

Decreased Levels of Lipoxin A4 and Annexin A1 in Wheezy Infants

Hatice Eke Gungor^a Fulya Tahan^a Selma Gokahmetoglu^b Berkay Saraymen^c

Departments of ^aPediatric Allergy, ^bMedical Microbiology and ^cBiochemistry, Erciyes University School of Medicine, Kayseri, Turkey

Key Words

Annexin A1 · Asthma · Lipoxin A4 · Wheezy infant

Abstract

Objective: Wheezing is a common and challenging health issue in infancy and early childhood. Asthma diagnosis is frequent in patients with a history of recurrent wheezing. A relationship has been reported between asthma and anti-inflammatory mediators such as lipoxin A4 and annexin A1. However, this remains uncertain in wheezy infants. The aim of the present study was to determine lipoxin A4 and annexin A1 levels in wheezy infants. **Materials and Methods:** Eighty-seven patients aged 6–36 months were included in this study. Demographic characteristics, clinical features, laboratory data, clinical diagnoses, and treatments, if present, were recorded. Patients were divided into 2 groups: patients with wheezing (n = 59) and healthy controls (n = 28). Blood samples were taken and lipoxin A4 and annexin A1 levels were evaluated by ELISA. **Results:** Lipoxin A4 and annexin A1 levels were significantly lower in the wheezing group than in the control group (p < 0.05). A significant correlation was found between the serum total immunoglobulin E (IgE) level and the percentage and absolute number of eosinophils (p < 0.05). No significant correlation was found in terms of lipoxin A4 and annexin A1 levels, the serum total IgE level, and the percentage and absolute number of eosinophils among groups (p > 0.05). **Conclusion:** This is the first study to assess lipoxin A4 and annexin A1 levels in

wheezy infants. The levels of lipoxin A4 and annexin A1 were found to be low in wheezy infants. We hope that these results will lead to novel therapeutic options for asthma in cases where an optimal treatment modality is lacking.

© 2014 S. Karger AG, Basel

Introduction

Wheezing, a widespread symptom in preschool children, is a persistent high-pitched sound, with a musical quality, spreading from the chest throughout expiration [1]. The term ‘wheezing infant’ is used for those who have 3 or more wheezing attacks but do not have any underlying disease upon examination [2]. In children, the onset of wheezing may occur at various ages and may disappear over time in some cases. However, it may have a persistent course and may progress to asthma. Asthma is one of the most important chronic inflammatory diseases, characterized by variable airflow obstruction and airway hyperresponsiveness in childhood. A large number of mediators that affect the airways play a role in the pathogenesis of asthma [3]. Lipoxins are the first agents determined to be anti-inflammatory endogenous lipid mediators involved in the resolution of inflammation [4].

Lipoxin and their analogs could support the resolution of inflammation through various mechanisms, including inhibition of the biosynthesis of proinflammatory lipid mediators, cytokine and chemokine production, leuko-

cyte recruitment and activation, stimulation of the the clearance of apoptotic leukocytes, and blocking of edema formation [4, 5]. Lipoxin A4 (LXA4) blocks both airway hyperresponsiveness and pulmonary inflammation in anti-inflammatory receptors named ALX that are expressed on both leukocytes [6] and airway epithelial cells [7]. This results in decreased leukocytes and mediators, including interleukin-5, interleukin-13, eotaxin, prostanooids, and cysteinyl leukotrienes [8]. It has been reported that a decrease in LXA4 occurs in severe asthma and downregulation of the expression of genes involved in LXA4 formation has been observed in lung biopsies from severe asthmatics [9, 10].

Annexins are a group of intracellular proteins with several cellular roles, which can bind calcium and phospholipids and have no hydrophobic signal sequences [11]. Annexin A1 (ANXA1) and its derivatives exert anti-inflammatory effects by inhibiting eicosanoid synthesis, preventing leukocyte migration, and stimulating the apoptosis of inflammatory cells as seen in glucocorticoids [12, 13]. Reduced or defective annexin production has been reported in smokers and patients with inflammatory conditions such as rheumatoid arthritis, cystic fibrosis, or asthma [14–16]. In experimental asthma models, it has been shown that exogenous ANXA1 has an anti-inflammatory effect [16]. However, the status of these two mediators is unknown in babies with wheezing. The aim of the present study was to determine the levels of LXA4 and ANXA1 in infants with wheezing.

