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Immunobiology 215 (2010) 264–274
Immunobiology
www.elsevier.de/imbio

B-1 cell participation in T-cell-mediated alloimmune response

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Received 15 April 2009; received in revised form 21 May 2009; accepted 22 May 2009

Abstract

B-1 and B cells are important producers of natural antibodies in mice and humans and, therefore, are considered as the first line of defense against pathogens. Because of that, their role in T-cell-mediated immune responses is commonly underrated. However, recent studies have described the participation of B-1 cells in immediate and delayed-type hypersensitivity. The present work assessed the role of B-1 cells in the rejection of allografts in mice, an immune reaction mainly orchestrated by T cells. We have transplanted allogeneic skin and heart to wild-type and B-1-cell-deficient mice, and followed rejection kinetics. Skin graft-infiltrating cells were analyzed by flow cytometry.

We observed a delay in rejection kinetics of B-1-cell-deficient mice when compared to wild-type mice. Adoptive transfer of B-1 cells into B-1-cell-deficient mice abrogated this delay. The longer survival observed in the absence of B-1 cells correlated with less $CD8^+$ T cells infiltrating the grafts, as well as with more mast cells. Collectively, our results show the participation of B-1 cells in the allograft rejection process in mice and collaborate to the understanding of B-1 cell biology.
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Keywords: Alloimmunity; B-1 cells; Graft-infiltrating cells; Mast cells; Transplant rejection

Introduction

The biological functions of B-1 cells in innate and adaptive immunity are issue of debate. Although descriptions of B-1 cells date from the early 1980s (Hayakawa et al. 1983; Manohar et al. 1982), a more detailed morphological characterization of these cells was only published in 2003 (Abrahao et al. 2003). This fact is representative of how scattered and disconnected is the information about these cells. B-1 cells, which represent the main B-cell population of peritoneal and pleural cavities in mice (Hayakawa et al. 1986), differ from conventional B lymphocytes (B-2) in respect to many features such as surface markers, antibody repertoire, developmental pathways and B-cell receptor signaling (Hayakawa and Hardy 2000). B-1b cells are known to express CD11b, CD19, CD45 and immunoglobulin M (IgM), whereas B-1a cells also express CD5. Besides the difference in CD5 expression, B-1a and B-1b cells have also distinct functions in the immune system (Alugupalli et al. 2004; Choi and Baumgarth 2008).

It is well known that B-1 cells are the major producers of natural self-reactive IgM (Herzenberg et al. 1986)

Abbreviations: DTH, delayed-type hypersensitivity; EDTA, ethylenediamine tetracetic acid; FACS, fluorescence-activated cell sorter; RPMI, Roswell Park Memorial Institute; Xid, X-linked immunodeficient.

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doi:10.1016/j.imbio.2009.05.007

and, because of that, they have been linked to early innate resistance to infection (Baumgarth et al. 2005) and autoimmunity (Murakami et al. 1995). Therefore, B-1 cells are commonly thought as part of innate, T-cell-independent humoral immunity. However, it has recently been described by others (Szczepanik et al. 2003) and our laboratory (De Lorenzo et al. 2007) that B-1 cells also participate in T-cell-mediated immune reactions such as immediate and delayed-type hypersensitivity (DTH).

Transplant rejection share some features of DTH, such as tissue damage elicited by T cell-activated macrophages. In view of that, the present work assessed the role of B-1 cells in the rejection of allografts, a phenomenon mainly orchestrated by T cells. Previous studies have documented that mouse B-1 cells secrete natural IgM against the carbohydrate epitope α1,3 Gal transferase, which is largely expressed in pig cells (Kawahara et al. 2003). It is strongly suggested that these antibodies would mediate hyperacute rejection in pig-to-primate transplantation, as well as in other xenografts (Cramer 2000). Following the same path, human B-1 cells have also been shown to produce natural antibodies against blood group A carbohydrate determinant (Zhou et al. 2003), a fact that poses a barrier to ABO-incompatible transplantation. Both xeno and ABO-incompatible transplantation rejections, as opposed to allograft rejection, are mediated by natural antibodies and complement activation (Cramer 2000). Thus, although B-1 cells have been linked to transplant rejection, their described mechanisms of action remain based on naturally existing antibodies.

In order to assess if B-1 cells can influence T-cell-mediated allograft rejection, we transplanted major histocompatibility complex-incompatible skin and heart to wild-type and X-linked immunodeficient (Xid) mice. The Xid deficiency in mice is caused by a mutation in Bruton's tyrosine kinase (Btk), which results in a lack of B-1 cells (Khan et al. 1995).

In the present work, Xid mice took longer to reject allografts and this delay was abolished by adoptive transfer of B-1 cells, which were able to regulate the infiltration of inflammatory cells, namely $CD8^+$ T and mast cells, into the graft.

Methods

Mice

Female BALB/c, BALB/Xid ($H\text{-}2^d$) and C57BL/6 ($H\text{-}2^b$) were purchased from the animal facility of Universidade Federal de São Paulo (CEDEME) and maintained in specific pathogen free environment. With exception to neonate heart donors, all animals were 6 weeks old at the time of transplantation.

Skin and heterotopic heart grafting

For all transplants, receptors were either BALB/c or BALB/Xid and donors were either C57BL/6 (allografts) or BALB/c (isograft control).

Skin grafts were performed as described (Billingham and Medawar 1951). Briefly, a full-thickness $1\,cm^2$ tail skin piece was removed from the donor and transplanted to the dorsal region of the receptor. The graft was covered in protective bandage for 5 days to allow healing. In survival experiments, grafts were daily monitored and rejection was defined when $>80\%$ of graft area exhibited necrotic aspect.

Heterotopic heart grafts were performed according to Fey's method (Fey et al. 1998). In brief, a heart from neonate donor was excised, longitudinally split in two and put in a Petri dish with cold saline solution. Receptor mouse was anesthetized and a 1 cm incision was made in the base of its ear pinna. From the incision, a subcutaneous pocket was gently opened with a steel hypodermic needle and the split-heart was then inserted into the pocket. A slight pressure was used to close the incision. The ears were daily monitored and rejection was defined using a score scale that comprehended four aspects: macroscopic observation of contractile activity (indicative of heart function), graft pigmentation (indicative of appropriate blood influx), loss of graft's original size and loss of graft's defined shape (indicatives of tissue damage).

In both techniques the observer was blinded to the groups and rejection was confirmed by histology.

Adherent peritoneal cell culture

The previously described method (Almeida et al. 2001) was adapted. Cells were harvested from the peritoneal cavity of mice by flushing with 8 mL RPMI 1640 medium (Sigma, Saint Louis, MO, USA). The cell suspension (5×10^5 cells/mL RPMI) remained for 40 min at 37 °C in a Petri dish in order to allow adherence. All supernatant was then discarded and the same volume of RPMI 1640 containing 10% heat-inactivated fetal calf serum was added to the adherent cells. Cultures were maintained for 5 days at 37 °C in 5% CO_2 atmosphere. At day 5 the floating cells were harvested for further experimental procedures. To obtain 10^7 floating cells, peritoneal cells from 8 to 10 mice were pooled together.

Analysis of cell phenotypes

Samples obtained from graft-infiltrating cells, adherent cell cultures or peritoneal *ex vivo* lavage were analyzed by flow cytometry. Cell density was adjusted to 10^7 cells/mL in phosphate buffered saline (PBS) with

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