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SOLUBLE PROGRAMMED DEATH-1 LIGAND-1 (PD-L1) IS DECREASED IN ALLERGIC RHINITIS PATIENT

Research

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CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

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ABSTRACT

Background:

Programmed death-1 ligand-1 (PD-L1) is a member of co-signaling molecules involved in fine-tuning of T-cell mediated inflammatory conditions. Soluble PD-L1 (sPD-L1) is recognized in healthy individuals and patients with some kinds of cancers or autoimmune diseases. However, a few studies have been conducted on the role of sPD-L1 in allergic disorders.

Objectives:

The aim of this survey was to evaluate the sPD-L1 level and its association with IgE in allergic rhinitis (AR) patients.

Patients and Methods:

Subjects with AR (n=43) were selected according to AR diagnostic criteria and considering the exclusive benchmarks. Patients' serum samples were prepared and stored at -20°C until measurements. Normal serum samples were also obtained from normal volunteers (n=18) with no symptoms or family history of allergy. The serum concentration of IgE and sPD-L1 in patients and normal individuals was determined by commercial ELISA kits.

Results:

The serum levels of sPD-L1 in AR patients (mean=3.73±2.05) were significantly (P=0.010) lower than normal individuals (mean=10.96±8.66). Moreover, sPD-L1 was not correlated with total IgE in AR patients (r=- 0.036, P=0.815).

Conclusion:

Soluble B7-H1 may present a down-regulatory function against allergic reactions in the course of allergic rhinitis development. Therefore, it can be assumed that the modulation of sPD-L1 may be an efficient therapeutic strategy together with current treatments in AR patients.

Keywords: Allergic Rhinitis; Soluble Programmed Death-1 Ligand-1; IgE

INTRODUCTION

Co-signaling molecules are involved in direction and modulation of T-cell receptor (TCR) signals, and function in fine-tuning of T-cell mediated inflammatory conditions(1). These modulatory molecules are mainly divided into two large families including B7-CD28 and tumor necrosis factor (TNF) receptors(2). Programmed death-1 ligand-1 (PD-L1) or B7 homolog 1 (B7-H1, CD274) is a member of B7 ligand family(3) acting both inhibitory and stimulatory functions controlled by its specific receptors such as programmed death-1 (PD-1) and B7-1 (CD80)(4-6). Dendritic cells, macrophages, and cells of immune privileged sites constitutively express PD-L1 (3). Inducible expression of B7-H1 is also observed in a variety of immune and non-immune cells stimulated by pro-inflammatory cytokines(3). Like some other members of B7 family, the soluble form of PD-L1 is produced, as well(7). Soluble PD-L1 (sPD-L1) was found in normal persons and in patients with some types of malignancies(8, 9) or autoimmune diseases(10, 11). There are few studies on sPD-L1 function in allergic disorders.

Allergic rhinitis (AR) is the leading cause of patients' reference to allergy clinics(12). Sneezing, coughing, rhinorrhea, redness of eyes or ocular pruritus constitute the main clinical manifestations of AR(13). This entity is considered as an important public health problem due to rising trend of its prevalence and imposing a noticeable socio-economic burden on different communities throughout the world(12). The purpose of this investigation was to evaluate the sPD-L1 level and its relationship to the IgE in AR patients.

SUBJECTS AND METHODS

Individuals and samples

In this cross-sectional survey patients with allergic rhinitis (n=43) were selected on the basis of history, physical examination and skin prick test by allergy specialists (Table 1). The suffering of the involved patients ranged from moderate to severe disease based on the Allergic Rhinitis and its Impact on Asthma (ARIA) classification. Non-allergic rhinitis, other acute or chronic inflammatory conditions (autoimmune or infectious), immunotherapy, and consumption of corticosteroids a month before admission constituted the exclusion criteria. These patients included 18 (41.86%) males and 25 (58.14%) females with mean age of 30.30 ± 5.89 . Normal sera were prepared from voluntary healthy individuals comprising 9 (50%) males and 9 (50%) females (mean age = 30.50 ± 5.71). They had no symptoms or family history of allergy as well as no other inflammatory diseases. Medical ethics committee of Academic Center for Education, Culture and Research (ACECR) approved the protocol of the study and written informed consents were obtained from all of the subjects. Characteristics of patients' reactivity to allergens in skin prick test are summarized in Table 2.

