Biochemical and Biophysical Research Communications 416 (2011) 318–324

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The lectin ArtinM binds to mast cells inducing cell activation and mediator release

Valéria Cintra Barbosa-Lorenzi [a], Patrícia Andressa de Almeida Buranello [a], Maria Cristina Roque-Barreira [a], Maria Célia Jamur [a], Constance Oliver [a], Gabriela Pereira-da-Silva [b,*]

[a] Departamento de Biologia Celular e Molecular e Bioagentes Patogênicos, FMRP/USP, Ribeirão Preto, SP, Brazil
[b] Departamento de Enfermagem Materno-Infantil e Saúde Pública, EERP/USP, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Article history:
Received 4 November 2011
Available online 15 November 2011

Keywords:
Mast cells
Activation
Degranulation
ArtinM

ABSTRACT

Mast cells are inflammatory cells that respond to signals of innate and adaptive immunity with immediate and delayed release of mediators. ArtinM, a lectin from *Artocarpus integrifolia* with immunomodulatory activities, is able to induce mast cell activation, but the mechanisms remain unknown. This study sought to further investigate the effects of the lectin on mast cells. We showed that ArtinM binds to mast cells, possibly to the high affinity receptor for immunoglobulin E (IgE) – FcεRI – and/or to IgE bound to FcεRI. Binding of the lectin resulted in protein tyrosine phosphorylation and release of the pre- and newly-formed mediators, β-hexosaminidase and LTB_4 by mast cells, activities that were potentiated in the presence of IgE. ArtinM also induced the activation of the transcription factors NFκB and NFAT, resulting in expression of some of their target genes such as IL-4 and TNF-α. In view of the established significance of mast cells in many immunological and inflammatory reactions, a better understanding of the mechanisms involved in mast cell activation by ArtinM is crucial to the pharmacological application of the lectin.

1. Introduction

Mast cells are hematopoietically derived, tissue-localized inflammatory cells that respond to signals of innate and adaptive immunity with immediate and delayed release of inflammatory mediators. Mast cells are central to the pathogenesis of immediate hypersensitivity diseases, and mediate tissue responses, fulfill effector and immunoregulatory functions and are implicated in tissue remodeling [1].

The major pathway for mast cell activation is through the high-affinity receptor for immunoglobulin E (FcεRI). FcεRI-dependent mast cell activation includes the rapid release of cytoplasmic granule-associated mediators, the secretion of *de novo*-synthesized lipid mediators as well as the synthesis and release of chemokines, and growth factors [2].

Lectins are included among the molecules that induce mast cell activation [3]. Results obtained by our group showed that ArtinM, a structurally well defined D-mannose-binding lectin from *Artocarpus integrifolia* [4], is able to induce the recruitment of rat mast cells from the bone marrow to the peritoneal cavity [5] and degranulate rat peritoneal mast cells [6].

Several functions have been described for ArtinM, including immunomodulatory activity [7,8]. The lectin induces IL-12 production by macrophages [7], which confers resistance to infections by intracellular pathogens [7,9]. In addition, ArtinM induces neutrophil migration mediated by the simultaneous interaction of its carbohydrate recognition domains (CRDs) with cell surface and extracellular matrix N-glycans [10]. An amplification of the neutrophil recruitment induced by ArtinM is provided by mast cell degranulation [6]. Moreover, ArtinM is able to accelerate the process of wound healing and epithelial tissue regeneration [11].

Due to the potential pharmaceutical applications of ArtinM and the role of mast cells in diverse immunological and inflammatory reactions, this study sought to further investigate the effects of the lectin on mast cells. The mechanisms involved in mast cell activation by ArtinM were examined. Our results demonstrate that ArtinM binds to mast cells, possibly through cell-bound IgE and/or FcεRI, leading to cell activation and release of mediators.

2. Materials and methods

2.1. ArtinM preparations

ArtinM was extracted from *A. integrifolia* seeds and purified by sugar affinity chromatography as described [4]. The protein concentration was determined by Bradford assay (Bio-Rad Laboratories, Inc., Canada). Biotinylation of ArtinM was performed as previously described [11].

* Corresponding author. Address: Departamento de Enfermagem Materno-Infantil e Saúde Pública, Escola de Enfermagem de Ribeirão Preto/USP, Av. Bandeirantes, 3900-14040-902-Ribeirão Preto, SP, Brazil. Fax: +55 16 36020518.
E-mail address: gbisson@eerp.usp.br (G. Pereira-da-Silva).

0006-291X © 2011 Elsevier Inc. Open access under the Elsevier OA license.
doi:10.1016/j.bbrc.2011.11.033

2.2. Cells

The rat mast cell line, RBL-2H3, was grown as monolayers as previously described [12]. The RBL-2H3 NFAT-GFP and the RBL-2H3 NFκB-GFP cell lines, produced as described [13], were kindly provided by Dr. Reuben Siraganian.

2.3. Cell sensitization and stimulation

RBL-2H3 cells were cultured overnight (ON) in the presence or absence of a 1:5000 dilution of mouse IgE anti-TNP ascites fluid. The cells were then incubated with 10 μg/ml of ArtinM for different times, as indicated. As a positive control, cells were sensitized with anti-TNP IgE and then stimulated with 50 ng/ml of DNP_{48}-HSA (Sigma–Aldrich). Cells that were not sensitized with IgE or not stimulated with DNP_{48}-HSA were used as negative controls.

2.4. Antibodies

The following primary antibodies were used: a pool of three mouse mAbs, BE2AB6, BE3D2, and BE5D1 (mAb BEs) [14] to the FcεRI β subunit, (1 μg/ml), and mAb 4G10-HRP conjugate (0.5 μg/ml, Millipore Corporation, Bedford, MA) against phosphotyrosine. Donkey anti-mouse IgG-HRP, (0.04 μg/ml; Jackson ImmunoResearch, USA) was used as the secondary antibody.

