Rapid Selective Priming of FcαR on Eosinophils by Corticosteroids

Willem ten Hove,*† Leo A. Houben,* Jan A. M. Raaijmakers,† Leo Koenderman,[1,2]* and Madelon Bracke[2]*†

Preactivation or priming of eosinophils by (proinflammatory) cytokines is important in the pathogenesis of allergic diseases. Several priming-dependent eosinophil responses, such as migration and adhesion, are reduced by treatment with corticosteroids. Many inhibitory effects of corticosteroids are mediated by the glucocorticoid receptor via genomic mechanisms, which are evident only after prolonged interaction (>30 min). However, also faster actions of corticosteroids have been identified, which occur in a rapid, nongenomic manner. In this study, fast effects of corticosteroids were investigated on the function of eosinophil opsonin receptors. Short term corticosteroid treatment of eosinophils for maximal 30 min with dexamethasone (Dex) did not influence eosinophil cell surface CD11b/CD18 expression, adhesion, and/or chemokinesis. In marked contrast, incubation with Dex resulted in a rapid increase in binding of IgA-coated beads to human eosinophils, showing that Dex can up-regulate the activation of FcαR (CD89). This priming response by Dex was dose dependent and optimal between 10^{-8} and 10^{-6} M and was mediated via the glucocorticoid receptor as its selective antagonist RU38486 (10^{-6} M) blocked the priming effect. In contrast to FcαR, eosinophil FcγRII (CD32) was not affected by Dex. Further characterization of the Dex-induced inside-out regulation of FcαR revealed p38 MAPK as the central mediator. Dex dose dependently enhanced p38 MAPK phosphorylation and activation in situ as measured by phosphorylation of its downstream target mitogen-activated protein kinase-activated protein kinase 2. The dose responses of the Dex-induced activation of these kinases were similar as seen for the priming of FcαR. This work demonstrates that corticosteroids selectively activate the FcαR on eosinophils by activation of p38 MAPK. *The Journal of Immunology,* **2006, 177: 6108–6114.**

Eosinophilic granulocytes play an important role in the pathogenesis of allergic diseases and the host immune defense against parasites (1). These cells belong to the most cytotoxic cells in the human body. Immune activation of these cells in vivo is therefore tightly controlled by interaction with pro- and anti-inflammatory cytokines and chemoattractants. By these mechanisms, the cells can adapt a primed or preactivated state, which results in enhanced responsiveness to distinct stimuli (2). Eosinophils of asthmatic and allergic patients exhibit a primed phenotype compared with controls, which is characterized by increased adhesion (3) and migratory responsiveness (4, 5). Priming of these and other eosinophil functions such as degranulation and respiratory burst activation can be achieved in vitro by Th2 cell-derived cytokines such as IL-4, IL-5, and GM-CSF (6–9) which are thought to be present in peripheral blood of allergic patients (10). In addition, priming of eosinophils can result in responsiveness toward factors such as IL-8, which do not function on unprimed cells (6).

Furthermore, priming of these cells by cytokines results in increased functionality of IgA and IgG receptors (11) and increased degranulation through these receptors in vitro (12). Freshly isolated eosinophils from asthmatic donors exhibit enhanced IgA binding compared with cells of healthy donors, suggesting that these cells have been primed in vivo (13). This priming of the IgA receptor functionality is thought to be important in asthmatics, because IgA is abundantly present in mucosal tissues and production of allergen-specific IgA occurs in patients with allergic diseases such as asthma (14, 15). In addition, IgA is a major trigger for eosinophil degranulation (16, 17).

Standard therapy in asthma aims at inhibiting inflammation by using inhaled corticosteroids (ICS)[3] (18, 19). It is generally agreed that activation and survival of eosinophils can be blocked by ICS although the amount of clear data regarding these issues is remarkably small (18, 20–23). It has also been described that corticosteroid treatment reduced eosinophil migration (24) and adhesion (22) but hardly affected Ig-induced degranulation in eosinophils (25). ICS are thought to exert their function by binding to the glucocorticoid receptor (GCR) and subsequent up- and down-regulation of anti-inflammatory and/or proinflammatory genes (19).

In addition to this well-accepted mechanism at the genomic level, recently rapid nongenomic actions of corticosteroids have been described in cellular systems and several diseases (26–30). The exact mechanisms underlying these rapid effects are, however, poorly defined. Several mechanisms have been proposed by which corticosteroids can induce these rapid effects (27, 28): 1) by specific interaction with the cytosolic corticosteroid receptor resulting in fast but poorly defined mechanisms (30–32); 2) by specific interaction with membrane bound corticosteroid receptors, which are not sufficiently characterized (27, 28, 33); and 3) as a result of

*Department of Pulmonary Diseases, University Medical Center Utrecht, Utrecht, The Netherlands; and †Department of Pharmacoepidemiology and Pharmacotherapy, Utrecht Institute of Pharmaceutical Sciences, Utrecht, The Netherlands

Received for publication March 28, 2006. Accepted for publication August 7, 2006.

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[1] Address correspondence and reprint requests to Dr. Leo Koenderman, Department of Pulmonary Diseases, University Medical Center, hpn. H.02.128, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. E-mail address: L.Koenderman@umcutrecht.nl

[2] Authors contributed equally to this article.

