A Novel Effect for Annexin 1-Derived Peptide Ac2-26: Reduction of Allergic Inflammation in the Rat

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ABSTRACT
Previous investigations have provided evidence that the N-terminal peptide of annexin 1 (peptide Ac2-26) has the capacity of reproducing the anti-inflammatory actions of the full-length protein in many systems. In the current study, we report the effectiveness of the peptide Ac2-26 as an antiallergic tool in a model of rat pleurisy and provide indication for some of the mechanisms involved. In rats inflamed by injection of ovalbumin into the pleural cavity 14 days postsensitization, peptide Ac2-26 (50–200 μg/cavity) inhibited mast cell degranulation, plasma protein leakage, and the accumulation of both neutrophils and eosinophils. Treatment with either peptide Ac2-26 (200 μg/cavity) or dexamethasone (1 mg/kg i.p.) inhibited ovalbumin-induced eotaxin release in the pleural effluents. In vitro, peptide Ac2-26 inhibited ovalbumin-evoked histamine release from subcutaneous tissue fragments obtained from sensitized rats (33–66 μM) and interleukin-13-evoked eotaxin generation from cultured rat mesothelial cells (16–33 μM) but not eosinophil chemotaxis. This work demonstrates that the annexin 1 mimicpeptide Ac2-26 prevents allergen-evoked eosinophilic inflammatory response in rats. Combined analysis of the in vivo and in vitro experiments presented herein suggests that the blockade of secretion of pivotal mediators for the allergic response, such as histamine and eotaxin, could be responsible for the inhibitory actions displayed by peptide Ac2-26.

A marked feature of the acute phase of the allergic inflammatory response is the activation of mast cells, resulting in the release of preformed and inducible proinflammatory mediators (Busse, 1998; Lemanske and Busse, 2003). Increased vascular permeability to plasma proteins is one of the major outcomes of the allergic mast cell activation, which is also believed to promote leukocyte recruitment via direct or indirect release of cytokines and chemokines (Metcalfe et al., 1997). An interesting aspect of the pathogenesis of allergic diseases is the accumulation of large numbers of tissue eosinophils. There is substantial evidence in the literature that eosinophil-derived mediators are strongly associated with the tissue damage observed in these diseases (Gleich, 2000). It is therefore presumed from these findings that drugs that can prevent mast cell activation and/or infiltration of eosinophils into inflamed tissue may have therapeutic potential to control allergic disorders such as asthma (Gleich, 2000).

Anti-inflammatory therapy with glucocorticoid agents is currently the most effective pharmacological approach for severe asthma, but its applicability is limited by the wide range of undesirable side effects associated with steroid therapy (Adamko et al., 2003). At present, it is understood that the glucocorticoid inhibitory effects are due to modulation of gene transcription. Binding of the hormone to its receptor is followed by translocation of the steroid-receptor complex, dimerization, and ligation of the dimer to the so-called "glu-

ABBREVIATIONS: IL, interleukin; PAF, platelet-activating factor; BSA, bovine serum albumin; DMEM, Dulbecco’s modified Eagle’s medium; RT, reverse transcriptase; PCR, polymerase chain reaction; bp, base pair(s); DNP, dinitrophenol.
corticoid-responsive elements" in the nuclear DNA, thus activating or inhibiting the transcription mechanism (Barnes, 1996). The suppressive effects of glucocorticoids on the transcription of a number of genes for inflammatory mediators, including interleukin (IL)-3, IL-4, IL-5, granulocyte-macrophage colony-stimulating factor receptor, and various chemokines, are well established (Barnes, 1996). However, glucocorticoids can induce the production of annexin 1, an anti-inflammatory protein of the superfamily of annexins, which has been implicated as an endogenous mediator of the glucocorticoid effect in many circumstances (Rovieszko et al., 2002). Annexin 1 (previously termed lipocortin 1) was originally described as an inhibitor of lipid mediator formation via blockade of phospholipase A₂ activity, thereby modulating many biological processes, including cellular growth and differentiation, central nervous system response to cytokines, neuroendocrine secretion, and tissue neutrophil accumulation (Flower and Rothwell, 1994; Hannon et al., 2003).

