Myeloid-Derived Suppressor Cells and Costimulatory Molecules in Children With Allergic Rhinitis

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Abstract

Objectives: The aim of this study is to assess the level of myeloid-derived suppressor cells (MDSCs) and the expression of costimulatory molecules CD80 and CD86 on monocytes and their ligands (CD28) on T-lymphocytes in children with allergic rhinitis (AR).

Methods: The study included 60 children with AR and 50 controls. Flow cytometry was performed to analyze MDSCs and the expression of costimulatory molecules CD80 and CD86 on monocytes and their ligands (CD28) on T-lymphocytes.

Results: The percentages of total and monocytic MDSCs and the expression of costimulatory molecule CD86 on monocytes were significantly higher in children with AR than in healthy controls. In addition, the expressions of CD28 on CD4+ and CD8+ were significantly elevated in AR patients.

Conclusion: The present study demonstrated that the percentages of MDSCs were significantly elevated in AR children. Moreover, the expressions of CD28 on CD4+ and CD8+ were significantly higher in children with AR.

Keywords

allergic rhinitis, children, CD28, CD86, myeloid-derived suppressor cells

Introduction

Allergic rhinitis (AR) is the most common allergic disorder of the airway. It is an allergic inflammation of the mucous membranes lining the nasal cavity.1,2 AR is a type I allergic disease of the nasal mucosa, characterized by watery rhinorrhea, repetitive paroxysmal sneezing, and nasal blockage. AR often shares the common risk factors of bronchial asthma, especially atopy.1,2 Genetic and environmental risk factors are involved in the pathogenesis of AR; however, the exact etiology remains to be identified.3 Currently, AR is a major health problem affecting 10% to 40% of children globally, which has a great influence on patients’ sleep and quality of life and can affect school achievement as well as increase the medical costs.2,3 T-lymphocytes are among the main factors that control and organize immune responses in allergic diseases. T-helper (Th)-1 T-cells release mainly interferon γ and interleukin (IL)-2 and are involved in the delayed hypersensitivity immune reactions, and Th2 T-cells release predominantly IL-4 and IL-5 and are predominantly involved in IgE-mediated allergic inflammation. AR is a disease with polarization toward Th2 and a defect of the regulatory T-cells.4 T-cell activation requires 2 signals5,6:

Signal 1 results from T-cell receptor recognition and binding to antigen presented by antigen-presenting cells.
(APCs); signal 2 is a costimulatory signal and results from binding of one of both molecules of the B7 family (CD80 and CD86) on APCs to receptors on T-lymphocytes (CD28). Costimulation is required for a productive immune response to occur. The lack of costimulation after engagement of T-cell receptor by antigen results in a state of antigen-specific unresponsiveness termed anergy. This places the costimulation pathway at a key location for controlling immune response.4,5 Previous studies have demonstrated an increase of costimulatory molecules B7.1 (CD80) and B7.2 (CD86), and CD28 in allergic and asthmatic patients6; however, the participation of these molecules in AR is still undefined. The myeloid-derived suppressor cells (MDSCs) are heterogeneous populations of bone marrow–derived myeloid progenitors and immature cells that have defects in differentiation into mature myeloid cells.6,7 They migrate out of the bone marrow and accumulate both in the blood and in peripheral lymphoid tissues, where their function can alter adaptive immunity. MDSCs significantly increase in severe infection, tumors, and acute inflammation, and they negatively regulate immune functions.8,9 Chronic inflammation is associated with induction and promotion of immune-suppressive mechanisms, such as MDSC accumulation.9 A previous study10 described MDSCs as being involved in Th2-driven airway hyperresponsiveness and asthma; however, the participation of MDSCs in AR is undefined. The aim of this study is to assess the levels of MDSCs and the expression of costimulatory molecules CD80 and CD86 on monocytes and their ligands (CD28) on T-lymphocytes of children with AR.

Methods

Compliance With Ethical Standards

This study was carried out in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki, 1989) for experiments involving humans. Written informed consents of legal caregivers of enrolled children were taken after explanation of the study objectives and its benefits for their children and others. Faculty of Medicine, Assiut University Ethical Committee approved the study protocol.