[3] Abbreviations used in this paper: ICS, inhaled corticosteroids; Dex, dexamethasone; GCR, glucocorticoid receptor; HSA, human serum albumin; MAPKAP-K2, mitogen-activated protein kinase-activated protein kinase 2.

0022-1767/06/$02.00

non-receptor-mediated physicochemical interactions with cellular membranes (34).

In asthma, rapid effects of budenoside have been shown in guinea pigs. In these experiments, this corticosteroid could inhibit asthma symptoms within 10 min (35). We obtained data showing similar rapid effects in human asthmatics treated with fluticasone (36). These data underscore the importance of nongenomic mechanisms involved in the effects of corticosteroids during treatment of allergic asthma. In addition, acute nongenomic vasoconstriction in the bronchial vasculature by corticosteroids (37, 38) is considered as an important mechanism in the resolution of acute asthma complaints by corticosteroid treatment (39).

We will show that short term treatment of human eosinophils with the corticosteroid dexamethasone (Dex) selectively primes the functionality of the FcαR on these cells through activation of the p38 MAPK signaling pathway. This occurs with no effect 1) on expression of adhesion molecules, 2) on adhesion and migration, and 3) on respiratory burst. These data demonstrate a complex mechanism by which corticosteroids can specifically modulate eosinophil function in mucosal immunity.

Materials and Methods

Reagents

Ficoll-Paque was obtained from Pharmacia. Human serum albumin (HSA) was from Sanquin. Recombinant human IL-5 was a gift from Dr I. Uings (GlaxoSmithKline). Dex and RU38486 (Mifepristone) were obtained from Sigma-Aldrich and diluted in ethanol. Abs used were: anti-phospho-p38 MAPK (Thr^{180}/Tyr^{182}), anti-phospho-ERK1/2 (Thr^{202}/Tyr^{204}) and anti-phospho-MAPKAP-K2 (Thr^{334}) from Cell Signaling, and anti-p38 from Santa Cruz Biotechnology. Directly labeled CD11b-PE and CD18-FITC and HRP-coupled swine anti-rabbit were from DAKO. Functionally blocking mAb IB4 (anti-β_2 integrin) and the control mAb W6/32 (anti-HLA-A, -HLA-B, and -HLA-C) were isolated from the supernatant of a hybridoma obtained from the American Type Culture Collection. The functionally blocking mAb My43 (anti-CD89) (40) was a gift from L. Shen (Dartmouth Medical School, Hanover, NH). Pharmacological inhibitor SB203580 was purchased from Kordia Life Sciences, and the inhibitors LY294002 and SP600125 were from Biomol.

Granulocyte isolation

Granulocytes were isolated from 100 ml of whole blood from healthy donors anticoagulated with trisodium citrate (0.4% w/v, pH 7.4). Blood was diluted 2.5:1 with PBS containing containing trisodium citrate (0.4% w/v, pH 7.4) and human pasteurized plasma-protein solution (4 g/L). Granulocytes and erythrocytes were isolated by centrifugation over Ficoll-Paque. Erythrocytes were lysed in isotonic ice-cold NH_4Cl solution followed by centrifugation at 4°C. After isolation, granulocytes were resuspended in PBS containing trisodium citrate (0.4% w/v, pH 7.4) and human pasteurized plasma-protein solution (4 g/L).

Eosinophils were isolated from the granulocyte fraction by negative selection using anti-CD16-conjugated microbeads (MACS; Miltenyi Biotec; Ref. 41). In addition, CD3- and CD14-conjugated microbeads (MACS; Miltenyi Biotec) were added to the granulocyte suspension to minimize mononuclear cell contamination. Purity of eosinophils was >97%.

Eosinophils were resuspended either in HEPES-buffered RPMI 1640 supplemented with L-glutamine (Invitrogen Life Technologies) and 10% FCS or in incubation buffer (20 mM HEPES, 132 mM NaCl, 6.0 mM KCl, 1.0 mM $MgSO_4$, 1.2 mM KH_2PO_4, supplemented with 5 mM glucose, 1.0 mM $CaCl_2$, and 0.5% w/v HSA).

Western blotting

Eosinophils (5×10^5 per sample) resuspended in HEPES-buffered RPMI 1640 supplemented with L-glutamine and 10% FCS were allowed to recover for 15 min at 37°C. Subsequently, cells were mock-stimulated or stimulated with Dex (10^{-6} M) for several time points (0, 15, 30, 60, and 120 min). In other Western blotting experiments, cells were mock-stimulated or stimulated with several concentrations of Dex (10^{-12}–10^{-6}M) or IL-5 (10^{-10} M) for 15 min at 37°C. After stimulation, cells were washed twice with PBS at 4°C. Cells were subsequently lysed in sample buffer (60 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, and 2% 2-ME) and boiled for 5 min.

