Synergy Between Dexamethasone and Interleukin-5 for the Induction of Major Histocompatability Complex Class II Expression by Human Peripheral Blood Eosinophils

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The hematopoietic growth factors interleukin-3 (IL-3), IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) regulate the survival, maturation, and activation of eosinophils. Corticosteroids in contrast have a negative effect both on the hematopoietic process and the function of eosinophils. We have unexpectedly observed synergy between IL-5 and glucocorticoids such as dexamethasone and hydrocortisone for induction of the MHC class II antigens HLA-DR and HLA-DP on eosinophils isolated from human blood. Similarly glucocorticoids enhanced GM-CSF and IL-3, but not interferon γ (IFNγ), induced expression of these antigens. Expression of a third MHC class II molecule, HLA-DQ, was not induced on eosinophils by any of the cytokines alone, but in one of three donors tested, IL-3 plus dexamethasone induced high levels of expression. Although cytokine-induced expression of the accessory molecule intercellular adhesion molecule 1 (ICAM-1) was partially inhibited by glucocorticoids, cytokine- and dexamethasone-treated eosinophils presented antigen more efficiently to a hemagglutinin peptide-specific T-cell clone than eosinophils treated with cytokine alone. These results highlight a potential new role for endogenous or exogenous glucocorticoid hormones in enhancing MHC class II expression by eosinophils.

EOSINOPHILS PLAY a central role in the pathology of allergic diseases such as asthma, where their release of granule proteins and lipid mediators is thought to be responsible for the tissue damage observed.[1,2] Although normal blood eosinophils do not express major histocompatability complex (MHC) class II antigens or the adhesion molecule intercellular adhesion molecule 1 (ICAM-1), their expression can be induced in vitro after stimulation with cytokines. These eosinophils are subsequently able to present antigen to and activate T lymphocytes.[3-5] Cytokines shown to mediate eosinophil activation in culture include IL-3, IL-5, GM-CSF, and IFNγ. Eosinophils in sputum samples from asthmatic individuals have been shown to express both HLA-DR and ICAM-1, reflecting the immune activation occurring during the disease process in vivo.[6,7]

Glucocorticoids are used as anti-inflammatory agents in the treatment of allergy where they prevent accumulation of eosinophils at the site of disease and reduce the numbers of circulating eosinophils.[8,9] In vitro experiments show inhibition of cytokine-mediated eosinophil survival by glucocorticoids[10-12] and of cytokine-induced upregulation of complement receptor type 3.[13]

We have recently described how dexamethasone acts in synergy with GM-CSF or IL-3 to upregulate HLA-DR, DP, and DQ antigen expression by purified human blood monocytes.[14,15] These cells respond weakly to either GM-CSF or IL-3 for upregulation of MHC class II antigen expression in comparison to the levels expressed after stimulation with IFNγ or IL-4. Because eosinophils are one of the few cell types reported to respond to these cytokines for increased MHC class II antigen expression, the present study was designed to assess whether glucocorticoid hormones similarly enhance MHC expression by blood eosinophils. We present studies on the effects of dexamethasone and hydrocortisone, as well as three nonglucocorticoid steroid hormones, on cytokine induction of MHC class II antigen expression, and other antigen presenting functions of highly purified human blood eosinophils.

MATERIALS AND METHODS

Monoclonal antibodies. The anti-CD16 antibody used for the eosinophil purification (mouse IgG2a, clone CLB-149) and the rat-antimouse IgG2a immunomagnetic microbeads were purchased from Eurogenetics (Teddington, UK). The following antibodies were used for flow cytometry: anti-CD9 (The Binding Site Ltd, Birmingham, UK), anti-ICAM-1 (1H4; a kind gift from Dr Andrew Boyd, Melbourne, Australia), anti-B7 (B7/BB1; generously provided by Dr E. Clark[16,17]). Anti-CD14-FITC, anti-CD20-FITC, control mouse IgG and IgG-FITC were obtained from Becton-Dickinson (Oxford, UK). Anti-HLA-DR (L243[18]), anti-HLA-DP (B7/21[19]), anti-HLA-DQ (SPVL3; a gift from Dr H. Spits, DNAX, Palo Alto, CA[20]), anti-CD3 (OKT3; from ECACC, Porton Down, Salisbury, UK) were purified in the Department using protein A-sepharose CL-4B (Pharmacia, Uppsala, Sweden) and conjugated to fluorescein (Sigma Chemical Company, Poole, Dorset) using standard methods.[21] The neutralizing antibodies anti-hGM-CSF and anti-hIL-3 were obtained from Genzyme Corp (Kent, UK).

Cytokines and steroids. Human recombinant IL-3, GM-CSF, and IFNγ were a kind gift from Dr A. O'Garra (DNAX). Human recombinant IL-5 was generously provided by Drs T. Wells and E. Zanders (Glaxo, Geneva, Switzerland). The steroids dexamethasone, hydrocortisone, β-oestradiol, dehydroisoandrosterone (DHEA) and vitamin D3 were obtained from Sigma. Hydrocortisone, β-oestradiol, vitamin D3, and DHEA were maintained as 1 mmol/L stocks in 100% ethanol. Dexamethasone was maintained in 50% ethanol, 50% RPMI 1640 medium supplemented with 10% fetal calf serum (FCS).

Eosinophil purification from peripheral blood. Blood was obtained from healthy donors and eosinophils purified using an immunomagnetic bead technique.[22] Briefly, 50 mL of heparinized blood was diluted 1:1 with PBS and layered onto a Percoll (Pharmacia) solution with a density of 1.082 g/mL and centrifuged for 30 minutes at 1000*g*. The mononuclear cells at the interface were carefully removed. The red blood cell pellet was transferred to a clean tube and the erythrocytes lysed by the addition of ice-cold isotonic 155 mmol/L ammonium chloride solution. The remaining granulocytes

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were then washed and incubated on ice for 30 minutes with anti-CD16 antibody. The cells were washed and incubated for a further 20 minutes with rat-antimouse IgG2a-coated immunomagnetic particles. The magnetically labeled neutrophils were depleted by passing the granulocytes through a magnetic cell separation column (Miltenyi Biotec, Berisch-Gladbach, Germany, and Becton-Dickinson). This procedure resulted in greater than 99.5% pure eosinophils ($CD9^+$ granulocytes) with no detectable lymphocyte or monocyte contamination, as determined by flow cytometry.