Table 1: Characteristics of allergic rhinitis patients.

	Male (n=18)	Female (n=25)
Age in years, mean$\pm$SD*	29.78 $\pm$ 6.51	30.68 $\pm$ 5.51
Comorbidities, % (n)		
Bronchial asthma	44.44(8)	40.00(10)
Atopic dermatitis	16.67(3)	28.00(7)
Food allergy	27.78(5)	24.00(6)
Drug allergy	11.11(2)	8.00(2)

Table 2:The frequency of allergen reactivity in allergic rhinitis patients on the basis of skin prick test

Allergen	Reactivity	Male		Female		Total	
		N	%	N	%	N	%
tree allergens	negative	2	11.11	6	24.00	8	18.60
	positive	16	88.89	19	76.00	35	81.40
grass/weed allergens	negative	1	5.56	2	8.00	3	6.98
	positive	17	94.44	23	92.00	40	93.02
animal allergens	negative	12	66.67	6	24.00	18	41.86
	positive	6	33.33	19	76.00	25	58.14
mite/mold allergens	negative	6	33.33	6	24.00	12	27.91
	positive	12	66.67	19	76.00	31	72.09
food allergens	negative	13	72.22	16	64.00	29	67.44
	positive	5	27.78	9	36.00	14	32.56

*SD: Standard deviation

N= Number, %=Percentage

Sample preparation

For measurement of IgE and sPD-L1, 1.5 ml of venous blood was collected in standard laboratory tubes from each subject (normal or patient) and was left at room temperature for 2 hours to clot. Then, the samples were centrifuged and separated sera were aliquoted, labeled and stored at -20°C until use.

The serum concentration of sPD-L1 and total IgE

The serum concentration of sPD-L1 and IgE were determined by commercial ELISA kits purchased from Cusabio Company (Cusabio Biotech Co. Ltd). Sensitivity of sPD-L1 kit was 3.9 pg/ml (Assay range for serum: 15.6-1000 pg/ml; Intra assay precision: < 8%; Inter assay precision: < 10%). Total IgE ELISA kit sensitivity was 3.640 ng/ml (Assay range for serum: 62.5-4000 ng/ml; Intra assay precision: < 8%; Inter assay precision: < 10%).

In summary, samples and different concentration of diluted standards were added to 96-well plates pre-coated with anti-human sPD-L1 or anti-human IgE antibody. After performing the needed incubations and washings as well as adding the conjugated antibodies, the optical densities (OD) of the wells were recorded at 450 nm by Bio-Tek Elx 800 ELISA reader. Finally, the standard curves were depicted by using the OD of standards. Then, sPD-L1 and IgE concentrations, related to each well, were determined by utilizing the relevant standard curve.

Statistics

Statistical analysis was made by using SPSS software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Logarithmic transformation was performed for IgE titers. After determination of the normality of quantitative variables, significant differences between patients and normal subjects were assessed by Mann-Whitney test. Spearman correlation coefficient was used for detection of probable association between IgE titers and sPD-L1 in normal individuals or involved patients. The significant level was considered <0.05 .

RESULTS

1. The serum levels of sPD-L1 in AR patients (mean= 3.73 ± 2.05) were significantly ($P=0.010$) lower than those of normal individuals (mean= 10.96 ± 8.66) (Figure 1). The geometrical mean for serum levels of total IgE in patients with AR (88.36, 95% CI: 61.36-127.26) was significantly ($P=0.013$) higher in comparison to that of control group (42.90, 95% CI: 29.63-62.11).
2. Possible correlation between sPD-L1 and total IgE in patients and normal persons were analyzed. There was no correlation between sPD-L1 and total IgE in AR patients ($r=-0.036$, $P=0.815$) or in normal individuals ($r=-0.057$, $P=0.823$).

