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Original Article

Plasma soluble CD30 level correlates negatively with age in children

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Background and Purpose: Atopic diseases are thought to be associated with cytokine-mediated immune dysregulation, for example, a T helper cell type 1/2 (Th1/Th2) imbalance. CD30 is proposed to be one of the surrogate markers for Th2 immunity. In this study, we investigated whether CD30 is a good marker for atopy and Th2 predominance in a pediatric population.

Methods: This study included 61 children with atopy and 27 normal controls. The expression of CD30 on the surface of T and B lymphocytes and soluble CD30 (sCD30) in plasma was determined.

Results: There was no difference in the surface expression of CD30 on B or T lymphocytes. Similarly, sCD30 levels in plasma were not different between the 2 groups. Nevertheless, we found a strong negative correlation between sCD30 and age in the control group ($r = -0.72$, $p<0.001$; sCD30 = $76.1 - 5.18 \times$ age) as well as in the atopy group ($r = -0.45$, $p<0.01$; sCD30 = $61.1 - 3.56 \times$ age).

Conclusions: An inverse relationship was found between age and sCD30 level in children. However, our findings suggest that CD30 is not a good marker for atopic disease and that further studies on sCD30 levels must take age into consideration.

Key words: Age factors; Antigens, CD30; Cytokines; T-lymphocytes

Introduction

Atopic diseases — bronchial asthma (BA), allergic rhinitis (AR), and atopic dermatitis (AD) — are caused by immunoglobulin E (IgE)-mediated allergic reactions, which involve acute as well as chronic inflammation. These atopic phenomena are especially relevant in children [1-3]. Cytokine-mediated immune dysregulation, for example, T helper cell type 1/2 (Th1/Th2) imbalance in response to environmental allergens is thought to be an important pathogenic mechanism of the disease [1-4]. Th2 cells predominantly produce interleukin (IL)-4, IL-5, and IL-13, thereby initiating allergic reactions by promoting IgE synthesis and eosinophil recruitment [5]. Indeed, predominance of Th2 cells in circulation and in the local lesions of patients with BA, AR, and AD have been demonstrated [6-10]. In contrast, non-atopic persons show mainly Th1 immunity, which inhibits the development of Th2 cells [11]. A deficiency in the ability of stimulated T cells from atopic patients to produce Th1, but not Th2 cytokines, has also been documented [12]. It is therefore expected that successful treatment of atopic disease would be accompanied by downregulation of Th2 cells, though little evidence has been presented showing changes in Th1/Th2 balance during the course of atopic diseases.

The primary immune response to allergens is the prototypic Th2 response, which occurs prenatally, favored by the normal Th2-skewed immune response of pregnancy. The immune system matures during the early years of life. Immune responses, primarily determined by genetic susceptibility, are also influenced by exposure to allergens and infections, which may reverse their direction [13].

The surface antigen CD30 is a 120-kD membrane-bound glycoprotein belonging to the tumor necrosis

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factor receptor superfamily [14,15]. It has been shown that surface CD30 is an activation marker of Th2-related cytokine-producing T cell clones [16,17]. The soluble form of the molecule (sCD30) is released into the blood after cellular activation, and elevated serum levels of sCD30 have been reported in patients with AD [18-20], but not in patients with AR or BA [21]. To the best of our knowledge, the only available report involving atopic children also showed elevated sCD30 levels in AD patients [21]. In this study, we collected complete atopy-related clinical and laboratory data, which we analyzed to investigate if surface and/or soluble CD30 levels are related to atopy or patient age.

Methods

Patients

From March to June 2001, we recorded and evaluated the atopy conditions, including BA, AR, and AD, of all patients visiting our outpatient clinic and those allowed to participate in complete studies were recruited. The diagnosis of BA was based on the global initiative for asthma guidelines [22]. AR was diagnosed by repeat symptoms, including congestion, rhinorrhea, itching, sneezing, and non-nasal symptoms and signs proposed by Meltzer et al [23]. AD patients were assessed using the severity scoring AD (SCORAD) index devised by the European Task Force on AD [24]. Essential laboratory tests included serum IgE, eosinophil count, eosinophil cationic protein, and peak expiration flow rate. Flow volume curve was optional. Our controls were recruited from the Pediatric Surgery Outpatient Department. One physician reviewed the atopy history as well as systemic condition of those patients scheduled for elective surgery, such as circumcision or herniorrhaphy. Only those with normal IgE levels and without a personal or family history of atopy were recruited as controls.

