

Osteopontin: A Potential Biomarker for Successful Bee Venom Immunotherapy and a Potential Molecule for Inhibiting IgE-mediated Allergic Responses

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ABSTRACT

Venom immunotherapy (VIT) is proven to be curative for insect allergy, but the mechanisms and the biomarkers associated with clinical efficacy remain elusive. We report herein the discovery of a leading candidate biomarker, osteopontin (OPN), for VIT. From cDNA microarray and clustering analyses, an increased expression of OPN was found in patients who completed 5–6 years of VIT and discontinued therapy for 3–6 years as compared with the untreated group. A significantly higher level of serum OPN was found in the completed treatment group as compared with the untreated group. Following VIT, kinetically increased levels of OPN associated with reduced venom specific IgE levels were noted in subjects with large local allergic reactions to venom. These findings together with the fact that OPN is involved in Th1-associated immune response strongly suggest a role of OPN as a functional biomarker for VIT.

KEY WORDS

biomarker, clustering, mechanism, microarray, osteopontin (OPN), venom immunotherapy (VIT)

INTRODUCTION

Venom immunotherapy (VIT) proves to be the most effective in insect allergy.^{1,3} However the regulatory mechanisms by which immunotherapy achieves its sustained clinical efficacy remain elusive, and the biomarkers associated with efficacious immunotherapy have not yet been identified. As part of a genomics study to identify candidate biomarkers associated with successful VIT, we have identified that a promising candidate, osteopontin (OPN), was up-regulated after the start therapy.^{4,5} In this review, we summarize the series of experiments in which we identified OPN as a potential biomarker for the efficacy of VIT through the use of cDNA microarray analysis. We also summarize the reported functions of OPN in immune responses, which suggest the potential involvement of OPN in the mechanisms of VIT and in the

IgE-mediated allergic responses.

VENOM IMMUNOTHERAPY

Allergen-specific immunotherapy is a method of modulating the clinical reactivity of the patient and is the most effective therapy for insect allergy. VIT is generally effective with less than 3 months' treatment, but persistent tolerance seems to develop after 4 years or more of treatment.^{1,2} There are no criteria to identify the point in time when protection becomes long-lasting. In addition, despite the remote possibility of severe reaction, the supervised sting challenge test is the only available means to evaluate the efficacy of VIT. For specific treatment to protect high-risk patients and for others to be able to avoid or stop treatment with impunity, there is a critical need to identify the markers that can be used to predict outcome or to derive biological insight into the mecha-

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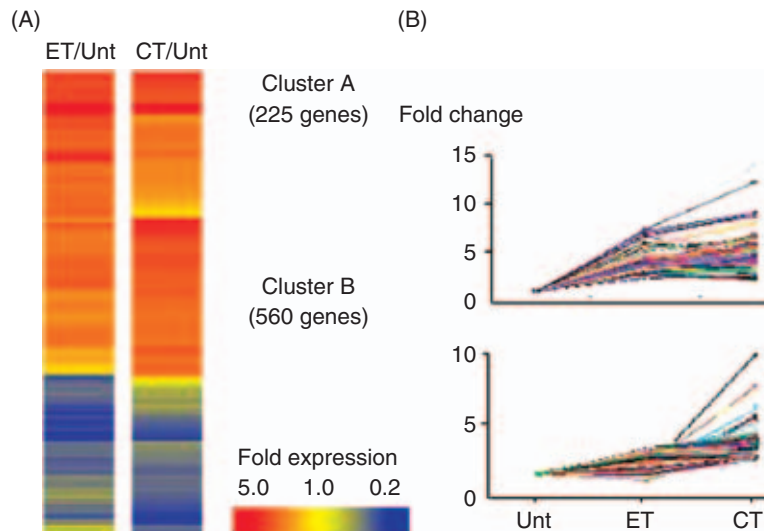


Fig. 1 Cluster analysis of 1751 genes with ≥ 2.0 -fold changes; early treatment (ET) versus untreated (Unt) or completed treatment (CT) versus untreated (Unt).

