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EFFECT OF DIFFERENT STIMULATORY AGENTS ON YIELD AND ACTIVATION OF PERITONEAL MACROPHAGES

EFEKAT RAZLIČITIH STIMULATORNIH AGENASA NA PRINOS I AKTIVACIJU PERITONEALNIH MAKROFAGA

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Macrophage activation, eliciting agents, p21

Ključne reči
Aktivacija makrofaga, stimulatorni agensi, p21

Abstract

Murine peritoneal cavity provides the most suitable source of resident macrophages needed for studies of macrophage activation. Stimulatory agents can be injected into the peritoneal cavity to increase macrophage yield although they may affect the physiology of these macrophages. In this study we provide evidence of the effect of 9% casein and 3% thioglycollate solution on yield and activation phenotype of macrophages derived from wild type and p21-deficient mice.

INTRODUCTION

Macrophages are considered to be critical effector cells of the immune system, present in virtually all tissues. They belong to the mononuclear-phagocyte system and originate from a common myeloid progenitor in the bone marrow that undergoes three divisions, into monoblasts, promonocytes and monocytes[1,2,3]. After their release into the circulation, monocytes enter the tissues and differentiate further into tissue-resident macrophages.

Depending on the local stimuli to which they are exposed, macrophages can acquire different physiological and morphological properties in different microenvironments[1,3]. This remarkable plasticity of phenotype reflects the specialization of function in both innate and adaptive immune responses, maintenance of tissue homeostasis, clearance of senescent and tumor cells, tissue repair and development[4]. This gives macrophages pivotal contribution to tumor cell growth and autoimmune pathologies, and makes them suitable targets in disease treatment. However, the ability of macrophages to dramatically change their physiology in response to activation stimuli may complicate studies of macrophage activation.

Murine peritoneal cavity provides the most suitable source of resident macrophages needed for studies of macrophage activation[5]. Under physiological conditions, the number of macrophages harvested from peritoneal cavity is insufficient for extensive studies. In order to increase macrophage yield, sterile eliciting agents are usually injected into the peritoneal cavity several days prior to cell harvest[6]. These agents increase monocyte migration into the peritoneum, however the physiologic characteristics of these macrophages may be altered.

p21 (WAF1/CIP1) is a cyclin-dependent-kinase inhibitor that controls cell-cycle [7]. The role of p21 has also been implicated in autoimmunity suppression and pathogenesis of lupus [8-10] and rheumatoid arthritis [11]. Furthermore, mice deficient in p21 display increased susceptibility to septic shock and it has been suggested that p21 plays a significant role in macrophage activation [12].

Better understanding of the effect of different stimulatory agents on peritoneal macrophage activation phenotype is crucial for development of new approaches in studies macrophage activation. Here, we investigate the effect of 9% casein solution and 3% thioglycollate medium, their efficiency in inducing macrophage migration to peritoneum as well as their influence on the expression of activation markers on macrophages. Because of the implication of p21 in macrophage activation, we sought to investigate the difference between p21-deficient and wild type mice in response to these eliciting agents.

MATERIALS AND METHODS

Mice

Mice were kept in the barrier zone of our animal facility to avoid contact with pathogens. Female C57BL/6 p21-/- and control C57BL/6 mice three months old were used for the experiments. p21-/- mice were obtained by F10 intercrossing and tested for microsatellite markers of C57BL/6 background contribution, as described previously[10]. All protocols complied with national and European norms, and were approved by the Centro National de Biotecnología Ethics Committee.

Both p21-/- and WT mice were divided into three groups: group I received no stimulatory agent (untreated); group II animals were treated with 1 ml of 9% casein (from bovine milk, sodium salt; Sigma) solution and group III animals were treated with 1 ml of 3% aged thioglycollate medium (DifcoTM Fluid). Both stimulatory agents were prepared as previously described [6,13] and injected intraperitoneally four days prior to call harvest.

Cell preparation and counting

Peritoneal exudates were harvested via lavage with 10 ml ice-cold PBS. Cells were centrifuged in a refrigerated centrifuge 10 min at 1000 rpm, 4°C. Cell pellets were resuspended in cold complete medium (RPMI 1640 with 10% FBS). Cell count was performed on Casy Cell Counter (Scharfe Sustem).

