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Semaphorin 3A: Is a key player in the pathogenesis of asthma

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ABSTRACT

Immune semaphorins are key players in regulating immune mediated inflammation. Semaphorin3A (sema3A) a secreted and membrane bound member of this family, is well reported for its properties in maintaining self-tolerance. Semaphorin3A was recognized to be a marker for T-regulatory cells (Tregs), and as such is a useful tool for assessing the status of these cells in preventing immune mediated diseases. This study was designed aiming to evaluate how sema3A is possibly involved in bronchial asthma. Here, we found sema3A serum levels and the expression of sema3A on Tregs significantly lower in patients with moderate to severe asthma when compared to healthy individuals. Co-culture of condition medium with 2 mcg/ml of recombinant human sema3A with CD4+ T cells, increased the expression of FoxP3 in Tregs, suggesting sema3A a potent immune-regulator of inflammation including that of asthma. Further in-vivo studies will better establish the beneficial effect of sema3A in regulating inflammation in asthma.

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1. Introduction

In many previous studies, semaphorins were appreciated for being involved in the regulation of immune mediated responses, thus identified as “immune semaphorins”. Semaphorin3A (sema3A), a secreted member of this family, is well established for its properties in suppressing immune mediated inflammation. It is expressed on many immune cells including B and T cells, but mainly on T regulatory cells (Tregs), leading to the inhibition of T effector cell proliferation and pro-inflammatory cytokine secretion [1,2]. In recent studies, sema3A was found to be involved in the pathogenesis of autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In this respect, the expression of sema3A on Tregs from patients suffering from active RA was altered. This expression was reversible and was increased in association with clinical remission [3]. Relevant to this finding, we were able to report on decreased serum level of sema3A in SLE patients, and here also this was found to be in correlation with SLE disease activity [4]. In one of the early studies in which Tregs were assessed in active asthma, it was shown that both FoxP3 expression and IL-10 production by Tregs were altered. However, both parameters increased following effective therapies such as corticosteroids. Tregs (CD4CD25highFoxP3+ cells) were also decreased in induced sputum of atopic asthmatics being negatively correlated with airway hyper-responsiveness. Finally, in a very recent study, FoxP3 expression in Tregs was down-regulated in asthma and negatively correlated with

IL-17 [5–7]. In this respect, the immuno-regulatory involvement of sema3A in atopic diseases including asthma could be of significant relevance. Hypothesizing that sema3A is important in the pathogenesis of asthma we designed this study: 1. to analyze whether serum levels of sema3A are lower in patients suffering from asthma than that in healthy individuals. 2. to assess whether sema3A expression is altered on Tregs from asthmatic patients. 3. Also, to evaluate the possible immune-modulatory effect of sema3A on regulatory markers following their co-culture of sema3A with Tregs.

2. Patients and methods

In order to evaluate the above objectives, we performed the following: Forty patients (27 women; 13 men; average age 41 ± 9 years) suffering from bronchial asthma, were recruited from our outpatient clinic (Bnai-Zion Medical Center) after getting the approval of our Helsinki committee. All studied asthmatic patients were classified as severe ($n = 21$ patients) or moderate ($n = 19$ patients) following detailed assessment which included: number of asthma attacks/week, decreased FEV1, therapeutic requirements. Severe asthmatics were those who were accordingly given a Gina score – 4 whereas moderate asthmatics were defined as having Gina score of 2–3. Twenty healthy individuals (sex and age matched) served as a control group. All individuals underwent the following: 1. Measurement of **sema3A serum levels** – Serum samples from asthmatic patients and from healthy controls were collected and subjected to analysis of sema3A levels using a commercial ELISA kit. 2. Analysis of **sema3A and Foxp3 expression in Tregs** – Mononuclear cells from both healthy controls and asthmatic patients were separated on ficoll from heparinized whole blood. Gating

