CD23, TH1/TH2 CYTOKINES IN BRONCHIAL ASTHMA, BRONCHIOLITIS AND BRONCHIAL PNEUMONIA IN PEDIATRIC PATIENTS.

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ABSTRACT

Background: CD23, a low affinity receptor for IgE, was investigated in children with allergy. We also determined CD23 in stool samples to examine the possibility of food allergy in children with asthma or bronchiolitis
Methods: CD23 was determined in buccal mucosa and stool samples, sCD23, Histamine release, total IgE and various cytokines were determined in blood samples of children suffering from bronchial asthma (n = 23), bronchiolitis (n = 20) and bronchial pneumonia (n =20) and age & sex matched normal children (n = 20) who were taken as controls.
Results: Serum sCD23 was significantly increased (p<0.01) in asthma (1209.8 ± 68.01 pg/mL), bronchiolitis (1455.52 ± 146.92 pg/mL) and bronchial pneumonia (1406.35 ± 98.26 pg/mL) when compared to controls (691.5 ± 74.94 pg/mL). Serum IgE and blood histamine levels were increased significantly (P<0.05) and IFN-γ (Th1 cytokine) was significantly lower (P<0.01) in both bronchial asthma and bronchiolitis than in controls. Serum IL-4 and CD23 either in buccal mucosa or stool samples were below detectable levels in all the subjects studied.
Conclusion: Our observations provide evidence on CD23 expression in children with and without asthma and a preferential activation of Th2 (IL-5) and suppression of Th1 (IFN- γ) in children with asthma. CD23 in buccal mucosa or stool samples were below detectable level suggesting lack of food sensitivity in the study subjects.

Keywords: CD23, Allergy, Cytokines, IgE, Histamine, Bronchial asthma, Bronchiolitis, Bronchial pneumonia

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular mast cells, eosinophils, and activated T lymphocytes. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli. Asthma can occur at any age but recurrent episodes of wheezing and airway obstruction manifest before the age of 6 years in most patients (Gern JE et al.,1999). Bronchiolitis is a common cause of illness and is the leading cause of hospitalization in infants and young children. It is a lower respiratory tract infection that occurs in children younger than two years old. It is usually caused by a virus which promotes the inflammation of the small airways. This inflammation partially or completely blocks the airways, which causes wheezing (Bush A and Thomson AH, 2007).

CD23, also called Fc€ RII, is a member of the C-type lectin super family that is the low-affinity Fc receptor for IgE, the antibody isotype that mediates allergic inflammation (Susan E and Coffin, 2005). The CD23 is expressed on B-lymphocytes, T-lymphocytes, monocytes, platelets and eosinophils (Conrad D.H, 1990; Mohamed Saleh Mohamed, 2008), which may play a role in triggering IgE mediated effector function (El-Helal et al., 2009). Previous studies have shown that increased serum levels of sCD23 in allergic patients suggesting CD23 as a potential diagnostic marker of atopic disease. T-cells control IgE production by B-cells and activate nonspecific effector cells (Di Lorenzo G et al.,1995). In atopic individuals, stimulation by allergens determines an increased synthesis of IgE and the up-regulation of CD23 (Di Lorenzo G et al., 1996).

From both clinical and experimental evidence, asthma is now viewed as an inflammatory airway disease involving lymphocyte activation and the release of pro-inflammatory cytokines (Harb Harfi, et al., 2010; Ishizaka K et al., 1966; Monteseirin J et al.,1993). It is associated with a predominant Th2 immune response. IL-4 has been anticipated that the CD4+ T cells that produce Th2 cytokines, including IL-4, IL-5, IL-9 and IL-13 which up regulate IgE production, play a pivotal role in the pathogenesis of the disease (Mathew et al., 2009). And IL-4 which is a primary product of Th2 cells is a most potent up-regulator of CD23 antigen.

Since CD23 and other allergic mediators play a crucial role in allergic diseases, we investigated the expression of CD23, total IgE, Th1/Th2 cytokines and histamine levels in children with bronchial asthma, bronchiolitis and bronchial pneumonia.

