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Beneficial effect of annexin A1 in a model of experimental allergic conjunctivitis

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ABSTRACT

Annexin A1 (ANXA1), a 37 kDa glucocorticoid-regulated protein, is a potent anti-inflammatory mediator effective in terminating acute inflammatory response, and its role in allergic settings has been poorly studied. The aim of this investigation was to evaluate the mechanism of action of ANXA1 in intraocular inflammation using a classical model of ovalbumin (OVA)-induced allergic conjunctivitis (AC). OVA-immunised Balb/c mice, wild-type (WT) and ANXA1-deficient ($AnxA1^{-/-}$), were challenged with eye drops containing OVA on days 14–16 with a subset of WT animals pretreated intraperitoneally with the peptide Ac_{2-26} (N-terminal region of ANXA1) or dexamethasone (DEX). After 24 h of the last ocular challenge, WT mice treated with Ac_{2-26} and DEX had significantly reduced clinical signs of conjunctivitis (chemosis, conjunctival hyperaemia, lid oedema and tearing), plasma IgE levels, leukocyte (eosinophil and neutrophil) influx and mast cell degranulation in the conjunctiva compared to WT controls. These anti-inflammatory effects of DEX were associated with high endogenous levels of ANXA1 in the ocular tissues as detected by immunohistochemistry. Additionally, Ac_{2-26} administration was effective to reduce IL-2, IL-4, IL-10, IL-13, eotaxin and RANTES in the eye and lymph nodes compared to untreated WT animals. The lack of ANXA1 produced an exacerbated allergic response as detected by the density of the inflammatory cell influx to the conjunctiva and the cytokine/chemokine release. These different effects observed for Ac_{2-26} were correlated with diminished level of activated ERK at 24 h in the ocular tissues compared to untreated OVA group. Our findings demonstrate the protective effect of ANXA1 during the inflammatory allergic response suggesting this protein as a potential target for new ocular inflammation therapies.

1. Introduction

Allergic conjunctivitis (AC) is defined as an inflammatory response initiated by type I (IgE-dependent and mast cell mediated) and/or type IV (mediated by T cells) hypersensitivity reactions following exposure to an allergen (Friedlaender, 2011; Marback et al., 2007). Binding of allergen to IgE cross-links its receptors on the surface of mast cells resulting in their degranulation and the release of inflammatory mediators, which may lead vasodilatation, increased vascular permeability and the infiltration of inflammatory cells into the conjunctiva. This process triggers the acute phase of the disease characterised by clinical manifestations and bilateral hyperaemia, chemosis, lid oedema and pruritus (Stern et al., 2005a,b; Bilkhu et al., 2012).

The pharmacological treatment of intraocular inflammatory conditions, especially allergic conjunctivitis, includes antihistamines, mast cell membrane stabilisers and corticosteroids which are used in the more severe forms of AC and have a greater risk of adverse effects such as increased intraocular pressure and cataractogenesis (McGhee et al., 2002; Friedlaender, 2011). In this respect, the discovery of new pharmacological agents that have high efficacy in controlling the inflammatory response with fewer side effects is critical.

As a potential anti-inflammatory agent we highlight the annexin

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A1 (ANXA1), a 37 kDa protein that downregulates the inflammatory response in experimental models of acute (Gastardelo et al., 2009; Girol et al., 2013), chronic (Oliani et al., 2008; Dalli et al., 2010) and systemic (Damazo et al., 2005) inflammation and is expressed in different immune cells, particularly mast cells and neutrophils (Oliani et al., 2000, 2001; Gil et al., 2006). However, the relationship between ANXA1 and ocular inflammation has not been well studied.

Studies performed in our laboratory using experimental models of endotoxin-induced uveitis (da Silva et al., 2011; Girol et al., 2013) and ocular toxoplasmosis (Mimura et al., 2012), both with predominant Th1 cytokine profile, revealed a modulation of endogenous expression of ANXA1 in inflammatory cells and retinal pigment epithelium and indicated that this protein, as well as its mimetic peptide Ac_{2-26}, is a key mediator in the homeostasis of inflammatory and infectious ocular processes.

In asthma, a Th2 cytokine profile disease, decreased levels of ANXA1 protein, but not mRNA, has been described in the lungs, which is likely due to protein cleavage to the 33 kDa isoform (Chung et al., 2004). In the bronchoalveolar fluid, the anti-allergic activity of Ac_{2-26} was associated with the inhibition of mast cell degranulation, histamine release and the influx of neutrophils and eosinophils into the lung, but not with altered levels of serum IgE and eosinophil chemotaxis in vitro (Bandeira-Melo et al., 2005; Wang et al., 2011).

Given these considerations and the common side effects of the current therapies used to treat conjunctivitis and other eye inflammatory processes (Read et al., 2006; Rosenbaum, 2010), we evaluated the mechanism of action of endogenous and exogenous ANXA1 protein in ovalbumin-induced conjunctivitis in WT and $AnxA1^{-/-}$ mice. These studies shed light on the genesis of ocular inflammatory disorders and may lead to new therapies for the treatment of ocular allergy.