2.5. ArtinM binding to RBL-2H3 cell surface

2.5.1. Flow cytometry

1×10^6 cells were incubated with biotinylated ArtinM (1.25–10.0 μg/ml) for 1 h at 4 °C. In some assays, the lectin was pre-incubated with 50 mM D-mannose (Sigma–Aldrich, St. Louis, MO, USA) or D-galactose (Sigma–Aldrich) for 1 h at 4 °C. After incubation with streptavidin-FITC (1:4000) (Sigma–Aldrich) for 30 min at 4 °C, cells were washed in PBS, fixed for 20 min with 2% formaldehyde (EM Sciences, USA) and analyzed with a BD FACS Calibur™ flow cytometer, using the CellQuest program (Becton–Dickinson Labware, USA).

2.5.2. Confocal microscopy

5×10^4 cells were cultured ON on coverslips and then incubated with biotinylated ArtinM (10 μg/ml) for 1 h at 4 °C. In some assays, the lectin was pre-incubated with 50 mM D-mannose or D-galactose (Sigma–Aldrich) for 1 h at 4 °C. The cells were fixed with 2% formaldehyde (EM Sciences) at RT for 20 min, washed in PBS and incubated with 5 μg/ml streptavidin-FITC (Jackson ImmunoResearch Laboratories, Inc., USA) at RT for 30 min. Then the cells were washed in PBS and rinsed in distilled water. The cover slips were mounted on slides with Fluormount-G (EM Sciences) and examined with a LEICA TCS-SP2 scanning confocal microscope (Leica Microsystems, Germany).

2.6. ArtinM binding to IgE

ArtinM (2.5–30 μg/ml), which in some assays was pre-incubated with 25 and 50 mM D-mannose (Sigma–Aldrich) for 1 h at 4 °C, was added to IgE-coated wells (1 mg/ml, rat IgE – Biosource, International, USA). The assay was performed as described [10] and binding was assessed by reading the absorbance at 450 nm (Power Wave X, Bio-Tek Instruments, Inc., USA).

2.7. ArtinM binding to FcεRI and tyrosine phosphorylation

IgE-sensitized or unsensitized cells were incubated for 15 min with ArtinM or DNP_{48}-HSA in DMEM. Total cell lysates were obtained as described [15]. The proteins were separated electrophoretically on 10% polyacrylamide gels under reducing conditions (3% β-mercaptoethanol – Sigma–Aldrich) and transferred to Hybond membranes (GE Healthcare Biosciences, USA). The membranes were blocked for 16 h at 4 °C in TTBS buffer containing 4% BSA and incubated for 1 h at RT with mAb BEs or with biotinylated ArtinM, or mAb 4G10-HRP. For FcεRI binding assays, the membranes were incubated with anti-mouse IgG-HRP secondary antibody or streptavidin-HRP for 30 min. The membranes were developed using chemiluminescence (ECL-GE Healthcare Biosciences). For stripping, the first immunoblot was immersed in stripping buffer (62.5 mM Tris–HCl, pH 6.8, 2% SDS, and 10 mM β-mercaptoethanol) for 1 h at 56 °C and the immunoblot was repeated as described. For quantification of tyrosine phosphorylation levels, optical density was determined using Photoshop 7.0 (Adobe Systems Incorporated, USA).

2.8. β-Hexosaminidase activity

IgE-sensitized or unsensitized cells were incubated for 45 min with ArtinM or DNP_{48}-HSA in Tyrode's buffer. In some assays, ArtinM was pre-incubated with 50 mM D-mannose (Sigma–Aldrich) for 1 h at 4 °C. Degranulation of RBL-2H3 cells was assessed by measuring the release of β-hexosaminidase activity [16].

2.9. Leukotriene B_4 and cytokine detection assays

IgE-sensitized or unsensitized cells were incubated for different times with ArtinM or DNP_{48}-HSA in DMEM. The concentrations of LTB_4, TNF-α and IL-4 in cell culture supernatants were measured by ELISA (Leukotriene B_4 EIA kit, Cayman Chemical Company, MI, USA; OPTEIA™ Rat TNF ELISA kit II; OPTEIA™ Rat IL-4 ELISA kit II – BD Biosciences) according to the manufacturer's instructions.

2.10. Flow cytometric measurements of NFAT and NFκB activation

GFP reporter was used as a marker of FcεRI-induced NFAT or NFκB activation. IgE-sensitized or nonsensitized cells were stimulated with ArtinM or DNP_{48}-HSA for 20 h (for NFAT activity) or 5 h (for NFκB activity). These periods of stimulation were found to result in maximal GFP expression. Fluorescence levels were measured using a Guava Personal Cell Analysis-96 System and data were processed by Guava InCyte Software (Millipore).

3. Results

3.1. Binding of ArtinM to mast cell surface occurs via CRDs

To study the binding of ArtinM to ligands on the surface of mast cells, RBL-2H3 cells were incubated with biotinylated ArtinM (1.25–10 μg/ml) and analyzed by flow cytometry. As shown in Fig. 1A, maximum binding of ArtinM to the cell surface was observed with 10 μg/ml of the lectin, where 97% of the mast cells were labeled. Inhibition of lectin binding by the specific sugar was assayed by flow cytometry and confocal microscopy. Flow cytometric analysis showed that ArtinM (10 μg/ml) binding to RBL-2H3 cells was abolished in the presence of D-mannose (50 mM), whereas D-galactose (50 mM) had no effect on binding (Fig. 1B). By confocal microscopy, ArtinM (10 μg/ml) was evenly distributed on the surface of mast cells. Binding of the lectin was inhibited in the presence of D-mannose, but not D-galactose (Fig. 1C). These results indicate that ArtinM binds to the mast cell surface via CRDs.