Flow cytometric analysis of lymphocyte cell surface antigens

The cell phenotype of lymphocytes was analyzed by flow cytometry performed on whole blood samples. Blood was obtained from patients and control subjects. Fresh blood (100 μL) was incubated with 20 μL of experimental monoclonal antibodies (fluorescence-labeled CD3, CD19, CD26, and CD30 antibodies [Becton Dickson, Franklin Lakes, NJ, USA]) for 30 min at room temperature. Erythrocytes were removed with fluorescence-activated cell sorting lysing solution (Becton Dickinson, Cowley, UK). Non-specific fluorescence was controlled by incubation with fluorescein isothiocyanate-IgG1 and phosphatidyl ethanolamine-IgG2 isotype-specific control antibodies (Becton Dickson, San Jose, CA, USA). Washed cells were analyzed on a flow cytometer (FACSCalibur; Becton Dickson, San Jose). Lymphocyte gating was determined on the basis of their forward and side scatters with identification of CD3CD26, CD3CD30, CD19CD26, and CD19CD30 (Leukogate; Becton Dickson, San Jose) expression. The data were analyzed using BD CellQuest Pro™ software (Pharmingen, Becton Dickinson Biosciences, San Diego, CA, USA) on a Macintosh computer.

Determination of sCD30

Plasma levels of sCD30 were measured by sandwich enzyme-linked immunosorbent assay using commercially available kits (Bender Med System, Vienna, Austria), according to the manufacturer's instructions. The detection limit of the assay was estimated to be 1.6 U/mL.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Student's unpaired *t* test was performed for comparison analysis between atopic patients and control subjects. Linear regression analysis was carried out to determine a possible correlation between values of CD26/CD30 and age. Differences were considered statistically significant when $p \leq 0.05$.

Results

We recruited 61 patients (31 males and 30 females; mean age ± SD, 6.34 ± 2.70 years). Their clinical diagnoses and case numbers were as follows: BA = 45, AR = 51, BA+AR = 35, BA+AD = 8, AR+AD = 9. Their plasma IgE level was 656.0 ± 669.8 KU/L. The 27 controls (19 males and 8 females; mean age ± SD, 5.58 ± 2.97 years) had normal plasma IgE levels with regard to their age (113.0 ± 88.4 KU/L). There was no difference in the total eosinophil count and eosinophil cationic protein between these 2 groups.

There was no difference in T and B lymphocyte CD30 expression or sCD30 levels between atopic children and controls, as shown in Table 1. sCD30 level showed a strongly negative correlation to age in both the control ($r = -0.72$, $p<0.001$) and patient ($r = -0.45$, $p<0.01$) groups. Fig. 1 and Fig. 2 show the distribution

Table 1. Comparison of soluble CD30, T and B cell surface CD30 between patient and control groups ($p>0.05$)

	Age (years; mean ± SD)	Soluble CD30 (U/mL; mean ± SD)	T cell surface CD30 (%; mean ± SD)	B cell surface CD30 (%; mean ± SD)
Patients	6.34 ± 2.70	38.8 ± 21.8	0.84 ± 0.59	0.37 ± 0.13
Controls	5.58 ± 2.97	47.0 ± 21.2	0.59 ± 0.4	0.5 ± 0.63

Abbreviation: SD = standard deviation

of the variables and calculated regression formula for the control and patient groups, respectively. On the other hand, neither T cell surface CD30 (r = –0.24 for patients, r = 0.036 for controls, $p>0.05$) nor B cell surface CD30 (r = –0.1 for patients, r = –0.33 for controls, $p>0.05$) had any correlation with age in these 2 groups (Table 2).

Discussion

In this study, we found that age and sCD30 level were significantly negatively correlated. One report pointed out the relationship between age and sCD30 but did not consider atopy status [25]. In that study, sCD30 levels in children less than 5 years old were higher than those in older children and adults. There was no correlation study mentioned. Cavagni et al [26] also noted higher sCD30 levels in non-atopic children than in adults. However, another study [27] found that sCD30 levels were not significantly age dependent. As shown in Fig. 1 and Fig. 2, the slope is steeper in case of the control subjects than in patients.