A, Cluster image of cDNA microarray expression profiles of 1751 genes. The ratio of gene expression levels is represented according to the color scale shown (red, upregulation; blue, downregulation).

B, Expression profiles of individual genes in cluster A and B. The X-axis indicates the fold change of upregulation or downregulation compared with the intensity of the untreated group.

nism of successful VIT.

During maintenance VIT, a modest reduction in serum allergen-specific IgE, concomitant with an increase in allergen-specific IgG,⁶ particularly of the IgG4 isotype,⁷ has been reported. However, the level of venom-specific IgG antibodies is not an absolute predictor of the outcome of a sting challenge, because in patients who are treated for more than 4 years, even those with low venom-IgG levels do not react to stings.⁸ This observation suggests that immune tolerance to venom becomes established through a non-IgG mechanism after extended maintenance therapy.

While the evidence accumulated to date has revealed that VIT is associated with many changes in immune cells and cytokines, such as Th2 to Th1 cytokine shift⁹ and the induction of T-cell anergy associated with elevated levels of IL-10,¹⁰ the mechanism of action has so far remained elusive.³ This is due, in part, to the fact that the focus has been on the cytokines and mechanisms associated with protection from sting reactions in the early stage of immunotherapy. Identification of relevant targets that are predictive of long-term clinical outcome is needed for the clinical utility and understanding of the mechanisms for the efficacy of VIT.

DISCOVERY OF A CANDIDATE BIOMARKER, OSTEOPOINTIN, FOR VENOM IMMUNOTHERAPY THROUGH THE USE OF CDNA MICROARRAY

To identify differentially expressed genes and potential biomarker(s) associated with VIT, gene expression profiles of peripheral blood mononuclear cells (PBMCs) from patients with and without VIT were compared using cDNA microarray analysis. The gene expression profiles from the untreated (Unt) group (3 patients) served as the baseline for comparisons with the early (3 subjects on VIT for less than 1 year) (ET) and completed (3 subjects who completed 5 to 6 years of VIT) (CT) treatment groups. A total of 1751 genes with ≥ 2.0 -fold changes were selected and subsequently used for hierarchical clustering. Candidate clusters were selected by querying the gene expression data set, derived from the study groups for genes that demonstrate an overall pattern of progressively increasing (or decreasing) expression concordant with different stages of VIT (Fig. 1). Among those genes differentially expressed, OPN emerged as the leading candidate with higher level of differential expression following VIT, particularly in subjects after 5 to 6 years of successful VIT (1.6-fold, ET versus Unt; 7.0-fold, CT versus Unt), which was validated by real-time PCR assays. Monocytes were the

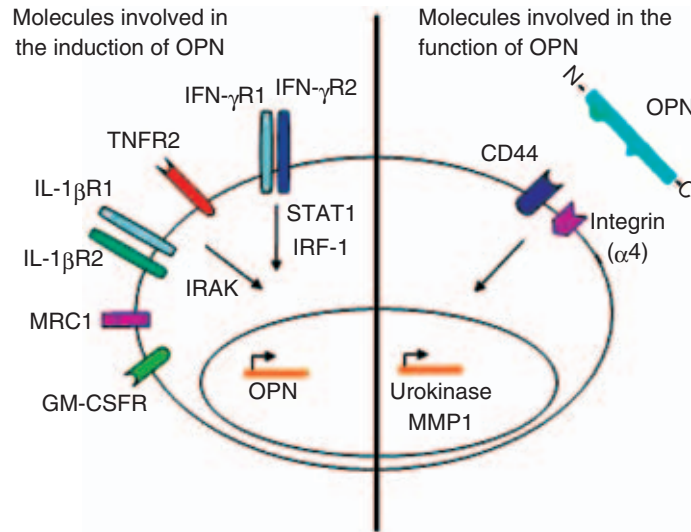


Fig. 2 Upregulated genes that are known to be associated with OPN. These genes were identified in clusters A and B shown in Figure 1.