3.2. ArtinM binds to IgE via CRDs and to FcεRI

As previously demonstrated [6], ArtinM is able to bind to IgE. Since the N-linked oligosaccharides present on IgE are potential

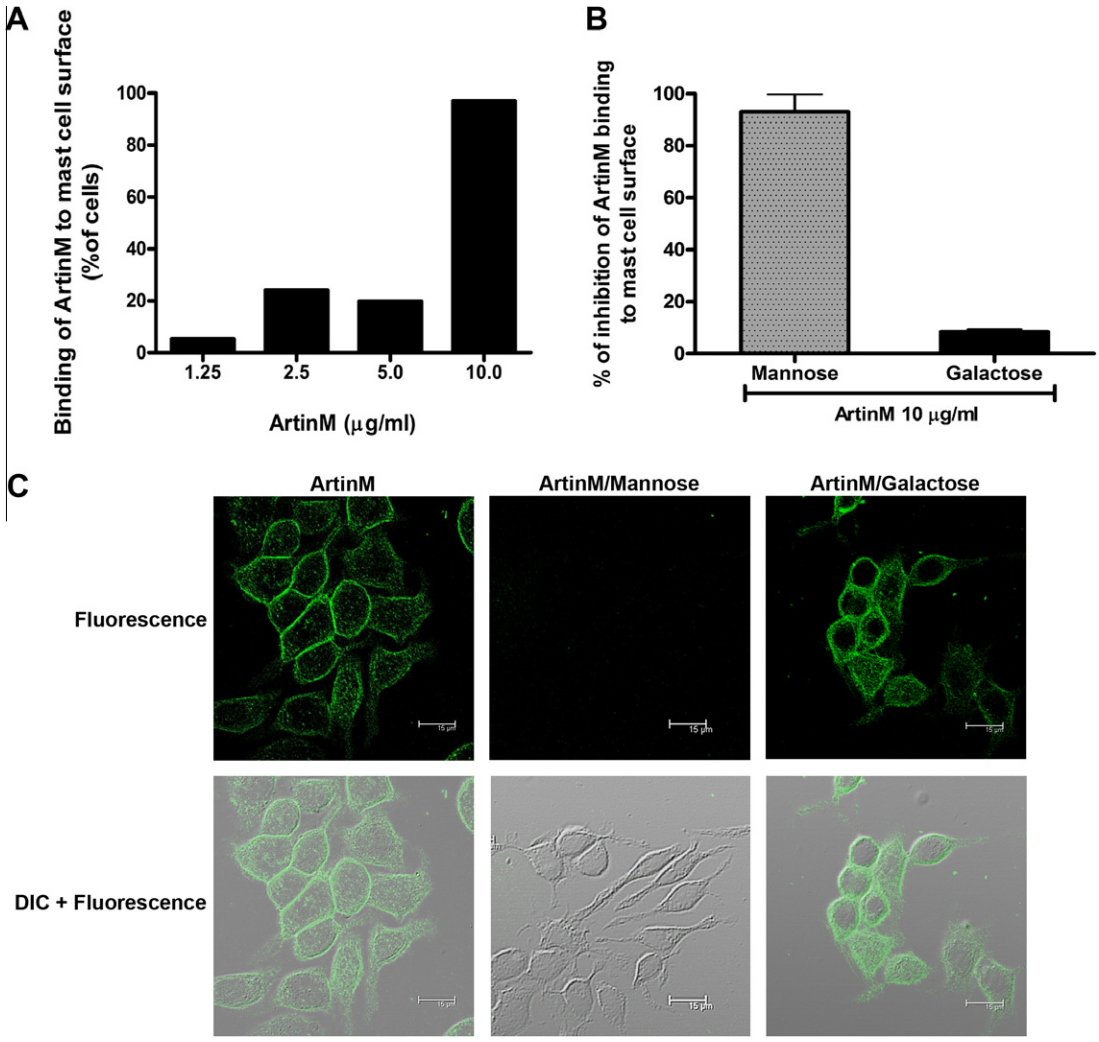

Fig. 1. ArtinM binds to the surface of mast cells via carbohydrate recognition domains. 1×10^6 cells were incubated with biotinylated ArtinM (1.25–10.0 μg/ml) for 1 h at 4 °C (A). ArtinM (10 μg/ml) was previously incubated, for 1 h at 4 °C, with 50 mM D-mannose or D-galactose (B). Cells were fixed, incubated with streptavidin-FITC and analyzed by FACS. Data are representative of three independent experiments. 5×10^4 cells were allowed to adhere to coverslips and were incubated with biotinylated ArtinM (10 μg/ml) for 1 h at 4 °C. In some assays, ArtinM was pre-incubated with 50 mM D-mannose or D-galactose. Cells were fixed, incubated with streptavidin-FITC and examined by confocal and DIC microscopy (C).

targets for recognition by the lectin, we investigated if ArtinM binding to the immunoglobulin occurs via CRDs of the lectin. We performed a microplate binding assay using biotinylated ArtinM (2.5–30.0 μg/ml) and also tested binding inhibition by D-mannose (Fig. 2). There was a significant increase in ArtinM binding between 2.5 and 5 μg/ml and a further increase at 10 μg/ml. There was no further increase in the binding after 10 μg/ml (Fig. 2A). Thus, 10 μg/ml of ArtinM was used in inhibition binding assays. ArtinM binding to IgE was reduced by 68% and 83% respectively when the lectin was previously incubated with 25 and 50 mM of D-mannose (Fig. 2B).

Structural features of FcεRI favor its recognition by lectins and so we investigated whether ArtinM would bind to the receptor. Total cell lysates immunoblotted with mAb BEs that recognize the β subunit of FcεRI, as well as those blotted with biotinylated ArtinM were compared. Among the various protein bands recognized by the lectin, the one with a molecular weight around 35 kDa was coincident to that recognized by the BEs (Fig. 2C and D). After stripping the membrane and reprobing with ArtinM, the same 35 kDa band was recognized by the lectin. The same was observed when the membrane incubated with ArtinM was stripped and reprobed with BEs (not shown).