Protein samples were analyzed on 10% SDS-polyacrylamide gels. Proteins were transferred to Immobilon-P (Millipore). The blots were blocked in hybridization buffer (10 mM Tris, 150 mM NaCl, and 0.3% Tween 20) containing 5% BSA for 1 h followed by incubation with anti-phospho-p38 MAPK (1/1000) and antiphospho-MAPKAP-K2 (1/1000), antiphospho-ERK 1/2 (1/1000), or anti-p38 MAPK (1/1000) in hybridization buffer with 5% BSA overnight at 4°C. After incubation with the first Ab, the blots were washed six times for 4 min in hybridization buffer. The second Ab (HRP-coupled swine anti-rabbit; 1/3000) was incubated in hybridization buffer with 5% BSA for 1 h at room temperature followed by five washings for 4 min in incubation buffer and a last wash step in PBS. Detection of all Western blots was performed by ECL plus (Amersham) and detected using a Typhoon 9410 (Amersham).

Fluorescent bead adhesion assay

The fluorescent bead adhesion assay was performed as described previously (42, 43). Fluorescent beads (TransFluorSpheres, 488/645 nm, 1.0 μm; Molecular Probes) were coated with ICAM-1 Fc fusion protein (derived from CHO-ICAM-1 Fc-producing cells provided by Professor Y. van Kooyk (VU University Medical Center Amsterdam, Amsterdam, The Netherlands) and containing an expression vector provided by Professor C. D. Buckley (University of Birmingham, Birmingham, U.K.). In short, 20 μl of streptavidin (5 mg/ml in 50 mM 2-(*N*-morpholino)ethanesulfonate buffer) was added to 50 μl of TransFluorSpheres, mixed with 30 μl of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.33 mg/ml), and incubated at room temperature for 2 h. The reaction was stopped by addition of glycine to a final concentration of 100 mM. Streptavidin-coated beads were washed three times with PBS and resuspended in 150 μl of PBS, 0.5% (w/v) BSA. Then, streptavidin-coated beads (15 μl) were incubated with biotinylated goat anti-human anti-Fc $F(ab')_2$ fragments (6 μg/ml) in 0.3 ml of PBS, 0.5% BSA for 2 h at 37°C. The beads were washed once with PBS, 0.5% BSA and incubated with ICAM-1 Fc supernatant overnight at 4°C. The ligand-coated beads were washed; resuspended in 100 μl PBS, 0.5% BSA, 0.01% sodium azide; and stored at 4°C.

For the fluorescent bead adhesion assay, eosinophils were resuspended in incubation buffer (5×10^4). Cells were preincubated with or without control anti-HLA-A, -HLA-B, and -HLA-C mAb W6/32 (10 μg/ml; American Type Culture Collection (ATCC) hybridoma), anti-β_2 integrin-blocking mAb IB4 (10 μg/ml; ATCC hybridoma); or the FcαR-blocking mAb My43 for 10 min at 37°C. The ligand-coated beads (40 beads/cell) were added in a 96-well V-shaped-bottom plate with several concentrations of Dex and/or IL-5. Next, the preincubated eosinophils were added and incubated for 15 min at 37°C. The cells were washed and resuspended in incubation buffer (4°C) and kept on ice until analysis. Binding of the fluorescent beads to the eosinophils was determined by flow cytometry using a FACSCalibur (BD Biosciences). Binding is depicted as the percentage of eosinophils that bind to ICAM-1-coated beads.

Preparation of Ig-coated magnetic Dynabeads

Ig-coated magnetic Dynabeads were prepared as described before (11). In short, Serum IgG and serum IgA were coated to uncoated magnetic Dynabeads (M-450; Dynal Biotech). Beads were washed twice with PBS, pH 8.5 and brought to a concentration of 45 mg/ml. IgG or IgA proteins were added at a final concentration of 1 mg/ml to the beads and mixed overnight at 4°C. The next day the beads were washed with borate buffer (0.5 M NaCl, 0.2 M H_3BO_3, and 0.02 M NaOH, pH 8.6) and blocked with 0.1 M lysine monohydrochloride, pH 8.6, in borate buffer for 2 h at room temperature. After two washes with 0.1 M acetate buffer, pH 4, beads were washed once with PBS with 1% w/v BSA. Until use, the beads were stored at 4°C in PBS-BSA at a concentration of 30 mg/ml (4×10^8 beads/ml). Before the rosette assay, beads were resuspended in 20% w/v HSA and left for 20 min at room temperature.

Rosette assay

Rosette assays were performed as described before (11). Purified eosinophils were washed with Ca^{2+}-free incubation buffer containing 0.5 mM EGTA and brought to a concentration of 8×10^6 cells/ml. A 50-μl cell suspension (0.4×10^6 cells) was incubated at 37°C. For priming, several concentrations of IL-5 and Dex were added at 1/10. Cells were incubated with IL-5, Dex, or IL-5 with Dex for 15 min at 37°C. After priming, the beads were added in a ratio of 3.5 beads/cell. Cells and beads were mixed briefly and pelleted for 15 s at 100 rpm. Eosinophils were incubated with beads during 15 min at 37°C. After incubation, cells were resuspended vigorously, and rosettes were evaluated under a microscope. All cells that bound two beads or more were defined as rosettes. One hundred cells were scored, and the number of beads that were bound to the cells was counted. The amount of beads bound to a total of 100 cells (bound and unbound to

beads) was designated as the rosette index. Previously, we have demonstrated that the rosetting method with magnetic beads is very specific given that 1) there is no appreciable background binding of eosinophils to control beads coated with OVA and 2) relevant blocking mAbs against Fc receptors inhibit the response (44).

