ELSEVIER

European Journal of Pharmacology 354 (1998) 73–81

ejp

Effects of glucocorticoids on apoptosis of infiltrated eosinophils and neutrophils in rats

Takeaki Nittoh [a], Hiroko Fujimori [a], Yoshiko Kozumi [a], Kenji Ishihara [a], Suetsugu Mue [b], Kazuo Ohuchi [a,*]

[a] *Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Sendai, Miyagi 980-8578, Japan*
[b] *Department of Health and Welfare Science, Faculty of Physical Education, Sendai College, Shibata, Miyagi 989-1693, Japan*

Received 22 April 1998; revised 29 May 1998; accepted 2 June 1998

Abstract

The effects of glucocorticoids on the survival of rat eosinophils and neutrophils infiltrated into the peritoneal cavity were examined. Glucocorticoids including dexamethasone, prednisolone and hydrocortisone inhibited the survival of rat peritoneal eosinophils at 10^{-6} M, whereas they prolonged survival of rat peritoneal neutrophils at 10^{-8} M. Sex steroids including estradiol and progesterone did not affect cell survival. Dexamethasone decreased the viability of eosinophils after 3 days of incubation and maintained the viability of neutrophils until 4 days after incubation concentration dependently. The EC_{50} of dexamethasone for inhibition of the survival of eosinophils was 1.5×10^{-8} M, and that for the spontaneous death of neutrophils was 6.4×10^{-10} M, suggesting that glucocorticoids at concentrations that inhibit eosinophil survival prolong neutrophil survival. Analysis of DNA fragmentation of cultured eosinophils and neutrophils revealed that glucocorticoids enhance eosinophil apoptosis but inhibit neutrophil apoptosis. The effects of dexamethasone on viability and DNA fragmentation were counteracted by the glucocorticoid receptor antagonist, mifepristone, concentration dependently. These findings indicate that glucocorticoids induce contradictory effects via the glucocorticoid receptor on rat eosinophils and neutrophils extravasated to an inflammatory locus such as the peritoneal cavity by modulating apoptosis.

Keywords: Glucocorticoid; Eosinophil; Neutrophil; Survival; Apoptosis; Mifepristone

1. Introduction

Eosinophils and neutrophils are effector cells protecting the body from invasion by microorganisms. The number of neutrophils increases rapidly on bacterial or viral infection whereas the number of eosinophils increases on parasitic infection or in response to allergic diseases. Both cell types differentiate from stem cells in bone marrow (Sanderson, 1992; Borregaard and Cowland, 1997), circulate for a few days destined to die by apoptosis, and finally are engulfed by macrophages (Savill et al., 1989; Stern et al., 1992). Survival rates for the cells are regulated by several factors including cytokines, inflammatory mediators, and cell surface molecules. Survival of peripheral eosinophils is prolonged by interleukin-3 (Rothenberg et al., 1988) or interleukin-5 (Rothenberg et al., 1989) but inhibited by transforming growth factor-β (Alam et al., 1994). On the other hand, survival of peripheral neutrophils is prolonged by granulocyte colony-stimulating factor (Colotta et al., 1992), C5a (Lee et al., 1993) or lipopolysaccharide (Colotta et al., 1992; Lee et al., 1993) but inhibited by tumor necrosis factor-α (Takeda et al., 1993) or interleukin-6 (Afford et al., 1992). Granulocyte–macrophage colony-stimulating factor (Lopez et al., 1986; Owen et al., 1987) and interferon-γ (Valerius et al., 1990; Colotta et al., 1992) act as survival factors for both cell types whereas Fas induces apoptosis of both types also (Iwai et al., 1994; Matsumoto et al., 1995; Tsuyuki et al., 1995; Druilhe et al., 1996; Liles et al., 1996).

Glucocorticoids have different effects on the number of peripheral eosinophils and neutrophils when administered systemically. Namely, administration of glucocorticoids to patients with hypereosinophilia induces a marked decrease in the number of circulating eosinophils, but increases neutrophil numbers (Schleimer, 1990). In in vitro experiments, glucocorticoids suppress the survival of human peripheral eosinophils cultured in presence of the eosinophil

* Corresponding author. Tel.: +81-22-217-6860; Fax: +81-22-217-6859; E-mail: ohuchi-k@mail.pharm.tohoku.ac.jp

