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The International Journal of Biochemistry & Cell Biology 37 (2005) 1805–1814
IJBCB
www.elsevier.com/locate/biocel

Pro-inflammatory effect of *Arum maculatum* lectin and role of resident cells

Veruska B.M. Alencar [a], Nylane M.N. Alencar [a], Ana M.S. Assreuy [b], Mário L Mota [b], Gerlly A.C. Brito [a], Karoline S. Aragão [c], Flávio S. Bittencourt [a], Vicente P.T. Pinto [d], Henri Debray [e], Ronaldo A. Ribeiro [a,1], Benildo S. Cavada [c,*]

[a] *Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceara, Brazil*
[b] *Mestrado em Ciências Fisiológicas, Universidade Estadual do Ceará, Fortaleza, Brasil*
[c] *BioMol-Lab, Department of Biochemistry, Federal University of Ceara, Sobral, Brazil*
[d] *BioMol-Lab, Faculty of Medicine, Federal University of Ceara, Sobral, Brazil*
[e] *Laboratoire de Chimie Biologique et Unité Mixte de Recherche N^0 8576 du CNRS, Université des Sciences et Technologies de Lille, France*

Received 5 July 2004; accepted 14 February 2005

Abstract

Arum maculatum agglutinin (AMA) is a monocot lectin isolated from tubers of *Arum maculatum* L. (Araceae) which exhibits different specificity towards oligo-mannosidic-type and *N*-acetyllactosaminic-type glycans. We have investigated the effect of this lectin on the cells of the immune system. Models of neutrophil migration in vivo, neutrophil chemotaxis in vitro and macrophage cultures were used to study the lectin inflammatory activity. When administered into rat peritoneal cavities, AMA (80, 200 and 500 μg/mL/cavity) induced significant and dose-dependent neutrophil migration. This effect was inhibited by incubation with α-methyl-D-mannoside. A 83% depletion in the number of resident cells following peritoneal lavage did not reduce the AMA-induced neutrophil migration, as compared to sham animals (not washed). However, pre-treatment with 3% thioglycolate which increases the peritoneal macrophage population by 236%, enhanced the neutrophil migration induced by AMA (200 μg/mL/cavity) (119%, $p < 0.05$). Reduction of peritoneal mast cell population by chronic treatment of cavities with compound 48/80 did not modify AMA-induced neutrophil migration. The neutrophil chemotaxy assay in vitro shows that the lectin (300 μg/mL) induces neutrophil chemotaxy (368% $p < 0.05$) compared to RPMI. Finally, injection into peritoneal cavities of supernatants from macrophage cultures obtained after stimulation with AMA (300 μg/mL) enhanced neutrophil migration (110%

* Corresponding author at: Federal University of Ceara, Biochemistry and Molecular Biology Department, Campus do Pici, s/n; Bloco 907, 60.455-970 Fortaleza-CE, Ceara, Brazil. Tel.: +55 85 288 98 18; fax: +55 85 288 98 18.
E-mail addresses: ribeiro@ufc.br (R.A. Ribeiro), bscavada@ufc.br (B.S. Cavada).
URL: www.biomol-lab.ufc.br.
[1] Present address: Depto de Fisiologia e Farmacologia, Faculdade de Medicina, Universidade Federal do Ceará, Brasil. Tel: +55 85 288 83 49; fax: +55 85 288 83 33.

1357-2725/$ – see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.biocel.2005.02.027

$p < 0.05$). Summarizing, our data suggest that *A. maculatum* agglutinin presents pro-inflammatory activity, inducing neutrophil migration by two ways, one which is independent on resident cells and another one dependent on the presence of these cells.

Keywords: Plant lectin; *Arum maculatum*; Neutrophil migration; Macrophage; Mast cell

1. Introduction

Lectins are (glyco) proteins of non-immune origin that interact reversibly and specifically with carbohydrates (Peumans & Van Damme, 1995). These proteins are widely distributed in nature such as in microorganisms, viruses and animals serving to mediate a variety of biological recognition events (Cavada et al., 1998; Gabius & Gabius, 2002; Moreira, Ainouz, Oliveira, & Cavada, 1991). In a recent review, our group reported that plants lectins can diverge considerably in their biological activities in vitro and in vivo (Cavada, Barbosa, Arruda, Grangeiro, & Barral Netto, 2001).

Inflammatory response is a complex phenomenon that involves vascular and cellular events. In cellular events the lymphocyte recirculation depends on the response to a cell surface signal (Cronstein & Weisssman, 1993; Malik & Lo, 1996; McEver, 1992). The interactions among macrophages, mast cells, lymphocytes, endothelial cells and neutrophils under inflammatory stimulus induce release of chemical mediators that are responsible for the development and control of the inflammatory reaction. These mediators are cytokines, components of the complement system, prostanoids and other cell chemoattractant factors (Rankin, Sylvester, Smith, Yoshimura, & Leonard, 1990; Ribeiro et al., 1997; Topley et al., 1996). It is known that neutrophil infiltration into inflamed tissues is an important cellular event for host defense and involves a sequential chain of complex events comprising spatial and temporal expression of adhesion molecules, both in leucocytes and in endothelial cell membranes (Cronstein & Weisssman, 1993; Malik & Lo, 1996; McEver, 1992). It has been described that plant lectins are able to induce in vitro lymphocyte proliferation with interferon γ production (Barral-Netto et al., 1992), mast cell histamine release (Gomes, Ferreira, Cavada, Moreira, & Oliveira, 1994), nitric oxide production in vivo and in vitro (Andrade et al., 1999) and apoptosis induction (Barbosa et al., 2001). Additionally, our group has originally demonstrated that, depending on the administration route used, plant lectins can exert pro- or anti-inflammatory actions activating or inhibiting neutrophil migration via interaction between the lectin domain of exogenous lectins with carbohydrate residues present in the inflammatory cell membranes (Alencar et al., 1999, 2003, 2004, 2005; Assreuy et al., 1997, 1999; Cavada et al., 2001). These effects were also shown to occur by an indirect mechanism, dependent on macrophage activation by lectins possibly releasing neutrophil chemoattractant factors (Alencar et al., 2003, 2005).

Thus, the aim of the present work was to investigate the effect of a monocot lectin from *Arum maculatum* (AMA) tuber on the neutrophil migration and the possible involvement of resident cells in the lectin mechanism of action.

2. Materials and methods

2.1. Animals

Male and female Wistar rats (150–200 g body weight) obtained from our own animal facilities were housed ($n = 5$ per group) in a temperature-controlled room and received water and food ad libitum. The experimental protocol was in accordance to the guidelines approved by the Council of American Psychological Society (1980) for the use of experimental animals.

2.2. Lectin

A. maculatum agglutinin (AMA) is a lectin that exhibits different specificity towards oligomannosidic-type and *N*-acetyllactosaminic-glycans (Allen, 1995; Van Damme et al., 1995). It was obtained from tubers of *A. maculatum* L. (Araceae) and purified by affinity chromatography on a column of asialofetuin immobilized on Sepharose 4B according to Van Damme et al. (1995).