Materials and Methods

This prospective study was conducted at the Department of Pediatric Allergy of Erciyes University (Kayseri, Turkey) from November 2011 to March 2012. A total of 87 children between the ages of 6 and 36 months were recruited. The study procedures were done in conformity with a protocol already approved by the Institutional Review Board of Erciyes University. Written informed consent was obtained from the parents of the children. Children who had had 3 or more wheezing attacks but did not have underlying diseases in their evaluations ($n = 59$) and healthy controls ($n = 28$) with a normal systemic examination and no known disease were included in this study. The exclusion criteria were: immunodeficiency, heart and lung diseases, treatment with corticosteroids during the preceding 1 month, and premature birth. The demographic, clinical, and laboratory data of the patients were recorded. Total immunoglobulin E (IgE) levels were measured via nephelometric methods and an eosinophil count was performed using a Siemens Acvia 2120 analyzer. Skin testing was done with 15 aeroallergens and 8 food allergens with positive and negative controls. Reactions with a wheal diameter >3 mm were considered positive. All of the children's blood samples were drawn into ethylenediaminetetraacetic acid (EDTA) tubes, and then plasma was re-

Table 1. Patients' characteristics

	Wheezy infants ($n = 59$)	Controls ($n = 28$)	p
Mean age ($\pm$ SD), months	22 $\pm$ 8.6	20.1 $\pm$ 10	$>0.05^a$
Sex, n (%)			
Female	21 (35.5)	17 (60.7)	
Male	38 (64.4)	11 (39.3)	$>0.05^b$
Eosinophil count, %	2 (0.3–12.10)	1.9 (0.2–3.9)	$>0.05^a$
Eosinophil count, n/mm^3	190 (20–1,460)	170 (10–380)	$>0.05^a$
IgE, kU/l	25 (5–1,980)	17 (5–127)	$>0.05^a$

Values are presented as medians (range) unless otherwise specified. ^a Mann-Whitney U test. ^b χ^2 test.

moved from the blood and plasma samples were stored at -80°C until analyzed.

LXA4 and ANXA1 Measurements

LXA4 levels were measured using enzyme-linked immunosorbent assay (ELISA) kits (Biomedical Research, Oxford, Mich., USA) following the manufacturer's instructions. Samples were measured in duplicate. Levels <10 pg/ml were not measured.

ANXA1 levels were also measured using ELISA kits acquired from USCNC (Life Science Inc., PR China) according to the manufacturer's instructions. Measurements varied between 0.312 and 20 ng/ml. The minimum measurable amount was 0.143 ng/ml.

Statistical Analysis

Statistical analysis was performed using SPSS 15 for Windows (Chicago, Ill., USA). LXA4 and ANXA1 levels in plasma were compared using the Mann-Whitney U test. Correlation analysis was performed with Spearman's tests. $p < 0.05$ was considered statistically significant.

Results

Patients' Characteristics

Of the 87 children included, 59 had wheezing while 28 were healthy controls. The age range was 6–36 months. Table 1 presents the patients' characteristics. No significant differences were detected between children with wheezing and healthy controls in terms of absolute eosinophil counts and the percent of eosinophils ($p > 0.05$; Mann-Whitney U test) (table 1).

No significant differences were detected between children with wheezing and controls in terms of total IgE ($p > 0.05$; Mann-Whitney U test) (table 1).

LXA4 Levels

When LXA4 levels were compared between children with wheezing and controls, they were found to be mark-

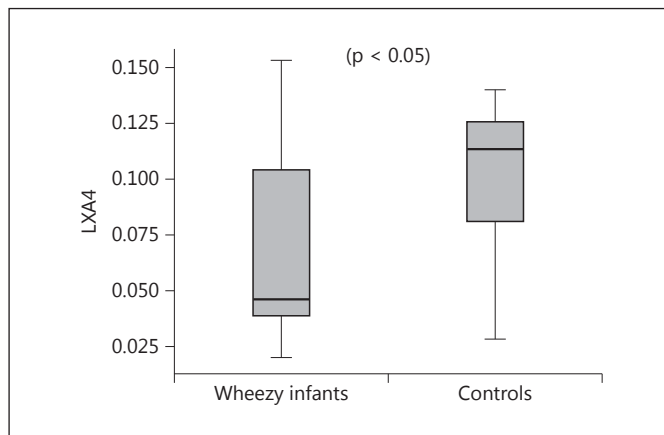


Fig. 1. LXA4 levels in infants with wheezing and healthy controls.