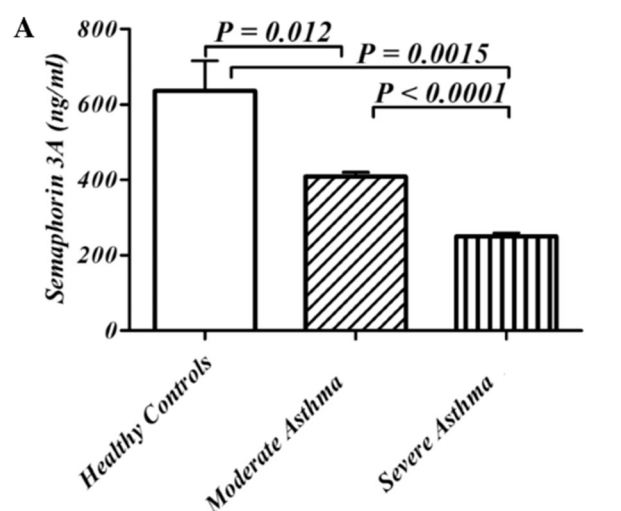
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on Tregs we assessed the expression of sema3A and Foxp3 using flow cytometry, expressed in mean fluorescent intensity (MFI). The effect of **soluble sema3A on cultured CD4 T cells**: CD4 T cells were purified from asthmatic patients and incubated with condition medium with or without recombinant human sema3A (2 mcg/ml). After 24 h of incubation with sema3A, CD4CD25high cells were gated and analyzed for the expression of FoxP3.

3. Results

Serum levels of sema3A were found significantly lower in both severe and moderate asthmatic patients when compared to that of healthy individuals (250; 409; and 636 ng/ml respectively; $p < 0.001$, and 0.01 respectively) (Fig. 1A). Sema3A expression on CD4 + CD25high cells in active asthma (during exacerbation) was significantly lower than that in normal controls (MFI; 2.6 vs. MFI; 3.5, $p < 0.006$, Fig. 1B). Also, FoxP3 expression in T regs was lower in patients with active asthma than that in normal controls (4.1 MFI vs. 6.5 MFI, $p < 0.002$, Fig. 2A). In



B
Sema3A expression on T regulatory cells (MFI)

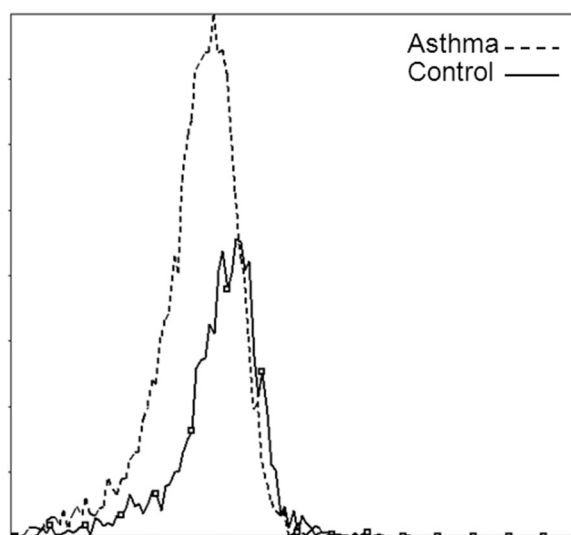


Fig. 1. A. Serum levels of semaphorin3A are significantly lower in asthma patients vs healthy individuals (250 ± 9.25 ng/ml, in severe asthma patients; 409 ± 11.34 ng/ml, in moderate asthma; and 636 ± 80.26 ng/ml in normal controls). B. A representative figure where expression of semaphorin3A on T regulatory cells in asthma patients was found to be significantly lower than that in healthy individuals (MFI; 2.6 vs. MFI; 3.61).