Inhibition of rosette assays with specific GCR and p38 MAPK inhibitors

For priming-inhibition studies, cells were preincubated with specific inhibitors before priming with cytokines. Cells were incubated with p38 MAPK inhibitor SB203580 (10^{-6}M) or GCR antagonist RU38486 (10^{-6}M) for 15 min at 37°C.

Procedure for staining eosinophils with CD11b-PE and CD18-FITC

Blood from healthy donors was collected in tubes containing sodium heparin as anticoagulant and placed at 4°C. Before stimulation, whole blood was incubated for 10 min at 37°C. Whole blood was subsequently stimulated with several concentrations of Dex for 15 min or with Dex 10^{-6} M for 5 or 30 min. Hereafter, the blood was placed at 4°C and stained with CD11b-PE (1/100) and CD18-FITC (1/100) for 30 min at 4°C. Erythrocytes were lysed, and leukocytes were resuspended in ice-cold PBS containing trisodium citrate (0.4% w/v, pH 7.4) and HSA (4 g/L). Flow cytometric evaluation of eosinophils labeled with CD11b and CD18 was conducted using a FACSVantage flow cytometer (BD Biosciences). Eosinophils were identified according to their specific side scatter and forward scatter signals (45). Data are reported as median channel fluorescence in arbitrary units.

Statistical analysis

The results are expressed as means ± SEM. Statistical analysis was performed using paired Student *t* tests (statistical software package SPSS version 11.0). $p < 0.05$ was considered statistically significant.

Results

Lack of direct effect of Dex on eosinophil adhesion and integrin expression on the cell surface

We investigated the effect of short term incubation of corticosteroids on the functionality of β_2 integrins expressed by human eosinophils. This functionality was investigated by measuring the binding of human eosinophils to ICAM-1-coated fluorescent beads (42, 43). As shown in Fig. 1, IL-5 (10^{-10} M) can induce binding of eosinophils to ICAM-1-coated beads, whereas unprimed cells are characterized by very low binding to these beads. Treatment with Dex (10^{-6} M; 15 min at 37°C) did not influence the binding of eosinophils to ICAM-1-coated beads in the presence or absence of IL-5. This binding was β_2 integrin dependent as a blocking Ab against β_2 integrins (mAb clone IB4, 10 μg/ml) completely blocked the binding of eosinophils to ICAM-1-coated beads, whereas a control Ab anti-HLA-A, -HLA-B, and -HLA-C (mAb clone W6/32, 10 μg/ml) did not affect any condition used in this assay (data not shown).

In addition to integrin activation, priming of eosinophils can lead to increased expression of integrin CD11b/CD18 (MAC-1) on its cellular surface (46). As shown in Fig. 2, short term treatment with several doses of Dex for 15 min did not influence CD11b or CD18 expression on eosinophils. Detailed time courses with Dex up to 30 min did not reveal a different result (data not shown).

In addition to integrin expression and activation, we also studied chemokinesis of eosinophils during short term corticosteroid treatment using a modified Boyden chamber assay (47). Corticosteroids had no rapid effect on eosinophil chemokinesis in the presence or absence of IL-5 (data not shown). Therefore, although corticosteroids inhibit eosinophil adhesion and migration after prolonged incubation, there was no such effect after short term treatment.

Selective enhancement of FcαR functionality by Dex

To investigate whether other opsonin receptors expressed by eosinophils could be influenced by corticosteroids, the effect of short term incubation of Dex on Ig binding was investigated. As shown in Fig. 3, *A* and *C,* short term Dex incubation resulted in increased binding of IgA to both IL-5- primed (Fig. 3*A*) and, surprisingly, unprimed (Fig. 3, *A* and *C*) eosinophils. This effect was dose dependent as shown for unprimed eosinophils (Fig. 3*C*) with an optimal corticosteroid concentration between 10^{-8} M and 10^{-6} M. The enhanced binding of IgA-coated beads to Dex- and IL-5-primed eosinophils was mediated by an enhanced functionality of the FcαRI (CD89) as the binding was significantly inhibited by the blocking mAb My43 (Fig. 3*D*). Time courses revealed that the rosette formation of Dex-primed eosinophils and IgA-coated beads was optimal after incubation for 30–45 min, including 15 min preincubation of the cells with Dex before adding beads. These kinetics are very similar when compared with cytokine-induced