These results indicate that ArtinM binds to IgE through its CRDs and possibly binds to the β-subunit of FcεRI expressed on mast cells.

3.3. ArtinM induces IgE mediated tyrosine phosphorylation and mediator release

Since ArtinM binds to FcεRI we therefore evaluated the ability of the lectin to stimulate tyrosine phosphorylation. RBL-2H3 cells were sensitized with anti-TNP IgE followed by stimulation with ArtinM or DNP_{48}-HSA. Similar to the response triggered by antigen induced FcεRI activation, increased levels of tyrosine phosphorylation were detected in IgE-sensitized cells upon stimulation with ArtinM (Fig. 3A). Indeed, there was an increase of approximately 50% in phosphotyrosine levels when IgE-sensitized cells were stimulated either with ArtinM or DNP_{48}-HSA (Fig. 3B).

The ability of ArtinM to induce degranulation of RBL-2H3 cells was investigated by β-hexosaminidase release assays. β-hexosaminidase activity in the supernatant by ArtinM-stimulated cells almost doubled in the presence of IgE, exceeding the values observed with antigen induced FcεRI activation (Fig. 3C). The production of the newly formed mediator leukotriene B_4 by mast cells in

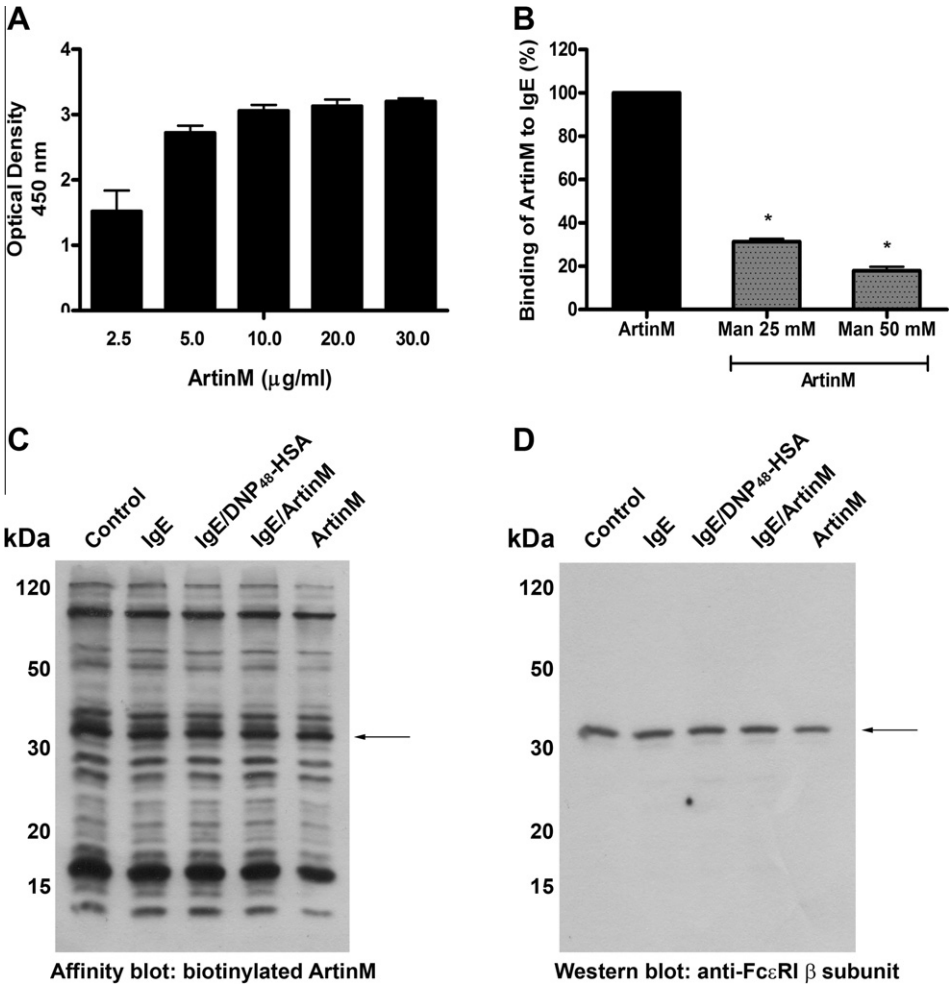

Fig. 2. ArtinM binds to IgE and to FcεRI. Biotinylated ArtinM (2.5–30.0 μg/ml) was incubated in IgE-coated (1 mg/ml) microwell plates for 2 h at RT (A). ArtinM (10 μg/ml) was pre-incubated with 25 or 50 mM D-mannose (B). After incubation with streptavidin-HRP and development with TMB, ArtinM binding to IgE was assessed by reading the optical density at 450 nm. The value provided by 10 μg/ml of ArtinM was considered as 100% of binding. Data represent the mean of three different experiments ± SD. $^{*}p < 0.05$ between ArtinM and samples incubated with D-mannose. Cell lysates were separated on a 10% SDS–PAGE gels and analyzed by blotting with biotinylated ArtinM (C) or with antibody against the β-subunit of FcεRI (D). Blots are representative of four independent experiments.

response to ArtinM stimulation was also investigated. In the presence of IgE, the lectin induced the release of LTB_4 at levels similar to those detected when cells were stimulated through FcεRI. ArtinM alone did not induce LTB_4 release (Fig. 3D). The release of pre-formed and newly formed mediators induced by ArtinM was inhibited by pre-incubation of the lectin with D-mannose (not shown).