0014-2999/98/$19.00

survival factor, interleukin-3, interleukin-5, or granulocyte–macrophage colony-stimulating factor (Her et al., 1991; Lamas et al., 1991; Wallen et al., 1991; Hallsworth et al., 1992). Glucocorticoids induce apoptosis in extravasated eosinophils in vivo in rats (Kawabori et al., 1991) and in patients with asthma (Woolley et al., 1996). In contrast, glucocorticoids prolong the survival of human peripheral neutrophils in in vitro culture (Cox, 1995; Liles et al., 1995). Recently, Meagher et al. (1996) reported that dexamethasone directly induces apoptosis of peripheral eosinophils and inhibits apoptosis of peripheral neutrophils isolated from healthy human donors. It has been reported that spontaneous apoptosis of extravasated neutrophils is more strongly suppressed than that of peripheral neutrophils (Tsuchida et al., 1995; Watson et al., 1997), because extravasated neutrophils receive intracellular signals through adhesion molecules on their surface during the extravasation of peripheral neutrophils (Coxon et al., 1996; Watson et al., 1997). However, the direct effects of glucocorticoids on the apoptosis of eosinophils and neutrophils that have infiltrated into the inflammatory locus remain obscure. Therefore, the present study was aimed to clarify whether glucocorticoids exert effects on the apoptosis of rat eosinophils and neutrophils that have been extravasated to an inflammatory locus, the peritoneal cavity.

2. Materials and methods

2.1. Preparation of rat peritoneal eosinophils

Male rats of the Sprague–Dawley strain (specific pathogen-free, Charles River Japan, Kanagawa, Japan) weighing 190 to 210 g received orally cyclophosphamide (Sigma, St. Louis, MO, USA) suspended in 0.5% (w/v) sodium carboxymethylcellulose (Wako, Osaka, Japan) at a dose of 100 mg/kg (day 0). Protein (4 mg) of *Ascaris suum* extract (Greer Laboratories, Lenoir, NC, USA) in saline (0.5 ml) containing aluminum hydroxide (5 mg) (Wako) as an adjuvant was divided into five portions. On day 2, a 0.1-ml aliquot of the antigen solution was injected intradermally at each of two nuchal and three lumbar sites, and 0.5 ml of the same solution was injected intraperitoneally. On day 12, protein (5 mg) of the antigen in saline (0.5 ml) containing aluminum hydroxide (5 mg) was injected intraperitoneally as a booster. Finally, on day 19, saline (30 ml) containing protein (8 mg) of the antigen was injected intraperitoneally. Forty-eight hours later, the rats were killed by cutting the carotid artery under diethylether anesthesia, and peritoneal fluid was collected. The peritoneal cells were obtained from each rat by centrifugation at $200 \times g$ and 4°C for 5 min and combined in one tube. The cells were washed twice with phosphate-buffered saline (PBS), suspended at a concentration of 1.5×10^7 cells/ml in RPMI-1640 medium (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% (v/v) fetal bovine serum (Flow Laboratories, McLean, VA, USA), and plated on 75-cm^2 plastic tissue culture flasks (Iwaki Glass, Tokyo, Japan). After incubation for 2 h at 37°C, non-adherent cells were collected. The non-adherent cells were layered over Percoll solution ($d = .080$) (Sigma), and centrifuged at $1000 \times g$ and 4°C for 30 min. Cells at the bottom of the centrifuge tube were collected, washed twice with PBS, and suspended in RPMI-1640 medium containing 10% (v/v) fetal bovine serum. The purity of eosinophils in this fraction was more than 95% as assessed by May–Grünwald–Giemsa staining, and the viability of eosinophils was more than 98% in the trypan blue exclusion test.

2.2. Preparation of rat peritoneal neutrophils

Rat peritoneal neutrophils were harvested 15 h after intraperitoneal injection of 40 ml of 1% (w/v) casein (from milk, vitamin-free, Wako) solution that had been sterilized by autoclaving at 120°C for 15 min (Edamatsu et al., 1997). The cells from each rat were combined in one tube, washed twice with PBS and suspended in RPMI-1640 medium containing 10% (v/v) fetal bovine serum. The purity of neutrophils was more than 97% as assessed by May–Grünwald–Giemsa staining, and the viability of neutrophils was more than 98% in the trypan blue exclusion test.