2. Materials and methods

2.1. Animals

Male wild-type (WT) and AnxA1-knockout ($AnxA1^{-/-}$) BALB/c mice (Hannon et al., 2003), weighing 20–25 g, were randomly distributed into 6 groups (n = 5/group). The animals were housed in a 12 h light–dark cycle and were allowed food and water ad libitum. All experimental procedures were submitted and approved by the Ethics Committee in Animal Experimentation of the Federal University of São Paulo – UNIFESP (No. 0076/12), and by the Ethics Committee on Animal Use of the School of Pharmaceutical Sciences, USP (No. CEUA/FCF/285) and the Internal Biosafety Commission (CIBio).

2.2. Experimental model of allergic conjunctivitis and treatment protocols

WT and $AnxA1^{-/-}$ mice (n = 5/group experimental) were immunised on days 0 and 7 with a subcutaneous injection of 5 μg of ovalbumin (OVA, grade V) and 15 mg/ml of aluminium hydroxide adjuvant (ALUM) diluted in 200 μL of sterile saline according to models from literature (Shoji et al., 2005; Chung et al., 2009) with modifications. On days 14, 15 and 16 after an intraperitoneal (i.p.) injection of anaesthesia with ketamine (100 mg/kg) and xylazine (20 mg/kg), animals received the direct instillation of 250 μg of OVA in 10 μL of sterile saline onto the conjunctival sac. Control animals received sterile saline alone.

To determine the therapeutic efficacy of the exogenous administration of ANXA1, WT sensitised mice were pretreated i.p. on days 14, 15 and 16 with its mimetic peptide Ac_{2-26} (1.0 mg/kg; Ac-AMVSEFLKQAWFIENEEQEYVQTVK - Invitrogen, São Paulo, Brazil) (Girol et al., 2013) or dexamethasone (1.0 mg/kg, Sigma–Aldrich) (Damazo et al., 2006), diluted in 0.1 ml of sterile saline, 15 min before the instillation of OVA.

Twenty-four hours after the last OVA challenge, mice were anaesthetised to obtain blood through cardiac puncture using a syringe with 10% EDTA for analysis of IgE levels and leukocyte quantification. The mice were then sacrificed and the eyes and cervical lymph nodes were collected.

2.3. Clinical score

After 20 min of OVA challenge, mice were examined on days 14, 15 and 16 in order to clinically verify the occurrence and severity of conjunctivitis. Four clinical signs were observed in a blind fashion: chemosis, conjunctival hyperaemia, lid oedema and tearing. Scoring similar to that described by Magone et al. (1998) was performed, and each parameter was graded on a scale ranging from 0 to 3+ (0 = absence; 1 = mild; 2 = moderate and 3 = severe symptoms). Thus, each animal received a total clinical score ranging from 0 to 12+, and the data were expressed as the mean ± standard error of the mean (SEM) for each group.

2.4. Blood leukocyte quantification

Aliquots of blood (10 μL) were diluted 1/20 in Turk's solution (0.1% crystal violet diluted in 3% acetic acid) and differential counting was obtained with a Neubauer chamber using a 40× objective on a light microscope. For this study blood cells were distinguished as neutrophils and eosinophils. Data were reported as the mean ± SEM of the number of cells × 10^5/mL.

2.5. IgE levels

Whole blood was centrifuged at 1500 rpm for 10 min to collect the plasma and determine the total IgE levels by ELISA at different experimental conditions. The concentration of IgE was measured using a commercially available mouse IgE immunoassay kit (BioLegend, San Diego, USA) with levels estimated according to the manufacturer's instructions. All estimations were made in duplicate and the data expressed as the mean ± SEM.

2.6. Histopathological analysis and quantification of inflammatory cells in ocular tissues

The right eyes were fixed in 10% buffered formalin for 24 h, washed in tap water, dehydrated in a decreasing ethanol series, and embedded in paraffin. Sections of 4 μm were obtained in a Leica RM2155 microtome (Leica Microsystems GmbH, Wetzler, Germany) and subsequently stained with haematoxylin–eosin or toluidine blue 0.5% (Taab Laboratories, UK) for histopathological analysis.

Inflammatory cells eosinophils, neutrophils and mast cells were quantified using a high power objective (63×) on an Axioskop 2-Mot Plus Zeiss microscope (Carls Zeiss, Jena, Germany). Three semi-serial sections of conjunctiva were analysed per animal and the area was determined using AxioVision software (Carl Zeiss). Values are expressed as the mean ± SEM of the number of cells per 0.1 mm^2.

2.7. Immunohistochemistry analysis

The detection of endogenous ANXA1 in WT ocular tissues was conducted in 4 μm sections of paraffin-embedded eyes. After an antigen retrieval step using citrate buffer pH 6.0, the endogenous peroxide activity was blocked and the sections were incubated