These results indicate that ArtinM activates IgE-sensitized RBL-2H3 cells, inducing protein tyrosine phosphorylation, and that ArtinM-induced release of the β-hexosaminidase and LTB_4 is potentiated by IgE.

3.4. ArtinM induces the activation of transcriptions factors and the expression of target genes

Since ArtinM triggers intracellular signaling in mast cells, we investigated whether transcription factors were activated. Initially, IgE-sensitized or non-sensitized RBL-2H3 cells transfected with NFκB- or NFAT-GFP were stimulated with ArtinM and then examined by flow cytometry for expression of NFκB-GFP and NFAT-GFP. As shown in Fig. 4A and B, ArtinM activated both NFAT and NFκB in the presence and in the absence of IgE.

We also observed that the activation of NFκB and NFAT was correlated with the expression of their target genes. In IgE-sensitized cells ArtinM stimulation led to an increased production of TNF-α and IL-4, whose synthesis is mediated by activation of NFAT and NFκB, respectively (Fig. 4C and D). These results indicate that ArtinM induces the activation of NFκB and NFAT, resulting in expression of some of their target genes.

4. Discussion

The present study demonstrates that mast cell activation induced by ArtinM culminates in degranulation and mediator release, activities that are significantly increased in the presence of IgE. Furthermore, our results indicate that the high affinity receptor for IgE and/or the IgE bound to FcεRI are candidate ligands for the lectin on the mast cell surface.

Previous studies from our group demonstrated that ArtinM binds to rat peritoneal mast cells, inducing degranulation of these cells [6]. In the current investigation we demonstrated that ArtinM binding to mast cells occurs via CRDs of the lectin, since ArtinM binding to RBL-2H3 cells was strongly inhibited by D-mannose.

Both IgE and FcεRI are glycosylated and thus are potential targets for recognition by lectins. Previous studies have reported binding of lectins to IgE [7,21–23] and to FcεRI [17]. Galectin-3, in turn, binds to both IgE and FcεRI [18–20]. Our results demonstrate that

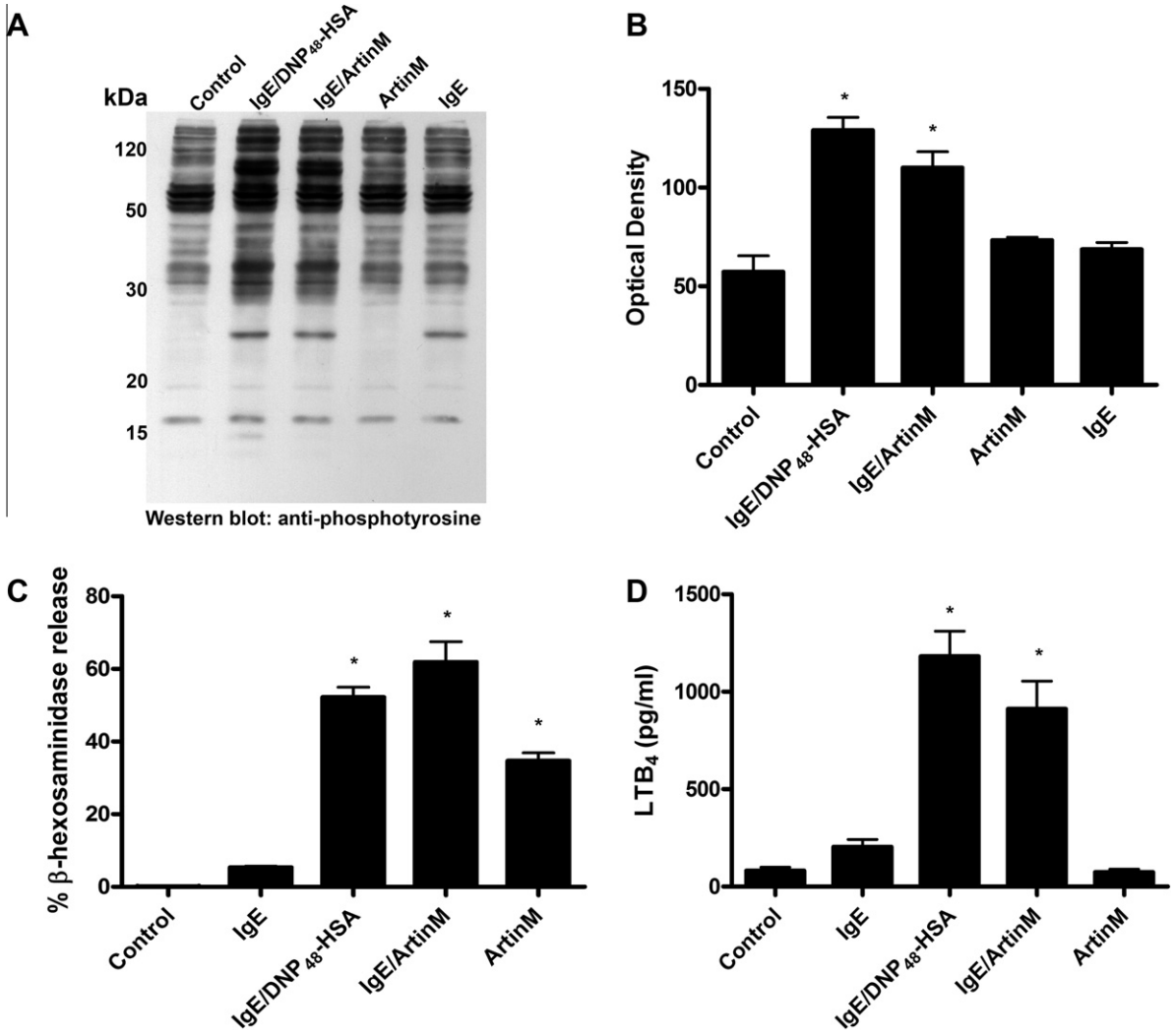

Fig. 3. ArtinM induces protein tyrosine phosphorylation and the release of β-hexosaminidase and leukotriene B_4 by mast cells. IgE-sensitized or unsensitized RBL-2H3 cells were stimulated with ArtinM (10 μg/ml) or DNP_{48}-HSA (50 ng/ml) for 15 min. Cell lysates were separated on a 10% SDS–PAGE gel and analyzed by blotting with anti-phosphotyrosine-HRP (A). A is representative of three independent experiments and B represents the mean of three different experiments ± SD. IgE-sensitized or unsentitized RBL-2H3 cells were stimulated with ArtinM (10 μg/ml) for 45 and 30 min and release of β-hexosaminidase activity (C) and LTB_4 (D) in the culture supernatant was determined, respectively. Data are expressed as mean ± SD and are representative of three different experiments. $^*p < 0.05$ between samples and the control.

