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The relationship between B7 homologous 1 with interleukin-4, interleukin-17 and interferon gamma in patients with allergic rhinitis

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Abstract

Background: Allergic rhinitis is the most common atopic disorder and the most common chronic allergic disease. The etiology of this disease includes an increase in cytokine levels, including IL- 4, IL- 13, IL- 17, and reduction in B7 homologous 1 (B7-H1) or programmed cell death-1 ligand-1 (PD-L1), a new member of the CD28: B7 stimulant molecule family. The aim of this study was to determine the relationship between cytokines and PD-L1.

Methods: In this experimental study, 80 patients with allergic rhinitis were enrolled according to inclusion and exclusion criteria. The severity and stage of the disease were determined by a specialist physician. A 5 cc venous blood sample was collected from the patients. IL-4, IL-17, INF γ and PD-L1 were measured using ELISA technique. The statistical analysis was performed using the SPSS15 software and correlation between levels of interleukins and PD-L1 and interferon gamma molecules was assessed using PERMANOVA test.

Results: The mean age of the subject was 28.5 ± 1.3 years. Out of the 80 subjects, 46 (57.5%) were male. There was a significant correlation between SPD-L1 and INF γ , IL-4 and IL-17 in allergic rhinitis patients ($P < 0.05$). Statistical analysis based on the severity of the disease (Mild, Moderate and Severe) showed a significant positive correlation between the SPD-L1 and INF γ in all three levels ($P < 0.001$). There was also a significant negative correlation between SPD-L1 and two cytokines IL-4 and IL-17 ($P < 0.001$). In regard to the duration of the disease (persistent or intermittent), a significant positive correlation was found between SPD-L1 and INF γ in both groups ($P < 0.001$).

Conclusion: Considering the negative correlation between the PD-L1 molecule and the levels of IL-4 and IL-17 as inflammatory cytokines, PD-L1 may have a protective role against allergic rhinitis, although the precise mechanism requires further detailed studies.

Key Words: B7 homologous 1, Interleukin-4, Interleukin-17, Interferon gamma, Allergic rhinitis

1. Introduction

Allergic rhinitis (AR) is a chronic inflammation of the upper airways that is mediated by immunoglobulin E (IgE) and is characterized by sneezing, rhinorrhea, pruritus, and nasal obstruction (1). This disease affects 10 to 20 percent of adults and 20 to 40 percent of children. The progression of AR is a combination of interactions between environmental factors, immune system and genetic disorders (1). Although, immune responses are generally considered to be the major contributors to the symptoms and tissue damage observed in AR patients, the multiplicity and diversity of the cells and molecules involved in these reactions as well as the multiplicity of communication between these cells, that are ultimately accompanied by other inflammatory disorders, have made its management and treatment difficult. Despite new strategies and approaches, patients with AR do not achieve the expected improvement. Needless to say, knowing how these processes and regulators / stimuli become more accessible will pave the way for achieving therapeutic approaches (2, 3). From the etiological point of view, most of the symptoms of AR are due to Type1 hypersensitivity, where T2 and T helper 2 (Th2) immunoglobulins (IgGs) play an essential role (4). While T helper (Th1) cells are seen in nasal mucus and epithelial tissues of healthy people, individuals Th2 cells are dominantly observed in the nasal mucus and epithelial tissues of the AR (5). Th2 lymphocytes, like other T-cells, perform their function through the production of specific cytokines. Cytokines play an important role in the mediation of allergic inflammation. The importance of TH2 secreted cytokines in the development of allergy and pathology of allergic reactions has been well documented(6).

Cytokines, are glycoproteins involved in regulating important cellular functions including survival, proliferation, differentiation and activity (7-10). Interleukin-4 (IL-4) is a type of cytokine essential for the differentiation of Th2 cells and the production of IgE and eosinophil recall as well as the growth and differentiation of lymphocytes, basophil, mastocytes and macrophage inactivation (11). Interferon γ (INF γ) is a cytokine that plays a central role in atopic diseases and prevents excessive production of IgE by IL-4 (12). Interleukin-17 (IL-17) is a pro-inflammatory cytokine produced by a group of auxiliary T cells called Th17 in response to stimulation by IL-23 (13, 14).