Flow cytometry

Peritoneal exudates were stained with fluorochrome-conjugated antibodies to CD11b (Mac1) (Beckman Coulter), F4/80 (eBioscience), CD40 (Pharmingen) and MHC class II (I-Ab) (AbD Serotec). Data were collected on a Cytomics FC500 MPL (Beckman Coulter).

RESULTS

The yield of total peritoneal cells and percentage of macrophages

As shown in Figure 1A the yield of peritoneal cells was significantly increased in animals treated with thioglycollate compared to both untreated control group ($p<0.05$) and casein-treated group ($p<0.01$). Yield from animals treated with casein showed no significant increase compared to control group. In all treatments there was no difference between wild type and p21- deficient mice in the number of cells obtained from peritoneal cavity.

Analyses of the macrophages surface markers F4/80 and CD 11b was used to distinguish macrophages from other cell types obtained from murine peritoneum. This analyses showed a significant change in the percentage of macrophages after thioglycollate treatment compared to both casein-treated and untreated groups (Fig.1B). We found no significant difference between wild type and p21-deficient mice regarding the macrophage yield in all treatments.

Activation phenotype of macrophages

We detected a substantial increase in macrophage activation in untreated p21-/- mice compared to wild type, as indicated by overexpression of CD40 and MHC class II antigens (I-Ab) (Fig. 2A). After treatment with casein, macrophages expressed higher MHC II levels than untreated ones, both in p21-/- and wild type mice, indicative of hyperactivation (Fig. 2B). Furthermore, we detected a substantial increase in macrophage activation in mice treated with thioglycollate both in respect to the casein-treated and untreated animals (Fig. 2B). In all treatments hyperactivation of p21-deficient macrophages compared to wild type established before any treatments, remained unaltered (Fig. 2C).

DISCUSSION

Stimulatory agents have been widely used to enhance the yield of peritoneal macrophages needed for extensive studies [5,6,12]. These elicited macrophages not only differ in number, but can be also functionally different from resident cells. The present study was designed to investigate the effect of 9% casein and 3% thioglycollate solution on yield and activation of macrophages derived from wild type and p21-deficient mice. After treatment with thioglycollate, statistical changes in yield of both total peritoneal cells and macrophages were observed (Fig. 1). However, there was no difference between wild type and p21-/- mice, which suggests that the absence of p21 does not affect monocyte migration into the peritoneum.

