The expression of receptors for IgE on human eosinophils remains controversial, as reviewed in a recent comprehensive editorial. 1 Although human eosinophils from subjects infected by *Schistosoma mansoni* were recently reported to express different types of receptors for IgE (high-affinity Fc-ε receptor [Fc-εRII]; 2 low-affinity Fc-ε receptor [Fc-εRIII], 3 and Mac-2a/βP), 4 murine eosinophils purified from hepatic granulomas of mice affected by *S. mansoni* parasitosis were shown not to express IgE receptors either by flow cytometry or by reverse transcriptase polymerase chain reaction analyses. 5 In this respect, further studies were encouraged by Kita and Gleich 1 to solve the existing controversies concerning the expression of Fc-εRII in human hypereosinophilic diseases.

We investigated the expression of CD23 antigen on peripheral blood eosinophils from 14 patients affected by vernal keratoconjunctivitis (VKC) with mild-to-moderate eosinophilia and from 10 matched normal controls. All patients showed skin reactivity to grass (n = 5), *Dermatophagoides pteronyssinus* (n = 5), *Parietaria officinalis* (n = 2), or multiple allergens (n = 2). In brief, 150 µL of unpurified fractionated peripheral blood were incubated for 30 minutes at 4°C with the following fluorescein isothiocyanate (FITC) or phycoerythrin (PE)-conjugated monoclonal antibodies (MoAbs): CD23 (EBVCS-5 clone, derived from BALB/c mice immunized with in vitro–transformed EBV cell line; IgG1), CD16 (NK15 clone; IgG1; Becton Dickinson, Mountain View, CA), and CD9 (MM2/57 clone; IgG2, Ylem, Rome, Italy) or isotype-matched FITC- or PE-conjugated irrelevant MoAb as negative controls; erythrocytes were lysed by adding NH4Cl/EDTA (Ortho Diagnostic, Raritan, NJ). Cells were subsequently fixed with 4% paraformaldehyde (F) and permeabilized with n-octyl-β-D-glucopyranoside (OG; Sigma, St Louis, MO) for 6 minutes at 20°C. The sequential fixation and permeabilization of unpurified peripheral blood allows the identification and electronic gating of eosinophils based on high side scatter signals, surface staining for CD9, and lack of CD16 expression, without altering surface antigen expression.6,8 After FOG treatment, cells were incubated with the EG2 MoAb (IgG1; Kabi Pharmacia Diagnostics, Uppsala, Sweden), recognizing the secretory form of eosinophil cationic protein (ECP) and were subsequently stained with FITC-conjugated F(ab)2 anti-mouse IgG. Unlabeled mouse IgG3 MoAb served as negative control. All samples were run through a FACScan flow cytometer (Becton Dickinson) equipped with an argon laser emitting at 488 nm. A minimum of 3,000 events were acquired in list mode using CellQuest software (Becton Dickinson). The purity of eosinophilic gate (>98%) was confirmed by sorting of CD9+ cells and subsequent microscopic analysis. Results were expressed as percent values obtained after channel-by-channel subtraction of test and control histograms; antigenic density was expressed as mean fluorescence intensity (MFI) ratio (MFI of test histogram: MFI of control histogram). The concentration of ECP in serum was measured by means of specific radio-immunoassays (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). Data were presented as median values and interquartile ranges. Statistical analyses were performed with Mann-Whitney U test for unpaired determinations. Correlations were examined with Spearman rank analysis. The criterion for statistical significance was defined as P < .05.

Fc-εRII/CD23 receptor could not be detected on eosinophils from normal subjects above background fluorescence, as previously reported by Hartnell et al.9 The eosinophil count in patients affected by VKC ranged from 100 to 800 cells/µL (median = 300/µL; interquartile range, 200 to 540). CD23 could be seen in 6 of 14 (43%) patients on 28% (10 to 54%) of circulating eosinophils. The percentage of CD23-expressing eosinophils positively correlated with the MFI ratio of the EG2 MoAb (r = .55; P = .042), recognizing the secretory form of ECP. Conversely, no correlation could be found between eosinophil absolute count and either the percentage of CD23+ eosinophils or CD23 staining intensity or between the percentage of CD23+ eosinophils and the degree of serum ECP level, total serum IgE, and conjunctival hyperemia.