B7-H1 or PD-L1 is a new member of the cluster of differentiation (CD) 28: B7 family stimulant molecules which play an important role in regulating the activity of T cells (15). B7-H1 is an inducible regulatory receptor which exists on activated T cells, B cells, regulatory T cells, dendritic cells, and monocytes (16). Even though it is suggested that PD-1 is a special inhibitory receptor, both stimulatory and inhibitory functions have been reported for PD-L1. Several important roles have been elucidated for PD-L1 in infectious diseases, autoimmunity or tumor immunity. However, few investigations are available regarding the effects of PD-L1 on allergic disorders (17, 18). Recently, the soluble form of this molecule (soluble PD-L1, sPD-L1) has been identified in serum (16). In our previous study, we found significant reductions in sPD-L1 in patients with AR compared to healthy controls. In addition, an inverse relationship was observed between sPD-L1 and the severity of disease and eosinophilia (19). In line with our previous works, this study was conducted in order to reveal the role and function of sPD-L1 in AR. The aim of this study was to evaluate the relationship between sPD-L1 and IL-4, IL-17 and IFN- γ cytokines in patients with AR.

2. Methods

2.1. Statistical population

In this experimental study, patients with AR were selected by a specialist based on AR diagnostic criteria. Sample size was calculated based on the Cochran formula.

$$n = \frac{Nz^2pq}{Nd^2 + z^2pq}$$

Based on this formula, the sample size was calculated to be 80.

2.2. Inclusion criteria

Being diagnosed with AR, being within the age range of 18 to 60 years old, and ability to give informed consent.

2.3. Exclusion criteria

Inflammatory diseases including autoimmune or infectious diseases, as well as receiving corticosteroids or immunotherapy within the past month, patients with other types of rhinitis, for example, infectious, drug-induced, hormonal rhinitis, positive history for any allergic diseases other than AR.

2.4. Performing tests

A total of 80 patients with AR who were diagnosed by a specialist physician through clinical examination were included in this study. Patients were divided into three groups, Mild, Moderate and Severe, based on the stage of the disease based on the modified AR protocol and its effect on asthma (ARIA). Five milliliters of venous blood was collected in anticoagulant tubes from all patients. The serum samples were centrifugated at 3000 rpm and kept at -70 ° C until the experiments were carried out. To measure B7-H1, IL-4, IL-17 and IFN- γ , the enzyme linked immune assay (ELISA) technique was performed using a kit from BD Biosciences company (USA) based on the manufacturer protocol. Briefly, the steps for measuring the four parameters were as follows; Preparation of samples, standard solutions and other necessary solutions, adding samples and standards to wells and incubation for recommended times, removing fluid from wells, adding antibody, incubation, aspirating wells and rinsing, adding HRP-avidin, incubation, aspiration of wells and washing, adding TMB, incubation (in dark), adding stopping solution and reading the optical absorption of wells at 450 nm, drawing a standard curve and obtaining the corresponding concentration for each well.

2.5. Ethical considerations

The study process was approved by the Ethical Committee of Kurdistan University of Medical Sciences. All patients were given comprehensive information on how the study was to be done, and then informed consent was obtained from all patients. Throughout the study, all patient information was confidentially collected and stored.

2.6. Statistical analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS) software version 18 and the significance level was defined at $P < 0.05$. The permutational multivariate analysis of variance (PERMANOVA) test was used to evaluate the relationship

between SPD-L1 and dependent variables, including INF, IL-4 and IL-17, due to homogeneity though non normal distribution of the data.

