

REVIEW

Role of osteopontin, a multifunctional protein, in allergy and asthma

S. Konno¹, M. Kurokawa², T. Uede³, M. Nishimura¹ and S.-K. Huang⁴¹First Department of Medicine, Hokkaido University School of Medicine, Sapporo, Japan, ²Department of Internal Medicine, Division of Respiratory Medicine and Allergology, Showa University School of Medicine, Tokyo, Japan, ³Division of Molecular Immunology, Institute for Genetic Medicine, Hokkaido University, Sapporo, Japan and ⁴Johns Hopkins Asthma and Allergy Center, Baltimore, MD, USA

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Summary

Osteopontin (OPN) is an extracellular matrix protein and immune modulator with a wide range of functions. OPN is recognized as a key cytokine in Th1 immune responses, yet its potential involvement in allergic/asthmatic responses has been investigated only recently. Current data from molecular and cellular studies and studies of OPN-deficient mice provide evidence that OPN plays multiple roles in the regulation of allergic responses, including regulation of IgE response, inflammatory cell migration, and the development of airway fibrosis and angiogenesis. These results suggest that OPN is a pleiotropic cytokine that functions both systemically and locally in tissue mucosa. Notably, OPN is able to exert its effects through different functional domains, and the secreted and intracellular forms of OPN may have distinct functions. Future research to elucidate all aspects of OPN function is needed to ultimately establish its role in the regulation of immune responses and various disease processes, including those critically involved in the development of allergies and asthma.

Correspondence:

Satoshi Konno, First Department of Medicine, Hokkaido University School of Medicine, Sapporo, Japan Kita-15 Nishi-7 Kita-Ku, Sapporo 060-8638, Japan.

E-mail: satkonno@med.hokudai.ac.jp

Introduction

Osteopontin (OPN), originally discovered in bone as an extracellular matrix protein, was later identified in many cell types in the immune system where it may be involved in the pathogenesis of inflammatory and immune diseases [1]. A number of studies using OPN-deficient mouse models and OPN-neutralizing antibodies (Abs) have demonstrated the importance of OPN in the development of several inflammatory diseases, particularly those involving CD4 T helper 1 (Th1)-associated immune responses [2–10]. In humans, high levels of OPN have been observed in several diseases associated with Th1 responses, such as sarcoidosis [11], tuberculosis [11], rheumatoid arthritis [12], Crohn's disease [13], and multiple sclerosis (MS) [14]. However, the role of OPN in Th2-associated diseases, rather than diseases involving Th-1, remains unclear. Its potential involvement in allergic/asthmatic responses has been investigated recently. In this review, we summarize research showing associations between OPN and several allergic diseases, and we also provide information on the role of OPN in the pathogenesis of IgE-mediated allergic responses and asthma.

Osteopontin modulates immunoglobulin E-mediated allergic responses

In 2000, since Ashkar et al. [15] found that OPN functions as a pro-inflammatory cytokine that induces Th1 cell-mediated immune responses, and subsequently, studies with various OPN-deficient mouse models have demonstrated the importance of OPN in the development of Th1-associated immune responses [2–10]. OPN induces IL-12 in macrophages [11, 16] and dendritic cells (DCs) [17, 18] *in vitro*. In addition, naïve Th cells stimulated by OPN-activated DCs produce Th1-polarized cytokines [19].

During our genomics studies to identify regulatory pathways important to the development of successful allergen-specific immunotherapy, we observed a significant increase in OPN expression in subjects who successfully completed venom immunotherapy and who showed a decrease in venom-specific IgE responses than in subjects who were hypersensitive to allergens and had not received venom immunotherapy [20, 21]. A Th1 cytokine, IFN- γ , is able to induce OPN [22], while two Th2 cytokines, IL-4 and IL-13, suppress it in human monocytes and monocyte-derived DCs [23]. Moreover, recent studies by Shinohara et al. [24] demonstrate that OPN expression in

activated T cells is regulated by T-bet, a member of the T box family of transcription factors, and that T-bet-dependent OPN expression in T cells is critical for skewing of naïve CD4⁺ T cells toward Th1 cell-lineage commitment. Regulation of Th1 vs. Th2 responses is often reciprocal; therefore, these results indicate that OPN may play a role in modulating the allergic responses via its Th1-inducing activity.