Eosinophilic Fc-εRII is homologous to CD23 differentiation antigen, expressed on activated B lymphocytes; low-affinity receptors for IgE (Fc-εRII/CD23) can be detected on a variety of cell types, including macrophages, monocytes, and platelets.10 Fc-εRII expressed on activated, low-density cells of hypereosinophilic patients might be involved in IgE-dependent cytotoxicity of helminths and in IgE-dependent release of eosinophilic granules and mediators, although no correlation between protein expression, as measured by flow cytometry, and Northern blot analysis of RNA, could be found by some investigators.11,12 In a recently published report, eosinophils from patients with hay fever were found to express Fc-εRII/CD23 at moderate levels, as detected by the BB10 MoAb, but failed to degranulate in response to anti-IgE.13

VKC is a severe allergic disease, characterized by activation of eosinophils in tears, conjunctival biopsies, and peripheral blood and by a dysregulated production of IgE.14,15 In our cohort of patients presenting with mild-to-moderate eosinophilia, the expression of CD23, restricted to a subgroup of patients and to a discrete subpopulation of circulating eosinophils, correlated with that of the secretory form of ECP, a well-established marker of eosinophilic activation in allergic

Importance of T-Cell Receptor δ-Chain Gene Analysis on CD7+ and CD56+ Myeloid/Natural Killer Cell Precursor Acute Leukemia

To the Editor:

Natural Killer (NK) cells differentiate from immature thymocytes under appropriate conditions in vitro and in vivo, and share cytotoxic activity and some surface antigens with T cells, indicating a close relationship with T-lineage.1 Recently, NK cells were found to develop from a population of CD34+, CD33+, CD56+ cells in vitro.2 Suzuki et al3 described six cases of CD7+ and CD56+ myeloid/NK cell precursor acute leukemia as a distinct hematolympoid disease entity. Based on Southern blot analysis of immunoassociated genes (except for T-cell receptor [TCR] β chain) and immunophenotypic and immunohistochemical staining, they neglected association of T-lineage for those cases, because they found germline configuration on Southern blot analysis for only TCR β and γ chain gene but not for TCR δ gene. Cytoplasmic CD3 (cyCD3) was positive in 50% of the cases.

TCR δ rearrangement occurs earlier in T-cell differentiation than that of other TCR genes. So far, δ rearrangement with joining region (J) gene is recognized in only T-cell leukemia or lymphoma but not any other lineage malignancies.4-6 Recently, it was shown that DDJ rearrangements require activation of a T-cell specific enhancer in contrast to VD rearrangements.7 Differences in TCR δ gene rearrangement patterns in B-cell precursor acute lymphoblastic leukemia (ALL) and T-cell precursor ALL (T-ALL) can be explained by this finding. We previously reported the high prevalence of DDJδ in 16 patients with CD7+ early T-ALL, which were negative for CD3/4/8/19/20, and myeloperoxidase (MPO).8 Fifteen patients (94%) had rearranged band(s) involving the J region of the TCR δ chain gene. Only DDJδ with no TCR β and γ rearrangement was shown in 9 patients. Four of 9 patients with DDJδ were negative for cyCD3δ. Therefore, it is suggested that DDJδ occurs at an early stage of T-cell differentiation and earlier than

diseases.7 The correlations between CD23 expression and severity score of VKC failed to reach statistical significance; further investigations are warranted to confirm the hypothesis that CD23 expression and/or shedding of CD23 into a soluble form might be correlated with the clinical picture. The hypothesis which can be proposed to explain these findings is that CD23 might be shed by cell surfaces into a soluble form and be no longer detectable on circulating eosinophils. Interestingly, recent investigations attributed to soluble CD23 an interesting role as a proinflammatory mediator, although its functional significance in hypereosinophilic diseases is not fully elucidated.9-11

In conclusion, Fc-εRI/CD23 receptor can be detected by flow cytometry on eosinophils from a subgroup of patients affected by VKC, a severe ocular inflammatory disease with tissue and peripheral eosinophilic activation, although its relevance to IgE-mediated effector functions and disease pathophysiology remains to be elucidated.
Fc-βRII/CD23 Receptor on Circulating Human Eosinophils

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