3. Results

3.1. Correlation between SPD-L1 with IFN- γ , IL-4 and IL-7 cytokines

A total of 80 patients with allergic rhinitis were studied in this research. The mean age of studied subject was 28.5 ± 1.3 years. Out of the 80 subjects, 46 (57.5%) were male. There was a significant correlation between SPD-L1 and IFN- γ , IL-4 and IL-7 variables in allergic rhinitis patients ($P < 0.05$). The correlation between SPD-L1, IFN- γ , IL-4 and IL-7 variables based on gender is presented in Table 1. Statistical analysis showed a significant positive correlation between SPD-L1 and IFN- γ in both sexes ($P < 0.05$). There was also a significant negative correlation between SPD-L1 and two cytokines (IL-4 and IL-17) ($P < 0.05$). There was a significant negative correlation between IFN- γ and IL-4 and IL-17 cytokines in both sexes ($P < 0.05$). There was a significant positive correlation between IL-4 and IL-17 ($P < 0.05$) (Table 1).

3.2. Correlation between SPD-L1 and IFN- γ , IL-4 and IL-17 cytokines in terms of disease severity

Table 2 shows the relationship between SPD-L1 and IFN- γ , IL-4 and IL-17 cytokines in terms of disease severity (Mild, Moderate, Severe). Statistical analysis showed a significant positive correlation between the SPD-L1 and IFN- γ at all three severity levels ($P < 0.001$). There was also a significant negative correlation between SPD-L1 and two cytokines (IL-4 and IL-17) ($P < 0.001$). There was a significant negative correlation between IFN- γ cytokine and two cytokines (IL-17 and IL-4) in Mild and Severe groups ($P < 0.001$ and $P < 0.05$ respectively). However, there was no correlation between IFN- γ and IL-17 in the Moderate group ($P > 0.05$). There was a significant positive correlation between IL-4 and IL-17 ($P < 0.01$ and $P < 0.05$ respectively) (Table 2).

3.3. Correlation between SPD-L1 and IFN- γ , IL-4 and IL-17 cytokines in terms of the duration of the disease

Statistical analysis showed a significant positive correlation between SPD-L1 and IFN- γ in both persistent and intermittent AR forms ($P < 0.001$). There was also a significant negative correlation between SPD-L1 and two cytokines (IL-4 and IL-17) ($P < 0.001$). A significantly negative correlation was found between INF- γ and IL-4 and IL-17 in both AR forms ($P < 0.001$). There was a significant positive correlation between IL-4 and IL-17 ($P < 0.001$) (Table 3).

3.4. Correlation between SPD-L1 and IFN- γ , IL-4, and IL-17 cytokines in terms of presence or absence of bronchial asthma

Statistical analysis showed a significant positive correlation between SPD-L1 and IFN- γ in both groups ($P < 0.001$). SPD-L1 was also found to have a significant negative correlation with IL-4 and IL-17 ($P < 0.001$). There was a significant correlation between IFN- γ and two cytokines (IL-17 and IL-4) in both groups ($P < 0.001$ and $P < 0.05$ respectively). There was a significant positive correlation between IL-4 and IL-17 ($P < 0.001$) (Table 4).

4. Discussion

The results of this study showed a negative correlation between SPD-L1 levels and IL-4 and IL-17 cytokines. There was also a positive correlation between SPD-L1 and INF- γ . This