Previous studies have emphasized that the N-terminal peptide of annexin 1 (peptide Ac2-26) can reproduce the anti-inflammatory actions of the full-length protein. This peptide of 25 amino acids inhibits tissue recruitment of neutrophils caused by different stimuli in vivo (Perretti et al., 1993). Peptide Ac2-26 can also reproduce the anti-inflammatory effect of annexin 1 in other processes, including hyperalgesia (Ferreira et al., 1997) and tissue injury caused by artery occlusion and reperfusion shock (Cuzzocrea et al., 1998). The hypothesis that the annexin 1 fragment might be able to affect allergen-induced eosinophil recruitment was previously investigated in a mouse skin model (Teixeira et al., 1998). The study clearly demonstrated that the inhibitory action of dexamethasone on eosinophil trafficking in cutaneous inflammatory reactions in the mouse was neither impaired by annexin 1-immune-neutralization nor mimicked by the annexin 1 N-terminal peptide Ac2-26, leading to the suggestion that the antieosinophilic effect of glucocorticoids was annexin 1-independent.

Recent research has shown a direct interaction of annexin 1 and its peptides with the specific seven transmembrane G protein-coupled lipoxin A₄ receptor (Perretti et al., 2002). Furthermore, stable analogs of lipoxin A₄ can block allergic airway inflammation, as shown by decreased eosinophil influx and release of mediators such as IL-4, IL-5, IL-13, and eotaxin, both in the mouse (Levy et al., 2002) and rat (Bandeira-Melo et al., 2000a). The current study was undertaken to evaluate the effect of the annexin 1 peptide derivative Ac2-26 on cell recruitment and protein extravasation triggered by antigen challenge in a rat model of allergic inflammation.

Materials and Methods

Animals. Wistar rats of both sexes weighing 150 to 200 g and guinea pigs of both sexes weighing 250 to 300 g were obtained from the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) breeding unit. The animals were housed in groups of five animals and maintained on a 12-h light/dark cycle with water and food ad libitum until use. The Ethics Committee for Care and Use of Animals of the Oswaldo Cruz Foundation approved the experimental protocols employed in this study (License 0085-02).

Ovalbumin-Evoked Pleurisy in Actively Sensitized Rats. Active sensitization was achieved by s.c. injection (0.2 ml) of a mixture containing 50 μg of ovalbumin and 5 mg of aluminum hydroxide in 0.9% NaCl solution (saline). Ovalbumin dissolved in sterile saline was administered intraperitoneally (i.p.) (12 μg/cavity) 14 days post-sensitization using a 27.5-gauge needle adjusted to 3 mm in length. All i.p. injections were performed under inhalation anesthesia and in a final volume of 100 μl. All solutions were always made fresh immediately before use. At distinct postchallenge time points, the rats were killed under CO₂ atmosphere, and the pleural cavity was rinsed with 3 ml of heparinized saline (10 IU/ml). The pleural effluent was collected, and its volume was measured with a graduated syringe and then used for cellular and mediator analysis.

Measurement of Pleural Leukocytes and Protein. Total leukocyte counts on samples of pleural effluent were determined in a Coulter Counter ZM (Beckman Coulter, Inc., Fullerton, CA) after red blood cell lysis using Zapoglobin (Beckman Coulter, Inc.). Differential analysis was performed under an oil immersion objective on cytocentrifuged smears stained with May-Grünwald-Giemsa dye. For the mast cell enumeration, pleural effluent samples collected 4 h postantigen challenge were diluted in toluidine blue dye solution (Mota and Peixoto, 1966) and evaluated in a Neubauer chamber using a light microscope. The effluent was then centrifuged at 1300g for 10 min, and the protein content of the supernatant was quantified in a spectrophotometer (540 nm) by the Biuret technique (Gornall et al., 1949).

Drug Treatments. To study the effect of human annexin 1 N terminus on allergic pleurisy, the peptide Ac2-26 (50–200 μg/cavity) was injected intraperitoneally 5 min before allergen challenge. In control groups, the peptide was replaced by the scrambled peptide Ac2-12 (200 μg/cavity) (La et al., 2001) or 0.9% saline. Alternatively, 1 mg/kg dexamethasone was injected intraperitoneally 1 h before challenge.

Quantification of Eotaxin by Enzyme-Linked Immunosorbent Assay. Eotaxin in rat pleural lavage fluid was measured by a murine eotaxin enzyme-linked immunosorbent assay as reported previously (Bandeira-Melo et al., 2000a). Rat eotaxin (97.3% sequence identity with murine eotaxin) (Williams et al., 1998) cross-reacts in this assay, but there was no detectable cross-reaction with any other chemokine tested, including human and guinea pig eotaxin.

