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B-1 cells modulate the kinetics of wound-healing process in mice ☆

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Abstract

Wound healing is a complex phenomenon whose mechanisms are not fully understood. Although inflammatory cells are recruited to the site of the lesion there are no reports concerning the participation of B lymphocytes in tissue repair. As demonstrated in our laboratory, B-1 cells migrate to a non-specific inflammatory focus and differentiate into a phagocyte. It has been reported that BALB/*Xid* mice are deficient in B-1 cells. Using this model, here we report that BALB/*Xid* mice have an increased inflammatory response, a delayed wound-healing process, a prominent neutrophilic infiltration of the lesion, and an increased neovascularization of the lesion as compared with BALB/c and BALB/*Xid* reconstituted with B-1 cells. The infiltration of B-1 cells into the wound was demonstrated. As B-1 cells secret and use interleukin 10 (IL-10) as an autocrine growth factor, the possible participation of this interleukin in the kinetics of wound healing was investigated. Results show that C57/BL6 IL-10 KO mice had an increased inflammatory response when compared with C57/BL6 and C57/BL6 IL-10 KO mice reconstituted with B-1 cells, thus suggesting that the observed effects of B-1 cells in the healing process is mediated by this interleukin. However, the mechanisms by which IL-10 influence these phenomena remain to be uncovered.

Keywords: B-1 cells; IL-10; Inflammation; Wound healing; BALB/*Xid* mice

Introduction

Aseptic and non-aseptic wound healing starts at the moment any kind of tissue from invertebrate or vertebrate subjects are injured. In vertebrates, this phenomenon can be divided into three overlapping phases: an inflammatory phase, characterized by inflammatory cell recruitment; a proliferative phase, characterized by collagen deposition and angiogenesis; and a maturation phase, characterized by resolution of inflammation and scar maturation (Martin et al. 2003). Neutrophils and macrophages arrive early in the wound. The major function of these cells is to remove foreign materials, bacteria, non-functional host cells and damaged matrix components (Sylvia 2003). By 48 h after injury, macrophages are the prominent inflammatory cells involved in the normal healing response (Engelhardt et al. 1998). Mast cells are another marker of interest in wound healing. They release granules

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filled with enzymes, histamine and other active amines and these mediators are responsible for the typical signs of inflammation around the wound site (Artuc et al. 1999).

The participation of T lymphocytes in the wound-healing process has been investigated although the role played by these cells in this phenomenon is not clear (Fishel et al. 1987). Less investigated yet is the possible participation of B lymphocytes in tissue repair.

In our laboratory, Almeida et al. (2001) have shown that B-1b cells were able to migrate from the peritoneal cavity to distant inflammatory sites, an observation confirmed by other investigators (Paciorkowski et al. 2000; Popi et al. 2008a). They also have demonstrated that B-1 cells proliferate in stationary cultures of normal mouse peritoneal cells and differentiate into mononuclear phagocytes *in vitro* and *in vivo* (Almeida et al. 2001; Popi et al. 2008b).

There are two subtypes of B lymphocyte, B-2 or conventional B cells and B-1 lymphocytes. B-1 lymphocytes differ from conventional B lymphocyte by their anatomical location, ontogeny, phenotypical and functional characteristics. These cells constitute a minor fraction of the B lymphocyte population in spleen and are not detected in lymph nodes of mice. However, they represent the main B lymphocyte population in the peritoneal and pleural cavities of these animals (Herzenberg and Kantor 1993).

B-1 lymphocyte can be divided into at least two subtypes: B-1a ($CD5^+$) and B-1b ($CD5^-$) (Kantor and Herzenberg 1993; Kantor et al. 1992a, b). B-1 lymphocyte expresses high levels of surface IgM and low levels of B220 and IgD, but not CD23, whereas conventional B-2 lymphocyte express CD23, $B220^{high}$, IgD and IgM^{low} (Herzenberg et al. 1986). B-1 lymphocytes also express low levels of Mac-1, and a subset designated B-1a has intermediate levels of CD5 expression on their surface (Almeida et al. 2001; Kantor and Herzenberg 1993).

As demonstrated in the literature, BALB/*Xid* mice have impaired production of B-1 lymphocytes (Narendran et al. 1993). Additional experiments provided support for this observation, considering that when peritoneal cells from BALB/*Xid* mice are cultured *in vitro*, B-1 cells do not proliferate in the culture medium (Popi et al., *personal communication*).

It is known that B-1 cells produce and use interleukin 10 as an autocrine growth factor (O'Garra et al. 1992). Popi et al. (2004) showed that IL-10 secreted by B-1 cells modulates the phagocytic activity of murine macrophages *in vitro*. Further, in our laboratory, De Lorenzo et al. (2007) described a novel role for B-1 cells as part of suppressor mechanisms in the immune system.

Herein we demonstrate that B-1 cells modulate the inflammatory response and the kinetics of wound-healing process via IL-10 secretion.

Materials and methods

Mice

BALB/c, BALB/*Xid*, C57BL/6, C57BL/6 IL-10 KO and C57BL/6 green fluorescent protein (GFP) labeled male mice, 6–8 weeks of age, were used. Mice were obtained from the animal facilities of Department of Immunology, Universidade de São Paulo, and CEDEME, UNIFESP, Brazil. This study was approved by Research Ethical Committee (1014/05) from Universidade Federal de São Paulo, Brazil.

Wounding

Mice were anesthetized by intraperitoneal injection of Ketanest/Rompun (Syntec, Cotia, Brazil), and dorsal or dorsal footpad lesions were made. After shaving, standardized circular excisional wound was made in the dorsal region of then animals. Digital photographs of lesions in BALB/c, BALB/*Xid* and B-1 cells transferred BALB/*Xid* mice were made to compare the wound-healing process. The images were evaluated by Metamorph software. The wound areas were standardized by comparison with the original wound size, expressed as percentage of wound closure: % wound closure = {(day 0 area−day N area)/day 0 area} × 100. Dorsal footpad lesions were made using a punch no. 2 (R-806-9-2 Richter, Brazil) and the thickness of footpad evaluated by dial thickness gage (Mitutoyo, Japan) after 0.5, 2, 4, 6, 24, 48 and 72 h.

B-1 cell cultures

B-1 cells were cultivated as described by Almeida et al. (2001). Briefly, cells were collected from the peritoneal cavity of mice by repeated lavage with RPMI-1640 medium (Sigma Sigma, St Louis, MO) and incubated at 37 °C in an atmosphere of 5% CO_2 for 40 min. After incubation, supernatants were aspirated to remove non-adherent cells. Subsequently, R10 medium (RPMI-1640 containing 10% of heat-inactivated fetal bovine serum) was added to adherent monolayer. Cultures were maintained at 37 °C in 5% CO_2 for 5 days. During this period B-1 cells grew as free-floating cells. These cells were submitted to FACS analysis or used in adoptive transference to BALB/*Xid* or C57BL/6 IL-10 KO mice.

Phenotypic analysis of B-1 cells by FACS

B-1 cells were obtained as described above and analyzed by fluorescence activated cell sorter (FACS). These cells were stained with the following antibodies: phycoerythrin (PE) rat anti-mouse CD19, fluorescein-isothiocianate (FITC) rat anti-mouse CD23, APC rat