To elucidate the role of OPN in IgE-mediated allergic responses, we compared the ovalbumin (OVA)-induced specific IgE levels between wild-type (WT) and OPN-deficient (OPN^{-/-}) mice, and found that OPN^{-/-} mice had significantly higher levels of OVA-induced IgE and systemic Th2 than WT mice [25]. In addition, treatment with recombinant OPN (rOPN) during intraperitoneal injection of OVA suppressed OVA-specific IgE production in WT and OPN^{-/-} mice.

In contrast, Xanthou et al. [26] also showed that Ag sensitization and pulmonary challenge of OPN^{-/-} mice resulted in higher Ag-specific IgE production compared with WT controls. In these experiments, however, administration of an anti-OPN Abs AF-808 during sensitization suppressed Ag-specific IgE production. They also showed that administration of anti-OPN Abs resulted in increased numbers of plasmacytoid DCs (pDC), which are considered to have a regulatory role of immune responses, in draining lymph nodes. These data are difficult to reconcile and probably reflect differing experimental protocols, but they clearly show that OPN has the potential to influence skewing of the T cell response to allergens in a variable fashion.

Nagasaka et al. [27] have demonstrated that OPN is produced by cultured mast cells, and that OPN induces chemotaxis of these cells as well as enhancing IgE-mediated degranulation. This OPN may have a role in regulating acute mast cell responses to allergens.

OPN is involved in multiple aspects of allergic/asthmatic responses in addition to IgE-mediated allergic responses, as described below. Contradictory and/or dual roles of this molecule in a single disease have been reported, suggesting that OPN has a multifunctional nature in allergic/asthmatic responses.

Role of osteopontin in aspects of asthmatic response

Expression of osteopontin in the airway of asthma

Induction of OPN by INF- γ [22] and suppression of OPN by IL-4/IL-13 in human monocytes and monocyte-derived DCs [23] suggests that OPN expression is lower in Th2-associated diseases, such as asthma, than in Th1-associated disease. However, the levels of OPN in sputum and bronchoalveolar lavage fluid (BALF) were higher in asthmatic patients than in healthy controls [28, 29]. Studies using a murine model of acute and chronic

allergic airway inflammation induced by OVA also reveal that OPN levels in BALF and lung tissue were higher in OVA-treated animals than in the control animals [28–31], and corticosteroids, which are commonly used to treat asthma, suppressed OPN expression [31]. OPN is expressed in a variety of cells, including bronchial epithelial cells and inflammatory cells around airways, such as T cells, CD11c-positive DCs, and eosinophils [27–31] in asthmatic airways. Although the factors responsible for the higher expression of OPN in asthma have not been elucidated, it is probable that several pro-inflammatory cytokines, such as TNF- α and IL-1 β , are involved in the induction of OPN in the airway [32, 33]. Increased levels of OPN were also observed in IgE-mediated allergic diseases other than asthma, such as allergic conjunctivitis [34] and allergic rhinitis [35], which is characterized by eosinophilic inflammation of upper airways, suggesting that OPN also plays a role in the pathogenesis of Th2-associated chronic inflammatory diseases.

Effect of osteopontin on eosinophilic airway inflammation

Eosinophils are found in abundance in the airways of asthmatic patients and have traditionally been considered the primary mediators of epithelial damage. They mediate epithelial damage via the release of preformed effector molecules, such as eosinophil major basic protein and eosinophil cationic protein [36]. Eosinophils are also implicated in the pathogenesis of airway remodelling observed in asthmatic airways [37, 38].

OPN also plays a role in the migration of inflammatory cells, such as monocytes [18], lymphocytes [5], and neutrophils [39, 40]. We [28] and Puxeddu et al. [29] recently demonstrated that OPN induces chemotaxis of eosinophils *in vitro*, and this effect is mediated by an interaction between the RGD and SVVYGLR domains of OPN and the α 4 integrin expressed on the surface of eosinophils. In addition, administration of an anti-OPN antibody that recognizes the RGD domain of OPN (preceding OVA challenge) significantly decreases the number of eosinophils in mouse BALF [28]. These results suggest a possible role for OPN in attracting eosinophils into asthmatic airways. The significant correlation between OPN protein level and the number of eosinophils in the sputum/BALF in asthmatic patients *in vivo* [28, 29] supports these results.