Anaphylactic Histamine Release from Rat Subcutaneous Tissue Fragments in Vitro. Actively sensitized rats were killed under CO₂ atmosphere, and fragments from dorsal subcutaneous tissue (~1 mg) were removed and placed in 48-well plates containing Hanks’ balanced salt solution (HBSS) with Ca²⁺ and Mg²⁺ in a final volume of 700 μl. The fragments were exposed to 300 μg/ml ovalbumin for 1 h, and then the plates were centrifuged at 150g for 10 min; the samples were collected and added to 0.8 N perchloric acid (1:2 dilution) as reported previously (de Oliveira Barreto et al., 2003). To test the potential effect of Ac2-26 (16–66 M) on ovalbumin-evoked histamine release, the tissue system was pretreated with the target substance or its vehicle at 37°C for 1 h. After centrifugation at 170g for 10 min, the supernatant was collected and stored at −20°C for histamine quantification as reported previously (Shore et al., 1959). Briefly, this method consists of sample dilution in 0.1 N HCl followed by 0.8 N NaOH and further addition of the substrate o-phthalaldehyde. After a 4-min incubation, the reaction was stopped with 3N HCl, and the fluorescence was measured in a Shimadzu RF1501 spectrofluorophotometer (Shimadzu, Kyoto, Japan) (excitation, 360 nm; emission, 450 nm). After collecting the medium, tissue fragments were dried in incubators at 40°C for 15 min, and the results were expressed as the amount of histamine released (nanograms) per milligram of dried tissue.

Eosinophil Chemotaxis. Eosinophils were isolated from the peritoneal cavity of normal rats using Percoll density gradient as previously reported (Martins et al., 1989). Eosinophil suspensions of 85 to 95% purity and 96% viability, as attested by the trypan dye exclusion test, were used throughout. Migration experiments were performed using 48-well microchemotaxis chamber (Neuro Probe, Inc., Gaithersburg, MD) and Toyo cellulose nitrate filters (3-μm
pore) according to the technique described previously (Richards and McCulloch, 1984).

To test the potential direct effect of peptide Ac2-26 on eosinophils, cells were preincubated with the peptide or its vehicle at 37°C for 1 h; then eosinophil suspension (2 × 10^6 cells; 50 μl) were placed in the upper compartment of the chamber, whereas the lower compartment was loaded with 1 μM murine eotaxin, 1 μM platelet-activating factor (PAF), and their vehicle (RPMI medium, pH 7.2, containing 0.1% bovine serum albumin (BSA)). The chemotaxis chamber was then incubated for 2 h at 37°C in a 5% CO2/95% O2 atmosphere. The filter was fixed and stained as described previously (Richards and McCulloch, 1984). Eosinophils migrated at 40 μm from the upper surface of the filter were counted in 15 consecutive high-power fields under an immersion objective (Martins et al., 1989).

Rat Peritoneal Mesothelial Cell Isolation and Cultures. Mesothelial cells were obtained from Wistar rats as described by Yang et al. (2004). Briefly, rats were killed in a CO2 atmosphere and injected in the abdominal cavity with 25 ml of phosphate-buffered saline containing 0.25% trypsin and 0.02% EDTA-Na2. Within 2 h, peritoneal fluid was removed under sterile conditions and centrifuged for 10 min at 1500 rpm. Pelleted cells were then washed with HBSS, suspended in DMEM/F-12 medium supplemented with 20% fetal calf serum, placed into 25-cm² tissue culture flasks, and incubated at 37°C in a humidified 5% CO2 incubator. The culture medium was changed every 3 to 5 days. Mesothelial cells were passaged by dissociating the monolayer with 0.25% trypsin/0.02% EDTA-Na2 and reseeded in 48-well plates (5 × 10⁴ cells/well). Subconfluent mesothelial cells were washed with phosphate-buffered saline pretreated with either Ac2-26 (16–66 μM) or dexamethasone (1–10 μM) in the presence of fetal calf serum-free DMEM/F-12 medium for 1 h. Cultured cells were then stimulated with 2 ng/ml hIL-13 for 72 h at 37°C in a humidified 5% CO2 incubator. The culture medium was collected and centrifuged at 2000g for 10 min at 4°C, and the supernatant was stored at −20°C until assayed for eotaxin.