Eosinophil culture. Purified eosinophils were cultured at a density of 1×10^6/mL in 96-well flat-bottomed plates (Nunc, Life Technologies, Paisley, Scotland) in a total volume of 200 μL RPMI 1640 medium supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (all purchased from GIBCO, Life Technologies, Paisley, Scotland) at 37°C in 5% CO_2. Cytokines and steroids were added at concentrations indicated in the results section. MHC class II expression on cultured eosinophils was usually assessed at day 5 or 6 of culture.

Flow cytometry. All flow cytometry was performed using an EPICS Profile II cell analyser (Coulter Electronics Limited, Luton, UK). Approximately 1×10^5 cells were incubated in round-bottomed 96-well plates in the presence of the appropriate antibody and 10 μg/mL goat IgG as a blocking agent for 1 hour at 4°C. The cells were then washed twice with phosphate-buffered saline containing 1% bovine serum albumin (Sigma). For unconjugated antibodies goat-antimouse IgG-FITC was added to the cells for a further 30-minute incubation. Live cells were gated according to forward- and side-light-scatter profile that had previously been determined for live cells by propidium iodide (Sigma) staining. Data was collected from 3,000 to 5,000 cells/sample. Gates for positively or negatively staining cell analysis were adjusted so ≈2% to 5% of the cells stained positive after treatment with a control mouse IgG antibody (see Fig 1, C and D). The same gate was used on cells treated with specific antibody to determine the percentage of positively staining cells. The log mean fluorescence intensity values were assessed for the whole cell population, an increase in the value indicating an increase in the cell surface antigen density at the population level.

Influenza virus hemagglutinin peptide-reactive T-cell clone. Cloning and maintenance of an influenza virus hemagglutinin peptide-reactive T-cell clone, HA1.7, has been previously described in detail.[23] This clone is specific for residues 307-319 (PKYVKQNTLKLAT) of hemagglutinin. Analysis of the HLA-D restriction of this clone has previously shown that this clone recognizes the HA peptide 307-319 in the context of DRB1*0101.[24]

HLA-DR expressing eosinophils and antigen presentation to HA1.7 T-cell clone. Highly purified eosinophils were cultured for 24 hours as described above in the presence of 50 U/mL GM-CSF with and without the presence of 10^{-8} mol/L dexamethasone. The eosinophils were then washed three times in medium and viable cells counted using trypan blue (Sigma). Eosinophils were resuspended at the appropriate cell concentration in RPMI 1640 medium supplemented with 5% screened, heat-inactivated human A^+ serum (National Blood Transfusion Service, Edgware), 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (GIBCO). T-cell proliferation assays were set up using round-bottomed 96-well plates with doubling dilutions of eosinophils ranging from 2.5×10^3 to 2×10^4 eosinophils/well. Cloned T lymphocytes were added at 2×10^4 cells/well in the presence or absence of 1 μg/mL HA peptide 307-319. The cells were cultured for 48 hours at 37°C in 5% CO_2 before pulsing with tritiated thymidine (1 μCi/well, Amersham International Plc, Amersham, UK) for the last 6 hours of culture. Proliferation was determined by ^{3}H-thymidine incorporation measured using a liquid-scintillation beta counter (Pharmacia).

RESULTS

Contrasting effects of dexamethasone on IFNγ- and GM-CSF–induced expression of HLA class II antigens by human eosinophils. Eosinophils were purified from peripheral blood by density-gradient centrifugation and use of a magnetic cell separator to deplete $CD16^+$ neutrophils. The cells were greater than 99.5% pure based on forward- and side-scatter analysis by flow cytometry (data not shown) and by staining with an antibody specific for CD9 that is expressed by eosinophils but not neutrophils (Fig 1A). These cells did not express MHC class II antigens (Fig 1B; less than 4% $HLA\text{-}DR^+$ in all experiments). To determine the effect of glucocorticoids on GM-CSF-induced expression of the three major MHC class II antigens, HLA-DR, -DP, and -DQ, eosinophils were cultured for 5 days with 50 U/mL recombinant human GM-CSF, a concentration of cytokine found to

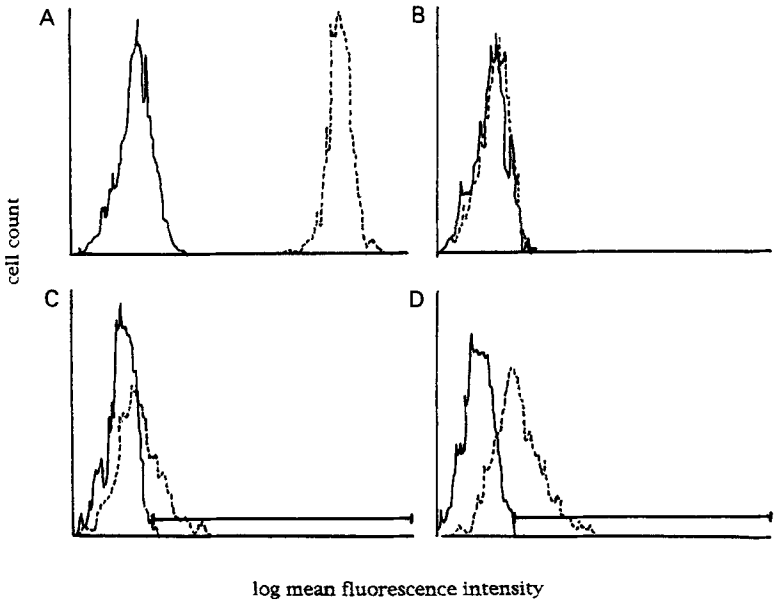

Fig 1. Dexamethasone enhances MHC class II expression by eosinophils cultured with GM-CSF. (A) Granulocytes depleted of $CD16^+$ cells using the MACS magnetic cell separation system are greater than 99.5% positive for the eosinophil marker CD9. (B) Purified blood eosinophils do not express HLA-DR before culture. (C, D) Eosinophils were cultured for 5 days with 50 U/mL GM-CSF in the absence (C) or presence (D) of 10^{-8} mol/L dexamethasone and then stained for expression of HLA-DR. (—, control staining; ....., specific staining). Data are presented as percent positively staining cells where cell staining with control mouse IgG-FITC is less than 2% as shown by the analysis gates in C and D. The log mean fluorescence intensity values are (C) control, 2.69; HLA-DR, 3.94; (D) control, 2.29; HLA-DR, 5.12.

