfer

B-1b cells were obtained as described by Almeida et al. [5]. Briefly, peritoneal cells were collected from mouse abdominal cavity by repeated lavage with 5 ml of RPMI-1640 medium (Sigma). Cells (2×10^6 cells/ml) were dispensed on glass dishes and incubated at 37 °C for 60 min. The culture supernatants were then aspirated to remove the non-adherent cell fraction. Adherent cell monolayers were rinsed with RPMI and subsequently covered with supplemented medium. Cultures were maintained at 37 °C in 5% CO_2 for 5 days. B-1 cells were purified from free-floating cells in the supernatant of these cultures using a magnetic bead system MiniMACS (Miltenyi Biotec, Germany). Approximately, 10^7 cells were incubated with rat antibody anti-mouse CD19 (Pharmingen, San Diego, CA, USA), followed by incubation with iron-coupled anti-rat IgG (Miltenyi Biotec, Germany). Cells were passed through a 25MS positive selection column (Miltenyi Biotec), were B-1b cells were retained. The purity of population was evaluated by FACS as described below, using anti-mouse CD19 and anti-mouse CD11b (Mac-1) phycoerythrin and fluorescein-conjugated, respectively (Pharmingen). It was recently demonstrated in our laboratory that B-1a cells do not attach to the glass surface (Thies et al., in preparation). Mice from NB-1 and IB-1 groups received 5×10^5 naive and OVA-primed B-1 cells, intraperitoneally, respectively.

2.1.3. Immunization with heat-coagulated hen egg white (HEW)

HEW was prepared as previously described [19,20]. Briefly, hen eggs were boiled in water for 30 min. The solid egg white was separated, washed in distilled water, dehydrated in 100% ethanol for 24 h and cut into small fragments of 4 mm × 2 mm × 2 mm (approximately 40 ± 2 mg each). The fragments were re-hydrated by immersion in phosphate-buffered saline (PBS) for 2 h at room temperature and subcutaneously implanted in the dorsum of control and B-1-populated mice.

2.1.4. Detection of serum anti-OVA IgM and IgG

ELISA was performed to detect anti-OVA antibodies in mouse serum. Plates were coated with 5 μg chicken egg albumin (Sigma) and maintained at 37 °C for 2 h and overnight at 4 °C. The blockage was made with PBS containing 1% BSA for 1 h at room temperature. Plates were washed three times with PBS containing 0.05% Tween-20 (A.G. Scientific, San Diego, CA). Serum samples were added (50 μl, 1:100) in duplicate and serial dilutions were performed up to 1:12,800. Plates were incubated at room temperature for 2 h. After washing, wells were coated with peroxidase-conjugated anti-IgG or anti-IgM monoclonal antibodies, obtained in our laboratory, and incubated at room temperature for 1 h. Plates were then washed and *o*-phenylenediamine (Sigma) was added as a peroxidase substrate. Optical density (OD) was determined at 492 nm.

2.1.5. Heat-aggregated ovalbumin

Type II ovalbumin (Sigma, St. Louis, MO, USA) was diluted in PBS and heated in a dry bath for 5 min at 100 °C. After centrifugation at 3000 × *g* for 10 min, the supernatant was discarded and the pellet resuspended in sterile PBS at a concentration of 20 mg/ml.