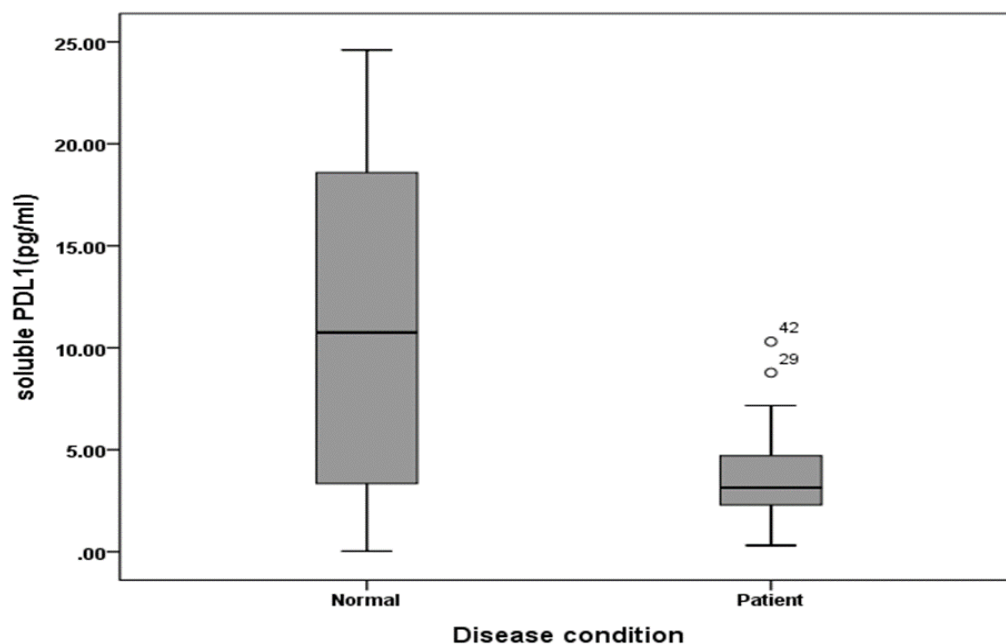


Figure 1: The serum levels of sPD-L1 in allergic rhinitis patients and normal individuals ($P=0.010$)

DISCUSSION

PD-L1 has pivotal role in regulating and modulating of immune cells responses(4). Extensive expression of PD-L1 in hematopoietic and non-hematopoietic cells represents its functional relevance(14). PD-L1 exerts its effects through two recognized receptors called PD-1 and B7-1. These signaling pathways (PD-1/PD-L1 or B7-1/PD-L1) mainly lead to down regulation and inhibition of T cells effector functions(4, 5, 15). However, some researchers have revealed the PD-L1 stimulatory functions by means of another unknown receptor(16, 17). Furthermore, a reverse signaling phenomenon has also been described for PD-L1 contributing T cell turn over and homeostasis(1). Moreover, the soluble form of PD-L1 can also be produced by activated mature dendritic cells (DCs) and many PD-L1⁺ cell lines(18). sPD-L1 retains receptor-binding ability similar to membrane-bound form (7). This soluble factor is found in normal individuals as well as in patients with some kind of cancers or autoimmune disorders(19, 20). However, limited investigations have been made regarding to sPD-L1 roles in allergic diseases. The present study evaluated the clinical significance of soluble B7-H1 (sB7-H1) in AR patients by determining the sB7-H1 levels in serum samples. In this survey, it was found that sPD-L1 is significantly lower in patients with AR in comparison to that of healthy persons. However, no significant correlation was detected between sPD-L1 and total serum IgE in these patients. Total IgE provides a global view of allergic reaction extent.