**RT-PCR Conditions.** RNA was extracted from 10⁷ resting mesothelial cells in 1 ml of TRIzol according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). Purified total RNA (1 μg) was used as a template for first-strand cDNA synthesis using SuperScript II reverse transcriptase (Invitrogen). PCR was performed using 2 μl of reverse transcription reaction (equivalent to 100 ng of starting RNA) as template, 200 μM dNTPs, 20 nM of each primer, and 2 U of Taq polymerase (Amersham Biosciences Inc., Piscataway, NJ) in a 50-μl reaction volume according to the enzyme manufacturer's instructions. The sequences of the primers (Alpha DNA, Montreal, QC, Canada) and the sizes of the amplified products were as follows: rat β-actin, 5'-TGACCCAGATCATGTTGAGAC-3’ and 5’-GGATTCCATACCCAGGAAGGA-3’ (458 bp); and rat ALXR, 5’-CCGTCATTAGAGGTCCTAC-3’ and 5’-TCATATTGCTTTTATATCATCAATGTT (343 bp). PCR conditions were as follows: a denaturing step at 94°C for 2 min; followed by 45 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 60 s; followed by a final extension step at 72°C for 7 min. A total of 20 μl of each PCR product was resolved on a 2% agarose gel and visualized with ethidium bromide. Primer sequences for rat β-actin were based on GenBank sequence accession number BC063166. Primer sequences for rat ALXR were the same as published previously (Chiang et al., 2003).

**Materials.** Peptide Ac2-26 (acetyl-AMVSEPLKQAWEENQQEYV-VQTVK, MW 3,050) and the control scrambled peptide Ac2-12 (acetyl-SVEQKMWALFA, MW 1,424) were prepared by the Advance Biotechnology Center (The Charing Cross and Westminster Medical School, London, UK) by using solid-phase stepwise synthesis. Purity was more than 90% as assessed by high-performance liquid chromatography and capillary electrophoresis (data supplied by manufacturer). Dinitropheolyalted albumin conjugate (DNP-BSA) was purchased from Calbiochem (San Diego, CA); murine immunoglobulin E anti-DNP monoclonal antibody was kindly provided by Dr. A. Provoust-Danon (Unite´ d’Immuno-Allergie, Institut Pasteur, Paris, France); PAF (1-O-hexade-nylated albumin conjugate (DNP-BSA) was purchased from Calbiochem (San Diego, CA); murine immunoglobulin E anti-DNP monoclonal antibody was kindly provided by Dr. A. Provoust-Danon (Unite´ d’Immuno-Allergie, Institut Pasteur, Paris, France); PAF (1-O-hexade-}

**Fig. 1.** Effect of peptide Ac2-26 (50 and 200 μg/cavity i.pl.) or scrambled peptide Ac2-12 (200 μg/cavity i.pl.) on pleural protein leakage (top) and neutrophil accumulation (middle) noted 4 h postchallenge, and eosinophil accumulation noted 12 postchallenge (bottom). All rats were administered with allergen (ovalbumin, 12 μg/cavity i.pl.) or scrambled allergen (ovalbumin, 12 μg/cavity i.pl.) and visualized with ethidium bromide. Results were expressed as means ± S.E.M. from at least five animals. *P < 0.001 compared with nonsensitized group. †P < 0.01 compared with allergen-challenged sensitized group.
Results

Effect of Peptide Ac2-26 on Allergen-Evoked Pleurisy. Ovalbumin pleural challenge (12 μg/cavity) in sensitized rats provoked an intense inflammatory reaction characterized by intense plasma leakage and leukocyte infiltration (Fig. 1). Analogous to our previous studies (Silva et al., 2001), this cellular infiltration consists markedly of neutrophils (Fig. 1, middle) in the early phase and eosinophils (Fig. 1, bottom) in the late phase of the process. Local treatment with peptide Ac2-26 (50–200 μg/cavity) dose-dependently inhibited plasma leakage (Fig. 1, top) as well as neutrophil (Fig. 1, middle) and eosinophil accumulation triggered by allergen provocation (Fig. 1, bottom). In contrast, treatment with ~4-fold excess of the negative control peptide (scrambled peptide Ac2-12, 200 μg/cavity) failed to alter these phenomena (Fig. 1). As shown in Table 1, the increased number of pleural mononuclear cells remained unchanged after peptide Ac2-26 treatment or control peptide.

Effect of Peptide Ac2-26 on Allergen-Evoked Mast Cell Degranulation and Histamine Release. In line with previous reports (Lima et al., 1991), the intrapleural challenge of sensitized rats with ovalbumin led to a significant reduction in the number of intact mast cells recovered from the pleural cavity 4 h postchallenge. As illustrated in Table 2, pretreatment with peptide Ac2-26, but not with the negative control peptide, significantly increased the number of intact mast cells recovered in the pleural effluent following allergen injection. Furthermore, in vitro exposure to peptide Ac2-26 (16–66 μM) also attenuated ovalbumin-evoked histamine release (Fig. 2), confirming the protective effect of peptide Ac2-26 on mast cell activation.