In contrast, Xanthou et al. [26] showed that the administration of an anti-OPN Ab, AF-808, before OVA challenge enhanced inflammation of allergic airways. Administration of anti-OPN Ab during OVA challenge caused a substantial increase in the recruitment of conventional DCs, which may have an immunogenic role, to the lung draining lymph nodes. These results contrast with our results [28]. Notably, Xanthou and colleagues used a different anti-OPN Ab, which recognizes different

functional domains of the OPN molecule, than we did; the differences in Abs and the epitopes they recognize might explain this discrepancy. OPN is known to perform diverse functions, some of which are mediated through an integrin-binding domain consisting of the RGD and SVVYGLR (SLAYGLR in mouse OPN) sequences [8, 41, 42], while others are mediated through ligation with the N- and C-terminal half of the protein [10, 18]. In fact, studies by Koguchi et al. [11, 16] demonstrated that the effects of OPN on IL-12 and IFN- γ induction in human PBMCs can be blocked by 10A16, a C-terminal region-specific Ab, but not by 2K1, an integrin-binding domain-specific Ab, indicating that ligation between the C-terminal half of OPN and an unidentified receptor(s) is involved in the induction of Th1 cytokines, while blockade of integrin binding is ineffective in regulating the Th1 immune responses.

Effect of OPN on airway remodelling

A number of studies have shown that OPN contributes to tissue fibrosis and wound healing by regulating cell-extracellular matrix interactions and modulating TGF- β 1 and matrix metalloproteinase (MMP) expression. These studies were conducted using several murine models of diseases associated with fibrosis and remodelling of specific tissues, including myocardium [43, 44], muscle [45], kidney [46], and skin [47]. In the case of pulmonary fibrosis, OPN was identified as one of the genes that distinguishes idiopathic pulmonary fibrosis from normal lungs in humans using oligonucleotide arrays [48, 49], and OPN is up-regulated in bleomycin-induced lung fibrosis in mice [50, 51].

Airway remodelling is a prominent pathophysiologic feature of chronic asthma. It is characterized by changes in tissue structural components, including damage and shedding of airway epithelium, mucus gland hypertrophy, increased myofibroblast number, subepithelial fibrosis, increased airway smooth muscle mass. In a murine model of allergen-induced airway remodelling, OPN expression is up-regulated in lung tissue and BALF and is correlated with collagen content and peribronchial smooth muscle area [30]. In addition, OPN significantly increases collagen deposition in a murine lung cell line *in vitro* [30]. OPN also plays an important role in angiogenesis [29, 52], which is also a prominent feature of airway remodelling [53–55]. Recently, Kohan et al. [56] and Simoes et al. [57] independently demonstrated that OPN^{-/-} mice chronically exposed to OVA exhibit reduced airway fibrosis in comparison with OVA-treated WT mice as characterized by subepithelial fibrosis, peribronchial smooth muscle area, increased airway mucus-producing cell number, and subsequent increases in airway resistance and airway hyperresponsiveness (AHR). Lung fibroblasts from OVA-treated OPN^{-/-} mice exhibit reduced proliferation,

migration, collagen deposition, and smooth muscle actin expression in comparison with WT lung fibroblasts [56]. Moreover, OPN induces increased expression of IL-13 and pro-matrix metalloproteinase-9 in the lungs [57]. These results indicate that OPN plays an important role during airway remodelling.

Osteopontin and disease severity in human asthma

Although for most asthma patients the asthma is well controlled with low doses of inhaled corticosteroids, in about 10% of asthma patients, those with severe asthma, the asthma remains poorly controlled despite treatment with high-dose inhaled corticosteroids and long-acting β_2 -agonists. These patients with severe asthma have a lower quality of life and account for the majority of total asthma-related costs, so new therapeutic approaches are needed to treat severe asthma. Recently, two reports have shown significant up-regulation of OPN in patients with severe asthma. Delimpoura and colleagues have shown that OPN protein levels in the sputum are significantly higher in patients with severe asthma than in patients with less severe forms of the disease. Moreover, OPN is associated with mediators involved in both inflammatory and remodelling processes, such as TGF- β 1, IL-13 and cysteinyl leukotrienes, only in severe asthmatic patients [58]. Samitas et al. [59] have shown that subepithelial inflammatory cells in severe asthmatic patients express significantly more OPN compared with those in patients with mild asthma, and OPN expression is correlated to reticular basement thickness. Severe asthma consists of effects of different aspects of the pathway leading to the increased severity [60–62], including intense inflammatory and remodelling processes, and OPN might be involved in these distinct aspects of severe asthma. Further research on OPN function and regulation in human asthma could lead to novel therapeutic approaches targeting the modulation of OPN expression and function *in vivo*.