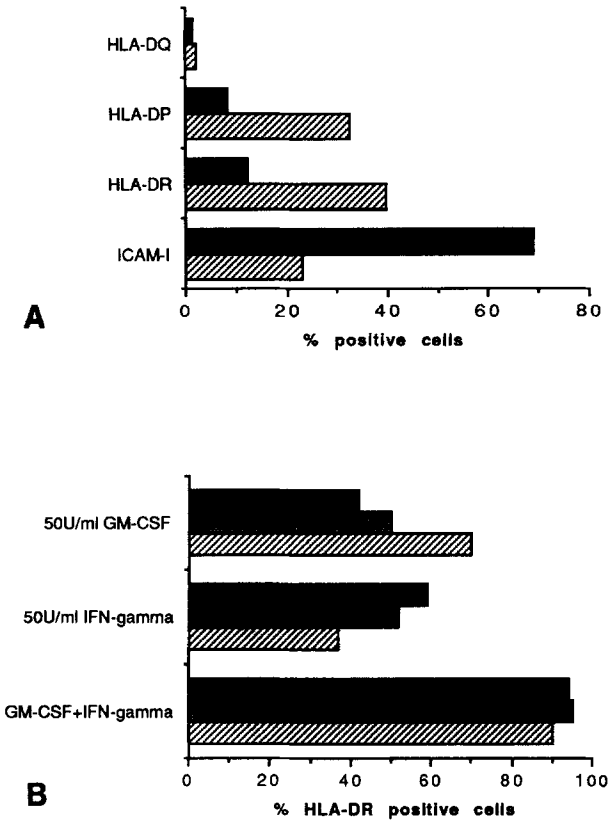

Fig 2. A comparison of the effects of dexamethasone on GM-CSF, IFNγ, and GM-CSF plus IFNγ-induced MHC class II expression. (A) Purified eosinophils were cultured for 6 days with 50 U/mL GM-CSF with (■) or without (■) 10^{-7} mol/L dexamethasone or (B) for 5 days with GM-CSF, IFNγ, or both cytokines at 50 U/mL in the presence of 10^{-9} mol/L (▨), 10^{-8} mol/L (■), or absence (■) of dexamethasone. Cells were stained for MHC class II and ICAM-1 expression as described in the Materials and Methods and data are presented as percent positive cells. Percent cells staining with control mouse IgG-FITC was less than 3% for all treatments.

give optimal cell survival in culture, in the presence or absence of dexamethasone. Stimulation with GM-CSF induced 28% of the eosinophils to express HLA-DR (Fig 1C), whereas addition of 10^{-8} mol/L dexamethasone enhanced expression to 50% positive cells (Fig 1D). In this experiment, no expression of HLA-DP or HLA-DQ was found with any of the treatments. In a second donor, GM-CSF induced both HLA-DR and HLA-DP expression (Fig 2A) which were enhanced by dexamethasone. Cells cultured for 5 days in medium or dexamethasone alone did not survive. The concentrations of glucocorticoids used in all experiments were found to have no effect on cell viability in the presence of cytokine.

Variability for the induction of MHC class II expression was noted amongst eosinophils isolated from different donors. GM-CSF induced HLA-DR expression in all donors tested, with levels varying from 21% to 94%. In 10 of 13 experiments, dexamethasone enhanced expression of HLA-DR, and in the three experiments where no enhancement was observed, expression induced by GM-CSF alone was very high, making it difficult to see over this baseline. GM-CSF-induced HLA-DP expression in six of seven individuals tested (range, 8% to 34%; day 0, less than 2% in all cases) with marked upregulation of HLA-DP antigen by dexamethasone being observed (Fig. 2A). One individual failed to show HLA-DP expression after any of these treatments when tested in five experiments over a 3-month period. GM-CSF, with or without dexamethasone, failed to induce HLA-DQ expression in all donors tested (Fig 2A).

IFNγ has previously been reported to induce MHC class II expression on eosinophils and to enhance GM-CSF upregulation of these antigens.[4] Although dexamethasone enhanced the expression of HLA-DR induced by GM-CSF (42% to 70%), induction by IFNγ was inhibited (59% to 37%). Cells cultured in the presence of both cytokines showed greater expression of HLA-DR than with either cytokine alone (94% positive cells); however, dexamethasone had little effect on this expression (90%).

Effect of dexamethasone on IL-3–induced expression of HLA class II antigens. The effect of dexamethasone on IL-3 induction of MHC class II antigens was also tested because this cytokine shares many biologic properties with GM-CSF, including the capacity to stimulate HLA-DR expression by eosinophils.[4] IL-3, used between 3 and 250 ng/mL, induced very high expression of HLA-DR in all three individuals tested. The further addition of dexamethasone did not significantly enhance the percentage of positively staining cells, but did induce a clear increase in the log mean fluorescence intensity. In a representative experiment, IL-3 alone induced a log mean fluorescence intensity of 41, which was increased to 92 in the presence of 10^{-8} mol/L dexamethasone.

Enhancement of HLA-DP expression by dexamethasone was also observed with all three donors tested. In a representative experiment for HLA-DP expression (Fig 3) IL-3 alone induced 59% of cells to express HLA-DP. Dexamethasone at 10^{-9} mol/L increased expression to 82% and at 10^{-8} mol/L to 92%. Although one individual was induced to express significant levels (50%) after stimulation with IL-3 plus dexamethasone (Fig 3), expression of HLA-DQ was not upregu-

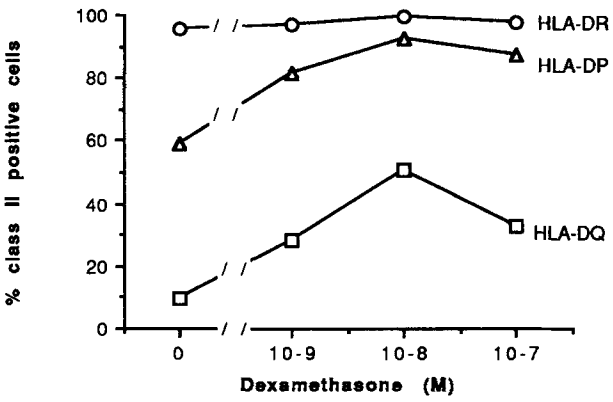

Fig 3. IL-3 induction of MHC class II antigens on purified eosinophils is enhanced by dexamethasone. Purified eosinophils were cultured for 6 days with 250 ng/mL IL-3 with or without dexamethasone at the concentrations indicated. HLA-DR (○), HLA-DP (△), and HLA-DQ (□) expression was analyzed by immunostaining. Control mouse IgG staining was less than 8%. Purified eosinophils stained before culture showed no expression of HLA-DR, HLA-DP, or HLA-DQ.

lated after exposure to IL-3 alone in any of the three donors tested. In a total of four experiments performed, HLA-DQ expression by eosinophils treated with IL-3 in the absence or presence of dexamethasone was as follows: donor 1, 6.3 > 8.0; donor 2, 9.9 > 50.5; donor 3, 3.6 > 5.2, and 3.4 > 2.8 percent positive cells. Thus, although IL-3 appears the more potent cytokine for MHC induction, dexamethsone appears to act in a similar fashion to enhance both IL-3 and GM-CSF-induced MHC class II expression by eosinophils.

