Interleukin-2 primes eosinophil degranulation in hypereosinophilia and Wells' syndrome

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Patients with hypereosinophilia frequently suffer from eosinophil-mediated damages of the heart, lungs, skin, and other organs, while some do not. The reason(s) for this difference is not known. We observed that eosinophils from most patients with hypereosinophilia express the α-chain of the IL-2 receptor (CD25), and that IL-2 enhances platelet-activating factor-stimulated release of eosinophil cationic protein from CD25-expressing but not from CD25-negative eosinophils. Such a "priming" effect has previously been described for eosinophil hematopoietins. These data suggest that patients with increased eosinophil surface CD25 expression are at higher risk of eosinophil degranulation and subsequent tissue damage when IL-2 is present at inflammatory sites.

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1 Introduction

Increased eosinophil numbers are often observed in allergic diseases and parasitic infections. Elevated eosinophil levels are also sometimes seen in patients with malignancies and in various idiopathic myeloproliferative disorders. Given the increased numbers of circulating eosinophils in these diseases, it seems likely that these cells directly or indirectly participate in the pathogenesis. The release of toxic mediators from eosinophils is considered to be important for both host defense [1] and tissue damage [2]. Besides their role as effector cells, eosinophils appear to be important immunoregulatory cells as they are able to produce and release cytokines following activation [3, 4].

Many attempts have been made to distinguish activated from resting eosinophils. Although many changes were observed after stimulation of eosinophils *in vitro*, there is no evidence to suggest that any of such activation markers is expressed on eosinophils under *in-vivo* conditions [5]. Similar to other surface structures, CD25 has been reported to appear on eosinophils after exposure to interleukin (IL)-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF) *in vitro* [6].

CD25 is the α-chain of the IL-2 receptor. A fully functional IL-2 receptor complex, as shown on lymphoid cells, is believed to consist of at least three molecularly distinct membrane proteins: p55 (α-chain, CD25), p75 (β-chain, CD122), and p64 (γ-chain, CD132) [7]. Besides their role in T cell activation, IL-2 receptors are also expressed by B cells, natural killer cells, and several nonlymphoid cells. It has been demonstrated that IL-2 affects cellular functions of fibroblasts [8], epithelial cells [9], monocytes [10], neutrophils [11], and eosinophils [12].

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Abbreviations: AD: Atopic dermatitis **BA:** Bronchial asthma **BAL:** Bronchoalveolar lavage **CA:** Cancer associated with hypereosinophilia **CTCL:** Cutaneous T cell lymphoma associated with hypereosinophilia **ECP:** Eosinophil cationic protein **IEE:** Idiopathic eosinophilic esophagitis **IHS:** Idiopathic hypereosinophilic syndrome **PAF:** Platelet-activating factor **WS:** Wells' syndrome

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In a large study on the pathobiology of idiopathic eosinophilia [13], we observed that some patients expressed high levels of surface CD25 on their blood eosinophils. In the present study, we compared CD25 levels on blood and tissue eosinophils in eosinophilic patients with different diseases (total number of patients and control individuals: 82) and investigated functional consequences *ex vivo*.

2 Results and discussion

2.1 CD25 surface expression on blood eosinophils is associated with hypereosinophilia and/or Wells' syndrome

Eosinophils from normal control individuals (mean blood eosinophil number: 99±23×10^6/l) and from most patients with atopic dermatitis (AD; 682±138×10^6/l), bronchial asthma (BA; 1374±260×10^6/l), or idiopathic eosinophilic esophagitis (IEE; 350±52×10^6/l) expressed no or only little CD25 protein on their surface (Fig. 1). Only some of these patients with eosinophil counts above 1,500×10^6/l had CD25-positive eosinophil subpopulations. This suggested that normal control individuals and allergic patients with mild eosinophilia do not have significant amounts of CD25-positive eosinophils in their blood. In contrast, patients with cutaneous T cell lymphoma (CTCL; 3215±1107×10^6/l), other cancer associated with hypereosinophilia (CA; 3916±2032×10^6/l), Wells' syndrome (WS; 1144±855×10^6/l), and idiopathic hypereosinophilic syndrome (IHS; 4976±607×10^6/l) had significantly higher numbers of CD25-positive eosinophils in their blood. With the exception of WS patients, CD25 expression on eosinophils was usually associated with hypereosinophilia (>1,500×10^6/l) [14].