Study Design

This was a 4-month, case-control study undertaken in the outpatient clinics for children at Assiut and Al-Azhar University Hospitals, Assiut, Egypt, during winter of 2016 (from September to December).

Patient Characteristics

Patients included 60 children with AR between the ages of 6 and 13 years. The diagnosis of AR was established by a senior ENT consultant before patients were recruited into the study. The diagnosis of AR was performed according to Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines.11 Classification and severity grading of patients were performed based on ARIA criteria such as intermittent (mild or moderate-severe) or persistent (mild or moderate-severe) rhinitis.11 All manifestations of AR, including rhinorrhea, sneezing, nasal itching, nasal obstruction, and facial features (shiners, allergic salute, horizontal nasal crease, mouth breathing, conjunctivitis, and infraorbital fold) were assessed by one of the authors (AA).

A total of 50 age- and sex-matched nonatopic, nonasthmatic healthy children were recruited as normal controls. Treatment with systemic corticosteroids and antihistamines was stopped at least 1 month before the study. The exclusion criteria were the following: (1) signs of allergic symptom exacerbation; (2) associated other allergic diseases—for example, asthma, sinusitis, acute respiratory tract infections, and nasal septum deformities; and (3) recent history of immunotherapy.

Laboratory Investigations

All patients and controls underwent laboratory tests, which included complete blood count with differential count. The levels of total IgE were measured by ELISA. All samples were measured in duplicate, in addition to flow cytometric detection of MDSCs and flow cytometric detection of the expression of monocyte costimulatory molecules (CD80 and CD86) and their ligand (CD28) on T-lymphocytes.

Flow Cytometric Detection of MDSCs. Fluorescein isothiocyanate (FITC)-CD11b, phycoerythrin (PE) conjugated CD15, PE-conjugated anti-CD14, peridinium-chlorophyll-protein (Per-CP)-conjugated CD33, and allophycocyanin-conjugated HLA-DR were used to detect MDSCs. All monoclonal antibodies were purchased from Becton Dickinson (BD) Biosciences (San Jose, California). A total of 50 µL of blood sample was incubated with 5 µL of CD33, CD11b, HLA-DR, and CD14 in one tube and CD33, CD11b, HLA-DR, and CD15 in another tube for 20 minutes at 4°C in the dark. Following incubation, red blood cell lysis (using lysing buffer from BD Biosciences; Catalog No. 555899) and washing with phosphate-buffered saline (PBS) were done. After washing, the cells were suspended in PBS and analyzed by FACs Cibiruf flow cytometry with Cell Quest software (BD Biosciences). An isotype-matched negative control was used for each sample. Total MDSC cells were defined as HLA-DR− CD33+ CD11b+ cells. Analysis of CD15 and CD14 expression on HLA-DR− CD33+ CD11b+ cells was done to detect their subset. Monocytic MDSCs (M-MDSCs) were identified as CD33+ CD11b+ CD14− HLA-DR+. Granulocytic MDSCs (G-MDSCs) were identified as CD33+ CD11b+ CD15+ HLA-DR− (Figure 1). The results of total MDSCs were expressed as a percentage of leukocytes.
M-MDSCs and G-MDSCs were expressed as percentages of total MDSCs.

**Flow Cytometric Detection of the Expression of Monocytes Costimulatory Molecules (CD80 and CD86) and Their Ligand (CD28) on T-lymphocytes.** Fluoroisothiocyanate CD4, PE-conjugated CD8, and Per-CP–conjugated CD28 were used to assess the expression of CD28 on T-lymphocytes, Per-CP–conjugated CD14, FITC-conjugated CD86, and PE-conjugated CD80. All monoclonal antibodies were purchased from BD Biosciences. A total of 50 µL of blood was incubated with 5 µL of CD4, CD8, and CD28 in one tube and with CD14, CD80, and CD86 in another tube for 15 minutes at 4°C in the dark. Following incubation, red blood cell lysis using lysing buffer from BD Biosciences, Catalog No555899, and washing with PBS were done. The cells were resuspended in PBS and analyzed by FACS Calibur flow cytometry with CellQuest software (BD Biosciences). An isotype-matched negative control was used with each sample. Forward and side scatter histograms were used to define the lymphocytes (R1) and monocytes (R2). Then, the expression of CD4 and CD8 within the lymphocytes was detected and then gated for further detection of the expression of CD28 on CD8+ and CD4+ lymphocytes. Also, the expressions of CD80 and CD86 were detected on monocytes, as shown in Figure 2. CD28, CD86, and CD80 levels were recorded as a percentage of expression and as a geometric mean of fluorescence intensity (MFI) for CD86 and CD80.