Osteopontin polymorphisms and serum total immunoglobulin E level

The human OPN gene is located on chromosome 4q22.1, and single-nucleotide polymorphisms of the gene are associated with the development and/or disease activity of several inflammatory diseases, including MS [63], systemic lupus erythematosus (SLE) [64], and type C hepatitis [65]. One report demonstrates an association of OPN gene polymorphisms and serum total IgE level in a Japanese population [66] with respect to asthma/allergy. Individuals carrying the C allele at position 5891 in exon 6, an allele that is more prevalent in patients with MS in Japanese populations [63], displayed significantly lower levels of total serum IgE compared with non-carriers of this allele. Individuals homozygous for the C allele at

position 7052 in exon 7, an allele associated with development of SLE [64], exhibited significantly higher total serum IgE levels compared with carriers of the 7052T allele. Patients with MS, a Th1-immune disease, display significantly fewer allergic symptoms, lower number of allergen sensitizations [67], and reduced risk of asthma [68] compared with individuals without MS. On the other hand, SLE is characterized by Th2 immune responses, and some reports have shown increased IgE production in SLE, thereby associating SLE with allergy [69]. These results indicate a potential relationship between OPN genetics and IgE-mediated allergic responses.

Therapeutic strategies against allergy and asthma based on osteopontin function

Based on our finding that rOPN inhibits allergen sensitization (production of allergen-specific IgE antibody) [25], OPN itself may be a promising therapeutic target for the treatment of IgE-mediated allergic diseases. In this context, increases in OPN levels, as shown in our previous reports [20, 21], may play a role in the efficacy of allergen immunotherapy. Moreover, neutralizing the effect of OPN is a potential therapeutic strategy for the treatment of allergy and asthma because OPN mediates various aspects of asthma and allergy pathogenesis, including mast cell degranulation [27], migration of eosinophils [28, 29], and airway remodelling [56, 57]. Therefore, in view of the pleiotropic effects of OPN on a variety of cell types and on various aspects of the asthmatic and allergic responses involved, therapeutic approaches using OPN or anti-OPN

antibody cannot be simplistic. A more physiologically relevant mouse asthma model, developed by inhalation of Der f without intraperitoneal injection [70, 71], would be necessary for evaluation of the potential therapeutic strategy of using rOPN or anti-OPN antibody. The effects of OPN may resemble the pleiotropic functions of TGF- β in allergy and asthma pathogenesis. TGF- β , like OPN, has immunosuppressive effects in addition to pro-fibrotic effects [72, 73]. Ideal therapeutic strategies would harness OPN's desirable therapeutic effects on specific cell types and particular aspects of allergic/asthmatic responses, while preventing any unwanted complications or side-effects elsewhere in the body.

Conclusions and future perspectives

In this review, we have summarized the roles of OPN in the pathogenesis of asthma/allergy. Although OPN had been recognized as a key cytokine involved in Th1 immune responses, recent findings demonstrate that OPN levels are also elevated in Th2-related diseases, including asthma. Moreover, OPN is involved in the regulation of allergic and fibrotic responses. Table 1 summarizes published reports that demonstrate the functional involvement of OPN in asthma and allergy. Taken together, data from many human and animal-model studies strongly support the notion that OPN is a central effector cytokine involved in critical steps of allergic inflammation. Thus, it is likely that a highly controlled release of OPN could be one important mechanism that maintains homeostasis and limits the extent of allergic and inflammatory responses.

Table 1. Summary of the previous reports showing association of OPN with asthma and allergy