relationship was observed in both sexes, in all three severity levels of the disease, in patients with bronchial / non-bronchial asthma, and persistent and intermittent forms of AR. The relationship between cytokines and AR has always been of interest to scientists (11, 20). It has been shown that AR is a Th2-dependent disease, and many studies indicate that cytokines, including IL-4 or IL-17, are associated with AR (11, 13, 20). In the study by Kırmaz et al., it was found that the levels of IL-4 and IL-17 increased in patients with AR compared to the control group. In their study, IFN- γ was also used to measure the Th1 immune response in AR patients. They reported a lower level of IFN- γ in patients with AR compared to control group. Subsequently, IL-4, IL-17, and IFN- γ play key roles in AR (20). Saba et al. observed that the level of IL-4 and IgE decreases in moderate form and increases in mild and severe forms of AR (7), although no precise mechanism for this process was identified. Similarly, in our study the relationship between IL-4 and PD-L1 was weaker in moderate form compared to the mild and severe forms. PD-L1 plays an important role in regulating the activity of T cells (21). Increased levels of this molecule have been observed in cancers and a number of autoimmune diseases (22). In previous studies, it was found that the PD-L1 level significantly decreased in patients with AR compared to normal individuals. Additionally, this PD-L1 was shown to be inversely correlated with eosinophil count in patients with AR (19). In our study, there was a negative correlation between sPD-L1 and IL-4 and a positive correlation between sPD-L1 and IFN- γ . In 2015, Zhao et al. showed that the sPD-L1 molecule has an inhibitory effect on T cell proliferation (23). Therefore, the correlation observed in our study was in line with the studies showing the inhibitory effect of sPD-L1 on cytokines. This inhibitory effect can be generated through inhibition of the transcriptional phase or protein translation phase but the exact mechanism has not been determined yet. One study suggests that the sPD-L1 molecule prevents the methylation of CpGs in the cytokine genes promoter and prevents their overproduction through epigenetic changes (24). Since IL-4 and IL-17 are inflammatory cytokines, inhibitory effects of sPD-L1 on these cytokines plays a significant role in preventing the aggravation of inflammation in AR. The results of the current study, also showed that the inverse correlation between sPD-L1 and IL-4 and IL-17, emphasize the role of sPD-L1 in the prevention of AR. On the other hand, in our study, there was a positive correlation between IFN- γ and sPD-L1 levels. As previously mentioned, IFN- γ , like the sPD-L1 molecule, prevents excessive production of inflammatory interleukins, including IL-4 and IL-17. IFN- γ has been shown to inhibit excessive production of IgE stimulated by IL-4 and thus prevent hypersensitivity. The reduction of IFN- γ and sPD-L1 level plays a central role in the etiology of AR.

Frigula et al. as well demonstrated the inhibitory effect of sPD-L1 on the secretion of cytokines and induction of apoptosis in T cells. The observed negative correlation between sPD-L1 and IL-4 and IL-17 levels in the current study was in line with the observations of Frigula et al. Thus, it can be hypothesized that one of the mechanisms involved in the development of AR may be reducing the amount of sPD-L1 and thereby reducing its inhibitory effect on the production of cytokines involved in AR, including IL-4 and IL-17(23).

Kataoka et al., reported an inhibitory effect for recombinant PD-L1 on neoplastic mast cell proliferation in human LAD2 cells (25). Additionally, Kubo et al. demonstrated that induction of PD-L1 expression in human B cells could lead to suppression of IL-5 and IL-13 production in

CD4 cells and stimulate allergies (24). The results of our study also confirmed the inhibitory effect of PD-L1 on production of cytokines, including IL-4 and IL-17. Since IL-4 and IL-17 are inflammatory cytokines that intensify allergy, PD-L1 mitigates their allergy promoting effect through the inhibition of the aforementioned cytokines. However, there are studies that violate the protective role of PD-L1 in immunity. For instance, Alees et al. showed that the presence of PD-L1 during the polarization of Th2 resulted in an increase in IL-4 production. This difference could be due to the various stages of the immune system, where probably the early stages of the disease includes the differentiation of both Th2 and production of cytokines; whereas severe stages of the disease functions through suppression of Th2 cytokines, inhibition of cytokines, and induction / activation of T cells (11, 19).

5. Conclusion

The results of this study indicate the potential role of IL-4 and IL-17 in inflammation and intensifying allergic reactions. Furthermore, due to the negative correlation between PD-L1 and IL-4 and IL-17 levels, it can be said that PD-L1 can have a protective role against AR although determining the precise underlying mechanism requires further studies. It seems that therapeutic interactions in the sPD-L1-dependent molecular pathway can be considered as a novel modality in the treatment of AR.

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Declaration of Interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties

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Table1: Correlation between SPD-L1, INFY, IL-4 and IL-7 variables by gender

Variables	Sex	SPD-L1	INFY	IL-4	IL-17
SPD-L1	Male	1.000	.730 ^{***}	-.338 [*]	-.994 ^{***}
	Female	1.000	.750 ^{**}	-.600 ^{**}	-.994 ^{**}
INFY	Male	.730 ^{**}	1.000	-.501 ^{**}	-.731 ^{**}
	Female	.750 ^{**}	1.000	-.496 ^{**}	-.733 ^{**}
IL-4	Male	-.338 [*]	-.501 ^{**}	1.000	.343 [*]
	Female	-.600 ^{**}	-.496 ^{**}	1.000	.623 ^{**}
IL-17	Male	-.994 ^{***}	-.731 ^{**}	.343 [*]	1.000
	Female	-.994 ^{***}	-.733 ^{**}	.623 ^{**}	1.000

