

CD30 Serum Levels and Response to *Hymenoptera* Venom Immunotherapy

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■ Abstract

Background: The glycoprotein CD30 is expressed and released by T lymphocytes that secrete type 2 helper cytokines of (T_H2). These molecules play a role in the pathogenesis of allergic diseases. Venom immunotherapy has proven to be very effective in hymenoptera venom allergy through a shift in cytokine production from T_H2 -type cytokines to T_H1 -type cytokines.

Objective: To evaluate the relationship between the soluble form of CD30 (sCD30) and venom immunotherapy in patients with hymenoptera venom allergy.

Materials and Methods: sCD30 levels were assayed by enzyme-linked immunosorbent assay in the sera of 61 healthy controls and 14 patients with hymenoptera venom allergy who had undergone immunotherapy before treatment and 1, 3, and 12 months after treatment started. Nine patients were allergic to *Apis* venom, 4 to *Vespula* venom, and 1 to *Polistes* venom.

Results: CD30 serum levels (median, interquartile range) were significantly higher in venom-allergic patients before treatment (33.6 U/mL; 14.8-61.6) than in controls (9.7 U/mL, 1.9-21.3) ($P < .000$). These levels decreased progressively during treatment in all patients except 2 ($P < .000$). At the third month of therapy, the levels reached statistical significance in comparison with baseline.

Conclusions: This study shows that sCD30 levels are significantly higher in patients with hymenoptera venom allergy and indirectly confirms a preferential T_H2 -type cytokine production in these patients. sCD30 expression decreases during immunotherapy, thus confirming the immunomodulatory role of this treatment in promoting a shift to T_H1 -type cytokines.

Key words: Hymenoptera venom. Allergy. CD30 serum levels. Immunotherapy.

■ Resumen

Antecedentes: La glicoproteína CD30 se expresa y se libera mediante los linfocitos T que secretan linfocitos T cooperadores del subtipo 2 ($Th2$). Estas citocinas participan en la patogenia de las enfermedades alérgicas. Se ha demostrado que la inmunoterapia con veneno es muy eficaz en la alergia al veneno de himenópteros porque produce un cambio en la producción de citocinas del tipo $Th2$ a citocinas del tipo $Th1$.

Objetivos: El objetivo fue evaluar la relación entre la forma soluble de CD30 (sCD30) y la inmunoterapia con veneno en pacientes con alergia al veneno de himenópteros.

Materiales y Métodos: Se analizaron las concentraciones séricas de sCD30 mediante enzimoanálisis de adsorción en 61 controles sanos y 14 pacientes con alergia al veneno de himenópteros que se habían sometido a inmunoterapia antes del tratamiento y 1, 3 y 12 meses después del inicio del tratamiento. Nueve pacientes resultaron ser alérgicos al veneno de *Apis*, 4 al veneno de la *Vespula* y 1 al de la *Polistes*.

Resultados: Las concentraciones séricas de CD30 fueron significativamente más altas en los pacientes alérgicos al veneno antes del tratamiento (mediana, 33,6 U/mL; rango, 14,8-61,6) que en los controles (9,7 U/mL, 1,9-21,3) ($P < 0,000$). Estos niveles disminuyeron progresivamente durante el tratamiento en todos los pacientes excepto en 2 ($P < 0,000$). Al tercer mes de tratamiento, los niveles alcanzaron una significación estadística en comparación con los valores de referencia.

Conclusiones: Este estudio muestra que las concentraciones de sCD30 son significativamente más elevadas en pacientes con alergia al veneno de himenópteros y confirma indirectamente la existencia de una citocina de tipo $Th2$ preferente en estos pacientes. La expresión de sCD30 disminuye durante la inmunoterapia, confirmando, por tanto, el papel inmunomodulador de este tratamiento cuando activa un cambio hacia una citocina de tipo $Th1$.

Palabras clave: Veneno de himenópteros. Alergia. Concentraciones séricas de CD30. Inmunoterapia.

Introduction

CD30 is a 105-120-kDa membrane glycoprotein belonging to the tumor necrosis factor receptor superfamily. It was originally described as a marker of Hodgkin and Reed-Stenberg cells in Hodgkin lymphoma [1]. A soluble form (sCD30) is released after activation by CD4⁺ and CD8⁺ T-cell clones that secrete type 2 helper T-cells (T_H2) cytokines [2]; thus, CD30 can be considered a marker of T_H2 response [1]. Indeed, increased expression of CD30 and the release of its soluble counterpart have been found in T_H2-related diseases such as Omenn's syndrome [3], several autoimmune disorders [4], and chronic infection by the hepatitis C virus [5] or human immunodeficiency virus (HIV) [6]. This process often occurs in correlation with disease activity.