**Figure 1.** Flow cytometric myeloid-derived suppressor cells in AR patients. (A) HLA-DR and side scatter histogram were used to select HLA-DR− cells (R1). (B) The expression of CD33 and CD11b were assessed in HLA-DR− cells to detect total myeloid-derived suppressor cells (HLA-DR−CD33+CD11b+). (C), (D) The expression of CD15 and CD14 were assessed in myeloid-derived suppressor cells to detect monocytic myeloid-derived suppressor cells (HLA-DR−CD33+CD11b+CD14+) and granulocytic myeloid-derived suppressor cells (HLA-DR−CD33+CD11b+CD15+).

Abbreviations: APC, antigen-presenting cell; FITC, fluoroisothiocyanate; PE, phycoerythrin; Per-CP, peridinium-chlorophyll-protein; FSC, Forward Scatter; SSC, Side Scatter.
Figure 2. Flow cytometric detection of the expression of monocyte costimulatory molecules (CD80 and CD86) and their ligand (CD28) on T-lymphocytes: (A) Forward and side scatter histograms were used to define the lymphocyte (R1) and monocyte populations (R2). (B) The expression of CD4 and CD8 within the lymphocytes was detected and then gated for further detection of the expression of CD28. Images (C) and (D) show the expression of CD28 on CD8+ and CD4+ cells, and (E) and (F) show the expression of CD80 and CD86 on monocytes. (G) and (H) show geometric mean of fluorescence intensity of expression of CD86 and CD80 on the monocytes. The positivity was defined as fluorescence (red histogram) higher than that of the isotype control (open histogram).

Abbreviations: FITC, fluoroisothiocyanate; PE, phycoerythrin; Per CP, peridinium-chlorophyll-protein; FSC, Forward Scatter; SSC, Side Scatter.
Data analysis was done using the Statistical Package for Social Sciences (SPSS 22, SPSS Inc, Chicago, Illinois). All data were expressed as the mean ± standard error of the mean. The differences between the groups were examined for statistical significance using the Mann-Whitney U test. The correlation coefficient was generated by Spearman’s rank correlation. Statistical significance was defined as $P < .05$.

### Results

Some demographic data, including age, gender, anthropometric and clinical data, and total IgE of all patients with AR and controls are presented in Tables 1 and 2. Regarding the MDSCs in our study, the percentages of total MDSCs and M-MDSCs were significantly increased in AR patients when compared with controls, whereas there was no significant difference in G-MDSCs between the patients and healthy controls (Table 3). The expression of costimulatory molecule CD86 on the monocytes was significantly higher in AR patients than in controls, but there was no significant difference in the expression of CD80. Also, the amount of CD86 per cell, represented by the MFI, was significantly higher in patients than in controls. The expressions of CD28 on CD4$^+$ and CD8$^+$ were significantly increased in patients when compared with controls (Table 4). There were positive correlations between total MDSCs and M-MDSCs and the expression of CD86 on monocytes. Moreover, there were positive correlations between total MDSCs and M-MDSCs and the expression of CD28 on CD4$^+$ and CD8$^+$ lymphocytes.