Table 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>Treatment</th>
<th>Mast Cell × 10^6/cavity</th>
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</thead>
<tbody>
<tr>
<td>Nonsensitized</td>
<td>-</td>
<td>7.4 ± 0.4</td>
</tr>
<tr>
<td>Sensitized</td>
<td>-</td>
<td>12.9 ± 1.4*</td>
</tr>
<tr>
<td>Sensitized</td>
<td>Ac2-26</td>
<td>9.7 ± 1.2</td>
</tr>
<tr>
<td>Sensitized</td>
<td>Scrambled</td>
<td>13.9 ± 1.4*</td>
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* Significantly different from the nonsensitized group (P < 0.01).

Discussion

In this study, we investigated the effect of the annexin 1 pharmacophore peptide Ac2-26 on the allergic reaction pro-
A number of studies have provided evidence for the involvement of this protein in the regulation by glucocorticoids of neutrophil migration (Perretti and Flower, 1993), inflammatory edema (Cirino et al., 1989), ischemic damage (Cuzzocrea et al., 1997), pain (Ferreira et al., 1997), and fever (Davidson et al., 1991). Nevertheless, it is clear that annexin 1 modulation is not the only mechanism of glucocorticoid action that can involve a variety of mechanisms, including direct and indirect genomic routes and other annexin 1-independent signaling effects (Hannon et al., 2003).

Recently, the peptide Ac2-26 and annexin 1 have been shown to activate the lipoxin A$_4$ receptor, suggesting that annexin 1 and lipoxin A$_4$ may act in concert at this G protein-coupled receptor to inhibit formation of mediators of inflammation and leukocyte recruitment (Perretti, 2003). Interestingly, the annexin 1/lipoxin A$_4$ receptor is also expressed in rats (Chiang et al., 2003), and several parameters of rat allergic pleurisy, including edema, eosinophil formation, and eosinophil accumulation, were inhibited by lipoxin A$_4$ stable analogs (Bandeira-Melo et al., 2000a,b). Of note, recent studies have positioned lipoxins as strong candidates for novel antiallergic therapies (Levy and Serhan, 2003). For these reasons, we have tested the putative influence of annexin 1 mimetics in a rat model of allergic inflammation.

For some time now, we have explored a model in which ovalbumin-sensitized rats react to ovalbumin intrapleural challenge with a rapid plasma leakage and edema that peaks from 1 to 4 h postchallenge. These changes are followed by neutrophil influx, peaking from 4 to 8 h, and mononuclear cell and eosinophil influx, first noted at 8 h but peaking from 12 to 24 h postallergen challenge (Lima et al., 1997). All these events were clearly sensitive to the systemic treatment with dexamethasone, whereas histamine and 5-hydroxytryptamine receptor antagonists inhibited plasma leakage without altering leukocyte infiltration (Martins et al., 1993). In our first set of experiments, we have shown that the local administration of peptide Ac2-26 reduced plasma leakage and neutrophil and eosinophil emigration into the rat pleural space, leaving intact the mononuclear cell enrichment caused by allergen challenge. These latter data differ from the glucocorticoid wide-ranging effects and could indicate a certain degree of selectivity in the actions of peptide Ac2-26. Thus, we next set out to investigate the Ac2-26 peptide-driven inhibitory mechanisms of both vascular and cellular changes caused by allergen challenge.

