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Original article

Inverse correlation of soluble programmed cell death-1 ligand-1 (sPD-L1) with eosinophil count and clinical severity in allergic rhinitis patients

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AR	allergic rhinitis
ARIA	Allergic Rhinitis and its Impact on Asthma
sPD-L1	soluble programmed cell death-1 ligand-1
ELISA	enzyme-linked immunosorbent assay
Th2	T helper 2

ABSTRACT

Background: T-cell response outcome is determined by co-stimulatory/inhibitory signals. Programmed cell death-1 ligand-1 (PD-L1) is a member of these co-signaling molecules with known soluble form in human serum. Soluble PD-L1 (sPD-L1) is also recognized in patients with some types of malignancy or autoimmune disorders, though there are few studies on sPD-L1 roles in allergic diseases. The purpose of this survey was to evaluate the association between sPD-L1 levels with eosinophil count as well as disease severity in allergic rhinitis (AR) patients.

Methods: 90 patients with AR were selected. Disease severity was determined by a modified Allergic Rhinitis and its Impact on Asthma (ARIA) classification as mild, moderate and severe. Whole blood samples were collected. Then eosinophil count and serum sPD-L1 were detected by a hematologic analyzer and a commercial ELISA kit.

Results: 13 (14.44%), 31 (34.44%), and 46 (51.12%) of patients had mild, moderate and severe disease, respectively. The mean levels of sPD-L1 and eosinophil count were ascertained 18.38 ± 14.42 ng/ml and 422.43 ± 262.26 cell/μl. A significant inverse correlation was determined between sPD-L1 levels and eosinophil count ($r = -0.364$, $P < 0.001$). Moreover, we detected a significant negative association between sPD-L1 levels and disease severity ($r = -0.384$, $P < 0.001$).

Conclusions: It is deduced that sPD-L1 can be used as a helpful marker to determine the severity of AR. Furthermore, this study indicated that sPD-L1 may have an inhibitory role in AR development, and its modulation may be considered as a useful accessory therapeutic approach for reduction of AR progression.

Introduction

IgE-mediated inflammatory reactions in nasal mucosa give rise to allergic rhinitis (AR), the most common allergic disease.[1] Major clinical manifestations of the disease include sneezing, nasal congestion/itching, rhinorrhea, and some ocular symptoms such as pruritus or redness.[1] According to the rising pattern of AR incidence, as well as its considerable effects on patients' quality of life, and its noticeable economic burden on communities, this entity has been turned into an important public health problem.[2]

T helper 2 (Th2) cell activation is a central phenomenon in development of an inflammatory allergic reaction.[3] In addition to T cell receptor (TCR) recognition of antigenic peptide-major histocompatibility (MHC) class II complex, appropriate function of T

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cells needs to co-stimulatory/inhibitory signals which determine the T cell response outcome.[4] Indeed, it is assumed that these co-stimulator and/or inhibitor molecules, which are mainly provided by antigen presenting cells (APCs), govern the T cell response direction.[4] The best known co-signalling molecules belong to two families: B7 and tumor necrosis factor receptors.[5] B7-homologues 1 (B7-H1, CD274) or programmed cell death-1 ligand 1 (PD-L1) is characterized as a member of B7 family. B7-H1 messenger RNA is expressed by a variety of human tissues, but B7-H1 protein could be found only on APCs in lymphoid tissues or peripheral organs. However, inflammatory cytokines, particularly interferon gamma (INF-γ), promote induction of B7-H1 molecule on many different lymphoid or non-lymphoid cells.[6] Moreover, functional soluble form of PD-L1 (sPD-L1) is also recognized in human serum.[7] B7-H1 is one of the two well-known ligands for programmed cell death-1 (PD-1); an inducible regulatory receptor on activated T cells, B cells, regulatory T cells, dendritic cells, and monocytes.[6] Even though it is suggested that PD-1 be a preferential inhibitory receptor, both stimulatory and inhibitory functions for PD-L1 are reported.[8–10] This indicates the presence of another probable receptor for PD-L1 to deliver stimulatory signals. Some important roles of PD-L1 in infectious diseases,[11] autoimmunity[12] or tumor immunity[13] have been elucidated. However, few investigations are available about PD-L1 effects on allergic disorders. The aim of this study was to find the relationship between sPD-L1 levels and eosinophil count or disease severity in allergic rhinitis patients.