Disease phenotype	Mouse/human	Modalities	Sample	Findings	References
Specific allergen immunotherapy	Human		PBMCs, serum	OPN expression was up-regulated after venom immunotherapy	[20,21]
Systemic allergen sensitization	Mouse	OPN ^{-/-} , rOPN		OPN inhibited systemic allergen sensitization	[25]
	Mouse	OPN Ab, OPN ^{-/-}		OPN Ab inhibited systemic allergen sensitization	[26]
Mast cell degranulation	Mouse, Human	OPN ^{-/-} , rOPN	FSMC	OPN augmented IgE-mediated degranulation, OPN-induced mast cell chemotaxis	[27]
Allergic conjunctivitis	Human		Tear	High OPN protein level in tear	[34]
Allergic rhinitis	Human		Nasal tissue	High OPN expression in nasal mucosa	[55]
Eosinophilic airway inflammation	Mouse	OPN ^{-/-} , rOPN, OPN Ab		OPN inhibited eosinophilic airway inflammation	[26]
	Mouse, human	rOPN, OPN Ab	Sputum	OPN-induced eosinophil chemotaxis	[28]
	Human	rOPN	BALF	OPN-induced eosinophil chemotaxis	[29]
	Airway remodelling	Mouse	rOPN	OPN increased collagen deposition	[30]
		Mouse	OPN ^{-/-} , rOPN	OPN-induced airway remodelling	[56, 57]
Severe/refractory asthma	Human		Sputum	High OPN protein level in sputum	[58]
	Human		Lung tissue	High OPN expression in lung tissue	[59]

OPN, osteopontin; PBMCs, peripheral blood mononuclear cells; OPN^{-/-}, OPN-deficient mice; rOPN, recombinant OPN; Ab, antibody; FSMC, fetal skin-derived mast cells; BALF, bronchoalveolar lavage fluid.

How resident tissue and inflammatory cells integrate OPN signals to limit the extent of the allergic responses may represent an important and novel mechanism for controlling allergic responses. Future challenges will likely include elucidation of the relative contribution of different OPN-expressing cell types to the expression of a disease. Equally important is an understanding of the molecular mechanisms by which OPN exerts its functions via different receptor-ligand axes. Also, detailed mechanism of functional activities mediated by intracellular (i-OPN) vs. secreted forms of OPN (s-OPN) requires further clarification because an i-OPN has been shown to exert distinct functions on several inflammatory diseases [74, 75] from s-OPN. In addition, recent studies demonstrate a role for OPN in Th17-related immune responses [75], which are involved in the pathogenesis of asthmatic airway inflammation [76]. Many of these issues may soon be resolved, and in conjunction with published data accumulated thus far, these insights would add a new dimension to our

understanding of the immune regulatory network of allergic diseases, where OPN is critical.

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References

- Morimoto J, Kon S, Matsui Y, Uede T. Osteopontin; as a target molecule for the treatment of inflammatory diseases. *Curr Drug Targets* 2010; 11:494–505.
- Ishii T, Ohshima S, Ishida T *et al.* Osteopontin as a positive regulator in the osteoclastogenesis of arthritis. *Biochem Biophys Res Commun* 2004; 316:809–15.
- O'Regan AW, Hayden JM, Body S *et al.* Abnormal pulmonary granuloma formation in osteopontin-deficient mice. *Am J Respir Crit Care Med* 2001; 164:2243–7.
- Chabas D, Baranzini SE, Mitchell D *et al.* The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. *Science* 2001; 294:1731–5.
- O'Regan AW, Chupp GL, Lowry JA, Goetschkes M, Mulligan N, Berman JS. Osteopontin is associated with T cells in sarcoid granulomas and has T cell adhesive and cytokine-like properties in vitro. *J Immunol* 1999; 162:1024–31.
- Yumoto K, Ishijima M, Rittling SR *et al.* Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced arthritis in mice. *Proc Natl Acad Sci USA* 2002; 99:4556–61.
- Kon S, Ikeshue M, Kimura C *et al.* Syndecan-4 protects against osteopontin-mediated acute hepatic injury by masking functional domains of osteopontin. *J Exp Med* 2008; 205:25–33.
- Yamamoto N, Sakai F, Kon S *et al.* Essential role of the cryptic epitope SLAYGLR within osteopontin in a murine model of rheumatoid arthritis. *J Clin Invest* 2003; 112:181–8.
- Diao H, Kon S, Iwabuchi K *et al.* Osteopontin as a mediator of NKT cell function in T cell-mediated liver diseases. *Immunology* 2004; 21:539–50.
- Fan K, Dai J, Wang H *et al.* Treatment of collagen-induced arthritis with an anti-osteopontin monoclonal antibody through promotion of apoptosis of both murine and human activated T cells. *Arthritis Rheum* 2008; 58:2041–52.
- Koguchi Y, Kawakami K, Uezu K *et al.* High plasma osteopontin level and its relationship with interleukin-12-mediated type 1T helper cell response in tuberculosis. *Am J Respir Crit Care Med* 2003; 167:1355–9.
- Xu G, Nie H, Li N *et al.* Role of osteopontin in amplification and perpetuation of rheumatoid synovitis. *J Clin Invest* 2005; 115:1060–7.
- Sato T, Nakai T, Tamura N *et al.* Osteopontin/Eta-1 upregulated in Crohn's disease regulates the Th1 immune response. *Gut* 2005; 54:1254–62.
- Comabella M, Pericot I, Goetsches R *et al.* Plasma osteopontin levels in multiple sclerosis. *J Neuroimmunol* 2005; 158:231–9.
- Ashkar S, Weber GF, Panoutsakopoulou V *et al.* Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. *Science* 2000; 287:860–4.
- Koguchi Y, Kawakami K, Kon S *et al.* Penicillium marneffei causes osteopontin-mediated production of interleukin-12 by peripheral blood mononuclear cells. *Infect Immun* 2002; 70:1042–8.
- Higuchi Y, Tamura Y, Uchida T, Matsuura K, Hijiya N, Yamamoto S. The roles of soluble osteopontin using osteopontin-transgenic mice in vivo: proliferation of CD4⁺ T lymphocytes and the enhancement of cell-mediated immune responses. *Pathobiology* 2004; 71:1–11.
- Kon S, Yokosaki Y, Maeda M *et al.* Mapping of functional epitopes of osteopontin by monoclonal antibodies raised against defined internal sequences. *J Cell Biochem* 2002; 84:420–32.
- Renkl AC, Wussler J, Ahrens T *et al.* Osteopontin functionally activated dendritic cells and induces their differentiation toward a Th1-polarizing phenotype. *Blood* 2005; 106:946–55.
- Konno S, Golden DBK, Schroeder J, Hamilton RG, Lichtenstein LM, Huang SK. Increased expression of osteopontin is associated with long-term bee venom immunotherapy. *J Allergy Clin Immunol* 2005; 115:1063–7.
- Konno S, Golden DBK, Schroeder J, Hamilton RG, Lichtenstein LM, Huang