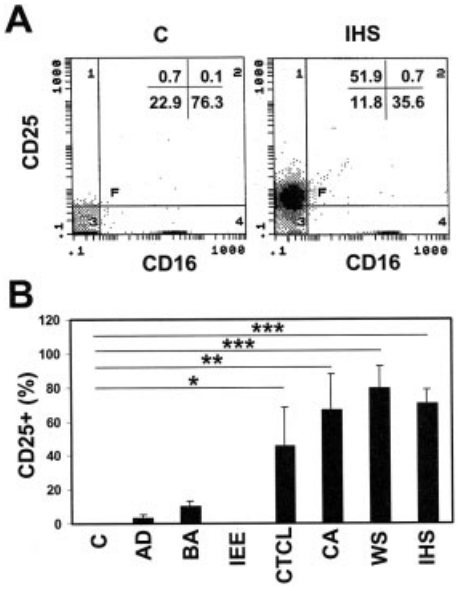

Fig. 1. CD25 expression by blood eosinophils. (A) Flow cytometry. CD16-negative (eosinophils) and CD16-positive (neutrophils) blood granulocytes do not express CD25 in control individuals (C). In contrast, eosinophils but not neutrophils express CD25 in patients with IHS. (B) Relative numbers of eosinophils expressing CD25. Bars represent the mean, error bars the SEM. C, control individuals, n=7; AD, n=10; BA, n=14; IEE, n=10; CTCL, n=5; CA, n=4; WS, n=7; IHS, n=15. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

2.2 Anti-eosinophilic therapies reduce CD25 expression on eosinophils

We performed follow-up studies on single patients receiving drug therapies that decreased eosinophil numbers in blood [15, 16]. Treatment of a steroid-resistant BA patient with 3×10^6 IU/day interferon-α and 75 mg prednisone, of a bladder CA patient with combined chemotherapy, and of an IHS patient with 25 mg prednisone resulted in decreased blood eosinophil numbers associated with decreased eosinophil CD25 surface expression (Fig. 2). The time period between the two investigations was approximately 1 week in each case. These observations furthermore suggested that CD25 expression on eosinophils correlates with eosinophil numbers and that an eosinophil differentiation factor, such as IL-5, might induce CD25 expression.

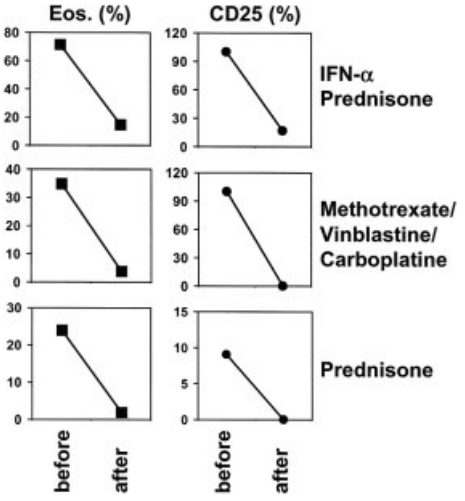

Fig. 2. Anti-eosinophilic pharmacological intervention decreases CD25 expression on eosinophils. Patients were treated for 1 week as indicated and described in the text. CD25 expression on eosinophils was analyzed as demonstrated in Fig. 1.

2.3 Tissue but not blood eosinophils from patients with BA and AD express CD25

Although blood eosinophils from patients with BA and AD do not usually express CD25, we observed that this molecule is expressed on eosinophils present at the inflammatory site of these patients (Fig. 3). In these experiments, we analyzed bronchoalveolar lavage (BAL) fluid eosinophils by flow cytometry and eosinophils in skin biopsies by immunohistochemistry. The immunohistochemistry data further suggested that eosinophils contain significant amounts of cytoplasmic CD25. The expression of CD25 on tissue but not blood eosinophils suggested that a local factor activates eosinophils at the inflammatory site. IL-5 might play a role, since its expression has been shown in bronchial tissues of asthmatic patients [17] and in skin lesions of AD patients [18].