2.3. Culture of eosinophils and neutrophils and measurement of viability

The purified eosinophils and neutrophils (5×10^6 cells of each) obtained as described above were suspended in 1 ml of RPMI-1640 medium containing 10% (v/v) fetal bovine serum and various concentrations of drugs and incubated at 37°C for the periods indicated in a humidified atmosphere containing 5% CO_2. Drugs used were dexamethasone, prednisolone, hydrocortisone, progesterone, estradiol (Sigma) and mifepristone (kindly supplied by Dr. Régine Sitruk-Ware, Laboratoires Exelgyn, France). The drugs were dissolved in ethanol and added to the medium. The final concentration of ethanol was adjusted to 0.1% (v/v). The control medium contained the same amount of ethanol. After incubation, the viability of the cells was determined by the ability to exclude trypan blue dye.

2.4. DNA fragmentation assay

After incubation, eosinophils and neutrophils (5×10^6 cells of each) were washed twice with PBS, and lysed by the addition of 0.5 ml lysis buffer (10 mM EDTA–2Na, 50 mM Tris–HCl, 1% sodium dodecyl sulfate, 100 μg/ml proteinase K, pH 8.0) at 55°C for 16 h. DNA was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1), and precipitated with 0.1 volume of 3 M sodium acetate and 2 vols. of ethanol at −20°C. Precipitated DNA was dissolved in TE buffer (1 mM Tris–HCl, 0.1 mM EDTA–

2Na, pH 7.5) containing 20 μg/ml of RNase, and incubated at 37°C for 30 min. Electrophoresis of DNA was performed in 1.4% agarose gel at 50 V for 3 h. After electrophoresis, DNA was visualized by ethidium bromide staining and photographed.

2.5. Statistical analysis

Results were analyzed for statistical significance by means of Student's unpaired *t*-test.

3. Results

3.1. Inhibition of the survival of rat eosinophils by dexamethasone

When rat eosinophils were incubated for 1 to 3 days in the presence of 10^{-6} M dexamethasone, their viability declined time dependently (Fig. 1a), and was significantly less than that of eosinophils incubated in the control medium at all time points studied. Dexamethasone inhibited the viability of eosinophils in a concentration-dependent manner (10^{-9} to 10^{-6} M) 3 days after incubation (Fig. 1b). Dexamethasone, 10^{-10} M, showed no significant effect on the viability of rat eosinophils (data not shown).

3.2. Prolongation of the survival of rat neutrophils by dexamethasone

In contrast to its effects on rat eosinophils, dexamethasone allowed neutrophils to recover from the spontaneous decrease in viability. As shown in Fig. 2a, when rat neutrophils were incubated in the control medium, the viability of the cells began to decrease 2 days after incuba-

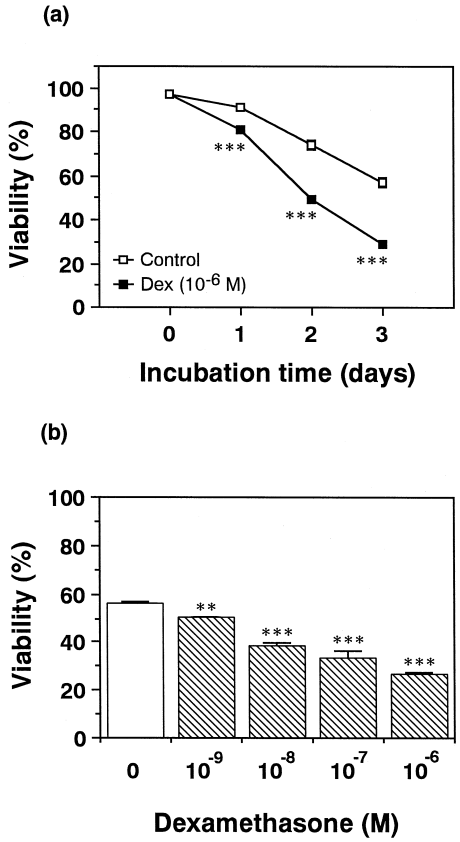

Fig. 1. Effects of dexamethasone on the survival of rat peritoneal eosinophils. (a) Rat peritoneal eosinophils (5×10^6 cells) were incubated for the periods indicated in the presence (closed squares) and absence (open squares) of 10^{-6} M dexamethasone (Dex). (b) Rat peritoneal eosinophils (5×10^6 cells) were incubated for 3 days in the presence of various concentrations of dexamethasone. After incubation, the viability of eosinophils was evaluated as the ability to exclude trypan blue dye. Values are the means from four samples with S.E.M. shown by vertical bars. Where bars are not shown, S.E.M. values are within the symbols. Statistical significance: $**P<0.01$, $***P<0.001$ vs. corresponding control.