Effect of dexamethasone on IL-5–induced expression of HLA class II on eosinophils. A previous study[5] has shown weak induction of HLA-DR on human eosinophils cultured for 48 hours with IL-5, and therefore, the effect of dexamethasone with this cytokine was tested. Eosinophils cultured in the presence of 0.5 to 50 ng/mL IL-5 showed modest induction of HLA-DR antigens (Fig 4A). The mean level of HLA-DR induced by IL-5 (32% ± 21%, n = 10) was considerably lower than that observed after stimulation with GM-CSF or IL-3 (53% ± 23%, n = 13; and 92% ± 8%, n = 4, respectively). However, in all cases, dexamethasone greatly enhanced IL-5-induced expression of HLA-DR with optimal enhancement occurring with 10^{-8} mol/L or greater concentrations of dexamethasone. Enhancement of IL-5-induced HLA-DR expression was seen in 10 experiments (seven donors) the mean ± SD induction with IL-5 alone being 32% ± 21%, and with IL-5 plus dexamethasone, 64% ± 20%.

Although IL-5 alone did not induce HLA-DP expression by eosinophils, dexamethasone plus IL-5 induced expression in six of seven donors (Fig 4B shows HLA-DP expression for a representative donor). IL-5 failed to induce HLA-DQ expression by any of the donors tested in the presence or absence of dexamethasone. Thus, a hierarchy appears to exist between the three cytokines for inducibility of MHC class II expression by eosinophils, with IL-3 the most potent and IL-5 the least. Although data are presented as percent positive eosinophils, this hierarchy is also apparent when data are expressed as log mean fluoresence intensity of class II expression. Thus, in a representative experiment, log mean fluorescence intensity for HLA-DR after optimal stimulation with IL-3, GM-CSF, and IL-5 were 99, 24, and 13, respectively, where staining with control antibody was less than 7 for all treatments.

When the kinetics of HLA-DR expression on eosinophils cultured with IL-5 was investigated, expression was barely detectable on the second day of culture (8% positive cells; GM-CSF-treated cells, 38%), although this was doubled by the presence of 10^{-7} mol/L dexamethasone (Fig 5A). IL-5-induced expression was maximal by day 4 of culture (21% positive cells) and this level was maintained at day 6. Dexamethasone increased the expression of HLA-DR at day 4 (53% positive cells) and this enhancement was maintained on day 6 of culture. The weaker induction of MHC class II expression by IL-5 may account for the much greater increase observed in the presence of dexamethasone as compared with GM-CSF and IL-3.

On the basis of the above results, we postulated that the effects of IL-5 may be indirect, perhaps by inducing the synthesis of other cytokines such as GM-CSF or IL-3 by eosinophils. To test this hypothesis and to prove that induction by GM-CSF and IL-3 was specific, neutralizing antibodies to these two cytokines were tested for their capacity to inhibit MHC class II expression. In the experiment shown in Table 1, IL-5 alone induced 7% of eosinophils to express HLA-DR, and IL-5 plus dexamethasone, 32%. Antibodies to IL-3 or GM-CSF, either alone or in combination, failed

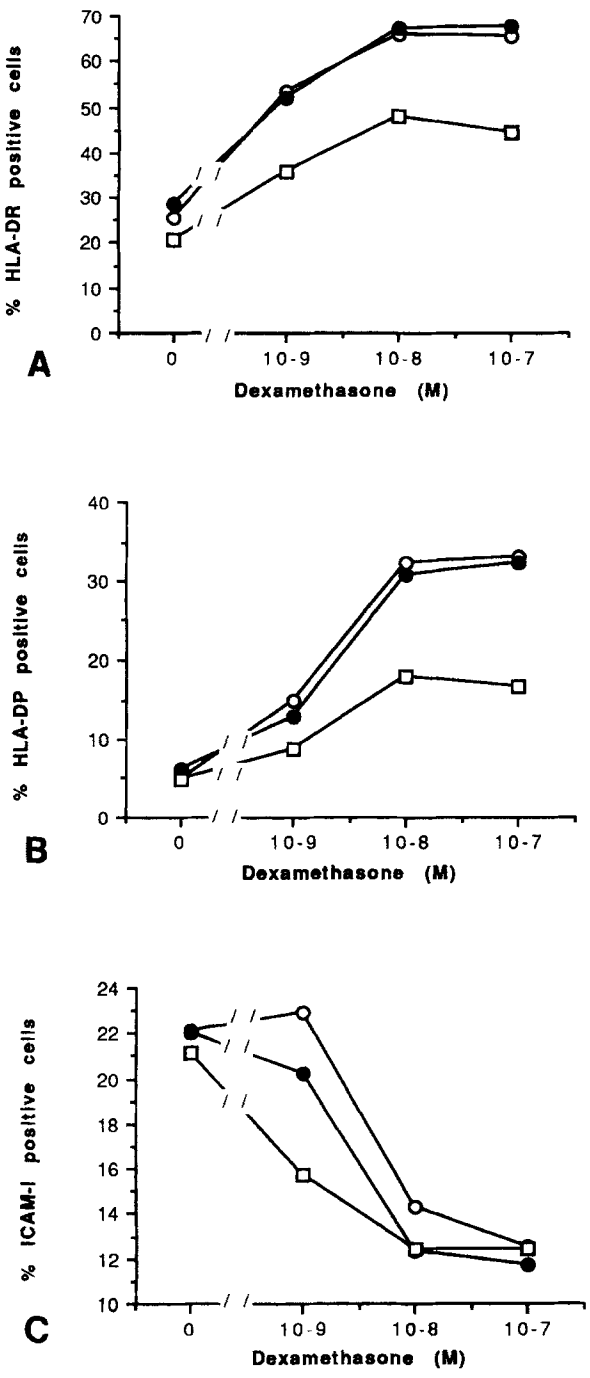

Fig 4. Dexamethasone and IL-5 exhibit synergy for induction of MHC class II antigen expression on eosinophils. Purified eosinophils were cultured for 6 days with 50 (○), 5 (●) or 0.5 ng/mL (□) IL-5 in the presence or absence of the indicated concentrations of dexamethasone and expression of HLA-DR (A), HLA-DP (B), and ICAM-1 (C) measured as described in Materials and Methods. Control staining with mouse IgG was less than 5% in all cases.