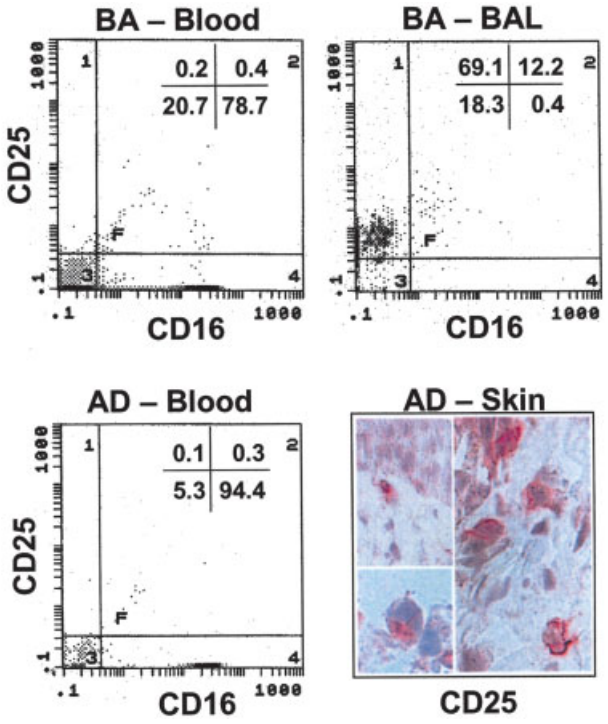

Fig. 3. CD25 expression by tissue eosinophils in BA and AD. Blood eosinophils do not express significant levels of CD25 as assessed by flow cytometry. In contrast, BAL eosinophils from a BA patients expressed CD25. This patient had 33% eosinophils and no neutrophils in the BAL fluid. Eosinophils in the skin of an AD patient demonstrated evidence for CD25 expression. A clear ring-like staining was sometimes observed; however, most of the CD25 appeared to be located within the cytoplasma. A control mAb was used and showed no staining under the same conditions (data not shown). The original magnifications of the presented photographs were as follows: upper left panel: ×400; lower left and right panel: ×630.

2.4 CD25 expression determines the capacity of IL-2 to prime eosinophils for eosinophil cationic protein release

In normal CD25-negative eosinophils, single 45-min activation of the cells with IL-2 (50 ng/ml), GM-CSF (50 ng/ml), or platelet-activating factor (PAF; 10^{-7} M) did not result in a significant release of eosinophil cationic protein (ECP; Fig. 4, upper panel). Activation of the cells with GM-CSF for 20 min and subsequent 25-min stimulation with PAF demonstrated the known priming effect of GM-CSF [19]. Although IL-2 did not show significant priming activity, ECP levels increased approximately two-fold. This would be in agreement with previously published work reporting that normal eosinophils express functional IL-2 receptors [12].

However, the priming activity of IL-2 was much higher on CD25-positive eosinophils. In fact, IL-2 acted equally efficient as priming factor as GM-CSF in CD25-positive eosinophils (Fig. 4, lower panel). Moreover, PAF stimulation alone induced a small but significant ECP release, indicating potential *in-vivo* priming of these cells. These data clearly demonstrate an increased susceptibility of CD25-positive eosinophils towards IL-2 compared to

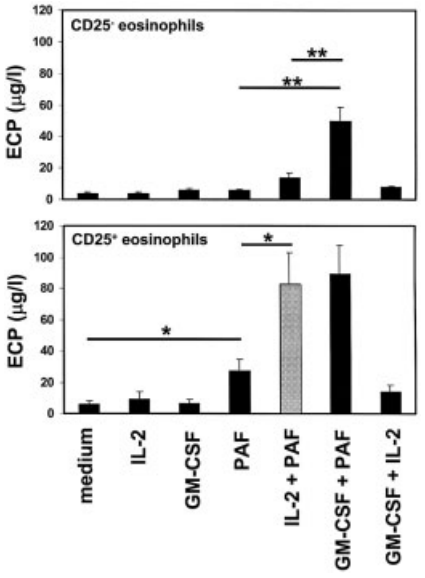

Fig. 4. Priming by IL-2 requires CD25 expression. CD25-negative (n=5; upper panel) and CD25-positive (n=7; lower panel) eosinophils were stimulated alone or in combination with IL-2, GM-CSF, and PAF, and released ECP was measured by an immunoassay. Bars represent the mean, error bars the SEM. Total ECP levels in both CD25-negative and CD25-positive eosinophils (1×10^6) ranged between 1,500 and 2,000 µg/ml. The gray bar demonstrates priming activity by IL-2. *$p<0.05$; **$p<0.01$.