A hypothetical mechanism that could account for the inhibitory effects of Ac2-26 might be the generation of pivotal mediators for the allergic response. Annexin 1 is a steroid-inducible protein that has been claimed to mediate a variety of anti-inflammatory properties of glucocorticoids (Roviezzo et al., 2002; Hannon et al., 2003).
bition of allergic inflammation by annexin 1-derived peptide is the down-regulation of resident mast cell activity. Mast cells play an important role in allergic inflammation by releasing preformed and inducible proinflammatory mediators (Lehmann and Busse, 2003). With particular reference to allergen-induced pleurisy in rats, activation of mast cells seems to be a primary event that may lead to both plasma leakage and leukocyte infiltration (Diaz et al., 1996; Lima et al., 1996). It is noteworthy that annexin 1 can be up-regulated by distinct stimuli in rat mast cells (Tasaka et al., 1994; Oliani et al., 2000) and correlated with down-regulation of compound 48/80-induced histamine release (Tasaka et al., 1994). In our study, local treatment with the peptide Ac2-26 attenuated ovalbumin-evoked mast cell degranulation in the rat pleural space, a finding that was also substantiated in in vitro settings. In fact, active concentrations of peptide Ac2-26 abrogated ovalbumin-evoked histamine release from subcutaneous tissue fragments collected from sensitized rats. Overall, these findings support the interpretation that the mast cell may be a relevant target for the antiallergic effect produced by the peptide Ac2-26 and agree with the previous original study in which human recombinant annexin 1 inhibited compound 48/80-induced edema in the rat paw (Cirino et al., 1989). The sensitivity of the rat mast cell to annexin 1 may also be the basis for the different findings reported in murine models, where dexamethasone inhibitory effects were neither modified by annexin 1-immune neutralization (Das et al., 1997; Teixeira et al., 1998) nor mimicked by the annexin 1 N-terminal peptide Ac2-26 (Teixeira et al., 1998). One can speculate that mouse mast cells are either less sensitive to annexin 1 or that the annexin 1/lipoxin A4 receptor may be differentially expressed on mast cells across species. Future studies will address this point. Of note, lipoxin A4 analogs did not inhibit allergen-evoked pleural mast cell degranulation in rats (Bandeira-Melo et al., 2000a). To explain such discrepancies, one can speculate that annexin 1-derived peptide and lipoxin A4 analogs activate ALXR expressed on rat pleural mast cells in a different fashion, coupling distinct signaling pathways as has been observed for other G protein-coupled receptors (Kenakin, 2004). On the other hand, one of these ALXR ligands could activate an alternative receptor in parallel (formyl peptide receptor or formyl peptide receptor-like-2, for example) (Ernst et al., 2004), interfering with ALXR-driven signaling in mast cells. In addition to the blockade of histamine release by pleural mast cells, a reduced production of other mediators critical to allergic inflammation could represent a complementary mechanism of the annexin 1-derived peptide-driven effects. Likewise, what was observed following treatment with lipoxin A4 stable analogs (Bandeira-Melo et al., 2000a), administration of either peptide Ac2-26 or dexamethasone clearly abolished allergen-evoked eosinophil generation in parallel with abrogation of eosinophil recruitment. This chemokine plays a crucial role in the mechanism of allergen-evoked pleural eosinophil accumulation in rats (Silva et al., 2001), supporting the interpretation that the antieosinophil effect of the peptide Ac2-26 may also be important in bringing about its anti allergic properties. While trying to identify the source of eosinotaxis and the putative target for the peptide Ac2-26 in this model, we found that rIL-13 is a positive stimulus for the induction of eosinotaxis in a primary culture of rat mesothelial cells, and this system is also sensitive to both peptide Ac2-26 and dexamethasone. Furthermore, primary rat mesothelial cells express message for ALXR, reinforcing its potential role as target for peptide Ac2-26 inhibition. Therefore, in the current model of allergic pleurisy, it is not unlikely that these agents are acting on pleural mesothelial cells to prevent eotaxin generation.

A final potential mechanism responsible for the antieosinophilic effect of annexin 1-derivative peptide could be the impairment of eosinophil locomotory functions; however, in vitro experiments revealed that peptide Ac2-26 failed to impair eotaxin- and PAF-induced eosinophil chemotaxis. This finding is in line with the inability of the annexin 1 fragment to affect mouse eosinophil migration in response to intradermal application of directly acting eosinophil chemokines such as chemokine macrophage inflammatory protein-1α (Teixeira et al., 1998). Of note, the refactoriness presented by eosinophils to peptide Ac2-26 is also observed for lipoxin A4 stable analogs (Bandeira-Melo et al., 2000a), indicating that either activation of the annexin 1/lipoxin A4 receptor may not regulate intracellular signaling events of eosinophil motility or even that rat eosinophils do not express such a receptor.

In conclusion, we demonstrate that local administration of the peptide Ac2-26 attenuates allergen-evoked eosinophilic inflammation in the rat. The inhibitory mechanism of plasma leakage and eosinophil enrichment seems to be closely associated with inhibition of histamine release from mast cells and local eotaxin generation, respectively. We believe that our results will stimulate further work for the development of annexin 1-derivatives as an innovative form of therapy for allergic dysfunction.

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References


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