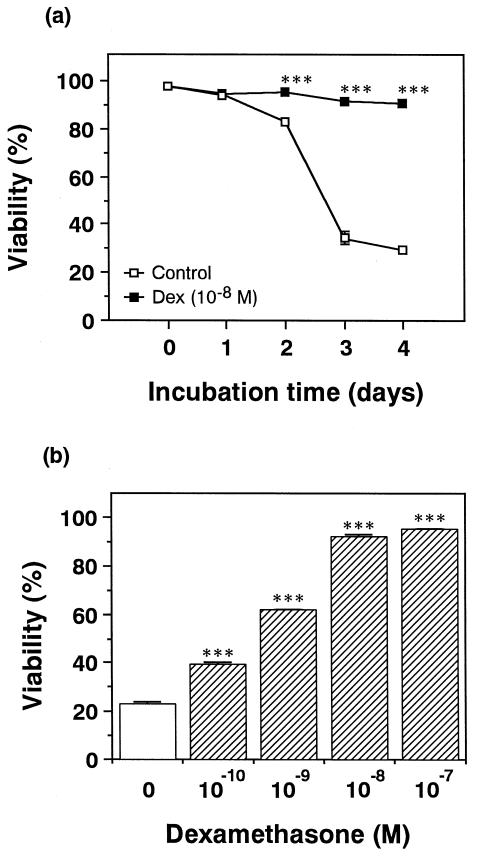

Fig. 2. Effects of dexamethasone on the survival of rat peritoneal neutrophils. (a) Rat peritoneal neutrophils (5×10^6 cells) were incubated for the periods indicated in the presence (closed squares) and absence (open squares) of 10^{-8} M dexamethasone (Dex). (b) Rat peritoneal neutrophils (5×10^6 cells) were incubated for 4 days in the presence of various concentrations of dexamethasone. After incubation, the viability of neutrophils was evaluated as the ability to exclude trypan blue dye. Values are the means from four samples with S.E.M. shown by vertical bars. Where bars are not shown, S.E.M. values are within the symbols. Statistical significance: $***P<0.001$ vs. corresponding control.

tion. However, the presence of dexamethasone in the medium at 10^{-8} M maintained viability at high levels until 4 days after incubation. Dexamethasone enhanced the survival of rat neutrophils in a concentration-dependent manner (10^{-10} to 10^{-8} M) and the effects reached a plateau at 10^{-8} M 4 days after incubation (Fig. 2b). It was shown that neutrophils (Fig. 2b) respond to much lower concentrations of dexamethasone than do eosinophils (Fig. 1b).

3.3. Effects of prednisolone and hydrocortisone on the survival of rat eosinophils and neutrophils

To find if glucocorticoids other than dexamethasone also affect the survival of eosinophils and neutrophils, the effects of prednisolone and hydrocortisone were examined. As shown in Fig. 3a, the viability of eosinophils cultured for 3 days in the presence of 10^{-6} M prednisolone or hydrocortisone was significantly lower than that of eosinophils cultured in the control medium, and the effects of prednisolone and hydrocortisone at 10^{-6} M were almost the same as that of dexamethasone at 10^{-6} M. When the effects of glucocorticoids were examined at 10^{-8} M, dexamethasone showed the most potent activity, followed by prednisolone and hydrocortisone (viability of eosinophils (% of control): dexamethasone, 68.9 ± 1.9; prednisolone, 88.6 ± 3.3; hydrocortisone, 100.1 ± 2.4, means $\pm$ S.E.M. from four samples). In contrast, when neutrophils were incubated in the presence of 10^{-8} M prednisolone or hydrocortisone for 4 days, their viability at 2 to 4 days were significantly greater than that of the control (Fig. 3b). At 10^{-8} M, the effects of prednisolone and hydrocortisone were almost the same as those of dexamethasone. However, when the effects of glucocorticoids were examined at 10^{-10} M, dexamethasone showed the most potent activity, followed by prednisolone and hydrocortisone (viability of neutrophils (% of control): dexamethasone, 169.3 ± 1.4; prednisolone, 114.3 ± 5.5; hydrocortisone, 109.3 ± 4.3, means $\pm$ S.E.M. from four samples). These findings suggested that glucocorticoids commonly prolong the lifespan of rat neutrophils and shorten that of rat eosinophils.