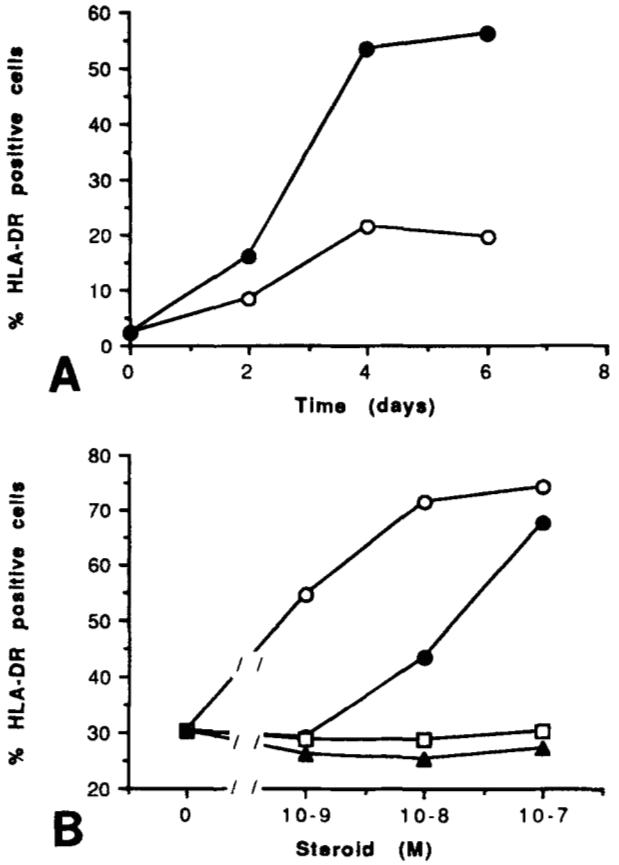

Fig 5. Eosinophil expression of IL-5 induced HLA-DR with time in culture. (A) Eosinophils were cultured with 5 ng/mL IL-5 in the presence (solid symbols) or absence (open symbols) of 10^{-7} mol/L dexamethasone and the cells immunostained for HLA-DR expression at the times indicated. (B) The effect of dexamethasone, hydrocortisone, β-oestradiol and DHEA on IL-5 induced expression of HLA-DR on cultured eosinophils. Purified eosinophils were cultured for 5 days in the presence of 5 ng/mL IL-5 and the indicated concentrations of dexamethasone (○), hydrocortisone (●), β-oestradiol (squares), or DHEA (triangles). HLA-DR expression was analyzed by immunostaining. Control mouse IgG-FITC staining was less than 3%.

to inhibit the level of expression induced with both IL-5 and dexamethasone (Table 1). In the same experiment, the effects of IL-3 and GM-CSF were inhibited by the appropriate antibody. Therefore, these experiments show that MHC class II induction by IL-5 in the presence of dexamethasone is not mediated by IL-3 or GM-CSF.

A comparison of the capacity of other steroid hormones to upregulate MHC class II expression. The specificity of the dexamethasone mediated enhancement of MHC class II expression was investigated by using a second glucocorticoid, hydrocortisone, and three nonglucocorticoid steroids. Dexamethasone and hydrocortisone both produced a dose-dependent enhancement of IL-5-induced expression of HLA-DR (Fig 5B). Similar results were obtained with GM-CSF-stimulated eosinophils (data not shown). However, in line with the known pharmacologic potency of these glucocorticoids, hydrocortisone appears to be 10- to 100-fold less active than dexamethasone. Two nonglucocorticoid steroids tested, β-oestradiol and DHEA, had no effect on class II expression when tested between 10^{-7} and 10^{-10} mol/L. In a separate experiment, a third nonglucocorticoid steroid, vitamin D3, was also found to have no effect on IL-5-induced expression of HLA-DR or DP (data not shown).

Effect of dexamethasone on cytokine-induced expression of ICAM-1. Previous studies have shown that sputum eosinophils from symptomatic asthmatics express HLA-DR and the adhesion molecule ICAM-1,[6] and that cultured peripheral blood-derived eosinophils can be induced to express ICAM-1 by cytokine stimulation.[4] These antigens are believed important in the documented capacity of eosinophils to present antigen to T lymphocytes in vitro.[4,5] The effect of dexamethasone on cytokine-induced expression of ICAM-1 was investigated in parallel with HLA-DR. Culture of eosinophils for 6 days with 50 U/mL GM-CSF induced 69% of the viable cells to express ICAM-1 in one donor (8% positive on day 0). Dexamethasone markedly inhibited GM-CSF-induced ICAM-1 expression in this experiment (23% positive cells), in contrast with the enhancement of MHC class II expression observed (HLA-DR, 12% and 40% positive cells; HLA-DP, 8% and 33% in the absence and presence of dexamethasone, respectively; Fig 2A). Dexamethasone inhibited GM-CSF induction of ICAM-1 expression: in 11 experiments, mean ICAM-1 expression by eosinophils cultured with cytokine, without or with 10^{-8} mol/L dexamethasone was 35% ± 23% and 15% ± 7%, respectively. Expression of ICAM-1 was also induced by both IL-3 and IL-5, and dexamethasone inhibited expression in a dose-dependent manner at each concentration of IL-5 tested (Fig 4C) and in two of three experiments where induction by IL-3 was studied (data not shown). Thus, dexamethasone shows contrasting effects on ICAM-1 and class II expression, two important components of a cell's capacity to present antigen to T cells.

Expression by eosinophils of a second costimulatory molecule, B7/BB1 (CD80), which has a clearly documented role in T-cell activation was also studied.[25] No constitutive

Table 1. Antibodies Specific for GM-CSF and IL-3 Do Not Affect IL-5–Induced Expression of MHC Class II by Eosinophils

	Antibody Concentration (mg/mL)	IL-5	IL-5 + Dexamethasone	IL-3	GM-CSF
No antibody	0	7	32	48	30
Anti-IL-3	100	—	37	*	—
Anti-GM-CSF	100	—	39	—	16
Anti-IL-3 and anti-GM-CSF	100	—	43	—	—
Anti-IL-3 and anti-GM-CSF	50	—	34	—	—

Purified eosinophils were cultured for 6 days with 5 ng/mL IL-5 in the presence or absence of 10^{-7} mol/L dexamethasone, or with 5 ng/mL IL-3 or 50 U/mL GM-CSF, and antibodies as shown in the table. HLA-DR was measured by immunostaining as described in Materials and Methods. Cells cultured with IL-3 and anti-IL-3 (*) did not survive in culture presumably because of neutralization of all the cytokine present in the culture. (—), represents not done in this experiment.