### Discussion

In the present study, our results showed for the first time that the percentages of total and M-MDSCs were significantly increased in children with AR than in healthy controls. MDSCs are a heterogeneous population of cells that grow in the course of inflammation, infection, and cancer and have a significant ability to suppress various T-cell functions.\textsuperscript{5,12} Recently, there has been accumulating evidence that MDSCs have an important role in the regulation of immune responses during cancer and other diseases. Furthermore, MDSCs regulate the innate immune responses by controlling the cytokine production of macrophages. MDSCs produce IL-10 and so reduce the level of IL-12 under severe inflammation.\textsuperscript{12} Other functions of MDSCs
have been described—for example, promotion of tumor cell invasion, tumor angiogenesis, and metastasis. A recent study investigated the levels of MDSCs, IL-10, and IL-12 in pediatric patients with asthma. MDSCs and serum IL-10 levels were significantly higher in asthmatic children when compared with the treated asthmatic group, normal healthy children, and pneumonia group (P < .05). The levels of IL-12 in asthmatic children were significantly lower when compared with the other groups. Furthermore, the percentages of MDSCs in the asthmatic group were positively correlated with IL-10 and negatively correlated with IL-12. In the same study, the authors found that MDSCs and IL-10 in an asthmatic mice model were significantly higher than in normal control mice (P < .05). Another research group investigated the frequencies of MDSCs and T-helper 17 (Th17) cells in 30 young children with recurrent wheezing, 20 children with pneumonia, and 23 controls. The percentages of MDSCs and Th17 cells in the wheezy group were significantly higher than in the pneumonia and control groups. It has been reported that MDSCs mediate lung inflammation and airway hyperresponsiveness through the production of reactive oxygen species, signifying multiple mechanisms through which MDSCs can cause asthma. In another study, MDSCs increased mast cell–mediated secretion of numerous inflammatory cytokines, including IL-13, IL-6, tumor necrosis factor, macrophage inflammatory protein-1α, and monocyte chemotactic protein-1. The interaction of MDSCs and mast cells enhanced the activities of each other, resulting in intensified inflammation and airway hyperresponsiveness. The precise role of MDSCs in the pathogenesis of allergic diseases, especially AR, remains to be defined.

Our results showed that the expression of costimulatory molecule CD86 on monocytes and its amount per cell were significantly higher in children with AR than in healthy controls. In addition, the expressions of CD28 on CD4+ T-lymphocytes and CD8+ T-lymphocytes were significantly elevated in AR patients than in controls. Interestingly, there were significant positive correlations between total and M-MDSCs and the expression of CD86 on monocytes and the expression of CD28 on CD4+ and CD8+ T-lymphocytes. Abnormal activation, recruitment, and differentiation of T-lymphocytes are significant elements in the pathogenesis of allergic diseases. T-cell stimulation needs the engagement of T-cell receptor/CD3 complex with external antigen and the costimulatory signals by the interaction between CD28 on T-lymphocytes and CD80 and CD86 on APCs. CD28 on T-lymphocytes interacts with either CD86 or CD80 on APC to start stimulatory signal for T-cell activation, cytokine production, and differentiation. CD86 is a type I transmembrane glycoprotein expressed on the surface of APCs. It provides important costimulatory signals for T-cell activation in response to inhaled allergens. CD86-CD28 signaling has been suggested for the induction of Th2 immune response and airway hyperresponsiveness. Allergen-specific T-cell proliferation and cytokine expression have a direct relation with CD28-CD86 costimulation in allergic patients. The effect of antigen recognition in atopic children is dependent on the complex interactions between costimulatory molecules (CD80 and CD86) on APCs with their ligands on T-cells. Furthermore, costimulation is involved in defining which effect profile will be acquired by the T-cells—Th1 or Th2 phenotype. CD80 or CD86 costimulation appears to be necessary for expression of Th2 cytokines in response to allergen in atopic children.

Our previous results suggested a potential pathogenic role of MDSCs for AR. In addition, the expression of costimulatory molecules (CD86 on the monocytes and the expression of CD28 on CD4+ and CD8+) may be potential markers for assessing children with AR. We support the need for further longitudinal studies with a larger cohort of AR patients to further investigate the role and functions of MDSCs and costimulatory molecules in AR children.

**Conclusion**

The present study demonstrated that the percentages of MDSCs were significantly elevated in AR children.
Moreover, the expressions of CD28 on CD4+ and CD8+ were significantly higher in children with AR.

Declaration of Conflicting Interests
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