- SK. Level of osteopontin is increased after bee venom immunotherapy. *J Allergy Clin Immunol* 2005; **115**:1317–8.
- 22 Li X, O'Regan AW, Berman JS. IFN-gamma induction of osteopontin expression in human monocytoid cells. *J Interferon Cytokine Res* 2003; **23**:259–65.
 - 23 Konno S, Eckman JA, Plunkett B et al. Interleukin-10 and Th2 cytokines differentially regulate osteopontin expression in human monocytes and dendritic cells. *J Interferon Cytokine Res* 2006; **26**:562–7.
 - 24 Shinohara ML, Jansson M, Hwang ES, Werneck MB, Glimcher LH, Cantor H. Tbet-dependent expression of osteopontin contributes to T cell polarization. *Proc Natl Acad Sci USA* 2005; **102**:17101–6.
 - 25 Kurokawa M, Konno S, Takahashi A et al. Regulatory role of dendritic cell-derived osteopontin in systemic allergen sensitization. *Eur J Immunol* 2009; **39**:3323–30.
 - 26 Xanthou G, Alissafi T, Semitekolou M et al. Osteopontin has a crucial role in allergic airway disease through regulation of dendritic cell subsets. *Nat Med* 2007; **13**:570–8.
 - 27 Nagasaka A, Matsue H, Matsushima H et al. Osteopontin is produced by mast cells and affects IgE-mediated degranulation and migration of mast cells. *Eur J Immunol* 2008; **38**:489–99.
 - 28 Takahashi A, Kurokawa M, Konno S et al. Osteopontin is involved in migration of eosinophils in asthma. *Clin Exp Allergy* 2009; **39**:1152–9.
 - 29 Puxeddu I, Berkman N, Ribatti D et al. Osteopontin is expressed and functional in human eosinophils. *Allergy* 2010; **65**:168–74.
 - 30 Kohan M, Bader R, Puxeddu I, Levi-Schaffer F, Breuer R, Berkman N. Enhanced osteopontin expression in a murine model of allergen-induced airway remodelling. *Clin Exp Allergy* 2007; **37**:1444–54.
 - 31 Kurokawa M, Konno S, Matsukura S et al. Effects of corticosteroids on osteopontin expression in a murine model of allergic asthma. *Int Arc Allergy Immunol* 2009; **149**:7–13.
 - 32 Selin DM, Kuang PP, Subramanian M et al. Interleukin-1 β induces osteopontin expression in pulmonary fibroblasts. *J Cell Biochem* 2006; **97**:519–29.
 - 33 Lu X, Zhang XH, Wang H et al. Expression of osteopontin in chronic rhinosinusitis with and without nasal polyps. *Allergy* 2009; **64**:104–11.
 - 34 Uchio E, Matsuura N, Kadonosono K, Ohno S, Uede T. Tear osteopontin levels in patients with allergic conjunctival diseases. *Graefes Arch Clin Exp Ophthalmol* 2002; **240**:924–8.
 - 35 Liu Y, Lu X, Yu HJ et al. The expression of osteopontin and its association with Clara cell 10-kDa protein in allergic rhinitis. *Clin Exp Allergy* 2010; **40**:1632–41.
 - 36 Rothenberg ME, Hogan SP. The eosinophil. *Annu Rev Immunol* 2006; **24**:147–74.
 - 37 Kariyawasam HH, Robinson DS. The role of eosinophils in airway tissue remodeling in asthma. *Curr Opin Immunol* 2007; **19**:681–6.
 - 38 Humbles A, Lloyd CM, McMillian SJ et al. A critical role for eosinophils in allergic airways remodeling. *Science* 2004; **305**:1776–9.
 - 39 Koh A, da Silva AP, Bansal AK et al. Role of osteopontin in neutrophil function. *Immunology* 2007; **122**:466–75.