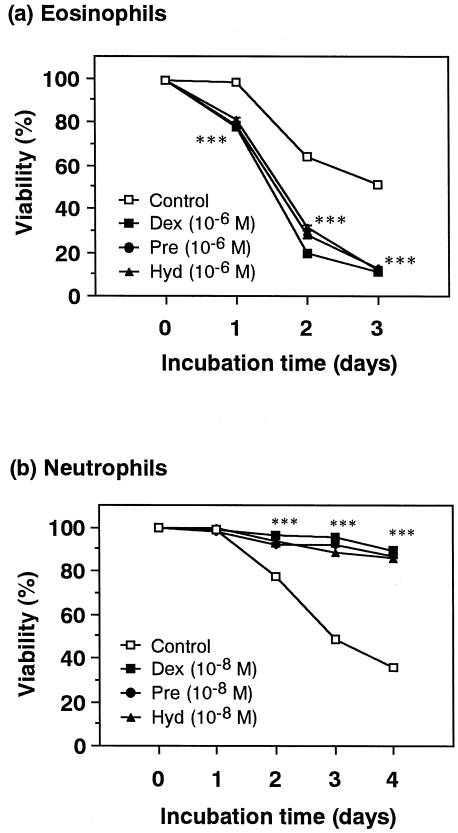

Fig. 3. Effects of various glucocorticoids on the survival of rat peritoneal eosinophils and neutrophils. Rat peritoneal eosinophils (5×10^6 cells) (a) and neutrophils (5×10^6 cells) (b) were incubated for the periods indicated in the absence (open squares) and presence of 10^{-6} M (a) and 10^{-8} M (b) of dexamethasone (Dex, closed squares), prednisolone (Pre, closed circles) or hydrocortisone (Hyd, closed triangles). After incubation, the viability of the cells was evaluated as the ability to exclude trypan blue dye. Values are the means from four samples with S.E.M. shown by vertical bars. Where bars are not shown, S.E.M. values are within the symbols. Statistical significance: $***P < 0.001$, control group vs. corresponding Dex, Pre, or Hyd group.

3.4. Effects of sex steroids on the survival of rat eosinophils and neutrophils

To investigate whether non-glucocorticoid hormones show the same effects as glucocorticoids, the effects of estradiol and progesterone on the survival of rat eosinophils and neutrophils were examined. Estradiol and progesterone, both 10^{-6} M, had no effect on the survival of rat eosinophils (Fig. 4a), and at 10^{-8} M showed no effect on the survival of rat neutrophils (Fig. 4b). These observations suggested that the non-glucocorticoid hormones did not affect the survival of rat eosinophils and neutrophils.

3.5. Effects of dexamethasone on DNA fragmentation of rat eosinophils and neutrophils

Because dexamethasone had different effects on the survival of rat eosinophils and neutrophils (Figs. 1 and 2), we investigated whether dexamethasone affects DNA fragmentation of rat eosinophils and neutrophils. After incubation for the periods indicated in the presence or absence of dexamethasone, DNA was extracted from eosinophils and neutrophils, and fragmentation of DNA due to internucleosomal cleavage was determined. Incubation of eosinophils in the absence of dexamethasone induced faint fragmentation of DNA at 12 h, which became prominent at 24 h (Fig. 5a). However, in the presence of dexamethasone (10^{-6} M), the typical ladder patterns characteristic of apoptotic cells were observed at 12 h (Fig. 5a), suggesting

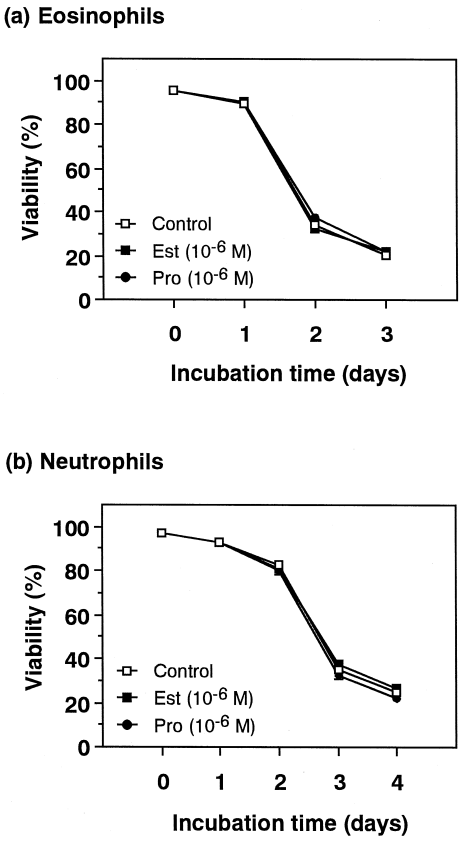

Fig. 4. Effects of estradiol and progesterone on the survival of rat peritoneal eosinophils and neutrophils. Rat peritoneal eosinophils (5×10^6 cells) (a) and neutrophils (5×10^6 cells) (b) were incubated for the periods indicated in the absence (open squares) and presence of 10^{-6} M (a) and 10^{-8} M (b) of estradiol (Est, closed squares) or progesterone (Pro, closed circles). After incubation, the viability of the cells was evaluated as the ability to exclude trypan blue dye. Values are the means from four samples with S.E.M. shown by vertical bars. Where bars are not shown, S.E.M. values are within the symbols.