ArtinM, like galectin-3, binds to IgE and to FcεRI. The IgE Cε3 chain has 7N-linked glycosylation sites [21] and the Asn-394 of this chain is occupied by oligomannose structures [21,22]. This pattern of glycosylation may explain the binding of ArtinM to IgE. Furthermore, binding of the lectin was inhibited by D-mannose. FcεRI belongs to the multichain immune receptor superfamily [23]. Among the subunits of this receptor, only the α-subunit is N-glycosylated [24]. We observed that ArtinM does not bind to this subunit (not shown), but instead it binds to the β-subunit of FcεRI, a chain that is not directly involved in binding to IgE.

Each of the subunits of the FcεRI has an individual function. The α-chain is involved in IgE binding while the β- and γ-chains are involved in signal transduction [25]. Upon aggregation of FcεRI, the tyrosine residues in the ITAMs of the β- and γ-chains are phosphorylated by the action of receptor-associated tyrosine kinases of the Src family (PTKs), which allows it to be bound by the Src homology 2 domains present in a number of signaling molecules [26]. The activated PTKs phosphorylate diverse signaling molecules. This activation ultimately culminates in degranulation, the *de novo* synthesis of proinflammatory lipid mediators and the synthesis and secretion of cytokines [27]. Thus, activation of tyrosine kinases is central to the ability of FcεRI to transmit downstream signaling events required for the regulation of mast cell activation [28].

In this study, exposure to ArtinM resulted in tyrosine phosphorylation of proteins in IgE-sensitized mast cells, suggesting that ArtinM binds to IgE bound to FcεRI, inducing crosslinking of the receptor and triggering signal transduction. Lectin binding to mast cells also resulted in degranulation, in the presence and in the absence of IgE. In the presence of IgE, however, the release of β-hexosaminidase almost doubled. These findings confirm the ability of ArtinM to activate either unsensitized or IgE sensitized mast cells [6] and are in agreement with our results that demonstrate that the lectin binds to IgE and to the FcεRI. In both cases, binding of ArtinM may result in FcεRI aggregation, thus inducing mast cell degranulation. It is plausible that binding of the lectin directly to FcεRI or to IgE bound to FcεRI determine quantitatively and/or qualitatively different cell responses. We speculate that in the presence of IgE, ArtinM could induce extensive crosslinking of the receptors, thereby resulting in increased phosphorylation of FcεRI and further downstream signaling. As observed for phosphorylation of proteins induced by ArtinM, the production of leukotriene B_4 in response to ArtinM also occurred only when the