MATERIALS AND METHODS

Subjects and controls

Children visiting the Allergy clinic, Niloufer hospital Hyderabad, suffering from Asthma, bronchiolitis, bronchial Pneumonia (total no. of patients 63) were recruited and age (1-5yrs) & sex matched siblings of the patients (N=20) were taken as controls. Blood samples were obtained from all of the above patients after standard questionnaire to estimate the serum CD23, histamine release, total IgE and various cytokines. CD23 was also determined from buccal mucosa and stool samples. Informed consent was obtained from the parents of the patients and control subjects. Ethical approval was taken from the hospital management for collection of the samples and to carry out the project.

Measurement of cytokines, CD23, total IgE and Histamine

Cytokines IL-4, IL-5 and IFN-γ in the serum were measured on fluorescent-coded beads known as microspheres, which are then read in a compact analyzer (Luminex xMAP technology, Milliplex, Millipore). The sCD23 estimated in using Immunoassay kit (Quantikine Human CD23/ Fc€RII) which employs the quantitative sandwich enzyme immunoassay technique. Histamine release test was performed according to Immunotech EIA histamine kit instructions. Serum IgE levels were estimated by a solid phase ELISA (UBI MAGIWEL Total IgE Quantitative ELISA kit) based on the sandwich technique. Biotek multimode (micro well) detector system was used to estimate the concentration of IgE at 450nm.

Statistical analysis

Kruskal-Wallis one way ANOVA was done to see the difference between groups for different parameters. Results were presented as mean± Standard error of the mean (SEM). Pearson's correlation coefficient was done to see relation between the parameters.

RESULTS

Controls

The mean total IgE, total histamine and CD23 were 191.1 (IU/mL), 44.38 (nM/mL) and 691.5 (pg/mL) respectively in the controls. IFN-γ and IL-5 were detectable in all the children, whereas IL-4 was below detectable range in all the children studied. The mean IL-5 and IFN-γ were 1.61 (pg/mL) and 57.4 (pg/mL) respectively (Table 1). The Pearson's correlation coefficient showed a significant (P<0.05 r= -0.61) inverse correlation between total IgE and IFN- γ.

Table 1: Serum CD23, Total Histamine & Cytokine profile in children with Bronchial asthma, Bronchiolitis and Bronchial pneumonia

Parameters	Control (n=20)	Bronchial Asthma (n=23)	Bronchiolitis (n=20)	Bronchial Pneumonia (n=20)
Total IgE (IU/mL)	191.12 ±40.96** (85.82, 296.41)	462.52 ± 48.33 (360.54, 564.5)	429.72 ± 10.69 (293.84,565.61)	42.66 ± 16.68 (23.26, 62.06)
Total Histamine (nM/mL)	44.38 ± 7.16** (21.59, 67.16)	63.27 ± 2.63 (57.55, 69)	54.37 ± 6.69 (33.09, 75.66)	49 ± 1.2 (33.72, 64.27)
Serum CD23 (pg/mL)	691.5 ± 74.94## (452.99, 930)	1209.8 ± 68.01 (1065.62,1353.98)	1455.52 ±146.92 (987.96,1923.08)	1406.35 ± 98.26 (1093.63,1719.07)
Cytokines IL4 (pg/mL)	ND	0.06 ± 0.06 (-0.07, 0.19)	ND	ND
Cytokines IL5 (pg/mL)	1.68 ± 0.49* (0.92, 2.44)	3.95 ± 0.46 (2.9, 5.01)	1.6 ± 0.36 (0.46, 2.75)	1.14 ± 0.36 (0.23, 2.05)
Cytokines IFNγ (pg/mL)	57.49 ± 15.34 @ (8.66, 106.31)	9.37 ± 1.43 (6.26, 12.47)	35.66 ± 5.04 (13.96, 57.36)	101.28 ± 26.26 (11.7, 203.20)

Results were expressed as Mean ± SE, in the parenthesis range values (least, highest) were given, **ND**-Not detectable.

** p< 0.01 Significantly different from bronchial asthma and bronchiolitis
p <0.01 Significantly different from the rest of the groups
* p<0.05 Significantly different from bronchial asthma
@ p <0.01 Significantly different from bronchial asthma

Bronchial asthma group

The mean IgE and histamine were 462.5(IU/mL) and 63.3(nM/mL) respectively in children with bronchial asthma. Both Total IgE and total histamine were significantly higher when compared to control children. Serum CD23 ranged from 1065 to 1353 with mean of 1209.8(pg/mL) and was significantly higher in children with bronchial asthma when compared to controls. IFN-γ was significantly low and IL-5 was significantly higher in children with bronchial asthma. Pearson's correlation coefficient showed a significant (P<0.05, r=0.50) association between IL-5 and total serum IgE concentration, however, IFN- γ or CD23 were not correlated with IgE.