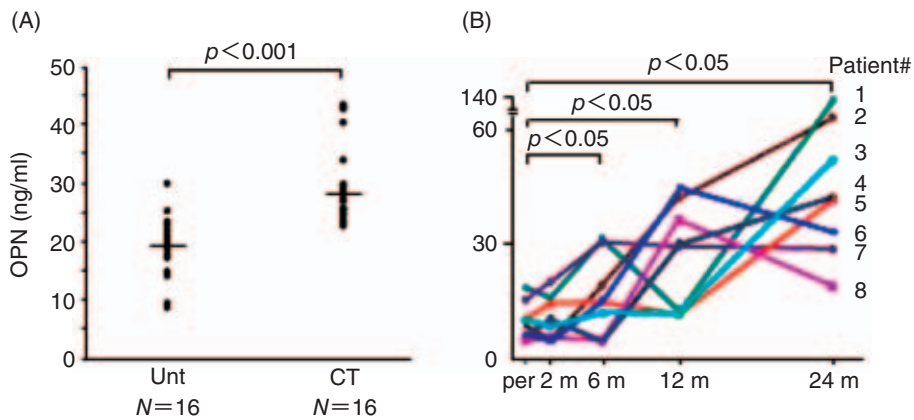


Fig. 3 (A) Serum OPN levels are higher in patients from the completed treatment group (CT) than in the untreated group (Unt). The line represents the median value of each patient group. (B) Kinetic changes of serum OPN levels after the start of VIT.

dominant cell population in the PBMCs expressing OPN, because depletion of monocytes significantly reduced the level of OPN expression. It is noted that a group of genes encoding products known to be involved in the OPN regulatory pathway was also identified in the same clusters (Fig. 2). For example, these include *CD44* (a receptor for OPN), *ITGA4* (integrin $\alpha 4$, a receptor for OPN), *IFNGR1*, *IFNGR2* (interferon- γ receptor), *MRC1* (mannose receptor, C type 1), and *PLAU* (urokinase type plasminogen activator, inducible by OPN). Further, the genes encoding cytokine receptors, such as *TNFR2* (TNF- α receptor), *CSF2RB* (GM-CSF receptor), and *IL1R1*, *IL1R2* (IL-1 receptor), and their associated signaling

molecules were also identified in these clusters (Fig. 2).

OSTEOPONTIN AS A POTENTIAL BIOMARKER FOR THE EFFICACY OF VENOM IMMUNOTHERAPY

To further confirm differential expression of OPN and to test the hypothesis that OPN is a functional biomarker of VIT, serum levels of OPN were analyzed in a panel of 16 subjects randomly chosen from the CT group and 16 from the Unt group. As seen in Figure 3A, significantly higher levels of OPN were found in the CT group than those seen in the Unt group (Mann-Whitney *U* test, $p < 0.001$).

To obtain longitudinal measurement of serum OPN levels after VIT, a total of 8 subjects with large local allergic reactions, but not systemic allergic reactions after sting challenge, were recruited. This group of patients received initial and maintenance VIT according to current practice parameters as previously reported,^{11,12} from which longitudinal data and specific outcome assessment (sting challenge) were available. This group of patients had demonstrable, but variable levels of reduction in skin reactivity to bee sting challenge after VIT, and the majority of patients responded well to VIT as early as 2 months after VIT. Serum samples were collected at various time points before VIT (Pre) and at 2 (2 mo), 6 (6 mo), 12 (12 mo), and 24 (24 mo) months after the start of VIT, and the levels of serum OPN were analyzed for all 8 subjects. As shown in Figure 3B, a significant increase in the levels of OPN with time-dependent upward trend was found over the 2-year period of VIT compared with those detected before VIT ($p < 0.05$ vs Pre in all cases). The difference was particularly significant at 12 mo and 24 mo, whereas no significant change was seen at an earlier time point (2 mo). It is often noted that the levels of serum IgE tend to increase shortly after the start of VIT and gradually decline over the course of long-term VIT. This trend was clearly evident for the majority of study subjects receiving VIT. It is noted that although the patterns of kinetic change were variable among the study subjects, the levels of OPN showed an inverse trend compared with the levels of IgE over the course of VIT (Fig. 3B).