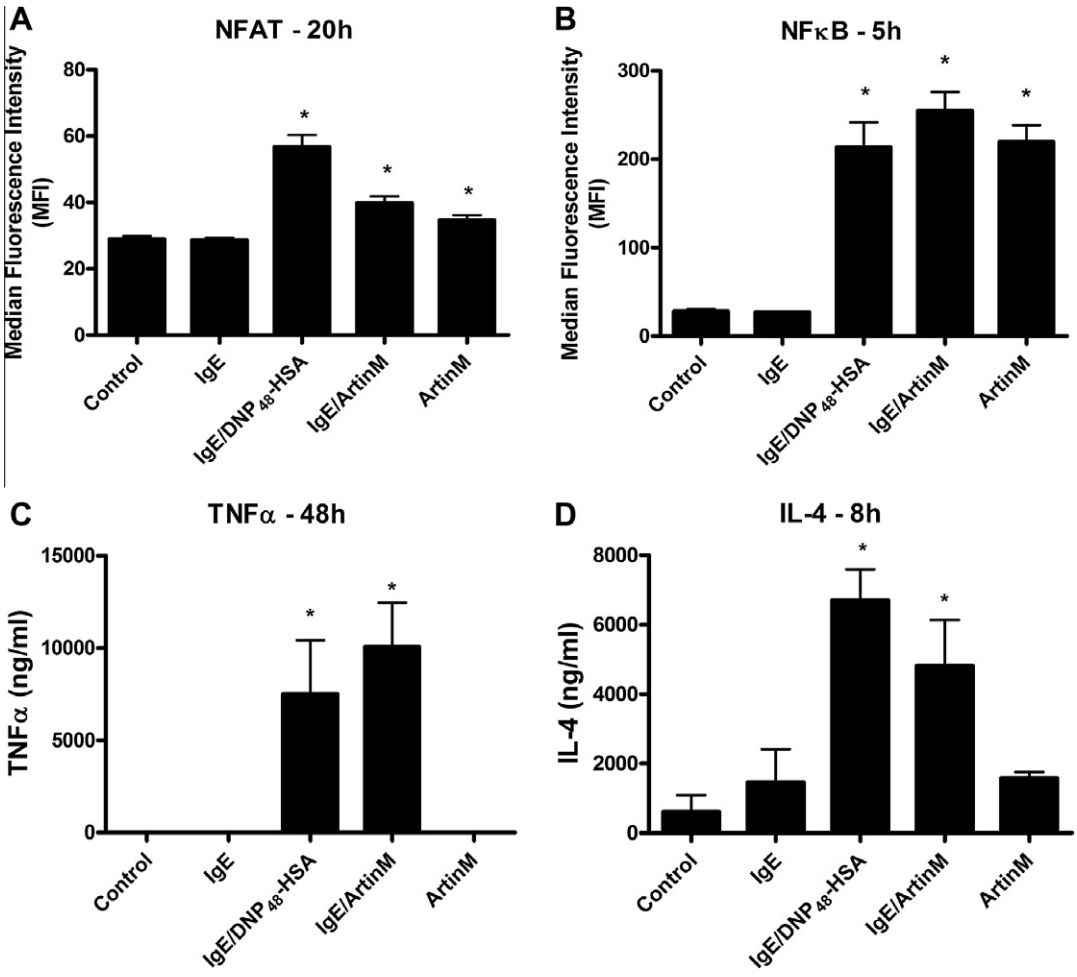

Fig. 4. ArtinM activates the transcription factors NFAT and NFκB and induces the release of TNF-α and IL-4. IgE-sensitized or unsentitized NFκB and NFAT-GFP transfected RBL-2H3 cells were stimulated with ArtinM (10 μg/ml) or DNP_{48}-HSA (50 ng/ml). Cells were analysed by flow cytometry for NFAT (A) and NFκB (B) driven GFP expression. IgE-sensitized or unsensitized RBL-2H3 cells were stimulated with ArtinM (10 μg/ml), the supernatants were collected and concentrations of TNF-α (C) and IL-4 (D) measured by ELISA. Data are expressed as mean ± SD and are representative of three different experiments. $^{*}p < 0.05$ between the experimental samples and the control.

mast cells were sensitized with IgE. Recent studies have demonstrated that the duration and the magnitude of the signal through FcεRI indeed determine different mast cell responses [29,30].

Receptor stimulation ultimately results in activation of specific genes encoding cytokines and growth factors, which may be mediated by members of the NFAT and/or NFκB transcription factor families [31]. As shown in this study, ArtinM stimulation led to the activation of NFAT and NFκB, inducing expression of some of their target genes such as TNF-α and IL-4, respectively. Although ArtinM-induced activation of transcription factors occurred whether cells were sensitized or not with IgE, the release of cytokines required previous sensitization. Recently, it has been reported that FcεRI activation can indeed result in nuclear signaling, as shown by NFAT activation, in the absence of mediator release [13]. Again, our findings suggest that different mast cell responses are the result of differences in signaling triggered by binding of ArtinM to FcεRI or to IgE bound to FcεRI. However, whether such bindings are interpreted by FcεRI as quantitative differences and are converted into qualitatively distinct outputs through respective signaling pathways remains to be investigated.

In conclusion, our results suggest that ArtinM, whether interacting with specific carbohydrates on cell-bound IgE or directly with FcεRI, activates mast cells, inducing degranulation and synthesis of newly formed lipid mediators and cytokines. In view of the well-established significance of mast cells in many immunological and inflammatory reactions, a better understanding of the mechanisms involved in mast cell activation by ArtinM is crucial to the pharmacological application of the lectin.

Acknowledgments

We thank Dr. Reuben P. Siraganian and Elsa H. Berenstein (Oral Infection and Immunity Branch, NIH) for providing the RBL-2H3 NFκB-GFP and NFAT-GFP cell lines; Mrs. Sandra M.O. Thomaz and Mr. Anderson R. Souza for technical support. Financial support from FAPESP (2006/54302-4 and 2007/00579-8).

References

[1] K.D. Stone, C. Prussin, D.D. Metcalfe, IgE, mast cells, basophils, and eosinophils, J. Allergy Clin. Immunol. 125 (2010) S73–80.
[2] J. Kalesnikoff, S.J. Galli, New developments in mast cell biology, Nat. Immunol. 9 (2008) 1215–1223.
[3] S.N. Pramod, Y.P. Venkatesh, P.A. Mahesh, Potato lectin activates basophils and mast cells of atopic subjects by its interaction with core chitobiose of cell-bound non-specific immunoglobulin E, Clin. Exp. Immunol. 148 (2007) 391–401.
[4] R. Santos-de-Oliveira, M. Dias-Baruffi, S.M. Thomaz, L.M. Beltramini, M.C. Roque-Barreira, A neutrophil migration-inducing lectin from Artocarpus integrifolia, J. Immunol. 153 (1994) 1798–1807.
[5] P.A. de Almeida Buranello, M.R. Moulin, D.A. Souza, M.C. Jamur, M.C. Roque-Barreira, C. Oliver, The lectin ArtinM induces recruitment of rat mast cells from the bone marrow to the peritoneal cavity, PLoS One 5 (2010) e9776.

[6] A.N. Moreno, M.C. Jamur, C. Oliver, M.C. Roque-Barreira, Mast cell degranulation induced by lectins: effect on neutrophil recruitment, Int. Arch. Allergy Immunol. 132 (2003) 221–230.

[7] A. Panunto-Castelo, M.A. Souza, M.C. Roque-Barreira, J.S. Silva, KM(+), a lectin from *Artocarpus integrifolia*, induces IL-12 p40 production by macrophages and switches from type 2 to type 1 cell-mediated immunity against *Leishmania major* antigens, resulting in BALB/c mice resistance to infection, Glycobiology 11 (2001) 1035–1042.

[8] K.A. Toledo, C. Scwartz, A.F. Oliveira, M.C. Conrado, E.S. Bernardes, L.C. Fernandes, M.C. Roque-Barreira, G. Pereira-da-Silva, A.N. Moreno, Neutrophil activation induced by ArtinM: release of inflammatory mediators and enhancement of effector functions, Immunol. Lett. 123 (2009) 14–20.