This index is influenced by several factors and mainly considered as an important screening allergy test (21). Nevertheless, a significant association was observed between allergic patients reactivity against some common allergens, such as pollens or house-dust mite, in the skin prick test and total serum IgE(22). In addition,

total serum IgE can be considered as an advantageous and significant laboratory indicator in diagnosis of AR (23). It is expected that increasing the number of studied patients or evaluating of a selected group of patients with higher levels of total IgE (> 400 IU/ml) would lead to a significant correlation. On the other hand, it can also be assumed that human sB7-H1, like mouse counterpart(24), has no or minimal effect on production of IL-4; the main cytokine for immunoglobulin class switching to IgE. Therefore, it may be concluded that sPD-L1 predominantly influences the cytokines produced by other T cell subsets, rather than T helper 2, being responsible for development of allergic reactions in AR.

Substantial decrease of sPD-L1 detected in AR patients indicates this regulatory ligand may have an inhibitory role in development and progression of AR. There are no reports regarding to the impact of human sPD-L1 in allergic processes. Nevertheless, the mentioned impression is consistent with Frigola findings that revealed the sB7-H1 produced by human renal cell tumor or human mature dendritic cells can induce the increase of apoptosis in $CD4^+$ or $CD8^+$ T cells, in vitro(18). Shi et al. also reported that sPD-L1 released by certain lung cancer cell lines was able to inhibit T cell proliferation, in vitro(25). Accordingly, in vivo previous studies on patients with malignancy demonstrated that sB7-H1 could be considered as an important suppressing factor in immunity against tumor growth and expansion(8, 19). On the basis of Frigola and Rossile researches, higher sB7-H1 predicted poorer prognosis for patients with renal cell cancer(26) and B cell lymphoma(19). Hence, it can be deduced that sB7-H1, the same as membrane-bound B7-H1, provides inhibitory signals for $CD8^+$ cytotoxic T lymphocytes and T helper 1 cells.

Conversely, the investigations made about patients with some kinds of autoimmune diseases elucidated opposite results. Wan et al. demonstrated the increased expression of sPD-L1 in serum and synovial fluid of rheumatoid arthritis (RA) patients(20). The same researchers found a positive significant correlation between serum concentration of sPD-L1 and titers of rheumatoid factor in evaluated patients, as well. They also showed that co-culture of $CD4^+$ T cells separated from RA patients with human PD-L1 fusion protein led to significant higher proliferation of T cells compared with that of control. In another study on diabetic patients, Shi et al. disclosed that the presence of sPD-L1 significantly enhanced proliferation of T cells isolated from patients(11). Furthermore, a significant association was reported between serum sPD-L1 and interferon gamma. Also, Patients with atherosclerotic macrovascular disease had elevated levels of sPD-L1 compared to diabetic patients without vascular disorders.

Inconsistent results observed in previous in vitro experiments (on RA or diabetic patients) may partly be due to applying the synthetic form of PD-L1 compared to another group of investigations performed by cell-derived sPD-L1. These two forms of PD-L1 do not have precisely equal structural characteristics(20). Therefore, they may differently engage the PD-1 receptor and induce different outcomes. Considering the reported both inhibitory and stimulatory effects for membrane-bound PD-L1(27), it is anticipated that sPD-L1 exhibits dual functions in different pathologic conditions, as well. This idea may explain above-mentioned conflicting in vivo findings.

Collectively, the present survey demonstrated the significant lower amounts of sPD-L1 in AR patients compared to control group. One can assume that modulation of this co-signaling molecule may be an efficient therapeutic strategy together with current treatments in AR patients. On the other hand, sPD-L1 can be considered as a possible advantageous factor for determining the severity or prognosis of this common allergic disease. More precise and extensive evaluations are needed to clarify these issues.

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