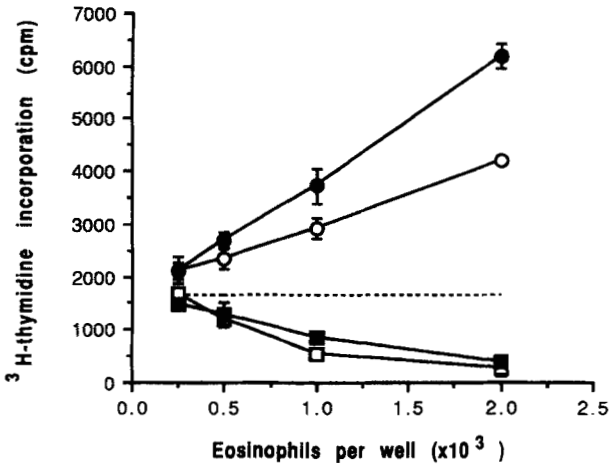

Fig 6. Dexamethasone enhances antigen presentation by eosinophils. Highly purified eosinophils from an HLA-matched (DR1, circles) and an HLA-mismatched (DR8, squares) donor were cultured for 24 hours with 50 U/mL GM-CSF in the presence (filled symbols) or absence (open symbols) of 10^{-8} mol/L dexamethasone. The eosinophils were then washed and cultured at the cell concentrations indicated with 2×10^4 T cells (HA1.7 clone) and 1 mg/mL influenza virus hemagglutinin peptide 307-319 for 48 hours as described in Materials and Methods. Proliferation of the T-cell clone, HA1.7, was measured as ^{3}H-thymidine incoporation and expressed as the mean ± SD of triplicate cultures. (----), Proliferation of T-cell clone with antigen alone. HLA-DR and ICAM-1 expression by eosinophils was measured at 24 hours by immunostaining as described in Materials and Methods. The DR1 donor showed 50% and 38% HLA-DR, and 46% and 59% ICAM-1$^+$ cells in the presence and absence of dexamethasone, respectively. The DR8 donor showed 27% and 22% HLA-DR, and 16% and 11% ICAM-1$^+$ cells in the presence and absence of dexamethasone, respectively.

expression of B7 on purified eosinophils was observed, despite strong immunostaining of Epstein-Barr virus-transformed B cells with the same reagent (data not shown). GM-CSF, in the presence or absence of dexamethasone, failed to induce B7 expression on eosinophils isolated from two separate donors (data not shown). Furthermore stimulation of eosinophils with GM-CSF or GM-CSF plus IFNγ in the presence or absence of dexamethasone failed to induce B7 expression.

The effect of dexamethasone treatment on the antigen presenting function of eosinophils. Dexamethasone partially inhibits ICAM-1 expression while enhancing MHC class II expression. To assess the potential functional relevance of exposure of eosinophils to cytokine and dexamethasone, the capacity of the different eosinophil populations to present antigen to the T cell clone HA1.7 (DR1 restricted; specific for the hemagglutinin peptide 307-319) was examined. Eosinophils from a DR1 and a DR8 donor were stimulated for 24 hours with 50 U/mL GM-CSF in the presence or absence of 10^{-8} mol/L dexamethasone. Cytokine stimulated eosinophils from the DR1 donor presented peptide to the T-cell clone, and the amount of proliferation was proportional to the number of eosinophils present (Fig 6). Dexamethasone-treated eosinophils showed enhanced presentation of the peptide compared with cells stimulated by cytokine alone. Presentation appeared to be DR restricted because eosinophils purified from a donor of the inappropriate DR8 haplotype failed to present peptide. Although measurements were made after only 1 day of culture, GM-CSF induced HLA-DR expression was enhanced by dexamethasone in both donors, whereas ICAM-1 expression was partially inhibited. Thus, dexamethasone and GM-CSF increase the antigen-presenting activity of eosinophils corresponding to the increase in class II expression, despite the decrease in ICAM-1.

DISCUSSION

We have examined the effect of glucocorticoid hormones on cytokine-induced MHC class II antigen expression by human eosinophils. Three major conclusions can be drawn from our study. Firstly, glucocorticoids upregulate IL-3, IL-5, and GM-CSF induction of HLA-DR and -DP expression by eosinophils. Secondly, this effect appears to be glucocorticoid specific because dexamethasone and hydrocortisone, but not three nonglucocorticoid steroid hormones, mediate this effect. Finally, treatment with dexamethasone and cytokines augments the antigen-presenting cell function of eosinophils to cloned T lymphocytes.

IL-5 was observed to be a much weaker inducer of MHC class II antigen expression by eosinophils than IL-3 and GM-CSF, inducing low levels of HLA-DR, but no HLA-DP expression. Nevertheless, dexamethasone had a much greater effect on IL-5 than on IL-3- or GM-CSF-induced antigen expression, and it seems likely that this reflects the much weaker induction by cytokine alone. In this respect, it is noteworthy that similar to the effect of IL-5 on eosinophils, synergy is observed between GM-CSF or IL-3 and dexamethasone for upregulation of HLA-DR, HLA-DP, and HLA-DQ antigen expression on purified monocytes. On monocytes, these cytokines alone either fail to upregulate or induce very low levels of antigen expression.[14] In contrast with the enhancement by dexamethasone of hematopoietic growth factor-induced MHC class II expression, induction by IFNγ was inhibited by glucocorticoids under the same experimental conditions.