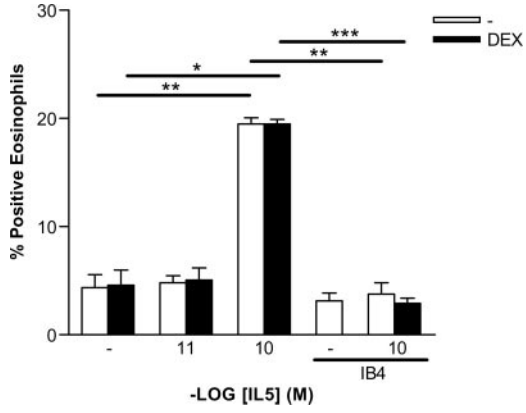

FIGURE 1. Lack of modulation of eosinophil adhesion by Dex as measured via β_2 integrin activation. Eosinophils were preincubated for 15 min with buffer or blocking Ab against β_2 integrins (mAb clone IB4; 10 μg/ml) as indicated. Subsequently, these eosinophils were added to wells containing ICAM-1-coated beads and buffer, IL-5 (10^{-11} M or 10^{-10} M), Dex (10^{-6}M), or Dex with IL-5. After 15 min at 37°C, the cells were washed, and the percentage of cells that were positive for beads was determined by flow cytometry. Mean values are presented ± SEM ($n = 3$). A paired Student *t* test was used to perform statistical analyses (*, $p < 0.05$; **, $p < 0.005$; and ***, $p < 0.0005$).

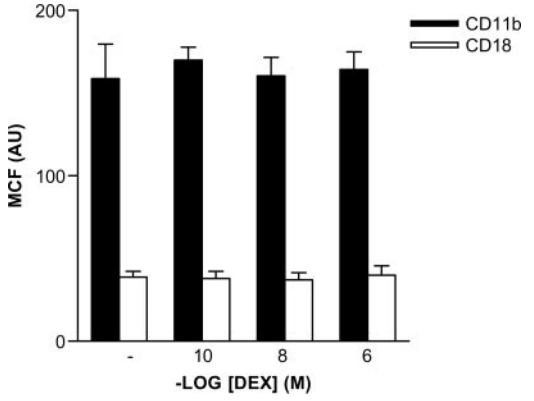

FIGURE 2. Absence of effect of Dex on the cell surface expression of CD11b/CD18 (MAC-1) on human eosinophils. Whole blood was preincubated at 37°C for 10 min and subsequently stimulated with several doses of Dex (10^{-10}–10^{-6} M). Whole blood was stained using directly labeled CD11b (■) and CD18 (□) and measured in a FACSVantage flow cytometer. Eosinophils were identified according to their specific side and forward scatter signals (45). Data are expressed as median channel fluorescence (MCF) in arbitrary units (AU) ± SEM ($n = 3$).