CD25-negative eosinophils. In contrast to GM-CSF and IL-5, however, IL-2 did not delay apoptosis of CD25-positive eosinophils (data not shown). Therefore, signaling pathways activated via the IL-2 receptor appear to be partially different from those initiated via GM-CSF or IL-5 receptors in eosinophils [20, 21].

2.5 CD25 surface expression on control eosinophils is induced by IL-5 and GM-CSF *in vitro*

The observation that increased CD25 levels on blood eosinophils are only seen in patients with hypereosinophilia (>1,500/μl, except patients with WS) and on tissue eosinophils of patients with allergic diseases suggested the eosinophil differentiation factor IL-5 as a potential candidate able to induce CD25 expression on eosinophils. Indeed, stimulation of eosinophils from control individuals with IL-5 or GM-CSF for 20 h *in vitro* induced CD25 expression on their surface (Fig. 5). These data are in agreement with previously published work demonstrating the ability of GM-CSF and IL-3 to induce CD25 surface expression on eosinophils [6]. Interestingly, we observed small amounts of cell surface CD25 even in the absence of cytokines that might result from activation of an intracellular pool (Fig. 3, lower right panel) under *in-vitro* culture conditions.

At least a subpopulation of eosinophils also expressed the β-chain (CD122) and the γ-chain (CD132) of the IL-2 receptor (Fig. 5). The expression of these two surface proteins did not change upon IL-5 or GM-CSF stimulation for 20 h. The levels of CD122 and CD132 on eosinophils from normal and eosinophilic donors were not different (data not shown). For comparison, the same experiments were performed using neutrophils. In contrast to eosinophils, GM-CSF did not induce CD25 expression on neutrophils. This suggests that neutrophils lack a certain component required for GM-CSF-mediated CD25 gene expression that is present in eosinophils. However, we noticed a significant increase of the expression of the γ-chain in GM-CSF-treated neutrophils.

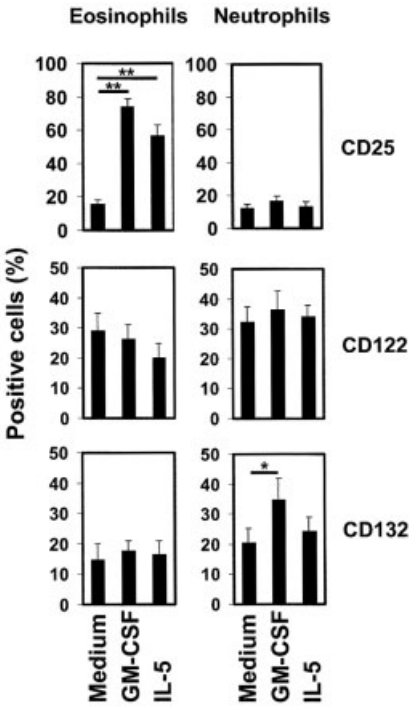

Fig. 5. IL-5 and GM-CSF induce CD25 expression on normal eosinophils but not neutrophils *in vitro*. Purified eosinophils and neutrophils from control individuals were stimulated with the cytokines for 20 h and subsequently flow cytometric analysis was performed. In contrast to CD25, CD122 and CD132 were not induced by IL-5 or GM-CSF in eosinophils. The small levels of surface CD25 in eosinophils not exposed to cytokines may result from the mobilization of intracellular stores due to the culture conditions (fresh cells do not express detectable surface CD25 levels, see Fig. 1). Interestingly, GM-CSF slightly increased CD132 expression on neutrophils.