IL-3, IL-5, and GM-CSF, but not IFNγ, belong to a family of cytokines that share many functional similarities. These cytokines share a common β chain of their heterodimeric cytokine receptor molecule that combines with unique α chains (IL-3α, IL-5α, and GM-CSFα) to confer specificity for each cytokine. These shared receptor properties are likely to represent a common signal transduction pathway distinct from that induced by IFNγ, and therefore, reflect a common mechanism of glucocorticoid action for enhancement of MHC antigen expression.[26,27] Although the mechanisms of regulation of MHC expression by glucocorticoids on eosinophils have not yet been defined, evidence from other experimental systems suggest that complex effects on both gene transcription and translation may occur.[28,29] For example, recent experiments by Celada et al[30] on murine B lymphocytes and macrophages suggest that repression of MHC IA expression by glucocorticoids is caused by prevention of binding of the X box binding protein to the X box DNA sequence located within the IAβ promotor and thereby inhibiting gene transcription.[30]

Our results highlight the increased potency of IL-3, in comparison with IL-5 or GM-CSF, for induction of MHC

class II antigens observed throughout this study and in at least one independent report.[4] This was apparent both when HLA-DR expression was expressed as percent positive cells, and more strikingly, when data were presented as mean fluorescence intensity values. In addition, IL-3, in the presence or absence of dexamethasone, was able to induce the greatest levels of HLA-DP in all individuals. Similarly, although none of the three hematopoietic growth factors alone induced expression of HLA-DQ, induction was observed in one donor when eosinophils were stimulated with IL-3 plus dexamethasone. These experiments show not only the greater capacity of IL-3 to induce MHC class II antigens, but also imply differential regulation of HLA-DQ as compared with HLA-DR and perhaps HLA-DP on eosinophils. The reason why it is more difficult to upregulate HLA-DQ expression and whether some individuals are completely unable to upregulate HLA-DQ remain important issues to address.

The enhancement of MHC class II expression was found to be glucocorticoid specific. Three nonglucocorticoid steroids, β-oestradiol, DHEA, and vitamin D3 had no effect on induction of MHC class II antigen expression by eosinophils. A second glucocorticoid, hydrocortisone, also enhanced HLA-D antigen expression induced by hematopoietic growth factors. However, hydrocortisone was considerably less potent than dexamethasone, requiring a 10- to 100-fold higher concentration than dexamethasone to achieve similar enhancement of MHC class II expression. These results are consistent with the known pharmacologic potency of these mediators.[31]

In contrast with the potent augmentative effects of glucocorticoids and cytokines on HLA-D antigens, expression of ICAM-1 was reduced. In addition, we were unable to detect expression of B7/BB1 by eosinophils with any of the treatments tested. Because all these functions are believed important for T-cell activation, the effect of glucocorticoid treatment on the antigen-presenting function of eosinophils was assessed. Glucocorticoid-treated eosinophils induced greater proliferation of an influenza peptide-specific T-cell clone than those treated with cytokine alone. Although glucocorticoids greatly enhance MHC antigen expression by eosinophils, the levels observed are still significantly lower than those on "professional" antigen-presenting cells such as macrophages, dendritic cells, or B lymphocytes. Considerable functional heterogeneity exists among different antigen-presenting cell populations that is likely to greatly influence the T-cell response that develops after antigenic challenge. Although dendritic cells may be better able to initiate primary immune responses, we envisage that other "nonprofessional" antigen-presenting cells, such as eosinophils, may be better able to present antigen to already-activated T cells, which require less costimulatory function, and thus, maintain ongoing immune responses.

The physiologic significance of the enhanced MHC class II expression and enhanced antigen-presenting cell function by glucocorticoids in vitro is intriguing. Glucocorticoids at the concentrations found to be effective in this study are within the levels thought to be active physiologically and achieved pharmacologically. At sites of allergic inflammation, mRNA specific for cytokines such as GM-CSF, IL-3, IL-4, and IL-5[32,33] has been detected and is thought to derive variously from T cells, macrophages, and eosinophils. However, T cells synthesising IFNγ, the other principal mediator inducing MHC class II expression, are absent. Because eosinophils recovered from inflammatory sites, such as the sputum of asthmatics, show significant expression of HLA-DR,[6,7] a role for hematopoeitic growth factors with either endogenous or exogenously derived glucocorticoids in regulating these molecules in vivo is possible.

The observation of synergy between glucocorticoids and hematopoeitic growth factors in the induction of MHC class II antigens and enhancement of antigen presentation is in contrast with the perceived role of glucocorticoids as anti-inflammatory and immunosuppressive mediators. These steroids are used as immunosuppressive agents for the treatment of conditions as diverse as leukemia, prevention of transplant rejection, systemic lupus erythematosus, and vasculitides. Their mode of action is thought, at least in part, to be caused by their potent inhibition of cytokine production including IL-1, IL-2, IL-3, IL-6, IL-8, GM-CSF, and TNF, but not M-CSF.[29,34-38] However, there is now a growing body of evidence, in addition to the present study, that glucocorticoids also have certain noninhibitory properties. These include the capacity to upregulate IL-6 receptor expression on epithelial cells, and IL-1 receptor expression on peripheral blood and bone marrow cells.[39,40] These data lead to the generation of a complex picture: treatment with glucocorticoids leading variously to downregulation of cytokine availability, including hematopoeitic growth factors and decreased recruitment of eosinophils to the inflammatory site. In contrast, glucocorticoids also mediate upregulation of cytokine receptor and enhancement of MHC class II expression. Studies on the effect of glucocorticoid therapy on MHC class II expression on sputum eosinophils from asthmatics may provide a clearer picture of the dominant effects of glucocorticoids in vivo.

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REFERENCES

1. Gleich GJ, Adolphson CR, Leiferman KM: The biology of the eosinophilic leukocyte. Annu Rev Med 44:85, 1993

2. Corrigan CJ, Kay AB: T cells and eosinophils in the pathogenesis of asthma. Immunol Today 13:501, 1992

3. Lucey DR, Nicholson-Weller A, Weller PF: Mature human eosinophils have the capacity to express HLA-DR. Proc Natl Acad Sci USA 86:1348, 1989

4. Hansel TT, De Vries IJM, Carballido JM, Braun RK, Carballido-Perrig N, Rihs S, Blaser K, Walker C: Induction and function of eosinophil intercellular adhesion molecule-1 and HLA-DR. J Immunol 149:2130, 1992

5. Weller PF, Rand TH, Barrett T, Elovic A, Wong DTW, Finberg RW: Accessory cell function of human eosinophils. J Immunol 150:2554, 1993

6. Hansel TT, Braunstein JB, Walker C, Blaser K, Bruijnzeel PLB, Virchow JC, Virchow C: Sputum eosinophils from asthmatics express ICAM-1 and HLA-DR. Clin Exp Immunol 86:271, 1991

7. Walker C, Rihs S, Braun RK, Betz S, Bruijnzeel PLB: Increased expression of CD11b and functional changes in eosinophils after migration across endothelial cell monolayers. J Immunol 150:4061, 1993