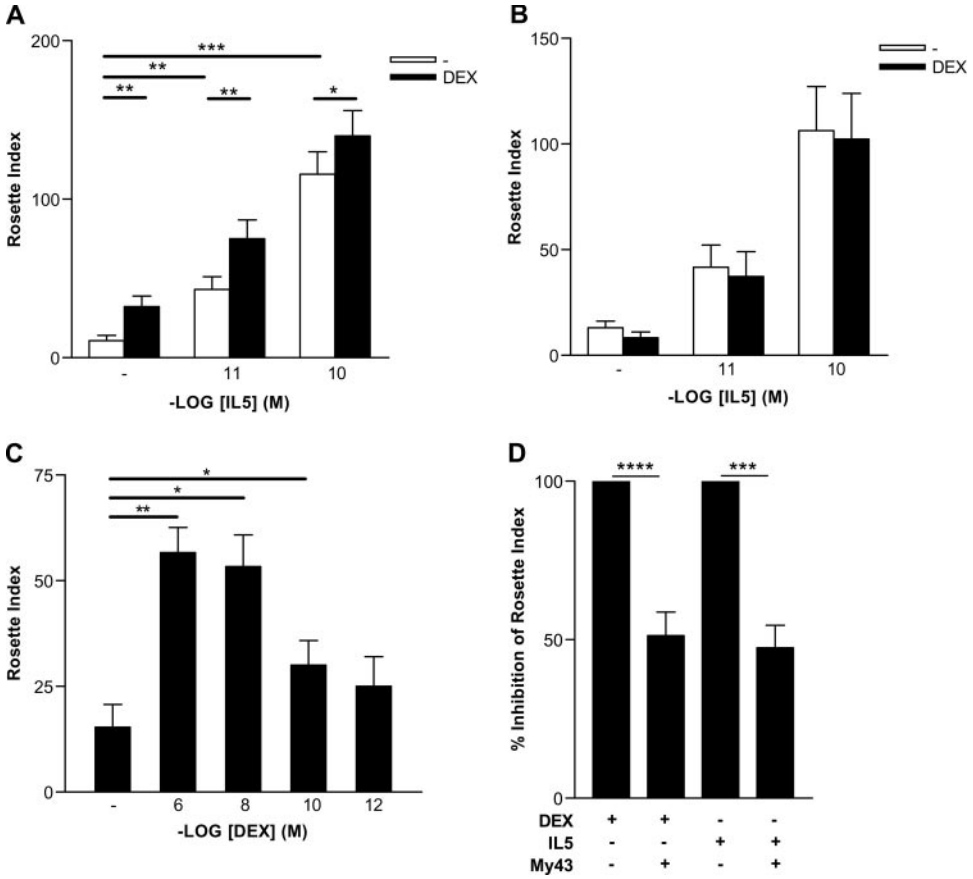

FIGURE 3. Specific enhancement of FcαR functionality by Dex. Eosinophils were preincubated for 15 min at 37°C with buffer (*A–C*) or mAb My43 (*D*) and subsequently stimulated at 37°C for 15 min with IL-5 (10^{-11} M or 10^{-10} M), Dex (10^{-6} M) or IL-5 together with Dex and incubated with IgA-coated beads (*A*) or IgG-coated beads (*B*) for 15 min. A dose-response curve of Dex (10^{-12}–10^{-6} M) was performed under the same conditions using IgA-coated beads (*C*). Specificity of the FcαR functionality toward IgA was evaluated using the blocking CD89 mAb My43 under conditions of IL-5 (10^{-10} M) or Dex (10^{-6} M) treatment (*D*). Binding of beads is expressed as a rosette index (number of beads per 100 cells; *A–C*) or presented as percentage inhibition from the Dex- or IL-5-induced rosette index (*D*). Mean values are presented ± SEM (*A, n* = 8; *B, n* = 3; *C, n* = 3; *D, n* = 6). A paired Student *t* test was used to perform statistical analyses. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$; and ****, $p < 0.00005$.

priming of FcαR on eosinophils (44). In marked contrast, binding of IgG-coated beads to resting or preactivated eosinophils was not affected by corticosteroid treatment (Fig. 3*B*), indicating the selectivity of Dex effects on FcαR activation on eosinophils.

Priming of FcαR by Dex is dependent on the GCR and is mediated by p38 MAPK and in part by PI3K

We first addressed the question whether the priming response induced by Dex was mediated by the cytosolic GCR. Therefore, IgA binding assays were performed in the absence or presence of the GCR antagonist RU38486. Admission of RU38486 inhibited the Dex-induced IgA binding, indicating that corticosteroids mediate the rapid effect of FcαR activation via the GCR (Fig. 4).

Previously, we have shown that in eosinophils activation of FcαR by cytokines is regulated via inside-out activation (11). We showed that cytokine-induced activation of FcαR is dependent on intracellular activation of p38 MAPK and PI3K, whereas FcγR regulation depends on activation of the ERK 1/2 pathway. Because Dex only induced IgA binding and did not affect IgG binding, we focused on the PI3K/p38 MAPK pathway. By using the specific p38 MAPK inhibitor SB203580 and PI3K inhibitor LY294002, we show that Dex-induced IgA binding could be significantly blocked by both inhibitors (Fig. 5), suggesting that the PI3K/p38 MAPK pathway is involved in Dex-mediated FcαR activation. In agreement with our previous study (11), the binding of IgA to IL-5-primed eosinophils could also be blocked using SB203580 or LY294002 (data not shown). Because JNK can also be induced by steroids (48), we evaluated the effect of inhibition of this kinase by the JNK inhibitor SP600125. As shown in Fig. 5*B,* this inhibitor did not significantly inhibit Dex-induced formation of IgA rosettes.

Rapid effect of corticosteroids on p38 MAPK activity in eosinophils

Inhibition of p38 MAPK by SB203580 results in clear inhibition of Dex-induced IgA binding by eosinophils, suggesting that the effect of Dex on FcαR is mediated via the p38 MAPK pathway. A short time course of p38 MAPK activation was performed to investigate whether p38 MAPK was activated by Dex. Indeed, Dex-induced p38 MAPK phosphorylation within 15 min in eosinophils (see Fig. 6*A*) as was also shown by Zhang et al. (48).

We next studied the Dex-induced activation of p38 MAPK in situ by evaluation of the phosphorylation of MAPKAP-K2, a specific downstream target for p38 MAPK (49–51). MAPKAP-K2 phosphorylation was clearly induced by Dex and was maximal after 15 min of stimulation (Fig. 6*A*). Similarly to formation of IgA rosettes Dex-induced phosphorylation of p38 MAPK was inhibited by the GCR antagonist RU38486 (results not shown). Dex-induced phosphorylation of p38 MAPK and MAPKAP-K2 was dose dependent (range, 10^{-12}–10^{-6} M) with an optimal dose range of 10^{-8}–10^{-6} M (Fig. 6*B*). This dose-response curve resembles the

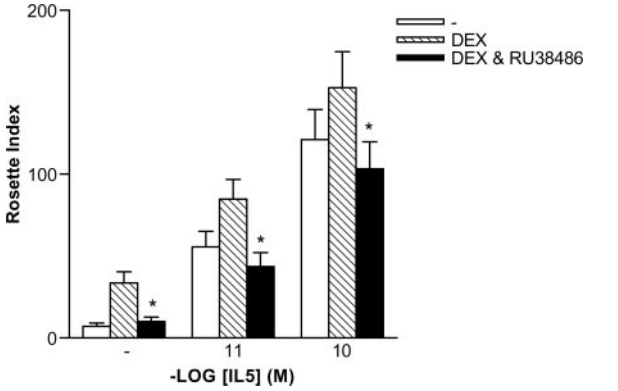

FIGURE 4. Enhancement of FcαR functionality by Dex is mediated by the GCR. Eosinophils were preincubated for 15 min at 37°C with buffer or GCR antagonist RU38486 (10^{-6} M). Subsequently, eosinophils were stimulated at 37°C for 15 min with IL-5 (10^{-11} M or 10^{-10} M), Dex (10^{-6} M), or IL-5 together with Dex and incubated with IgA-coated beads for 15 min. Binding of beads is expressed as a rosette index (number of beads per 100 cells). Mean values are presented ± SEM ($n = 4$). A paired Student t test was used to perform statistical analyses. *, $p < 0.05$ compared with samples without RU38486.

dose-response curve seen by Dex-induced IgA binding to eosinophils (Fig. 3*C*). In contrast to IL-5, which induced both the ERK and p38 MAPKs, Dex selectively induced p38 MAPK activation.

