

nAssessment of Costimulatory Molecules in Children with Allergic Asthma

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Abstract: Objective: Bronchial asthma is a complex heterogeneous disease of airway, which is associated with genetic and environmental factors. A complex network of enhancing and inhibitory costimulatory signals regulates T-cell differentiation and effector functions which play a central role in mediating the pathogenesis in allergic asthma. The aim of this study was to evaluate and clear the role of CD86 and cytotoxic T lymphocyte antigen-4 (CTLA-4) as a possible immunopathogenic and etiologic factors in asthmatic patients and to find the relationship of these proteins with each other.

Key words: Children, costimulatory molecules, allergic asthma.

INTRODUCTION

The prevalence of allergic asthma, especially with children, has been increasing worldwide. Allergic asthma is a chronic inflammatory disease of airway characterized by spontaneous airflow limitation lung tissue remodeling, increased plasma IgE concentration, and airway hyper responsiveness, with the infiltration of lymphocytes and eosinophils into the airway submucosa (Finkelman and Vercelli, 2007). It is characterized by an imbalanced T-helper 1 and T-helper (Th1/Th2) cytokine profile with a predominant production of Th2 cytokine, such as interleukin-4(IL-4), IL-5 and IL-13 (Owen, 2007).

Aberrant activation, recruitment, and differentiation of T lymphocytes are critical elements in the pathogenesis of allergies and asthma. Optimal T-cell activation requires not only the engagement of T-cell receptor (TCR)/CD3 complex with foreign antigen associating with major histocompatibility complex (MHC) classII molecules, but also costimulatory signals by the interaction between costimulatory molecules CD28 on T lymphocytes and its ligands B7 family molecules, B7.1(CD80) and B7.2 (CD86), on antigen presenting cells (APC) (Wong *et al.*, 2005). CD28, constitutively expressed on T lymphocytes, interacts with either CD80 or CD86 on the surface of APC to initiate stimulatory signal for T-cell activation, cytokine production, and differentiation. CTLA-4 is a member of immunoglobulin superfamily, which is structurally homologous to CD28. CTLA-4 is potently induced and expressed on activated T lymphocytes (Ipwk *et al.*, 2006) It binds to both CD80 and CD86 with 20-to100-fold higher affinity than CD28, and transmits an inhibitory signal which opposes the action of CD28 on T lymphocytes. CTLA-4 will preferentially bind to B7 molecules and therefore, its inhibitory interaction eventually predominates, leading to the downregulation of T-cells responses and induction of apoptosis as well as immunological anergy. CTLA-4 is also expressed on activated B lymphocytes and even activated monocytes. It may also have a negative regulatory role for both humoral immune response and monocyte functions (Ipwk *et al.*, 2005).

The B7- family molecule CD86 is a type I transmembrane glycoprotein expressed on the surface of pulmonary and thoracic lymph node APCs. It delivers essential co stimulatory signals for T-cell activation in response to inhaled allergens. CD86-CD28 signaling has been proposed for the induction of lung mucosal Th2 immune response and altered airway responsiveness (Corydon *et al.*, 2007).

Sayegh (1999) demonstrated that CD28, CTLA-4, and B7 co-stimulatory pathways play an important role in the development of airway inflammation and airway hyper responsiveness in asthma. These pathways provide key second signals regulating the activation, inhibition, and fine-tuning of T-cell responses. The surface expressions of costimulatory molecules on peripheral blood mononuclear cells (PBMCs) from asthmatic patients and their significance in the pathogenesis of asthma have been studied. Allergen-specific T-cell proliferation and cytokine expression were dependent on CD28-CD86 costimulation in allergic patients (Van Neerven *et al.*, 1998).

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MATERIALS AND METHODS

The present study was carried out on 20 children patients suffering from allergic bronchial asthma (14 males and 6 females). Their ages ranged from 2 to 11 with a mean (\pm SD) (5.85 ± 2.13) years.

- Ten, sex and age matched apparently healthy individuals (3 females and 7 males) were also included as a control group.
- All subjects were free from upper respiratory tract infection for 2 weeks preceding the study. The patients were selected from the inpatients in Al-Zahraa University Hospital Pediatric Department, others were recruited when they were attending outpatients pediatric clinic in Al-Zahraa University Hospital. Data were collected from October 2005 to January 2006.
- All patients were diagnosed according to the global strategy guidelines for diagnosis of asthma (GINA guideline) based on day time symptoms, nocturnal symptoms and lung functions (Global strategy for asthma management and prevention, 2002).
- All our studied asthmatic patients were on short acting bronchodilator as needed either orally or by inhalation.
- All patients neither had oral steroid intake nor theophylline therapy.