The presence of increased CD30 expression in T cells with elevated levels of this soluble marker has been reported in adult patients with allergic disease, such as atopic dermatitis or atopic asthma [7,8], which are associated with a T_H2 response.

Hymenoptera venom allergy is an immunoglobulin (Ig) E-mediated disease governed by activated, allergen-specific T cells that produce T_H2-type interleukins (IL), such as IL-4 and IL-5 [9, 10]. Studies have shown that venom immunotherapy (VIT) is able to induce specific anergy in T cells, which in turn decreases production of T_H2-type cytokines [11].

We attempt to demonstrate whether sCD30 levels increase in patients with hymenoptera allergy and how VIT is able to modify these levels.

Patients And Methods

Patients

Fourteen patients (8 male) with a median age of 47.5 years (range, 22-67 years) were included in the study. The patients were referred to our allergy unit with a history of systemic reaction caused by a hymenoptera sting. According to a Muller reaction classification [12], 5 patients exhibited a type II reaction, 4 a type III reaction, and 5 a type IV reaction. The presence of venom-specific IgE allergy was demonstrated by an intradermal skin test performed using endpoint titration and allergen-specific IgE. Nine patients were allergic to *Apis*, 4 to *Vespula*, and 1 to *Polistes* venom. No patients had HIV infection or lymphoproliferative disorder.

Skin Test

The skin prick tests were performed with standardized venom extract from *Vespula*, *Polistes*, and *Apis* (Stallergenes, Milan, Italy). Histamine dihydrochloride (10 mg/mL) and glycerol diluents were used as positive and negative controls, respectively. Skin tests were considered positive if a reaction occurred 20 minutes after allergen injection. The skin prick test reaction was positive if the wheal diameter reached at least half that of the wheal induced by a positive control solution.

Successive intradermal tests were performed with 0.01, 0.1, and 1 µg/mL concentrations of *Vespula*, *Polistes*, and *Apis* venom extract (Stallergenes, Milan, Italy), respectively, by injecting 0.02 mL of solution into the volar forearm. The

reaction was read after 20 minutes and considered positive when the diameter of the wheal elicited was ≥ 5 mm. The lowest concentration yielding a positive reaction was considered the endpoint.

Allergen-Specific IgE

Allergen-specific IgE against *Polistes*, *Apis*, and *Vespula* venom were measured in sera using a fluorescence immunoassay (CAP, Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions. All specific IgE antibody values of 0.35 kU/L or more were considered positive.

Immunotherapy

VIT was carried out according to a rush protocol up to a maintenance dose of 100 µg/mL of venom (Stallergenes, Milan, Italy). This was injected subcutaneously on the outer side of the upper arm, within 3 days of hospitalization. The maintenance dose of 100 µg/mL was then administered at 4-week intervals and vital signs were monitored constantly. No patient experienced a systemic reaction, although 2 patients presented a local reaction, which did not require therapy.

CD30 Assay

After the patient's informed consent was given, CD30 was measured in serum samples from all the patients before VIT and 1, 3, and 12 months after the start of the treatment. Sera from 61 healthy blood donors served as controls. CD30 was detected from sera samples that had been kept frozen at -20°C before testing. CD30 levels were determined by a sandwich enzyme-linked immunosorbent assay (CD30 K1 antigen, ELISA; Dako, Glostrup, Denmark) according to the manufacturer's instructions.

Statistical Analysis

Data were expressed as the median and interquartile range, and analyzed by nonparametric tests. The median was compared between groups using the Mann-Whitney test. Differences between values recorded at different times during the study were evaluated using the Wilcoxon test and the Friedman test. Correlations were derived using the Spearman rank correlation coefficient (ρ). A 2-tailed P value < .05 was considered to be statistically significant. Statistical evaluation was performed using SPSS 11.0 statistical software.

Results

Before treatment, sCD30 serum levels were significantly higher in venom-allergic patients (median 33.6 U/mL; range 14.8-61.6) than in controls (9.7 U/mL; 1.9-21.3) ($P < .000$) (Figure 1). The sCD30 levels decreased progressively during treatment in all patients except 2 ($P < .000$) (Figure 2). In these 2 patients, we observed a slight decrease, but the sCD30 levels were already low at the start of treatment.