3 Concluding remarks

Since CD25 expression on eosinophils is usually not observed in eosinophilic patients with blood eosinophil numbers below $1{,}500\times10^6$/l, it appears that this phenomenon requires very strong IL-5 production. The IL-5 levels produced in patients with allergic disorders seem to be sufficient to increase CD25 expression on tissue eosinophils at inflammatory sites, but do not appear to be sufficient for inducing the CD25 gene in blood eosinophils. Patients with WS show high numbers of CD25-expressing eosinophils without hypereosinophilia, suggesting that their eosinophils are either more susceptible towards IL-5 stimulation or that the cells receive additional signals, which decrease the threshold levels required for IL-5-mediated effects. Elevated CD25 levels on eosinophils from patients with WS and the subsequent increased susceptibility to release cationic proteins following exposure to IL-2 suggest a crucial role for eosinophils in the pathogenesis of the disease [22]. Indeed, anti-eosinophilic therapies rapidly improve the clinical situation of these patients [23].

Taken together, increased expression of eosinophil hematopoietins may not only accelerate eosinophil differentiation, but may also induce a complete and highly susceptible IL-2 receptor on the surface of eosinophils.

Since IL-2 can easily be induced in any event of T cell activation, reduction of eosinophil numbers and CD25 expression in those patients that show CD25 expression on their eosinophils using drugs that reduce IL-5 expression is strongly recommended.

4 Materials and methods

4.1 Patients

A total of 75 patients with eosinophil-associated diseases and seven control individuals were evaluated. Patient groups were as follows: AD, *n*=20; BA, *n*=14; IEE, *n*=10; CTCL, *n*=5; CA, *n*=4; WS, *n*=7; and IHS, *n*=15. From some patients, we obtained BAL fluid or skin biopsies.

4.2 Reagents

Complete culture medium was RPMI 1640 (Life Technologies, Inc., Basel, Switzerland) supplemented with 2 mM L-glutamine, 200 IU/ml penicillin/100 μg/ml streptomycin, and 10% fetal bovine serum (all from Life Technologies). IL-2 and IL-5 were purchased from R&D Systems (Abingdon, GB), and GM-CSF was a kind gift from Dr. T. Hartung (Konstanz, Germany). PAF was from Calbiochem (Lucerne, Switzerland). Fluorescein-conjugated anti-CD16 monoclonal antibody (mAb) and phycoerythrin-conjugated anti-CD25 mAb were from Coulter (Hialeah, FL). Fluorescein-conjugated anti-CD122 mAb was obtained from Serotec Ltd. (Oxford, GB) and R-phycoerythrin-conjugated anti-CD132 mAb from PharMingen (San Diego, CA). Non-conjugated anti-CD25 mAb (Act-1) was provided by Dr. H. Stein (Berlin, Germany). Unless stated otherwise, all other reagents were from Sigma.

4.3 Cell purifications

Neutrophils [24, 25] and eosinophils [26] were purified as previously described. The resulting cell populations contained >95% neutrophils and >98% eosinophils, respectively.

4.4 Immunofluorescence analysis

CD25 expression on blood and BAL eosinophils was measured by two-color flow cytometry on gated granulocytes after staining of blood leukocytes with mAb against CD16 and CD25 (Coulter). All CD16-negative cells within the granulocyte population were essentially eosinophils [26]. CD25, CD122, and CD132 expression on cultured eosinophils and neutrophils was measured by single-color flow cytometry following 20-h stimulations with 25 ng/ml IL-5 and GM-CSF, respectively. Isotype-matched control mAb were used as controls.

4.5 Immunohistochemistry

CD25 expression in biopsies from lesional skin of patients with AD was performed using the Act-1 mAb and the alkaline phosphatase-anti-alkaline phosphatase method [27].

4.6 ECP release

Purified eosinophils were cultured at 1×10^6/ml in the presence and absence of IL-2, IL-5, GM-CSF (all 25 ng/ml), and PAF (10^{-7}M). In the two-signal stimulation experiments, the second signal was added 20 min after the first signal [19]. Total stimulation times were 45 min. Total cellular ECP levels were analyzed as previously described [19]. ECP levels were measured in eosinophil supernatants in duplicates using the Pharmacia UniCAP System (Pharmacia & Upjohn, Dubendorf, Switzerland) according to the manufacturer's instructions.

4.7 Statistical analysis

Statistical analysis was performed using Student's *t*-test. A *p* value of <0.05 was considered statistically significant. Mean levels are presented together with standard errors of the mean (SEM).

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