All Patients and the Control Group Were Subjected to the Following Procedures:

1. Full medical history and clinical examination.
2. Lmonary function tests:
3. Plain chest X-ray: postero anterior and lateral views
4. Laboratory investigations for sputum smear for acid fast bacilli, Tuberculin skin test.
5. A fresh peripheral blood sample is collected by vein – puncture for:
6. Complete and differential blood count.
7. Erythrocyte sedimentation rate (ESR).
- 8 .Liver and kidney function tests.
9. Estimation of total IgE level in serum by ELISA.
10. Estimation of sCTLA-4 level in serum by ELISA.
11. Detection of CD86 expression on PBMCs in whole blood with EDTA by flow cytomet.

sCTLA-4 Level and Total IgE Estimation:

Serum sample was separated and stored at -20°C for detection of sCTLA-4 level and total IgE done by ELISA technique using a kit supplied by Bender Med Systems.

Determination of CD86 by flow cytometry:

Flowcytometric analysis was performed by kit supplied by DAKO using Becton Dickinson FACS caliber. In briefly; $10\mu\text{l}$ of fluorescein isothiocyanate (FITC) conjugated monoclonal mouse antihuman CD86 antibodies were added and mixed with $100\mu\text{l}$ of fresh EDTA blood sample and was incubated at dark for 30 min. Then, a lysing reagent was added in order to lyse the red cells and separate the mononuclear cells. Then washed twice with phosphate buffered saline (PBS) containing 2% bovine serum albumin. Supernatant was removed and the cells were suspended in an appropriate fluid, vortexed, 0.3ml of 1% paraformaldehyde in PBS were added to the sedimented cells as a fixative. Negative control was included in each run to determine cut – off value between negative and positive population of cells. Analyzed on flow cytometer within, 1hour using forward & side scatter. Gating was set on monocyte.

RESULTS AND DISCUSSION

This work was carried out on twenty patients suffering from bronchial asthma. Their ages ranged from 2 to 11 with a mean (\pm SD) value of 5.85 ± 2.13 years. Asthmatic patients were non steroid patients, who treated with bronchodilator only and ten apparently healthy individuals were also included in the study as a control group. There was a highly significant increase of the total IgE, sCTLA-4 levels and CD86 expression in asthmatic non steroid patients group as compared to the control group as seen in Table (1) and Figs. (1-3).

Table 1: Comparison between the control group and asthmatic patients on non steroid therapy.

| Parameters | Control group Mean \pm SD N=10 | Asthmatic non steroid group Mean \pm SD N=20 | P | Significance |
|-----------------|----------------------------------|--|-------|----------------|
| Total IgE Iu/ml | 33.96 \pm 16 | 96.74 \pm 27.68 | 0.001 | H.S \uparrow |
| sCTLA-4ng/ml | 1.17 \pm 0.40 | 5.84 \pm 2.09 | 0.001 | H.S \uparrow |
| CD86% | 44.50 \pm 10.39 | 62.45 \pm 13.35 | 0.001 | H.S \uparrow |

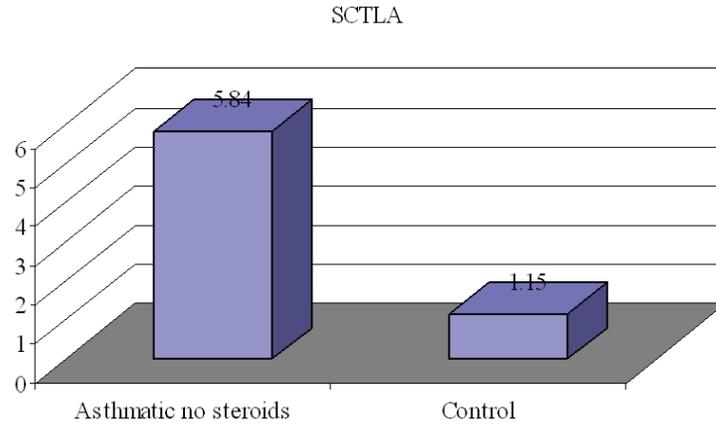


Fig. 1: Comparison of sCTLA between asthmatic non-steroid patients and control group.