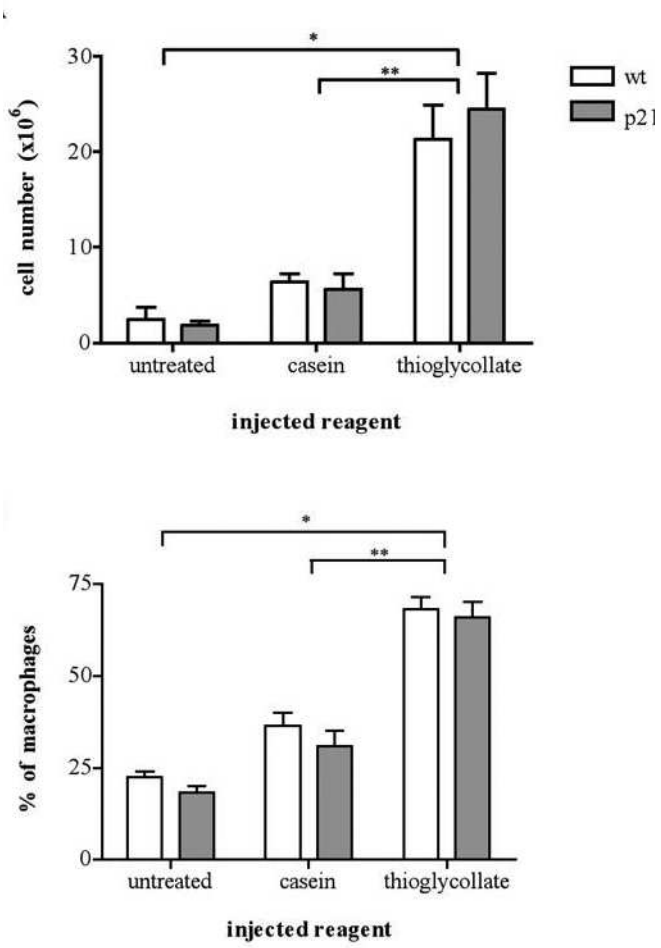

FIGURE 1. (A) Total peritoneal cells yield (x 106) from wild type and p21-deficient mice after treatments with stimulatory agents. (B) Percentage of macrophages among total peritoneal cells obtained after treatments with stimulatory agents. Data are expressed as the mean ± SD (n=2 mice/condition) and are representative of three experiments.

Expression of CD40 and MHC class II antigens has been used to measure the level of macrophage activation [12,14], so we have also followed the expression of these markers to monitor effects of treatment with eliciting agents. Our results confirmed previous data [12] where it has been described that in untreated animals p21-deficient peritoneal macrophages are more activated compared to wild type, as indicated by overexpression of CD40 and MHC class II antigens (I-Ab) (Fig. 2A). After both casein and thioglycollate treatment, we detected enhanced MHC class II expression in both wild type and p21-deficient macrophages. However, p21-/- macrophages retained initial hyperactivation compared to wild type during both treatments. This result suggests that, even though p21 influences macrophage activation, it is not involved in macrophage response to this kind of treatments. Therefore, it may be concluded that both casein and thioglycollate can be used as eliciting agents in the studies of the role of p21 in macrophage activation.

In conclusion, our study indicates that treatment with thioglycollate is very efficient in increasing the peritoneal macrophage yield and also does not affect the initial difference in activation between wild type and p21-deficient mice. This finding might be important for any study conducted to understand the mechanism by which p21 regulates macrophage activation and design of therapeutic strategies directed at inflammation.

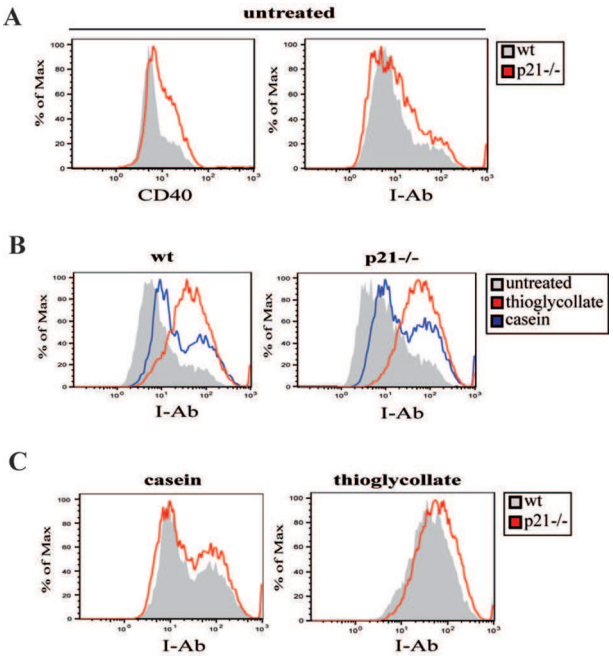

FIGURE 2. (A) Increased macrophage activation in p21-/- mice before treatment with stimulatory agents. (B) Activation phenotype of wild type and p21-/- mice after treatment with stimulatory agents. (C) Hyperactivation of p21-/- mice remained unaltered after treatment with stimulatory agents.

Apstrakt

Mišja peritonealna šupljina predstavlja najpogodniji izvor rezidentnih makrofaga koji se koriste u studijama o aktivaciji makrofaga. Kako bi se povećao prinos makrofaga, stimulatorni agensi se ubrizgavaju u peritonealnu šupljinu. Međutim, oni mogu znatno uticati na fiziologiju ovih ćelija. U ovom istraživanju pružamo podatke o uticaju 9% rastvora kazeina i 3% rastvora tioglikolata na prinos i aktivaciju makrofaga dobijenih iz peritonealne šupljine p21-nokaut miševa i miševa divljeg tipa.

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