 - 40 Senger DR, Perruzzi CA, Papadopoulos-Sergiou A, Van de Water L. Adhesive properties of osteopontin: regulation by a naturally occurring thrombin-cleavage in close proximity to the GRGDS cell-binding domain. *Mol Biol Cell* 1994; **5**:565–74.
 - 41 Kitamura M, Iwabuchi K, Kitaichi N et al. Osteopontin aggravates experimental autoimmune uveoretinitis in mice. *J Immunol* 2007; **178**:6567–72.
 - 42 Yamamoto N, Nakashima T, Torikai M et al. Successful treatment of collagen-induced arthritis in non-human primates by chimeric anti-osteopontin antibody. *Int Immunopharmacol* 2007; **7**:1460–70.
 - 43 Singh M, Foster CR, Dala S, Singh K. Osteopontin: role of extracellular matrix deposition and myocardial remodeling. *J Mol Cell Cardio* 2010; **48**:538–43.
 - 44 Matsui Y, Jia N, Okamoto H et al. Role of osteopontin in cardiac fibrosis and remodeling in angiotensinII-induced cardiac hypertrophy. *Hypertension* 2004; **43**:1195–201.
 - 45 Vetrone SA, Montecio-Rodriguez E, Kudryashova E et al. Osteopontin promotes fibrosis in dystrophic mouse muscle by modulating immune cell subsets and intramuscular TGF- β . *J Clin Invest* 2009; **119**:1583–94.
 - 46 Wolak T, Kim H, Ren Y, Kim J, Vaziri ND, Nicholas SB. Osteopontin modulates angiotensin II-induced inflammation, oxidative stress, and fibrosis of the kidney. *Kidney Int* 2009; **76**:32–43.
 - 47 Mori R, Shaw T, Martin P. Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring. *J Exp Med* 2008; **205**:43–51.
 - 48 Pardo A, Gibson K, Cisneros J et al. Up-regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. *PLoS Med* 2005; **2**:e251.
 - 49 Kelly MM, Leigh R, Gilpin SE et al. Cell-specific gene expression in patients with unusual interstitial pneumonia. *Am J Respir Crit Care Med* 2006; **174**:557–65.
 - 50 Berman JS, Serlin D, Li X et al. Altered bleomycin-induced lung fibrosis in osteopontin-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 2004; **286**:L1311–18.
 - 51 Takahashi F, Takahashi K, Okazaki T et al. Role of osteopontin in the pathogenesis of bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2001; **24**:264–71.
 - 52 Leali D, DellEra P, Stabile H et al. Osteopontin (Eta-1) and fibroblast growth factor-2 cross-talk in angiogenesis. *J Immunol* 2003; **171**:1085–93.
 - 53 Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: new insights. *J Allergy Clin Immunol* 2003; **111**:215–25.
 - 54 Vignola AM, Kips J, Bousquet J. Tissue remodeling as a feature of persistent asthma. *J Allergy Clin Immunol* 2000; **105**:1041–53.
 - 55 Jeffery PK. Remodeling in asthma and chronic obstructive lung disease. *Am J Respir Crit Care Med* 2001; **164**:S28–38.
 - 56 Kohan M, Breuer R, Berkman N. Osteopontin induces airway remodeling and lung fibroblast activation in a murine model of asthma. *Am J Respir Cell Mol Biol* 2009; **41**:290–6.
 - 57 Simoes DC, Xanthou G, Petrochilou K, Panoutsakopoulou V, Roussos C, Gratziau C. Osteopontin deficiency protects against airway remodeling and hyperresponsiveness in chronic asthma. *Am J Respir Crit Care Med* 2009; **179**:894–902.
 - 58 Delimpoura V, Bakakos P, Tseliou E et al. Increased levels of osteopontin in sputum supernatant in severe refractory asthma. *Thorax* 2010; **65**:782–6.

