CD23 surface density on B cells is associated with IgE levels and determines IgE-facilitated allergen uptake, as well as activation of allergen-specific T cells

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Background: Increasing evidence suggests that the low-affinity receptor for IgE, CD23, plays an important role in controlling the activity of allergen-specific T cells through IgE-facilitated allergen presentation.
Objective: We sought to determine the number of CD23 molecules on immune cells in allergic patients and to investigate whether the number of CD23 molecules on antigen-presenting cells is associated with IgE levels and influences allergen uptake and allergen-specific T-cell activation.
Methods: Numbers of CD23 molecules on immune cells of allergic patients were quantified by using flow cytometry with QuantiBRITE beads and compared with total and allergen-specific IgE levels, as well as with allergen-induced immediate skin reactivity. Allergen uptake and allergen-specific T-cell activation in relation to CD23 surface density were determined by using flow cytometry in combination with confocal microscopy and T cells transfected with the T-cell receptor specific for the birch pollen allergen Bet v 1, respectively. Defined IgE-allergen immune complexes were formed with human monoclonal allergen-specific IgE and Bet v 1.
Results: In allergic patients the vast majority of CD23 molecules were expressed on naive IgD^+ B cells. The density of CD23 molecules on B cells but not the number of $CD23^+$ cells correlated with total IgE levels ($R_S = 0.53$, $P = .03$) and allergen-induced skin reactions ($R_S = 0.63$, $P = .008$). Uptake of allergen-IgE complexes into B cells and activation of allergen-specific T cells depended on IgE binding to CD23 and were associated with CD23 surface density. Addition of monoclonal IgE to cultured PBMCs significantly ($P = .04$) increased CD23 expression on B cells.
Conclusion: CD23 surface density on B cells of allergic patients is correlated with allergen-specific IgE levels and determines allergen uptake and subsequent activation of T cells. (J Allergy Clin Immunol 2017;139:290-9.)

Key words: *CD23, allergy, IgE, low-affinity IgE receptor, B cell, allergen*

IgE is known to have 2 different receptors, the high-affinity receptor FcεRI and the low-affinity receptor CD23 (FcεRII). Cross-linking of FcεRI-bound IgE by allergens mediates degranulation of mast cells and basophils and thus leads to immediate symptoms of allergic disease.[1] In addition, FcεRI is expressed on eosinophils[2] and antigen-presenting cells (APCs; eg, monocytes and dendritic cells) and was shown to be involved in IgE-facilitated allergen presentation to T cells.[3,4] Interestingly, expression of FcεRI on mast cells, basophils, and even APCs is upregulated by increasing IgE levels,[5,6] and it was found that omalizumab, an anti-IgE antibody, prevents IgE binding to FcεRI and thereby also downregulates FcεRI expression.[7]

Expression of the low-affinity receptor for IgE (CD23), a 45-kDa calcium-binding protein belonging to the family of C-type lectins on various cell types, has been investigated mainly by using cells cultured under various conditions. These studies have shown that CD23 is expressed on B cells,[8] monocytes,[9] T cells,[10] dendritic cells,[11] platelets,[12] and neutrophils.[13] However, expression of the numbers of CD23 molecules on these cell types has not been studied in detail by using *ex vivo* isolated cells from allergic patients. CD23 has an important function in IgE-facilitated allergen presentation to T cells.[14,15] In fact, IgE-facilitated antigen presentation strongly activates allergen-specific T cells and secretion of proinflammatory and T_H2-driving cytokines.[14-17] It has been shown that facilitated antigen presentation can be inhibited with a therapeutic anti-CD23 antibody[18] and by allergen-specific IgG antibodies induced by allergen-specific immunotherapy.[19] An association between improvement of symptoms after specific immunotherapy with a reduction of allergen-IgE binding to CD23 (facilitated antigen binding) on B cells by enhanced levels of blocking IgG antibodies has been demonstrated by using facilitated antigen-binding assays.[20,21]

Despite the importance of CD23 in activating allergen-specific T cells, several aspects of its biology have not been investigated as

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Abbreviations used
APC: Antigen-presenting cell
MFI: Mean fluorescence intensity
NK: Natural killer
PE: Phycoerythrin
R: Pearson correlation coefficient
R_s: Spearman rank correlation coefficient

meticulously as for FcεRI. For example, there are no studies that have investigated the density of the expression of CD23 molecules on *ex vivo* isolated cells from allergic patients. Studies investigating CD23 mainly focused on the relative number and percentage of cells expressing CD23.[22-29] Therefore it has also not been studied whether the number of CD23 molecules on the cells is associated with total and allergen-specific IgE levels. Furthermore, there are no systematic studies in defined experimental human model systems that have analyzed whether and how the number of CD23 molecules on APCs has an effect on the magnitude of IgE-facilitated allergen presentation and subsequent T-cell activation.

In the present study we established a new technique for measurement of CD23 receptor molecule numbers on the surfaces of immune cells. We investigated the distribution frequency of CD23 on immune cells in allergic patients and whether and how this parameter is correlated with IgE levels. We also studied whether addition of IgE to PBMC cultures has effects on CD23 expression on B cells. Furthermore, we used CD23 cell lines expressing different numbers of CD23 molecules on their surfaces to study whether and how the density of CD23 molecules on APCs influences IgE-facilitated allergen uptake and allergen-specific T-cell activation.