8. Charlesworth EN, Kagey-Sobotka A, Schleimer RP, Norman PS, Lichtenstein LM: Prednisone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late-phase response to allergen. J Immunol 146:671, 1991

9. Schleimer RP, Claman HM, Oronsky A (eds): Antiinflammatory Steroid Action: Basic and Clinical Aspects. San Diego, CA, Academic, 1989, p 151

10. Lamas AM, Leon OG, Schleimer RP: Glucocorticoids inhibit eosinophil responses to granulocyte-macrophage colony-stimulating factor. J Immunol 147:254, 1991

11. Wallen N, Kita H, Weiler D, Gleich GJ: Glucocorticoids inhibit cytokine-mediated eosinophil survival. J Immunol 147:3490, 1991

12. Hallsworth MP, Litchfield TM, Lee TH: Glucocorticoids inhibit granulocyte-macrophage colony-stimulating factor-1 and interleukin-5 enhanced in vitro survival of human eosinophils. Immunol 75:382, 1992

13. Hartnell A, Kay AB, Wardlaw AJ: Interleukin-3-induced upregulation of CR3 expression on human eosinophils is inhibited by dexamethasone. Immunol 77:488, 1992

14. Sadeghi R, Hawrylowicz CM, Chernajovsky Y, Feldmann M: Synergism of glucocorticoids with granulocyte macrophage colony stimulating factor (GM-CSF), but not interferon gamma (IFN-γ) or interleukin-4 (IL-4) on induction of HLA class II expression on human monocytes. Cytokine 4:287, 1992

15. Sadeghi R, Feldmann M, Hawrylowicz C: Upregulation of HLA class II, but not intercellular adhesion molecule 1 (ICAM-1) by granulocyte-macrophage colony stimulating factor (GM-CSF) or interleukin-3 (IL-3) in synergy with dexamethasone. Eur Cytokine Netw 3:373, 1992

16. Yokochi T, Holly RD, Clark EA:B lymphoblast antigen (BB-1) expressed on Epstein-Barr virus-activated B cell blasts, B lymphoblastoid cell lines, and Burkitt's lymphomas. J Immunol 128:823, 1982

17. Linsley PS, Clark EA, Ledbetter JA: T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB1. Proc Natl Acad Sci USA 87:5031, 1990

18. Lampson LA, Levy R: Two populations of Ia-like molecules on a human B cell line. J Immunol 125:293, 1980

19. Watson AJ, de Mars R, Trowbridge IS, Bach FH: Detection of a novel human class II HLA antigen. Nature 304:358, 1983

20. Spits H, Keizer G, Borst J, Terhorst C, Hekman A, de Vries JE: Characterisation of monoclonal antibodies against cell surface molecules associated with cytotoxic activity of natural and activated killer cells and cloned CTL lines. Hybridoma 2:423, 1983

21. Harlow E, Lane D: Antibodies-A Laboratory Manual. Cold Spring Harbor, 1988, p 310

22. Hansel TT, De Vries IJM, Iff T, Rihs S, Wandzilak M, Betz S, Blaser K, Walker C: An improved immunomagnetic procedure for the isolation of highly purified human blood eosinophils. J Immunol Methods 145:105, 1991

23. Lamb J, Eckels D, Lake P, Woody J, Green N: Human T cell clones recognise chemically synthesised peptide of influenza hemagglutinin. Nature 300:66, 1982

24. Rothbard J, Lechler R, Howland K, Bal V, Eckels D, Sekaly R, Long E, Taylor W, Lamb J: Structural model of HLA-DR1 restricted T cell antigen recognition. Cell 52:515, 1988

25. Clark EA, Ledbetter JA: How B and T cells talk to each other. Nature 367:425, 1994

26. Lopez AF, Elliott MJ, Woodcock J, Vadas MA: GM-CSF, IL-3 and IL-5: Cross-competition on human haemopoietic cells. Immunol Today 13:495, 1992

27. Kishimoto T, Taga T, Akira S: Cytokine signal transduction. Cell 76:253, 1994

28. Beato M: Gene regulation by steroid hormones. Cell 56:335, 1989

29. Kern JA, Lamb JL, Reed JC, Daniele RP, Nowell PC: Dexamethasone inhibition of interleukin 1 beta production by human monocytes. Post-transcriptional mechanisms. J Clin Invest 81:237, 1988

30. Celada A, McKercher S, Maki RA: Repression of major histocompatibility complex IA expression by glucocorticoids: The glucocorticoid receptor inhibits the DNA binding of the X box DNA binding protein. J Exp Med 177:691, 1993

31. Gilman AG, Rall TW, Nies AS, Taylor P (eds): Goodman and Gilman's The Pharmacological Basis of Therapeutics (ed 8), Elsmford, NY, Pergamon, 1990, p 1431

32. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB: Predominant T_{H2}-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 326:298,1992

33. Broide DH, Firestein GS: Endobronchial allergen challenge in asthma: Demonstration of cellular source of granulocyte macrophage colony-stimulating factor by in situ hybridization. J Clin Invest 88:1048,1991

34. Waage A, Bakke O: Glucocorticoids suppress the production of tumour necrosis factor by lipopolysaccharide-stimulated human monocytes. Immunol 63:299, 1988

35. Tanabe O, Akira S, Kamiya G, Wong GG, Hirano T, Kishimoto R: Genomic structure of murine IL-6 gene: High degree conservation of potential regulatory sequences between mouse and human. J Immunol 141:3875, 1988

36. Mukaida N, Shiroo M, Matsushima K: Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. J Immunol 143:1366, 1989

37. Tobler A, Meier R, Seitz M, Dewald B, Baggiolini M, Fey MF: Glucocorticoids downregulate gene expression of GM-CSF, NAP-1/IL-8, and IL-6 but not M-CSF in human fibroblasts. Blood 79:45, 1992

38. Arya SK, Wong-Staal F, Gallo RC: Dexamethasone-mediated inhibition of human T cell growth factor and gamma interferon messenger RNA. J Immunol 133:273, 1984

39. Snyers L, De Wit L, Content J: Glucocorticoid up-regulation of high-affinity interleukin 6 receptor on human epithelial cells. Proc Natl Acad Sci USA 87:2838, 1990

40. Akahoshi T, Oppenheim JJ, Matsushima K: Induction of high-affinity interleukin 1 receptor on human peripheral blood lymphocytes by glucocorticoid hormones. J Exp Med 167:924, 1988