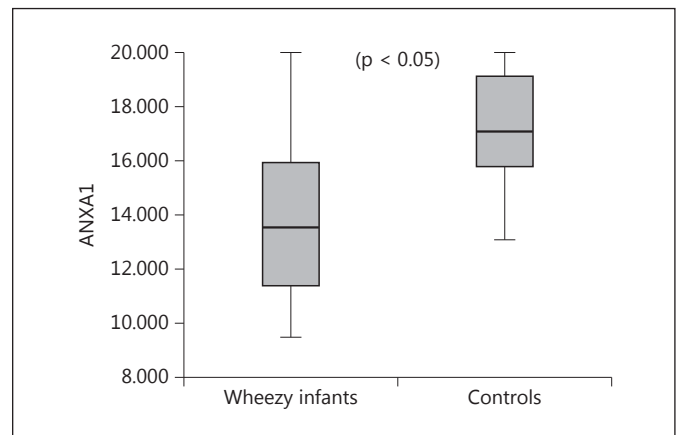


Fig. 2. ANXA1 levels in infants with wheezing and healthy controls.

edly lower in the wheezing group (66 ± 35 pg/ml) than in the control group (120 ± 11 pg/ml) ($p < 0.05$; Mann-Whitney U test) (fig. 1).

ANXA1 Levels

When ANXA1 levels were compared between children with wheezing and controls, they were found to be markedly lower in the wheezing group (14.45 ± 3.26 ng/ml) than in the control group (16.40 ± 1.94 ng/ml) ($p < 0.05$; Mann-Whitney U test) (fig. 2).

Correlation Analysis

When a correlation analysis was performed for the wheezing group and the control group, a correlation was detected between total IgE levels and eosinophil counts in the wheezing group ($r = -0.223$; $p = 0.02$; Spearman's test) (fig. 3).

No correlation was detected between groups when LXA4, ANXA1, total IgE levels, and the absolute count and percent of eosinophils were analyzed ($p > 0.05$; Spearman's test).

Discussion

LXA4 and ANXA1 are mediators which have anti-inflammatory effects [12, 17]. In human studies, it has been shown that the level of LXA4 is low in severe asthma [9, 10, 18]. In addition, experimental studies have shown that ANXA1 is associated with asthma development [16]. However, levels of LXA4 and ANXA1 have not been evaluated in children with recurrent wheezing.

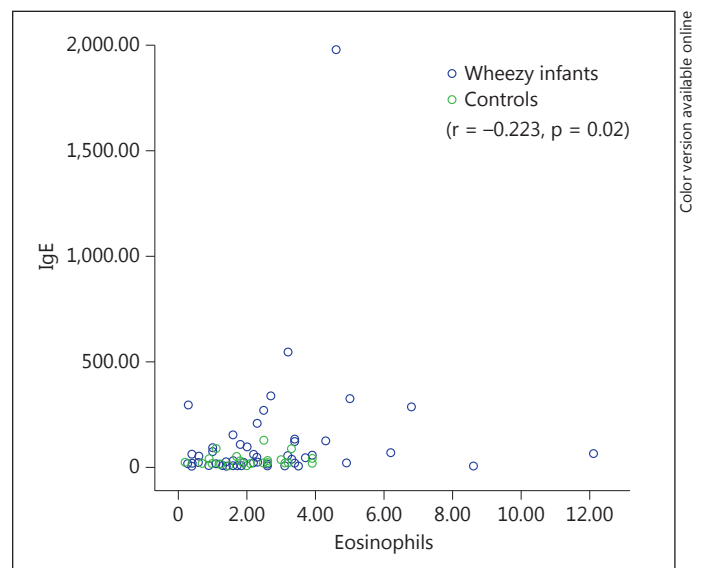


Fig. 3. Correlation between total IgE levels and eosinophil counts (%) in wheezing and control groups.

In previous studies, increased serum IgE levels and eosinophilia have been found to be associated with asthma, persistent wheezing, and early sensitization [19]. In a study by Karakoc et al. [20], eosinophilia was linked to persistent asthma independently of atopia during childhood. This finding suggests that there is a strong correlation between parental asthma and eosinophilia; thus, genetics can be an important determinant of the eosinophilic response [20]. In our study, higher serum IgE levels and eosinophil counts were detected in the wheezing

group compared to controls, although this finding was not statistically significant. It was thought that increased IgE levels and eosinophilia related to asthma and persistent wheezing may be associated with decreased LXA4 and ANXA1 levels, but no association was detected.