Discussion

Priming is a process by which cytokines influence the functionality of innate immune cells in normal immunity as well as under pathological conditions, e.g., chronic inflammatory diseases such as allergic asthma (2, 4, 5, 13, 52). Priming rapidly up-regulates responsiveness toward multiple inflammatory stimuli compared with nonprimed cells. This priming response is involved in the tight control of innate immune cells (such as neutrophils and eosinophils) because these belong to the most cytotoxic cells in the body.

In this study, we focused on the mechanisms of priming of eosinophil receptors involved in adhesion and in binding to opsonins such as Igs (FcRs) and complement fragments (MAC-1; CD11b/CD18). These processes were studied in the context of glucocorticoids because these drugs are routinely used to inhibit inflammatory responses in chronic inflammatory diseases such as asthma (18, 19). Previous studies have shown that prolonged (at least several hours) corticosteroid treatment: 1) reduces eosinophil infiltration into the lung and the residence time at its mucosal surfaces (53); and 2) reduces eosinophil adhesion (22) and migration (24) in vitro. Interestingly, corticosteroid treatment hardly affected IgA- and IgG-mediated degranulation of eosinophils (25).

Our experiments show that in several functional assays, short term corticosteroid treatment did not influence: 1) expression of CD11b/CD18 on the cell surface of eosinophils; 2) β_2 integrin functionality as measured by binding to ICAM-1-coated beads; and 3) chemokinesis measured in the absence and presence of IL-5 (Figs. 1 and 2 and data not shown). Also, we did not detect a rapid effect of Dex on the respiratory burst of eosinophils (data not shown). These data are in contrast to studies focused on the long term effects of corticosteroids within these parameters (22, 24).

In marked contrast, a clear up-regulation of the functionality of the FcαR on eosinophils was shown by short term Dex exposure with no effect on FcγRII. The priming of the functionality of FcαR by corticosteroids was additive and not synergistic to the IL-5 induced IgA binding. This process was mediated by FcαRI as blocking this receptor with the mAb My43 (40) significantly inhibited both Dex- and IL-5-induced rosette formation (Fig. 3*D*). Priming of the FcαR by corticosteroids was dependent on p38 MAPK and to a lesser extend on PI3K activation as the response was blocked by the specific p38 inhibitor SB203580 as well as the PI3K inhibitor LY294002 (Fig. 5). This is in line with our previous findings showing the pivotal role of both kinases in cytokine-induced inside-out regulation of FcαR (11). Zhang et al. (48) also showed that Dex can directly activate JNK. However, activation of this kinase does not seem to be essential in signal transduction leading to activation of FcαR (CD89), because the specific inhibitor SP600125 did not significantly inhibit the response. Dex did not influence FcγR, which fits with our previous findings that p38 MAPK is not involved in control of FcγRII (Ref. 11; see Fig. 3*B*). Although p38 MAPK was also suggested to be important in eosinophil migration and adhesion (54), our data indicate that short term activation of p38 MAPK by corticosteroids is not sufficient for modulation of adhesion and/or chemokinesis.