The sCD30 levels reached statistical significance after the third month of therapy (7.2 U/mL; 11.0-25.2) ($P < .001$). They decreased significantly between the first month (27.4 U/mL; 12.6-56.0) and the third month (7.2 U/mL; 11.0-25.2), and

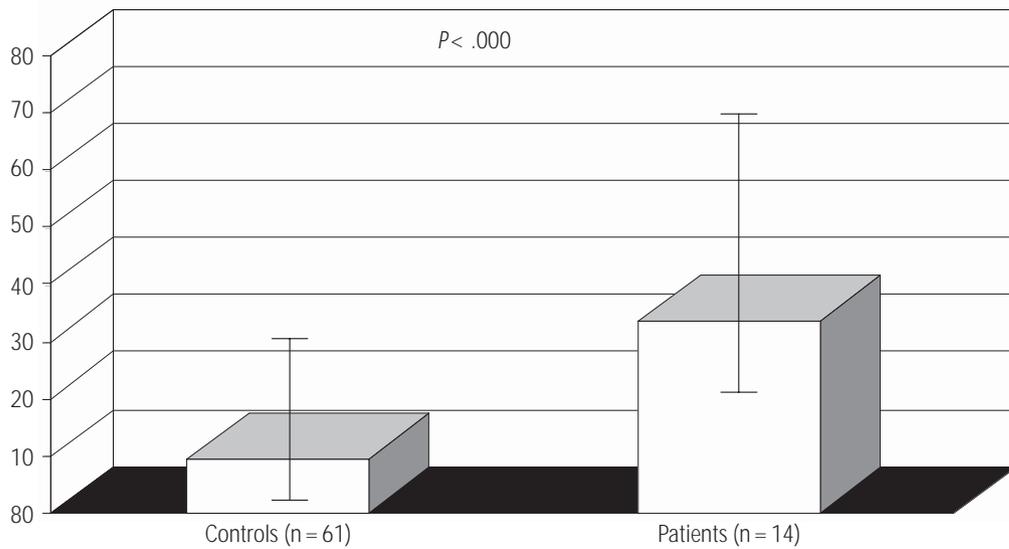


Figure 1. sCD30 Level in Control and in Patients.

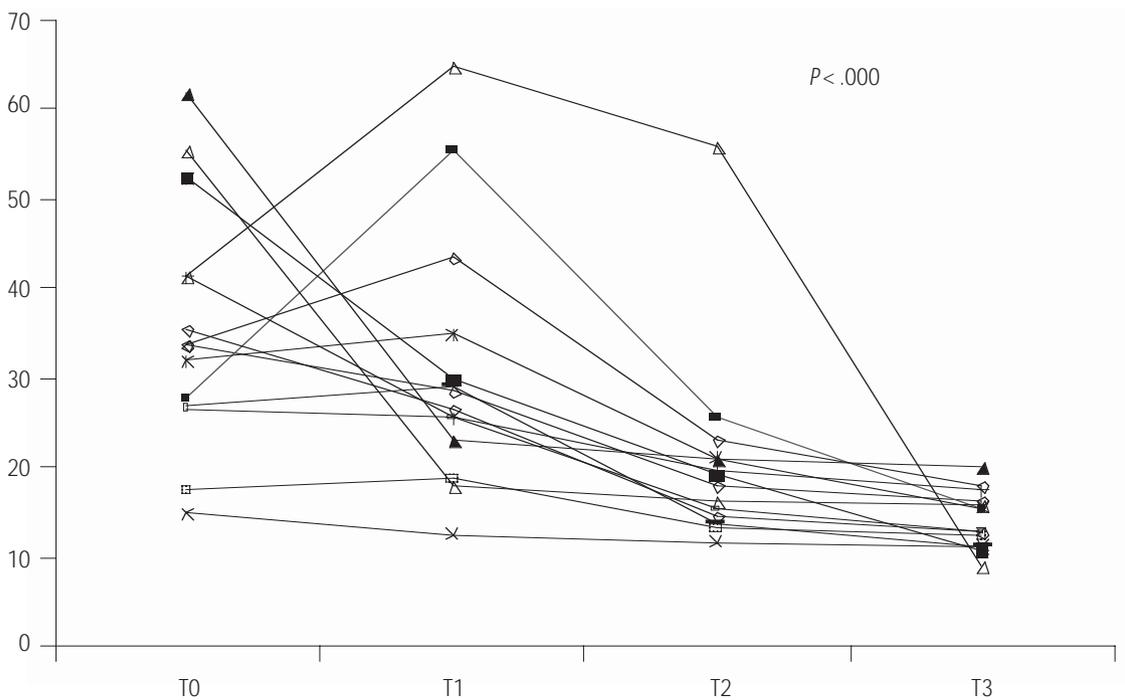


Figure 2. sCD30 Levels in Patients at Different Times After Venom Immunotherapy.

between the third month and the twelfth month (14.0 U/mL; 9.0-20.0) ($P < .001$). No significant decrease was observed between the start of the treatment and the first month of treatment.