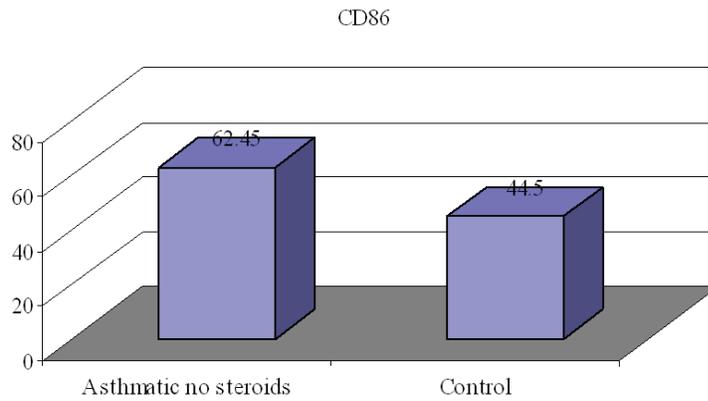


Fig. 2: Comparison of CD86 between asthmatic non-steroid patients and control group.

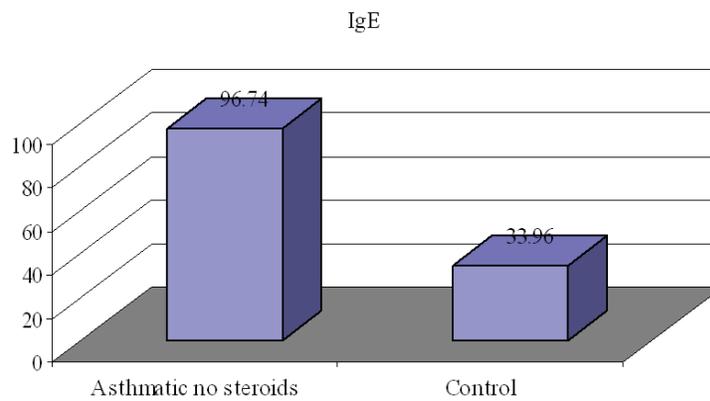


Fig. 3: Comparison of s IgE between asthmatic non-steroid patients and control group.

Table 2: Correlation study between sCTLA-4 and other studied parameters in asthmatic non steroid patients group.

| Parameters | r | Significance |
|------------|-------|--------------|
| CD86 | 0.658 | +ve HS |
| Total IgE | 0.673 | +ve HS |

There was a highly significant positive correlation between sCTLA-4 level and CD86 and the total IgE in asthmatic non steroid patients group.

Discussion:

Bronchial asthma is a complex disease resulting from interactions between multiple genes and environmental factors. Asthma is one of the most common disorders encountered in clinical medicine in both children and adult (Chan *et al.*, 2006). The prevalence of asthma among Egyptian school children aged 5-15 years was found to be 8.2% (Esmat *et al.*, 2006). The asthmatic response to allergens is characterized by elevated production of IgE, cytokines, chemokines, mucus hyper-secretion, airways obstruction, eosinophilia and enhanced hyper reactivity (AHR) (Ishikawa *et al.*, 2006).

A complex network of enhancing and inhibitory co stimulatory signals regulates T-cell differentiation and effector functions. Activation, differentiation, and production of TH2 cytokines as interleukine (IL)-4, IL-5 and IL-13 over TH1 cytokines such as interferon- γ may play a central role in mediating the pathogenesis in allergic asthma (Kallinich *et al.*, 2007).

CTLA-4 is a member of the immunoglobulin super family and a structural homologue of CD28. It is expressed only on activated Th cells and plays a negative regulatory role in T cells by transmitting an inhibitory signal to terminate immune response (Beier *et al.*, 2007). sCTLA-4 mRNA has been shown to be constitutively expressed on non stimulated T cells, and its expression is down regulated after activation (Wong *et al.*, 2005).

The B7- family glycoprotein expressed on the surface of pulmonary and thoracic lymph molecule CD86 is a type I transmembrane node APCs. It delivers essential co stimulatory signals for T-cell activation in response to inhaled allergens. CD86-CD28 signaling has been propose for the induction of lung mucosal Th2 immune response and altered airway responsiveness (Corydon *et al.*, 2007).

In the current study, the diagnosis of asthma was assessed by the total IgE level as a markers of allergy and our results showed that there was a highly significant increase of serum total IgE level (mean96.74 \pm 27.68 Iu/ml) in all asthmatic patients (P<0.01) as well as the non steroid group (P<0.001) when compared to control group.