that apoptosis of eosinophils was enhanced by dexamethasone. In contrast, DNA fragmentation of neutrophils in the absence of dexamethasone was faint at 24 h and became prominent 36 h after incubation (Fig. 5b). In the presence of dexamethasone (10^{-8} M), DNA fragmentation of neutrophils was inhibited at 24 and 36 h, suggesting that dexamethasone inhibits apoptosis of rat neutrophils.

3.6. Effects of various steroid hormones on DNA fragmentation of rat peritoneal eosinophils and neutrophils

Because dexamethasone was shown to enhance DNA fragmentation of eosinophils (Fig. 5a) and inhibit that of neutrophils (Fig. 5b), the effects of other glucocorticoids, prednisolone and hydrocortisone, and sex steroids, estradiol and progesterone were examined. Among the steroids examined, glucocorticoids including dexamethasone, prednisolone, and hydrocortisone at 10^{-6} M enhanced DNA fragmentation of rat peritoneal eosinophils at 12 h (Fig. 6a). However, at 10^{-6} M, progesterone and estradiol which are not glucocorticoid hormones, showed no effect on DNA fragmentation of eosinophils at 12 h (Fig. 6a).

In contrast, DNA fragmentation of neutrophils was inhibited by the glucocorticoid hormones, dexamethasone, prednisolone and hydrocortisone at 10^{-8} M when examined 36 h after incubation (Fig. 6b) while, at 10^{-8} M, progesterone and estradiol, which are not glucocorticoid hormones, showed no effect on DNA fragmentation of neutrophils (Fig. 6b). These findings are strong indications that glucocorticoid hormones enhance apoptosis of rat peritoneal eosinophils and inhibit apoptosis of rat peritoneal neutrophils.

3.7. Effects of mifepristone on the viability and DNA fragmentation of rat eosinophils and neutrophils

To clarify whether the effects of glucocorticoids on the viability and DNA fragmentation of eosinophils and neu-

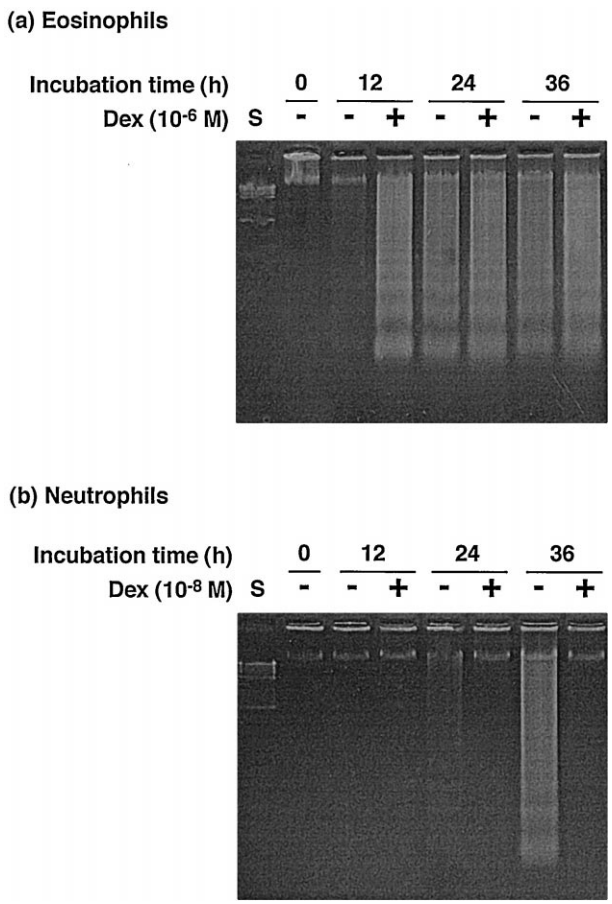

Fig. 5. Effects of dexamethasone on DNA fragmentation of rat peritoneal eosinophils and neutrophils. Rat peritoneal eosinophils (5×10^6 cells) (a) and neutrophils (5×10^6 cells) (b) were incubated for the periods indicated in the absence (−) and presence (+) of the concentrations of dexamethasone (Dex) as shown. After incubation, total genomic DNA was extracted from the cells and electrophoresed in a 1.4% agarose gel. DNA size marker standards (S, λ phage DNA digested by *Hin*dIII) were electrophoresed with the genomic DNA. Similar results were obtained in three independent experiments.