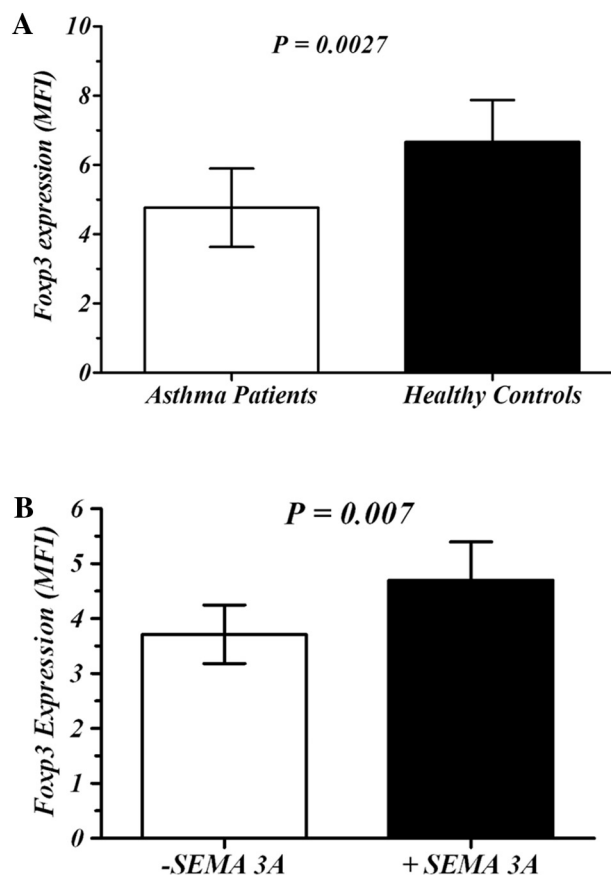


Fig. 2. A. The expression of FoxP3 in T regulatory cells of asthma patients is significantly lower than that in T regulatory cells of healthy individuals (In asthma patients – 4.1 ± 0.38 MFI vs. 6.6 ± 0.38 MFI in healthy individuals). B. The co-culture of sema3A with T regulatory cells of asthma patients enhanced significantly the expression of FoxP3 in these cells (3.7 ± 0.53 MFI at baseline vs. 4.98 ± 0.69 MFI following the addition of sema 3A).

order to examine whether sema3A has any regulatory effect on T regs we incubated purified CD4 + T cells from these patients with recombinant human sema3A. Up-regulation of FoxP3 expression in gated CD4 + CD25high cells was demonstrated significantly after 24 h (3.94 MFI vs. 4.98 MFI, $p = 0.007$, Fig. 2B).

4. Discussion

A great deal of attention was recently given to regulatory immune responses, the normal function of which was proven to suppress inflammation. Of the early regulatory cells one should mention T regulatory, and the production of inhibitory cytokines such as IL-10 and TGF- β . However, as the interest in these mechanisms increased, other regulatory cells were appreciated, namely B regulatory cells and regulatory molecules such as semaphorins in maintaining immune mediated homeostasis. In recent years, there is a growing body of data regarding the involvement of semaphorins in regulating immune mediated inflammation and therefore identified as “immune semaphorins”. In allergen-treated sema4A –/– mice, eosinophilic airway infiltration and bronchial epithelial cell inflammatory response were significantly higher than that in wild-type (WT) mice. Inflammatory responses were associated with increased levels of IL-13 and lower regulatory (Treg) numbers in bronchoalveolar lavage (BAL). These data defined sema4A as an important regulator of Th2-driven allergic asthma [8]. In a later study, airway hyper-reactivity (AHR), and Th2-type allergic responses were enhanced in sema4A –/– mice compared to WT mice when immunized and challenged with OVA. In vivo systemic administration of sema4A-Fc during the challenge period, ameliorated AHR,

lung inflammation and reduced the production of Th2-type cytokines in WT mice. These results demonstrate again that sema4A plays an inhibitory role in Th2-type allergic diseases, such as asthma [9]. Sema3A another member of immune semaphorins, was proven to be a potent immuno-regulator during all immune response stages, namely, the early initiation and the late phase of inflammatory processes [10]. The expression of sema3A and its receptors NP-1 were found to be increased on differentiating macrophages and on activated T cells, suggesting that they have a role in modulating inflammatory conditions [11]. Becoming a frontier player in the regulation of immune responses sema3A was also reported to be involved in a murine model of airway inflammation. Decreased expression of sema3A in the nasal epithelium was shown to contribute to hypersensitivity in animal models of allergic rhinitis. In rescue experiments, intranasal administration of recombinant sema3A to these mice alleviated sneezing and nasal rubbing symptoms [12]. Our study is a further contribution suggesting sema3A to be involved in the pathogenesis of bronchial asthma in humans. Being decreased in the serum and of lower expression on Tregs in severe asthmatics we may suggest that sema3A is crucial in regulating inflammation in asthma. Furthermore we show that the co-culture of sema3A with Tregs could increase their regulatory properties, suggesting that it could effectively immuno-modulate asthma and other atopic diseases. Further in-vitro and future in-vivo studies will better establish how sema3A is indeed a key regulator of inflammation in asthma.

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