Lipoxins are anti-inflammatory eicosanoids, derived from 15-LO, which are endogenously produced in the human body [21]. A study by Christie et al. [22] showed that nebulized LXA4 protects against LTC4-mediated bronchoconstriction. It has also been reported that there is a significant relationship between lipoxin biosynthesis and asthma severity, and that the basal LXA4 level is lower in severe asthma compared to moderate asthma [9, 10, 18]. This suggests that endogenous production is insufficient in these patients. LXA4 levels have been found to be lower in the BAL fluid of patients with severe asthma or aspirin intolerance compared to those without severe asthma [9, 10]. Moreover, FPR2/ALX gene and protein expressions have been found to be significantly decreased in the granulocytes of patients with severe asthma [9, 10]. In a study on children with asthma by Wu et al. [23] it was shown that 15-LO expression in leukocytes and blood LXA4 levels gradually decrease when moving from mild to severe on the asthma spectrum.

In a study by Ni et al. [24], 69 patients were assigned to one of the following 3 groups: acute bronchiolitis, bronchial asthma, or acute gastroenteritis. LXA4 levels were measured in all patients and the lowest levels were detected in patients with asthma, but no significant difference was found among the groups. In the same study, it was reported that LXA4 levels changed depending on age and LXA4 levels were not lower in adult patients with asthma or in the older group with asthma [24]. That study included children younger than 24 months of age with acute bronchiolitis while our study included children younger than 36 months of age with recurrent bronchiolitis. In addition, it was reported that LXA4 levels increased with age in that study. The difference with our results may be attributed to the older age of the subjects and recurrent bronchiolitis. LXA4 levels are unknown in infants with wheezing. In our study, LXA4 levels were found to be lower in infants with wheezing compared to controls. The finding that LXA4, an anti-inflammatory mediator, was decreased in infants with wheezing, which is an inflammatory condition, is considered to be in agreement with previous studies in asthmatic patients [8, 9, 10, 25].

In recent years it has been shown that ANXA1 and its derivative peptide Ac2-26 activate LXA4 receptors [26]. ANXA1 and LXA4 receptors are expressed in rats, and some allergic conditions such as eosinophil accumula-

tion, edema, eotaxin formation, and allergic pleurisy are inhibited by LXA4 analogs [27]. Recent studies have indicated that lipoxins are strong candidates for antiallergic treatments and, in turn, studies have been conducted evaluating the effects of ANXA1 in this context [28]. ANXA1 is abundantly released into airway secretions [12]. In smokers [14] and those with an inflammatory condition of the lungs such as cystic fibrosis [15], ANXA1 with a molecular weight of 33 kDa is released rather than ANXA1 with a molecular weight of 37 kDa. This suggests that inflammatory conditions increase the release of defective ANXA1 [16]. A study by Ng et al. [16] showed that the exogenous ANXA1 N-terminal peptide exerts anti-inflammatory effects in Th2 cell-weighted ANXA1 knockout rats with pulmonary inflammation [16]. Bandeira-Melo et al. [28] used peptide Ac2-26 as an antiallergic agent for rats sensitized via ovalbumin administration to the pleural cavity. This peptide inhibited mast cell degranulation and neutrophil and eosinophil recruitment, and it prevented the release of histamines and eotaxins which are precursor mediators. This condition suggests that derivatives of ANXA1 can be used in the presence of allergic inflammation [28, 29].