OSTEOPONTIN

OPN, also known as Eta-1 (early T lymphocyte activation protein 1) or as SPP1 (secreted phosphoprotein 1) contains functional domains for extracellular matrix adhesion. It has been implicated in a number of physiological and pathological events and have recently been shown to play a role in T cell-mediated immunity.¹³⁻¹⁶ While the mechanistic aspect of OPN function remains to be defined, it appears that OPN mediates cell-matrix interactions and cellular signaling through binding with integrin and CD44 receptors,¹⁶ involving both RGD-dependent and RGD-independent interactions. Expression of OPN is constitutive in bone and at epithelial surfaces, and it is upregulated in activated T cells, natural killer (NK) cells, macrophages, Kupffer cells and tumor cells in models of inflammation, ischemia-reperfusion, bone resorption, and tumor progression.¹⁷⁻¹⁹ Also, high levels of OPN have been observed in several Th1-associated diseases, including rheumatoid arthritis²⁰ and multiple sclerosis (MS).²¹ Increased expression of OPN has also been found in granulomatous responses,²² a typical Th1 immune response, and in pulmonary fibrosis.²³

EFFECT OF OSTEOPONTIN ON IgE-MEDIATED ALLERGIC RESPONSES

Given the fact that a reciprocal regulation of Th1 vs Th2 responses is a well-documented phenomenon, it is tempting to speculate that the known Th1-inducing activity of OPN may potentially play a role in modulating the allergic responses via its Th1-inducing activity. However, the direct involvement of OPN in Th2-associated allergic responses has not been investigated.

Although studies of various OPN-deficient mouse models have provided evidence for the importance of OPN in the development of Th1-associated immune responses,^{21,24-26} the precise mechanism is still unclear. It is noted that in primary cultures of PBMCs, we found no functional effects of OPN (native or recombinant form) on Th1/Th2 cytokine expression *in vitro* using human PBMCs.²⁷ We concluded that reported cytokine-inducing activity of OPN was entirely due to the contamination of endotoxin. In addition, human IgE synthesis from purified B cells *in vitro* was also unaffected by the addition of varying doses of OPN (unpublished data). Further studies are still needed to clarify the pathophysiological mechanisms by which OPN affects Th1/Th2 immune responses.

Of interest, a significant reduced level of OPN is associated with hyper-IgE syndrome.²⁸ Moreover, we identified that OPN gene polymorphisms are associated with serum total IgE level in a Japanese population.²⁹ Individuals carrying the C allele at position 5891 in exon 6, which is more prevalent in patients with MS in the Japanese population³⁰ 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, which is associated with development of SLE,³¹ exhibited significantly higher total serum IgE levels than carriers of the 7052T allele. Patients with MS, a Th1-immune disease, display significantly fewer allergic symptoms, a lower number of allergen sensitizations³² and reduced risk of asthma³³ than individuals without MS. Conversely, SLE is characterized by Th2 immune responses, and some reports have shown increased IgE production in SLE and have associated SLE with allergy.³⁴ The results of these genetic studies indicate the potential involvement of OPN in IgE-mediated allergic responses.

CONCLUSION

OPN is now recognized as a key cytokine involved in Th1 immune responses. However, the data exploring the direct role of OPN in IgE-mediated allergic responses are scanty. The data shown in this review suggest a potential role for OPN in the IgE-mediated allergic responses. Further studies with large numbers of patients are required to evaluate the utility of serum OPN as a functional marker of VIT. Further

functional studies are also clearly needed to define the role of OPN in allergic responses.

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