The results within hand are in agreement with Borish *et al.* (2005) who reported that level of serum IgE was considerably higher in asthmatics than non asthmatic controls. They found that level of serum IgE was more than 100 Iu/ml in 30% of patients. Since IgE is considered to play a crucial role in allergic immune responses, the reduction of free IgE level has been an attractive target in the treatment of allergic diseases (Szeffler *et al.*, 2005).

As regards to sCTLA-4 the present study showed that there was a highly significant increase in the level of serum sCTLA-4 (mean 5.84 \pm 2.09 ng/ml) in all asthmatic patients (P<0.001) as well as the non steroid when compared with control.

These results were consistent with those of Ipwk *et al.* (2006) who found that the serum sCTLA-4 protein level was significantly elevated in patients with asthma and this level correlated with the severity of asthma. Wong *et al.* (2005) stated that plasma sCTLA-4 concentration was significantly higher in asthmatic patients treated or not treated with steroids than that of control subjects. It is possible that the elevation of sCTLA-4 in allergic asthmatic patients is derived from the cleavage of mCTLA-4 along with increasing disease severity. Regarding the expression of CD86 molecule, most of the studies investigated its cell surface expression on eosinophils, alveolar macrophages, DCs and B lymphocytes in patients with allergic asthma. However, the cell surface expression of CD86 on PBMCs has not been well investigated in adult patients with allergic asthma (Wong *et al.*, 2005).

The present study showed that there was a highly significant increase in CD86 expression on PBMC (measured by FCM) (mean 62.45 \pm 13.35%) in all asthmatic patients (P<0.001) as well as non steroid asthmatic group (P<0.01) when compared with the control group. The results were consistent with those of Zhu *et al.* (2004) who found an increase in the expression of CD86 on PBMC in children with acute asthma and they suggested that CD86 and imbalance of TH subset might play an important role in the occurrence of asthma. Chen *et al.* (2006) examined the expression of CD86 on mature DCs (mDCs). They found that CD86 expression on mDCs from allergic asthmatics was higher than that from healthy control, so they hypothesized that mDCs from allergic asthmatics preferentially priming naïve T cells towards Th2- cell development might be due to increased expression of CD86 and reduced production of IL-12 and IL-10.

Many studies were done on serum sCD86 Ipwk *et al.* (2006) concluded a significantly increased level of serum sCD86 protein after allergen inhalation in sensitized asthmatic subjects, which correlated with the severity of asthma and down regulated by prednisolone therapy. The sCD86 was most probably derived from monocytes in the peripheral blood. Corydon *et al.* (2007) have documented that CD86 gene polymorphism might be a novel etiological factor in the development of asthma and related allergic disorders.

The present study demonstrated that there was a highly significant positive correlation between sCTLA-4 level and CD86 and total IgE level non steroid group ($r = 0.658$), ($r = 0.673$) respectively. These results were supported by Wong *et al.* (2005) who reported that sCTLA-4 level was correlated positively and significantly with serum total IgE concentration in the serum of patients with bronchial asthma. The study showed that there was a highly significant positive correlation between CD86 and total IgE level in non steroid group ($r=0.856$), and this agreement with Zhu *et al.* (2004) who reported a significant positive correlation between CD86 expression on PBMC and total IgE level in plasma of children with bronchial asthma. On the other hand, in contrast to our finding, Wong *et al.* (2005) found a non significant negative correlation between sCTLA-4 level and sCD86 level in the asthmatic patients. This contradiction may be due to difference in the technique used, as we estimated CD86 surface expression on PBMC by FCM technique while Wong *et al.* (2005) estimated sCD86 level in plasma using ELISA technique.

Conclusion:

In conclusion, plasma CTLA-4 and expression of CD86 may be potential markers for assessing asthmatic children. Our study has reflected the roles of costimulatory molecules in T- cell activation in allergic asthma, and supported the need for further longitudinal studies with a larger cohort of patients. In addition to plasma, other clinical samples such as induced sputum, bronchoalveolar lavage, or bronchial biopsy may be useful to further investigate the roles and functions of costimulatory molecules in asthmatic children. The increased expression of CD86 and sCTLA-4 in children with allergic asthma may reflect the dysregulation of T cell activation, contributing to the immunopathogenesis of allergic asthma. There were highly significant increase in sCTLA-4, total IgE and CD86 expression on mono-nuclear cells (PMNC) in asthmatic children when compared with controls ($P < 0.001$). Additionally, there was a highly significant correlation between sCTLA-4 level and CD86 expression in asthmatic children.

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