The ANXA1 protein is present in the BAL fluid of patients with asthma. However, the role of ANXA1 in the pathogenesis of allergy and asthma is unclear. ANXA1 knockout rats were sensitized with ovalbumin. Then they were provoked using aerosolized ovalbumin, and airway resistance, lung compliance, and total and allergen-specific antibody responses were evaluated. In a test using inhaled metacholine, airway hyperresponsiveness was detected in ANXA1 knockout rats. The presence of increased allergens, the specific and total antibody responses and airway hyperresponsiveness in the ANXA1-deficient rats suggest that ANXA1 plays a role in asthma development [16]. In our study, ANXA1 levels were found to be lower in infants with wheezing compared to controls. This is promising for the prevention of asthma using annexin derivatives to inhibit inflammation. Human neutrophils express formyl peptide receptor-like (FPR2)/LXA4 receptors (ALX). These receptors bind serum amyloid A, an acute-phase reactant, and LXA4, 15-epi-LXA4, and ANXA1 [30]. Recent data have indicated that there are two endogenous anti-inflammatory mechanisms which have an effect via FPR2/ALX receptors [31, 32]. ANXA1 and ANXA1-mimetic peptide AC2-26 and 15-epi-LXA4 reduce neutrophil infiltration and mediator release from bronchioles. In addition, LXA4 and Ac2-26 facilitate the phagocytosis of apoptotic neutrophils by macrophages. These findings indicate that LXA4, an en-

ogenous anti-inflammatory agent, interacts with ANXA1 for the resolution of inflammation [30]. In our study, decreased levels of LXA4 and ANXA1 in the wheezing group versus the control group suggest the interaction of these two anti-inflammatory mechanisms.

Our results are the first demonstration of lower levels of LXA4 and ANXA1 in wheezy infants. We believe these re-

sults are important for understanding the pathophysiology of wheezy infants. Reduced endogenous LXA4 and ANXA1 biosynthetic capability may be one of the reasons for airway inflammation in wheezy infants. LXA4 and ANXA1 mimetics and related compounds could provide novel therapeutic approaches for the treatment of wheezy infants.