Bronchiolitis group

The mean IgE, histamine and CD23 were 429.7 (IU/mL), 54.3 (nM/mL) and 1455.5(pg/mL) respectively in children with bronchiolitis. Both total IgE and total histamine were significantly higher (P<0.01) when compared to control group. The serum CD23 was significantly higher (P<0.01) in bronchiolitis when compared to control group. However the cytokines such as IL-5 and IFN- γ of bronchiolitis children were comparable to control group. The Pearson's correlation coefficient showed a significant (P<0.05 r=-0.95) inverse correlation between IL-5 and IFN-γ. However total IgE and CD23 did not show any correlation with IL-5 and IFN-γ.

Bronchial Pneumonia group

The mean IgE, histamine and CD23 in bronchial pneumonia are 31(IU/mL), 49.0 (nM/mL), and 1406.35 (pg/mL) respectively. The total IgE levels, total histamine levels in bronchial pneumonia group were comparable, where as serum CD23 levels were significantly (P<0.01) higher when compared to control group and were comparable to the groups with bronchial asthma and bronchiolitis, however, IL-5 and IFN-γ were comparable to controls. The Pearson's correlation coefficient showed significant correlation between IL-5 and Total IgE (P<0.01, r= 0.98) and Total IgE & IFN-γ (P<0.01, r= 0.90).

DISCUSSION

Allergic diseases are complex disorders in which inflammatory and immunological mechanisms are involved (Gould et al., 2003). The activity of IgE is associated with a network of proteins; prominent among these are its two principle receptors, FcεRI (high-affinity Fc receptor for IgE) and CD23 or FcεRII (Sheng-Chin Liao., 2004; Junior et al., 2011). Elevated levels of CD23 expression has been observed in humans with asthma, non allergic asthma and rhinitis (Sánchez-Guerrero et al., 1994). We found enhanced response of serum CD23 in bronchial asthma, bronchiolitis and bronchial pneumonia (Fig. 1B), where as CD23 was not secreted in stool or expressed on buccal mucosal cells. Though CD23 has been suggested as a marker to identify allergic condition, we found high levels of CD23 even in bronchial pneumonia, thus suggesting non specific response of CD23.

It was earlier anticipated that reduced microbial exposure in early life is responsible for a shift of the Th1/Th2 balance in the immune system towards the pro-allergenic Th2 response, This Th1/Th2 imbalance results in the clinical expression of allergy and/or asthma. Studies on mice and humans have shown Th2 cytokines [IL-4, IL-13, and IL-5] as major contributors to allergy and asthma (Ngoc et al., 2005). In our study serum IL-4 was below detectable level in all the subjects, nevertheless IL-5, an IgE stimulating cytokine was associated with bronchial asthma children (Fig. 1C).

We found a strong association of IL-5 with serum IgE in bronchial asthma. However, the bronchiolitis children showed an inverse correlation between IL-5 and IFN- γ. In normal children IFN-γ appears to down regulate the production of IL-5 and IgE but in Bronchial Asthma IL-5 contributes to increased IgE production.