Because all the patients included in the study were between 2 and 14 years of age, this regression formula may not be applicable to those beyond these ages. The forecasted sCD30 level at birth was higher in the control than in the patient group. This forecast can only be confirmed by checking the stored cord sera and looking for atopy development in the corresponding, at least 2-year-old, children.

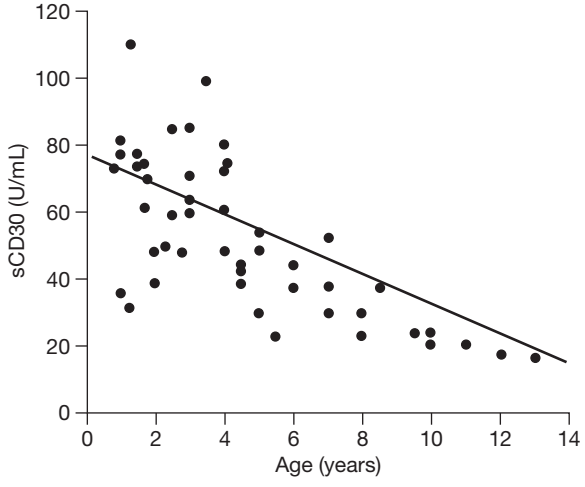

Fig. 1. Regression curve showing the relationship between soluble CD30 (sCD30) levels and age in the control group (regression formula: Y = –5.18 X + 76.1).

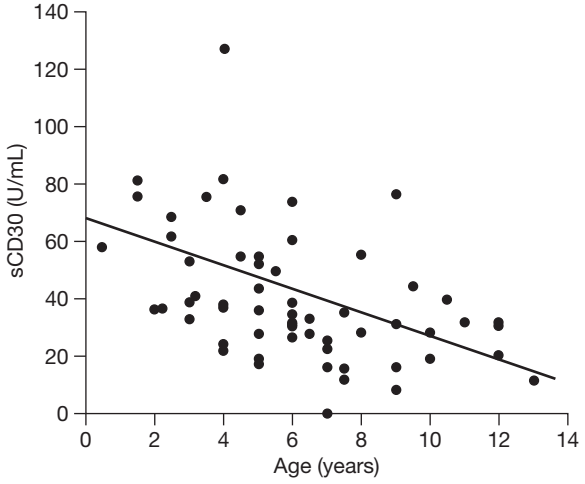

Fig. 2. Regression curve showing the relationship between soluble CD30 (sCD30) levels and age in the patient group (regression formula: Y = –3.56 X + 61.1).

We tried to obtain complete data using strict criteria to allocate cases into patient or control groups. Our results were not found to be consistent with our hypothesis or the limited available reports [7,12,14,15,17, 23-25]. We found no difference between control subjects and patients in terms of soluble or surface CD30 expression. One explanation is the difference in patients' atopic conditions. Most reports [17,18,24] dealt

Table 2. Correlation coefficients between parameters

		Soluble CD30	T cell surface CD30	B cell surface CD30	Age
Soluble CD30	Patients	–0.13	0.33	–0.72[a]	
	Controls	–0.06	0.28	–0.46[a]	
T cell surface CD30	Patients		–0.06	–0.1	
	Controls		0.04	0.04	
B cell surface CD30	Patients			–0.24	
	Controls			–0.33	

[a] $p<0.05$.

with AD cases. In contrast, the data on AD children in our study were quite limited and there was no case of isolated AD. Only 10 children had moderate-to-severe AD, with SCORAD from 10 to 96, and the sCD30 levels of those 10 patients were not elevated. Furthermore, the authors of another study [19] mentioned that elevated sCD30 levels are associated with AD, but not with respiratory atopy.

The second possibility was the difference in age between the patient and control groups. Several reports [18,26] did not provide the ages of the control subjects, and another report [19] did not compare the difference in age. We observed no difference in sCD30 levels after adjusting for the age of the control group.

In summary, our study suggests that CD30 is not a good marker for atopic disease. However, we found an inverse relationship between age and sCD30 level in children that increases understanding of sCD30. We suggest that further studies on sCD30 levels must take age into consideration.

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