METHODS

Patients

Blood samples from 17 study participants with a positive history suggestive of grass pollen allergy and a positive skin prick test reaction with grass pollen extract were analyzed. Apart from their allergy, none of the subjects had a history of a chronic or current acute disease. Subjects were included in the study during the grass pollen season (ie, during the months of June/July in Vienna). The presence of symptoms of grass pollen allergy (rhinitis, conjunctivitis, and asthma) was recorded at that time. Furthermore, a history of other allergies was obtained. No patients were analyzed who had a contraindication against skin prick testing or were receiving long-term treatment with systemic corticosteroids, immunosuppressive drugs, tranquilizers, or psychoactive drugs. Before the study, patients were not allowed to use oral antihistamines for 3 days and local (in the skin test area) and systemic corticosteroids for 14 days. Blood samples were analyzed in an anonymized manner with approval of the Ethics Committee of the Medical University of Vienna (EK508/2011) after written informed consent was obtained from the patients.

Skin prick tests

Skin test solutions (positive control, timothy grass pollen extract; negative control solution, codeine phosphate; Stallergenes, Antony, France) were applied to the lower arms of patients and were pricked with commercial prick lancets (Allergopharma, Reinbek, Germany). After 20 minutes, the wheal reaction was surrounded with a felt pen and transferred to paper by using adhesive tape. The size of the wheal reactions was measured by using planimetry, as previously described.[30]

Blood samples and total and allergen-specific IgE measurements

Immediately after venipuncture from the antecubital vein, cells from heparinized blood samples were assessed for CD23 expression. Serum was obtained from clotted blood samples by means of centrifugation and stored at −20°C until use. Total IgE and timothy grass pollen–specific IgE levels were measured with the Phadia CAP system (Thermo Fisher, Uppsala, Sweden).

Data analysis

All clinical data (patient history and skin prick test results) were obtained by a clinical investigator and deposited in a database. Measurement of total and specific IgE levels was performed by an independent external laboratory, which was unaware of clinical data and CD23 measurements. Measurement of CD23 levels on different cell types was performed by another independent investigator who was not in contact with the study participants and who was blinded regarding total and specific IgE levels and clinical data (ie, results from skin prick tests and clinical symptoms). After all 3 independent data sets (ie, IgE levels, clinical data, and CD23 measurements) were completed, they were submitted to a database, and correlations were analyzed.

Blood sample preparation and flow cytometry

Red blood cell lysis solution (155 mmol/L ammonium chloride, 10 mmol/L potassium bicarbonate, and 12 mmol/L EDTA) was applied to heparinized blood samples from patients. For flow cytometry, the following surface markers of cells were stained: T cells (positive with anti-CD3, clone OKT3), natural killer (NK) cells (negative with anti-CD3 and positive with anti-CD56, clone TULY56), B cells (positive with anti-CD19, clone HIB19), monocytes (positive with anti-CD14, clone 61D3), platelets (positive with anti-CD61, clone VI-PL2, and with anti-CD41a, clone HIP8), basophils (positive with anti-CD123, clone 6H6, and anti-CCR3, clone 5E8-G9-B4), neutrophils (granulocytes negative with anti-CD49d, clone HP2/1), eosinophils (granulocytes positive with anti-CD49d, clone HP2/1, and negative with anti-CD19), dendritic cells (lineage cocktail negative, positive with anti-CD11c, clone 3.9), naive B cells (positive with anti-CD19, clone J3-119, and anti-IgD, clone 11-26), memory B cells (positive with anti-CD19, clone J3-119, and positive with anti-CD27, clone O323). All cells were additionally stained with anti-CD23 (clone EBVCS2). Matching nonbinding isotype antibodies were used as controls. All antibodies were obtained from eBioscience (San Diego, Calif), except for anti-CD49d, anti-CD19 for naïve/memory staining (Beckman Coulter, Brea, Calif), and lineage cocktail lin1 (BD Biosciences, Franklin Lakes, NJ). Aliquots of 1.5×10^6 cells were used for each staining. Before staining, cells were blocked with 10% vol/vol mouse serum (Life Technologies, Carlsbad, Calif). Dead cells were excluded from the analysis with eFluor 780 Fixable Viability Dye (eBioscience). Flow cytometric analysis was performed on a Beckman Coulter FC 500 flow cytometer (Beckman Coulter). Depending on the cell type, 3×10^5 (T cells, B cells, NK cells, and monocytes), 1×10^6 (basophils), or 5×10^5 (all other cell types; ie, neutrophils, eosinophils, dendritic cells, and platelets) events were recorded. FlowJo Software 7.5 (TreeStar, Ashland, Ore) was used for data analysis. Gates were set according to the matching nonbinding isotype control of each antibody for each cell type.

Measurement and calculation of CD23 surface density

Quantification of CD23 expression was performed with BD QuantiBRITE PE beads (BD Biosciences), according to the manufacturer's instructions. Briefly, beads with different intensity levels in the phycoerythrin (PE) channel FL-2 and defined numbers of surface PE molecules were used as a standard in flow cytometry and used for back-calculation of CD23 stained with a PE-labeled anti-CD23 antibody. FlowJo Software 7.5 (TreeStar) was used for data analysis. Molecule density on cells was calculated only when more than 20 cells of the assessed cell type were positive for CD23.