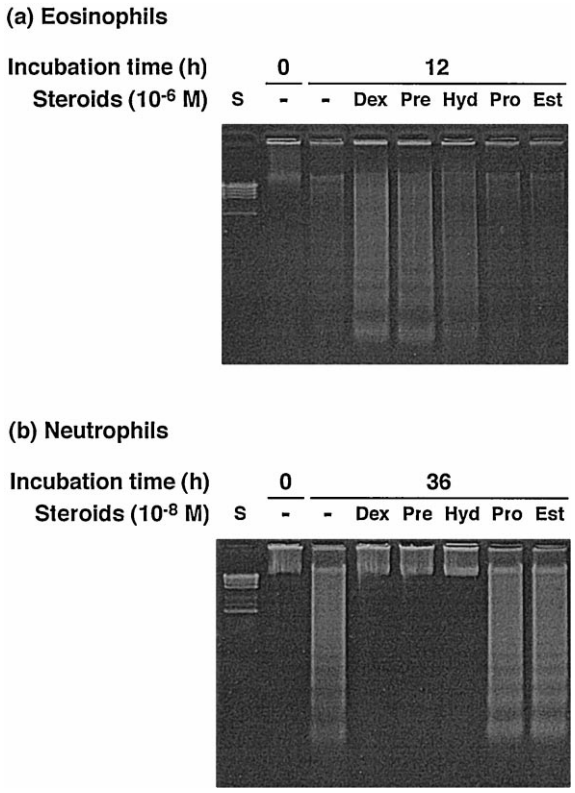

Fig. 6. Effects of various steroids on DNA fragmentation of rat peritoneal eosinophils and neutrophils. Rat peritoneal eosinophils (5×10^6 cells) (a) and neutrophils (5×10^6 cells) (b) were incubated for the periods indicated in the absence (−) and presence (+) of the concentrations of dexamethasone (Dex), prednisolone (Pre), hydrocortisone (Hyd), estradiol (Est), or progesterone (Pro) as shown. After incubation, total genomic DNA was extracted from the cells and electrophoresed in a 1.4% agarose gel. DNA size marker standards (S, λ phage DNA digested by *Hin*dIII) were electrophoresed with the genomic DNA. Similar results were obtained in three independent experiments.

trophils are mediated via the glucocorticoid receptor, we examined the effects of mifepristone, a glucocorticoid receptor antagonist. As shown in Fig. 7a, incubation of eosinophils in the presence of dexamethasone at 10^{-6} M for 3 days decreased the viability of eosinophils, but in the presence of dexamethasone (10^{-6} M) and mifepristone, the dexamethasone-induced decrease of eosinophil viability was alleviated by mifepristone at 10^{-6} M and 10^{-5} M, in a concentration-dependent manner. At 10^{-5} M, mifepristone completely blocked the effect of dexamethasone. Mifepristone itself showed no effect on the viability of eosinophils at 10^{-6} M and 10^{-5} M (Fig. 7a). DNA fragmentation of rat eosinophils induced by dexamethasone (10^{-6} M) at 12 h was also inhibited by mifepristone at 10^{-5} M (Fig. 8a).

On the other hand, the greater viability of rat neutrophils maintained by dexamethasone 10^{-8} M for 4 days was decreased by mifepristone 10^{-7} M and 10^{-6} M in a concentration-dependent manner (Fig. 7b). Mifepristone 10^{-6} M completely blocked the effect of dexamethasone 10^{-8} M. Mifepristone itself, at 10^{-7} M and 10^{-6} M, showed no effect on the viability of neutrophils (Fig. 7b). Treatment with dexamethasone at 10^{-8} M for 36 h suppressed DNA fragmentation of neutrophils, but in the presence of mifepristone at 10^{-6} M and dexamethasone at 10^{-8} M, DNA fragmentation of neutrophils was induced (Fig. 8b). The effects of prednisolone and hydrocortisone at 10^{-6} M on the viability and DNA fragmentation of rat eosinophils were also antagonized by mifepristone at 10^{-6} M (data not shown). In addition, effects of prednisolone and hydrocortisone at 10^{-8} M on the viability and DNA fragmentation of rat neutrophils were also antagonized by mifepristone at 10^{-6} M (data not shown). These findings indicate that the effects of dexamethasone on the viability

