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Modulation of $P2X_7$ receptor expression in macrophages from mineral oil-injected mice

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Abstract

$P2X_7$ receptor activation is involved in a number of pro-inflammatory responses in macrophages and other immune cells. Their expression can be positively modulated with lipopolysaccharide (LPS) and TNFα, reinforcing their role during inflammation. We investigated the effect of substances capable of recruiting macrophages into the peritoneal cavity of mice (mineral oil and thioglycolate) on $P2X_7$ receptor expression and function, addressing whether these stimuli can interfere with multinucleated giant cell (MGC) formation, ATP-induced apoptosis, plasma membrane permeabilization and nitric oxide production. It was demonstrated that mineral oil treatment reduces $P2X_7$-dependent MGC formation, whereas thioglycolate treatment does not. Mineral oil treatment reduced $P2X_7$ receptor expression, down-modulating ATP-induced apoptosis, permeabilization and nitric oxide production. In conclusion, mineral oil down modulated $P2X_7$ expression and consequently $P2X_7$-associated phenomena, but thioglycolate did not. These effects might be associated with the unpleasant side effects already described during long-term administration of mineral oil for cosmetic purposes or as a laxative and could be useful in understanding the mechanism of recycling and modulation of P2 receptors present in other situations of immunopathological interest.

Keywords: Apoptosis; ATP; Mineral oil; Nitric oxide; $P2X_7$ receptors; Permeabilization

Introduction

$P2X_7$ receptors are ATP-activated receptors expressed in the cell membrane of most immune cells including macrophages (Coutinho-Silva and Persechini, 1997), phagocytic cells of thymic reticulum (Coutinho-Silva et al., 1996), dendritic cells (Coutinho-Silva et al., 1999) and B lymphocytes (Adinolfi et al., 2002; Klapperstuck et al., 2000; Sluyter et al., 2001). The activation of $P2X_7$ receptors induces the opening of cationic channels (Markwardt et al., 1997) and, a few seconds later, the permeabilization of the plasma membrane to molecules up to 900 Da (Persechini

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et al., 1998; Steinberg et al., 1987). The $P2X_7$ receptor is known to regulate various inflammatory functions of macrophages such as maturation and secretion of pro-inflammatory cytokines (Solle et al., 2001), apoptosis (Coutinho-Silva et al., 1999; Schulze-Lohoff et al., 1998), elimination of intracellular parasites (Lammas et al., 1997) and induction of cell fusion generating multinucleated cells (Chiozzi et al., 1997; Falzoni et al., 2000). $P2X_7$ receptors can be positively modulated by inflammatory modulators such as lipopolysaccharide (LPS), M-CSF, IL-2, TNF-α, IL-6 (Zhang et al., 2005).

Mineral oils (MOs) and thioglycolate are well-known recruiters of macrophages to the peritoneal cavity. They have been employed indiscriminately as a source of a large number of inflammatory macrophages for use in general functional or biochemical studies. They were considered good elicitor substances but not macrophage activators as originally described by Mackaness (North, 1978). Therefore there are various descriptions showing that inflammatory macrophages obtained as a result of the use of thioglycolate and MO are somehow different from resident macrophages, presenting differences in phagocytic and microbicidal activities, superoxide anion production and in antibody-dependent cell-mediated cytolysis (Cohn, 1978; Leijh et al., 1984; Leu et al., 1989; Johnston et al., 1978; Shaw and Griffin, 1982).

In particular, MO has also been used as a vaccine adjuvant, as an antibiotic, for cosmetic purposes or as a laxative agent in humans (Williams and Mahaguna, 1998; Meza-Perez and Rodiguez, 2004).

Depending on the profile of environmental stimuli, macrophages are generally either classically induced (by IFNγ plus TNFα or Toll-like receptor ligands) or alternatively activated (induced by IL-4 and IL-13 or phagocytosis of apoptotic cells) (Taylor et al., 2005). Since $P2X_7$ receptors are up-regulated by pro-inflammatory mediators and participate in the immune response by shaping lymphocyte sub-type polarization via secretion of cytokines (Bours et al., 2006), we wondered what the effect of MO and thioglycolate on the expression and function of $P2X_7$ receptors on macrophages would be.

Therefore, in this study we addressed the question of whether $P2X_7$ expression can be modulated by substances capable of recruiting macrophages (MO and thioglycolate) and if so, whether its modulation can interfere with multinucleated giant cell (MGC) formation and ATP-induced apoptosis, cell permeabilization, and nitric oxide (NO) production. We demonstrated that MO treatment reduces MGC formation and $P2X_7$ receptor expression. Finally, we discuss the possible consequences of this phenomenon during inflammatory conditions.

Materials and methods

Animals

Breeding, maintenance and killing of the animals used in this study followed the principles of good laboratory animal care and experimentation in compliance with Brazilian national law and regulations. Animals were kept at a constant 12 h/12 h light–dark cycle with free access to food and water. This study was carried out with 10-week adult male Swiss-Websters, BALB/c, C57 Bl6, and the $P2X_7$-receptor knockout mice $P2X_7^{-/-}$. The $P2X_7^{-/-}$ derived from Pfizer (Groton, CT, USA) generated by Solle et al. (2001) were supplied by Dr. A. Gabel and bred at the Transgenic Mice laboratory in the Biophysics Institute Carlos Chagas Filho at the Federal University of Rio de Janeiro. The animals were killed by exposure to an increasing dose of carbon dioxide. Death was confirmed by cervical dislocation. All animals were housed and maintained in a federally approved animal facility and the Animal Care and Use Committee of the Biophysics Institute approved all protocols used in this work.

Preparation of macrophages from peritoneum

Mouse peritoneal macrophages were obtained by lavage of the intraperitoneal cavity with cold balanced salt solution (PBS). Cells were transferred to PBS medium containing 5% fetal bovine serum (FBS) for immediate immunostaining. The mononuclear cells were enriched by centrifugation on a Ficoll density gradient Histopaque 1083 (Sigma St. Louis, MO, USA). Cell viability following this procedure was over 95% in all cases, as measured by Trypan blue exclusion. The enriched mononuclear cells were adjusted to 5×10^6 cells/ml and transferred to RPMI-1640 medium (Gibco BRL, Paisley Scotland) containing 5% heat-inactivated FCS, 2 g/l sodium bicarbonate, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were plated in 35 mm Petri dishes. After 1-h incubation at 37 °C in a 5% CO_2 humidified atmosphere, non-adherent cells were removed by vigorous washing and the adherent cells were kept in the same conditions for 24–72 h until use.

For flow cytometry experiments, freshly harvested intraperitoneal cell population was used directly prior to the adhesion step.

Reagents

DMEM medium (Dulbecco's Modified Eagle Medium), FBS and penicillin/streptomycin were purchased from GIBCO. Ethidium bromide (EB) and Lucifer yellow (LY) were obtained from Molecular Probes