- 59 Samitas K, Zervas E, Vittorakis S *et al.* Osteopontin expression and relation to disease severity in human asthma. *Eur Respir J* 2011; **37**:331–41.
- 60 Holgate ST, Polosa R. The mechanism, diagnosis, and management of severe asthma in adults. *Lancet* 2006; **368**:780–93.
- 61 Wenzel SE, Busse WW. National Heart, Lung and Blood Institute's Severe Asthma Research Program. Severe asthma: lessons from the Severe Asthma Research Program. *J Allergy Clin Immunol* 2007; **119**:14–21.
- 62 Anderson GP. Endotyping asthma: new insights into key pathogenic mechanism in a complex, heterogenous disease. *Lancet* 2008; **372**:1107–19.
- 63 Niino M, Kikuchi S, Fukazawa T, Yabe I, Tashiro K. Genetic polymorphisms of osteopontin in association with multiple sclerosis in Japanese patients. *J Neuroimmunol* 2003; **136**:125–9.
- 64 Forton AC, Petri MA, Goldman D, Sullivan KE. An osteopontin (SPP1) polymorphism is associated with systemic lupus erythematosus. *Hum Mutat* 2002; **19**:459.
- 65 Mochida S, Hashimoto M, Matsui A *et al.* Genetic polymorphisms in promoter region of osteopontin gene may be a marker reflecting hepatitis activity in chronic hepatitis C patients. *Biochem Biophys Res Commun* 2004; **23**: 1079–85.
- 66 Tanino Y, Hizawa N, Konno S *et al.* Sequence variants of the SPP1 gene are associated with total serum IgE levels in a Japanese population. *Clin Exp Allergy* 2006; **36**:219–25.
- 67 Oro AS, Guarino TJ, Driver R, Steinman L, Umetsu DT. Regulation of disease susceptibility: decreased prevalence of IgE-mediated allergic disease in patients with multiple sclerosis. *J Allergy Clin Immunol* 1996; **97**:1402–28.
- 68 Neukirch F, Lyon-Caen O, Clanet M, Bousquet J, Feingold J, Druet P. Asthma, nasal allergies, and multiple sclerosis. *J Allergy Clin Immunol* 1997; **99**:270–1.
- 69 Shahar E, Lorber M. Allergy and SLE: common and variable. *Isr J Med Sci* 1997; **33**:147–9.
- 70 Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009; **15**: 410–6.
- 71 Lajoie S, Lewkowich IP, Suzuki Y *et al.* Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. *Nat Immunol* 2010; **11**: 928–35.
- 72 Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and its role in asthma. *Pulm Pharmacol Ther* 2003; **16**:181–96.
- 73 Howell JE, McAnulty RJ. TGF-beta: its role in asthma and therapeutic potential. *Curr Drug Targets* 2006; **7**:547–65.
- 74 Shinohara ML, Lu L, Bu J *et al.* Osteopontin expression is essential for interferon-alpha production by plasmacytoid dendritic cells. *Nat Immunol* 2006; **7**:498–506.
- 75 Shinohara ML, Kim JH, Garcia VA, Cantor H. Engagement of the type I interferon receptor on dendritic cells inhibit T helper 17 cell development: role of intracellular osteopontin. *Immunity* 2008; **29**:68–78.
- 76 Wakashin H, Hirose K, Maezawa Y *et al.* IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 2008; **178**:1023–32.