References

- Bhatt JM, Smyth AR: The management of pre-school wheeze. *Paediatr Respir Rev* 2011; 12:70–77.
- Bisgaard H, Szefer S: Prevalence of asthma-like symptoms in young children. *Pediatr Pulmonol* 2007;42:723–728.
- Bonnans C, Chanez P, Chavis C: Lipoxins in asthma: potential therapeutic mediators on bronchial inflammation? *Allergy* 2004;59: 1027–1041.
- Serhan CN, Chiang N, Van Dyke TE: Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008;8:349–361.
- Gilroy DW, Lawrence T, Perretti M, Rossi AG: Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov* 2004;3:401–416.
- Takano T, Fiore S, Maddox JF, Brady HR, Petasis NA, Serhan CN: Aspirin-triggered 15-epi-lipoxin A4 (LXA4) and LXA4 stable analogues are potent inhibitors of acute inflammation: evidence for antiinflammatory receptors. *J Exp Med* 1997;185:1693–1704.
- Fukunaga K, Kohli P, Bonnans C, Fredenburgh LE, Levy BD: Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. *J Immunol* 2005;174:5033–5039.
- Levy BD, De Sanctis GT, Devchand PR, Kim E, Ackerman K, Schmidt BA, Szczeklik W, Drazen JM, Serhan CN: Multipronged inhibition of airway hyperresponsiveness and inflammation by lipoxin A4. *Nat Med* 2002; 8:1018–1023.
- Levy BD, Bonnans C, Silverman ES, Palmer LJ, Marigowda G, Israel E: Diminished lipoxin biosynthesis in severe asthma. *Am J Respir Crit Care Med* 2005;172:824–830.
- Planagumà A, Kazani S, Marigowda G, Haworth O, Mariani TJ, Israel E, Blecker ER, Curran-Everett D, Erzurm SC, Calhoun WJ, Castro M, Chung KF, Gaston B, Jarjour NN, Busse WW, Wenzel SE, Levy BD: Airway lipoxin A4 generation and lipoxin A4 receptor expression are decreased in severe asthma. *Am J Respir Crit Care Med* 2008;178:574–582.
- Christmas P, Callaway J, Fallon J, Jones J, Haigler HT: Selective secretion of annexin 1, a protein without a signal sequence, by the human prostate gland. *J Biol Chem* 1991;266: 2499–2507.
- Ferlazzo V, D'Agostino P, Milano S, Caruso R, Feo S, Cillari E, Parente L: Anti-inflammatory effects of annexin-1: stimulation of IL-10 release and inhibition of nitric oxide synthesis. *Int Immunopharmacol* 2003;3:1363–1369.
- Parente L, Solito E: Annexin 1: more than an anti-phospholipase protein. *Inflamm Res* 2004;53:125–132.
- Smith SF, Tetley TD, Guz A, Flower RJ: Detection of lipocortin 1 in human lung lavage fluid: lipocortin degradation as a possible proteolytic mechanism in the control of inflammatory mediators and inflammation. *Environ Health Perspect* 1990;85:135–144.
- Vishwanatha JK, Davis RG, Rubinstein I, Floreani A: Annexin I degradation in bronchoalveolar lavage fluids from healthy smokers: a possible mechanism of inflammation. *Clin Cancer Res* 1998;4:2559–2564.
- Ng FS, Wong KY, Guan SP, Mustafa FB, Kajiji TS, Bist P, Biswas SK, Wong WS, Lim LH: Annexin-1-deficient mice exhibit spontaneous airway hyperresponsiveness and exacerbated allergen-specific antibody responses in a mouse model of asthma. *Clin Exp Allergy* 2011;41:1793–1803.
- Bonnans C, Chanez P, Chavis C: Lipoxins in asthma: potential therapeutic mediators on bronchial inflammation? *Allergy* 2004;59: 1027–1041.
- Vachier I, Bonnans C, Chavis C, Farce M, Godard P, Bousquet J, Chanez P: Severe asthma is associated with a loss of LX4, an endogenous anti-inflammatory compound. *J Allergy Clin Immunol* 2005;115:55–60.
- Sherrill DL, Stein R, Halonen M, Holberg CJ, Wright A, Martinez FD: Total serum IgE and its association with asthma symptoms and allergic sensitization among children. *J Allergy Clin Immunol* 1999;104:28–36.
- Karakoc F, Remes ST, Martinez FD, Wright AL: The association between persistent eosinophilia and asthma in childhood is independent of atopic status. *Clin Exp Allergy* 2002; 32:51–56.
- Serhan CN, Hamberg M, Samuelsson B: Trihydroxytetraenes: a novel series of compounds formed from arachidonic acid in human leukocytes. *Biochem Biophys Res Commun* 1984;118:943–949.
- Christie PE, Spur BW, Lee TH: The effects of lipoxin A4 on airway responses in asthmatic subjects. *Am Rev Respir Dis* 1992;145:1281–1284.
- Wu SH, Yin PL, Zhang YM, Tao HX: Reversed changes of lipoxin A4 and leukotrienes in children with asthma in different severity degree. *Pediatr Pulmonol* 2010;45:333–340.
- Ni CM, Yang W, Wan KS: Serum levels of lipoxin A(4) do not predict the development of subsequent asthma among young children with acute bronchiolitis. *J Asthma* 2011;48: 576–580.
- Tahan F, Saraymen R, Gumus H: The role of lipoxin A4 in exercise-induced bronchoconstriction in asthma. *J Asthma* 2008;45:161–164.
- Perretti M: The annexin 1 receptor(s): is the plot unravelling? *Trends Pharmacol Sci* 2003; 24:574–579.
- Chiang N, Takano T, Arita M, Watanabe S, Serhan CN: A novel rat lipoxin A4 receptor that is conserved in structure and function. *Br J Pharmacol* 2003;139:89–98.
- Bandeira-Melo C, Bonavita AG, Diaz BL, E Silva PM, Carvalho VF, Jose PJ, Flower RJ, Perretti M, Martins MA: A novel effect for annexin 1-derived peptide ac2–26: reduction of allergic inflammation in the rat. *J Pharmacol Exp Ther* 2005;313:1416–1422.
- Wang LM, Li WH, Xu YC, Wei Q, Zhao H, Jiang XF: Annexin 1-derived peptide Ac2–26 inhibits eosinophil recruitment in vivo via decreasing prostaglandin D₂. *Int Arch Allergy Immunol* 2011;154:137–148.
- El Kebir D, József L, Filep JG: Opposing regulation of neutrophil apoptosis through the formyl peptide receptor-like 1/lipoxin A4 receptor: implications for resolution of inflammation. *J Leukoc Biol* 2008;84:600–606.
- Gavins FN, Sawmynaden P, Chatterjee BE, Peretti M: A twist in anti-inflammation: annexin 1 acts via the lipoxin A4 receptor. *Prostaglandins Leukot Essent Fatty Acids* 2005;73: 211–219.
- Maderna P, Cottell DC, Toivonen T, Dufton N, Dalli J, Perretti M, Godson C: FPR2/ALX receptor expression and internalization are critical for lipoxin A4 and annexin-derived peptide-stimulated phagocytosis. *FASEB J* 2010;24:4240–4249.