Methods

Patients

In this cross-sectional study allergic rhinitis patients (n = 90) were selected by an allergist/immunologist on the basis of history, physical examination, and skin prick test which was conducted by an allergy specialist utilizing the international standard mixed allergens (Greer Laboratories, Lenoir, NC, USA) of tree, grass, weed, mite and mold. Non-allergic rhinitis, affection to chronic or acute inflammatory diseases including autoimmune or infectious disorders and also receiving corticosteroids or immunotherapy within a month of admission were considered as exclusion criteria. Before patients' enrollment, in a decision-making process, voluntary written informed consents were obtained from all of them. The protocol of this survey was approved by the medical ethics committee in the Faculty of Medicine, Kurdistan University of Medical Sciences.

Classification of disease severity

Severity categorization of participants was determined on the basis of a modified Allergic Rhinitis and its Impact on Asthma (ARIA) classification.[14] Sleep, daily activities/sport, work/school, and troublesome symptoms include 4 ARIA items for severity of AR. In the current survey, disease severity was categorized into mild, moderate and severe. In patients with mild disease none of the mentioned items were affected. Impairment of one, two or three items characterized the moderate severity. Severe disease was also defined as patients in whom all 4 items were disturbed. Characteristics of patients with mild, moderate and severe disease were shown in Table 1.

Determination of nasal symptom score

Patients' nasal symptoms (nasal obstruction, nasal itching, rhinorrhea, and sneezing) were assessed on a 4-point scale basis (0 = no symptom, 1 = mild, 2 = moderate, 3 = severe). Total nasal symptom score (TNSS) was accessed from the sum of all 4 points for each patient. Therefore, the possible score that could be attained to a patient had a range of 0 (no symptom) to 12 (the highest symptom intensity) (Table 2).

Table 1
Characteristics of mild, moderate and severe allergic rhinitis patients.

	Mild (n = 13)	Moderate (n = 31)	Severe (n = 46)
Gender, % (n)			
Female	8.33 (4)	43.75 (21)	47.92 (23)
Male	21.43 (9)	23.81 (10)	54.76 (23)
Age in years, mean ± SEM[†], (median)	28.38 ± 2.27, (26)	29.19 ± 1.52, (26)	29.39 ± 1.15, (27)
Comorbidities, % (n)			
Bronchial asthma	2.44 (1)	29.27 (12)	68.29 (28)
Atopic dermatitis	0	14.29 (4)	85.71 (24)
Food allergy	0	5.13 (2)	94.87 (37)
Drug allergy	0	0	100.00 (10)
Total serum IgE, mean ± SEM, (median)	37.85 ± 8.01, (34.40)	86.07 ± 19.59, (48.60)	213.98 ± 24.89, (183.25)
Eosinophils percentage, mean ± SEM, (median)	1.49 ± 0.39, (1.04)	3.92 ± 0.46, (3.54)	9.54 ± 0.48, (8.65)

[†] SEM, Standard error of the mean

Measurement of eosinophil count and sPD-L1

For measurement of hematologic indices, whole blood sample (1.5 ml) from each patient was drawn into standardized tubes containing anticoagulant (ethylene diamine tetra acetic acid). Then the counts of blood cells were estimated by an automatic hematologic analyzer (MEK-7300K, Nihon Kohden Corporation, Tokyo, Japan).

The required serum samples for sPD-L1 assessment were also prepared by collecting the venous blood (1.5 ml) from each participant in conventional laboratory tubes, allowing the blood tubes to clot at room temperature for approximately 2 h, and then centrifuging at 3000 rpm for 15 min. Separated serum samples were aliquated in microtubes, labeled and stored at −20 °C until use. Determination of sPD-L1 was performed by commercial ELISA kits (Uscn Life Science Inc., Wuhan, China) according to the manufacturer's instructions. The detection sensitivity of this sPD-L1 ELISA experiment was typically less than 0.117 ng/ml (Assay range for serum: 0.313–20 ng/ml, Intra-assay precision: CV < 10%, Inter-assay precision: CV < 12%).

Table 2
Scoring and intensity of nasal symptoms in allergic rhinitis patients (n = 90).

Definitions of symptoms score	
Guideline	Score (Grade)
No symptom is evident	0 (None)
Symptom is clearly present but easily tolerated	1 (Mild)
Symptom bothersome but tolerable	2 (Moderate)
Symptom difficult to tolerate-interferes with activities	3 (Severe)
Nasal symptom score	**Mean (SD[†])**
Total score	9.17 (3.28)
Sneezing	2.60 (0.76)
Rhinorrhea	2.35 (0.95)
Nasal obstruction	2.37 (0.92)
Nasal itching	1.84 (1.19)
Symptoms intensity	**% (n)**
Very mild symptoms (0–2 points)	6.67 (6)
Mild symptoms (3–6 points)	10.00 (9)
Moderate symptoms (7–9 points)	26.67 (24)
Severe symptoms (10–12 points)	56.67 (51)

[†] SD, Standard deviation