*p < .05, **p < .01, ***p < .001, SPD-L1: programmed cell death-1 ligand-1, INFY: Interferon gamma, IL-4: Interleukin -4, IL-17: Interleukin -17

Table 2: The association between SPD-L1 and cytokines INFY, IL-4 and IL-17 in terms of severity of disease

Variables	Disease severity	SPD-L1	INFY	IL-4	IL-17
SPD-L1	Mild	1.000	.934***	-.725**	-.999***
	Moderate	1.000	.788***	-.407*	-.987***
	Severe	1.000	.574***	-.453**	-.996***
INFY	Mild	.934***	1.000	-.758**	-.933***
	Moderate	.788***	1.000	-.652***	-.771***
	Severe	.574***	1.000	-.198	-.556***
IL-4	Mild	-.725**	-.758**	1.000	.710**
	Moderate	-.407*	-.652***	1.000	.425*
	Severe	-.453**	-.198	1.000	.473**
IL-17	Mild	-.999***	-.933***	.710**	1.000
	Moderate	-.987***	-.771***	.425*	1.000
	Severe	-.996***	-.556**	.473**	1.000

*p < .05, **p < .01, ***p < .001, SPD-L1: programmed cell death-1 ligand-1, INFY: Interferon gamma, IL-4: Interleukin -4, IL-17: Interleukin -17

Table 3: The relationship between SPD-L1 and cytokines INF γ , IL-4 and IL-17 in terms of the duration of the disease

Variables	Duration of Disease	SPD-L1	INF γ	IL-4	IL-17
SPD-L1	Intermittent allergic rhinitis	1.000	.718***	-.493***	-.997***
	Persistent allergic rhinitis	1.000	.786***	-.502***	-.993***
INF γ	Intermittent allergic rhinitis	.718***	1.000	-.557***	-.721***
	Persistent allergic rhinitis	.786***	1.000	-.446**	-.756***
IL-4	Intermittent allergic rhinitis	-.493***	-.557***	1.000	.498***
	Persistent allergic rhinitis	-.502***	-.446***	1.000	.514***
IL-17	Intermittent allergic rhinitis	-.997***	-.721***	.498***	1.000
	Persistent allergic rhinitis	-.993***	-.756***	.514***	1.000

*p < .05, **p < .01, ***p < .001, SPD-L1: programmed cell death-1 ligand-1, INF γ : Interferon gamma, IL-4: Interleukin -4, IL-7: Interleukin -17

Table 4: The relationship between SPD-L1 and the INFY, IL-4, and IL-17 cytokines in terms of the presence or absence of bronchial asthma

Variables	Duration of Disease	SPD-L1	INFY	IL-4	IL-17
SPD-L1	Bronchial asthma	1.000	.700 ^{***}	-.312 [*]	-.996 ^{***}
	None bronchial asthma	1.000	.769 ^{***}	-.645 ^{***}	-.994 ^{***}
INFY	Bronchial asthma	.700 ^{***}	1.000	-.337 [*]	-.687 ^{***}
	None bronchial asthma	.769 ^{***}	1.000	-.616 ^{***}	-.760 ^{***}
IL-4	Bronchial asthma	-.312 [*]	-.337 [*]	1.000	.334 [*]
	None bronchial asthma	-.645 ^{***}	-.616 ^{***}	1.000	.645 ^{***}
IL-17	Bronchial asthma	-.996 ^{***}	-.687 ^{***}	.334 [*]	1.000
	None bronchial asthma	-.994 ^{***}	-.760 ^{***}	.645 ^{***}	1.000

*p < .05, **p < .01, ***p < .001, SPD-L1: programmed cell death-1 ligand-1, INFY: *Interferon gamma*, IL-4: Interleukin -4, IL-17: Interleukin -17