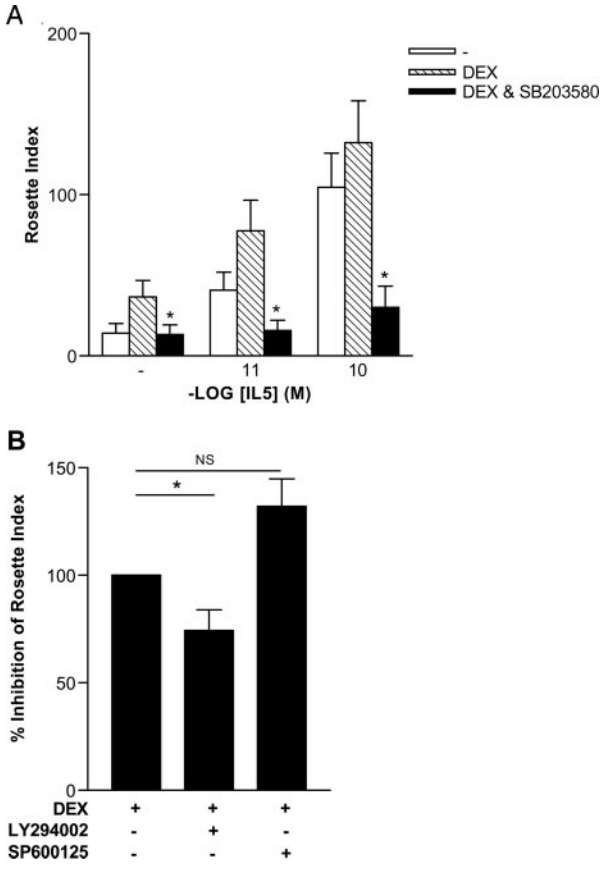

FIGURE 5. Dex-induced enhancement of FcαR functionality is mediated by activation of PI3K and p38 MAPK, but not via activation of JNK. Eosinophils were preincubated for 15 min at 37°C with buffer, SB203580 (10^{-6} M; *A*), LY294002 (10^{-6}M; *B*), or SP600125 (10^{-5} M; *B*). Subsequently, eosinophils were stimulated at 37°C for 15 min with IL-5 (10^{-11} M or 10^{-10} M), Dex (10^{-6} M), or IL-5 together with Dex and incubated with IgA-coated beads. Binding of beads is expressed as a rosette index (number of beads per 100 cells; *A*) or presented as percentage inhibition from Dex-induced rosette index (*B*). Mean values are presented ± SEM of at least four experiments. The paired Student t test was used to perform a statistical analysis. *, $p < 0.05$ compared with samples without inhibitors.

These functional findings prompted us to study the effect of short term addition of corticosteroids on the p38 MAPK signaling pathway in eosinophils. Addition of corticosteroids to eosinophils led to a fast and transient phosphorylation of p38 MAPK as visualized by Western blot analysis with phosphor-specific Abs. This

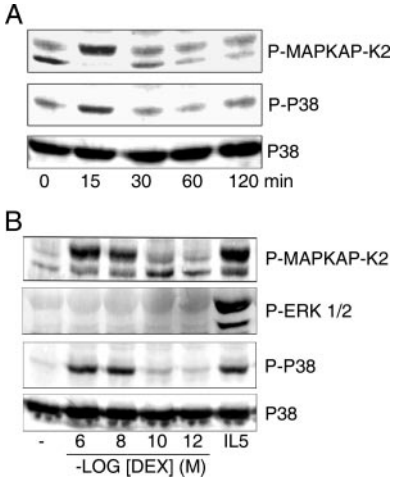

FIGURE 6. Time- and dose-dependent induction of p38 MAPK phosphorylation and activation in eosinophils by Dex. Eosinophils were preincubated for 15 min at 37°C and subsequently stimulated with Dex (10^{-6} M) for 15, 30, 60, or 120 min at 37°C (*A*) or stimulated with several concentrations of Dex (10^{-12}–10^{-6} M) or IL-5 (10^{-10} M) for 15 min (*B*). After stimulation, cell lysates were prepared in sample buffer. The blots were incubated with Abs to phosphor (P)-p38 MAPK, phospho-ERK 1/2 (*B*), and phospho-MAPKAP-K2 and reprobed with an Ab to total p38 MAPK as a loading control. The experiments shown are representative for at least three experiments.

effect was time and dose dependent (Fig. 6) and in line with the data published by Zhang et al. (48). Phosphorylation of p38 MAPK on Thr^{180} and Tyr^{182} in a Thr-Gly-Tyr loop is associated with activation of the kinase (49, 50). A better indication for the activation of p38 MAPK in situ is the determination of the phosphorylation of p38 MAPK downstream target kinase MAPKAP-K2 (49–51). Indeed, Dex also induced phosphorylation of MAPKAP-K2, demonstrating that apart from phosphorylation, p38 MAPK is also activated in situ by this corticosteroid. Maximal p38 MAPK and MAPKAP-K2 phosphorylation and activation were found with Dex concentrations in the range 10^{-8}–10^{-6}M within 15 min after addition of the corticosteroid. This time frame makes it unlikely that genomic actions of the ligand-bound corticosteroid receptors were involved (27, 28).

To gain more insight into involvement of the classical GCR in eosinophils, the different responses were studied in the context of its competitive inhibitor RU38486. Because this inhibitor blocked Dex-induced binding of IgA as well as phosphorylation of p38 MAPK in eosinophils, it is likely that the effect of corticosteroids on the FcαR was mediated via the cytosolic GCR.

In conclusion, glucocorticoids apart from their clear inhibitory effects on various effector mechanisms of innate immune cells can influence these cells in another and unique way. Both eosinophils and IgA are implicated in mucosal immunity, and it is striking that corticosteroids specifically prime the interaction between this ligand and these cells in vitro. Eosinophils from asthmatic patients even show increased FcαR activity compared with healthy donors most likely caused by an up-regulated PI3K pathway (13). The precise mechanisms underlying the inside-out control of FcαR are still poorly understood but converge at the phosphorylation of a C-terminal serine 263 (55). By up-regulation of this pathway, these cells are enhanced sensitive for stimuli that engage p38 MAPK such as TNF-α and corticosteroids. Glucocorticoids are first choice therapy in treatment of the (mucosal) inflammation in chronic inflammatory diseases such as allergic asthma (56). It is, however, unclear at this moment whether this short term corticosteroid induced up-regulation of the eosinophil response is beneficial or detrimental for the natural course of these diseases.

Disclosures

The authors have no financial conflict of interest.

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