In the third month, no significant differences were found between patients and controls. There were no statistically

significant differences between the *Apis* and *Vespula* groups and no correlation was observed between specific IgE, total IgE, type of reaction, type of venom, and sCD30. VIT was well tolerated and there were no severe reactions. Seven patients underwent spontaneous rechallenge with no problems. One patient, who was atopic, had an increased level of total IgE.

Table . Patient Characteristics

Patient	Muller Reaction	SPT (+) Lowest Concentration, $\mu\text{g/mL}$	RAST (class)	Total IgE	sCD30 (U/mL) ^a	Venom Allergy	Challenge (Number)
1	III	0.1	II (1.70)	104	33.52	<i>Vespula</i>	2
2	IV	0.1	II (2.37)	9	27.72	<i>Apis</i>	
3	III	0.1	II (3.01)	19	17.40	<i>Apis</i>	
4	II	0.1	II (2.6)	133	14.85	<i>Apis</i>	3
5	II	0.1	III (11.5)	42	26.56	<i>Vespula</i>	1
6	III	1	II (0.79)	145	33.87	<i>Polistes</i>	
7	IV	0.1	IV (18.4)	135	32.02	<i>Apis</i>	1
8	IV	1	I (0.4)	27	41.53	<i>Vespula</i>	1
9	II	0.1	II (1.61)	16	26.80	<i>Apis</i>	1
10	II	0.01	III (9.1)	233	35.61	<i>Apis</i>	
11	II	0.1	III (7.4)	150	61.60	<i>Vespula</i>	
12	III	1	0 (0.35)	5	55.45	<i>Apis</i>	1
13	IV	0.1	II (2.3)	19	41.30	<i>Apis</i>	
14	IV	0.1	V (67.5)	1170	52.43	<i>Apis</i>	

Abbreviations: IgE, immunoglobulin E; RAST, radioallergosorbent test; sCD30, soluble CD30; SPT, skin prick test.

Discussion

In the present study, we demonstrated high sCD30 levels in patients with hymenoptera venom allergy, as reported elsewhere for other atopic conditions [7]. However, exactly what regulates CD30 expression remains unknown, although this molecule is preferentially expressed by type 2 helper T cells [7]. This hypothesis is confirmed by the evidence that IL-4, a type 2 cytokine, is able to induce CD30 expression on activated CD4⁺ cells, whereas INF- γ antagonizes this induction [11]. In atopic dermatitis, the CD4⁺CD30⁺ T-cell count was higher in skin biopsies, and this increase correlates strongly with the ability of these cells to produce IL-4 and IL-5 [13].

However, the correlation between CD30 and certain cytokine profiles is still controversial [7,14,15]; some authors suggest that these molecules may not be used as a definitive marker T_H2 cells [7] and there is evidence that CD30 activation can induce apoptosis in the eosinophils of allergic asthmatic patients [16]; therefore, further studies are necessary to identify the immunoregulatory mechanisms of this pleiotropic molecule.

Nevertheless, many studies have demonstrated that CD30 and the release of its soluble counterpart are enhanced in T_H2-related diseases but not in TH1-related diseases [17]. Our data demonstrate the increase in serum CD30 levels in venom allergy and indirectly confirm the presence of preferential T_H2-type response in patients with venom allergy.

VIT is an effective treatment for hymenoptera venom

allergy [18]—a condition characterized by activated, allergen-specific T_H2 cells—and VIT is able to modify the immune response to the allergen. In fact, some authors have demonstrated the capacity of VIT to promote the T_H1 pattern of cytokine production [19,20]. We confirm an immunomodulatory effect of VIT in patients with venom allergy. It is interesting that VIT is able to modify the immune response in a few months; in fact, sCD30 levels decrease significantly after only 3 months. These data suggest that VIT has a fast immunomodulatory action and indirectly confirm a protective role against hymenoptera stings. In 2 patients, we observed a slight decrease in CD30 levels, but this observation could be related to the low levels of sCD30 at the start of treatment.

The limited number of patients does not allow us to establish a correlation between sCD30 levels and the severity of the reaction or other parameters. Nevertheless, it highlights the need for further studies to explore the complex immune system of patients with venom allergy.

To date, the only way to evaluate the effectiveness of immunotherapy is a sting challenge, but the danger involved means that this practice remains controversial for ethical reasons [21]. Therefore, a suitable laboratory test is urgently required. The increased levels of CD30 in hymenoptera venom allergy and the low levels observed after immunotherapy suggest a possible role for the circulating level of these molecules as a surrogate marker in response to VIT.

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