[9] K.C. Coltri, L.L. Oliveira, C.F. Pinzan, P.E. Vendruscolo, R. Martinez, M.H. Goldman, A. Panunto-Castelo, M.C. Roque-Barreira, Therapeutic administration of KM+ lectin protects mice against *Paracoccidioides brasiliensis* infection via interleukin-12 production in a toll-like receptor 2-dependent mechanism, Am. J. Pathol. 173 (2008) 423–432.

[10] G. Pereira-da-Silva, A.N. Moreno, F. Marques, C. Oliver, M.C. Jamur, A. Panunto-Castelo, M.C. Roque-Barreira, Neutrophil activation induced by the lectin KM+ involves binding to CXCR2, Biochim. Biophys. Acta 1760 (2006) 86–94.

[11] P.-C.A. Pinto-da-Silva LL, de Souza Goldman MH, Roque Barreira, d.-O.R. MC, Dias-Baruffi M, Blanco de Molfetta Machado, J., WIPO, Patent, 2004.

[12] E.L. Barsumian, C. Isersky, M.G. Petrino, R.P. Siraganian, IgE-induced histamine release from rat basophilic leukemia cell lines: isolation of releasing and nonreleasing clones, Eur. J. Immunol. 11 (1981) 317–323.

[13] A.C. Grodzki, K.D. Moon, E.H. Berenstein, R.P. Siraganian, FcepsilonRI-induced activation by low antigen concentrations results in nuclear signals in the absence of degranulation, Mol. Immunol. 46 (2009) 2539–2547.

[14] M. Benhamou, N.J. Ryba, H. Kihara, H. Nishikata, R.P. Siraganian, Protein-tyrosine kinase p72syk in high affinity IgE receptor signaling. Identification as a component of pp72 and association with the receptor gamma chain after receptor aggregation, J. Biol. Chem. 268 (1993) 23318–23324.

[15] R.O. de Castro, J. Zhang, M.C. Jamur, C. Oliver, R.P. Siraganian, Tyrosines in the carboxyl terminus regulate Syk kinase activity and function, J. Biol. Chem. 285 (2010) 26674–26684.

[16] M.C. Jamur, A.C. Grodzki, E.H. Berenstein, M.M. Hamawy, R.P. Siraganian, C. Oliver, Identification and characterization of undifferentiated mast cells in mouse bone marrow, Blood 105 (2005) 4282–4289.

[17] R.M. Helm, A. Froese, Binding of the receptors for IgE by various lectins, Int. Arch. Allergy Appl. Immunol. 65 (1981) 81–84.

[18] L.G. Frigeri, F.T. Liu, Surface expression of functional IgE binding protein, an endogenous lectin, on mast cells and macrophages, J. Immunol. 148 (1992) 861–867.

[19] F.T. Liu, L.G. Frigeri, C.A. Gritzmacher, D.K. Hsu, M.W. Robertson, R.I. Zuberi, Expression and function of an IgE-binding animal lectin (epsilon BP) in mast cells, Immunopharmacology 26 (1993) 187–195.

[20] L.G. Frigeri, R.I. Zuberi, F.T. Liu, Epsilon BP, a beta-galactoside-binding animal lectin, recognizes IgE receptor (Fc epsilon RI) and activates mast cells, Biochemistry 32 (1993) 7644–7649.

[21] K.J. Dorrington, H.H. Bennich, Structure-function relationships in human immunoglobulin E, Immunol. Rev. 41 (1978) 3–25.

[22] J. Baenziger, S. Kornfeld, Structure of the carbohydrate units of IgA1 immunoglobulin I. Composition, glycopeptide isolation, and structure of the asparagine-linked oligosaccharide units, J. Biol. Chem. 249 (1974) 7260–7269.

[23] U. Blank, C. Ra, L. Miller, K. White, H. Metzger, J.P. Kinet, Complete structure and expression in transfected cells of high affinity IgE receptor, Nature 337 (1989) 187–189.

[24] J. Baenziger, S. Kornfeld, S. Kochwa, Structure of the carbohydrate units of IgE immunoglobulin I. Over-all composition, glycopeptide isolation, and structure of the high mannose oligosaccharide unit, J. Biol. Chem. 249 (1974) 1889–1896.

[25] J.P. Kinet, The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology, Annu. Rev. Immunol. 17 (1999) 931–972.

[26] R.P. Siraganian, R.O. de Castro, E.A. Barbu, J. Zhang, Mast cell signaling: the role of protein tyrosine kinase Syk, its activation and screening methods for new pathway participants, FEBS Lett. 584 (2010) 4933–4940.

[27] R.P. Siraganian, Mast cell signal transduction from the high-affinity IgE receptor, Curr. Opin. Immunol. 15 (2003) 639–646.

[28] A.M. Gilfillan, J. Rivera, The tyrosine kinase network regulating mast cell activation, Immunol. Rev. 228 (2009) 149–169.

[29] C. Gonzalez-Espinosa, S. Odom, A. Olivera, J.P. Hobson, M.E. Martinez, A. Oliveira-dos-Santos, L. Barra, S. Spiegel, J.M. Penninger, J. Rivera, Preferential signaling and induction of allergy-promoting lymphokines upon weak stimulation of the high affinity IgE receptor on mast cells, J. Exp. Med. 197 (2003) 1453–1465.

[30] S. Yamasaki, E. Ishikawa, M. Kohno, T. Saito, The quantity and duration of FcRgamma signals determine mast cell degranulation and survival, Blood 103 (2004) 3093–3101.

[31] A. Lorentz, I. Klopp, T. Gebhardt, M.P. Manns, S.C. Bischoff, Role of activator protein 1, nuclear factor-kappaB, and nuclear factor of activated T cells in IgE receptor-mediated cytokine expression in mature human mast cells, J. Allergy Clin. Immunol. 111 (2003) 1062–1068.