From a number of studies it seems that IFN-γ might be one of the appropriate candidate marker of the prediction of bronchial asthma and allergy. Production of IFN-γ has been used as potential marker for the post natal immune maturation processes that are associated with the subsequent risk for the development of bronchial asthma or allergic diseases (Hugo PS Van Bever et al., 2009). The plasma histamine levels of acute asthmatics were reported to be when compared with normal subjects, while, whole blood levels were unchanged and urinary levels were slightly, but not significantly reduced in a study conducted by zobeiri (Zobeiri et al., 2009). But in our study whole blood histamine levels were raised in children with asthma and bronchiolitis where as the levels were comparable in bronchial pneumonia (Fig. 1E). Histamine affects the maturation of dendritic cells and specifically regulates the development of Th1 and Th2 T cells (Jutel M et al.,2006; Helaly et al., 2009). Bronchial asthma, bronchiolitis and bronchial pneumonia are three most important and frequent diseases in children. Bronchiolitis is the most important risk factor for the development of asthma in infants (Bjorksten B et al., 1995). Previous studies have found lower IFN-γ production in bronchiolitis to be linked with abnormal pulmonary function and development of asthma at later life (Paolo M et al., 1999; Fahad N Et al., 2009; Ohshima Y et al., 1995; Amruta Pritam et al., 2007).
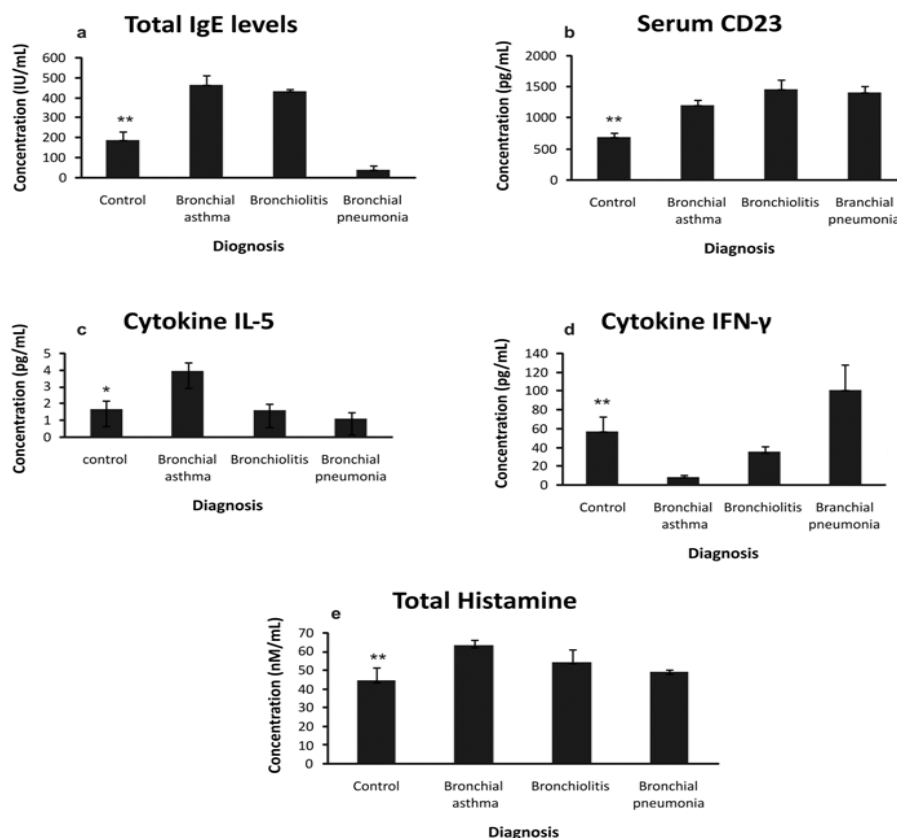
Fig. 1 Levels of total IgE, CD23, Cytokines and Blood Histamine. A) ** P < 0.01 significantly different from Bronchial Asthma and bronchiolitis, B) ** P < 0.01 significantly different from the rest of the groups, C) * P <0.05 Significantly different from Bronchial Asthma, D) ** P <0.01 Significantly different from Bronchial Asthma and E) ** P < 0.01 significantly different from Bronchial Asthma

In children with bronchial pneumonia where comparable to the levels in the control groups. Earlier studies have reported increased levels of CD23 in infants aged 7-12months, but not in older age groups. However, we found up regulation of CD23 in older allergic children as well. In addition, we found increased level of CD23 in bronchial pneumonia but the sample size was very low to conclude the involvement of CD23 in this condition. Patients were grouped into different clinical conditions based on clinical criteria following Nelson's pediatrics; whereas no viral or bacterial etiology was attempted. Nevertheless, all the children with bronchial pneumonia had high body temperature suggesting acute infection.

CONCLUSION

Our observations provide evidence on CD23 expression in children with and without asthma and a preferential activation of Th2 (IL-5) and suppression of Th1 (IFN- γ) in children with asthma. Absence of CD23 in stool samples suggests lack of food sensitivity in the study subjects.

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