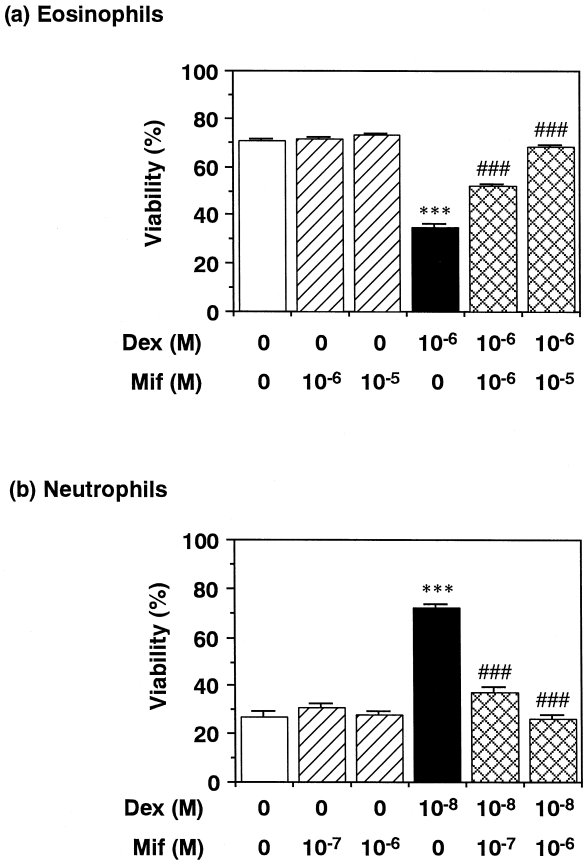

Fig. 7. Effects of dexamethasone and mifepristone on the survival of rat peritoneal eosinophils and neutrophils. Rat peritoneal eosinophils (5×10^6 cells) (a) and neutrophils (5×10^6 cells) (b) were incubated for 3 days (a) and 4 days (b), respectively, in the presence of the concentrations of dexamethasone (Dex) and mifepristone (Mif) as shown. After incubation, the viability of the cells was evaluated as the ability to exclude trypan blue dye. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: $***P<0.001$ vs. Dex 0 M and Mif 0 M, $^{###}P<0.001$ vs. corresponding Mif control.

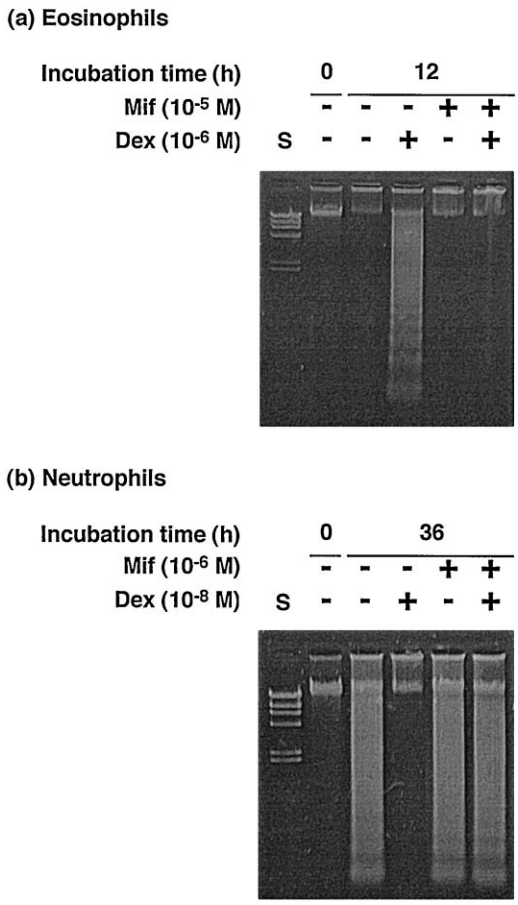

Fig. 8. Effects of dexamethasone and mifepristone on DNA fragmentation of rat peritoneal eosinophils and neutrophils. Rat peritoneal eosinophils (5×10^6 cells) (a) and neutrophils (5×10^6 cells) (b) were incubated for the periods indicated in the absence (−) and presence (+) of the concentrations of dexamethasone (Dex) and mifepristone (Mif) as shown. After incubation, total genomic DNA was extracted from the cells and electrophoresed in a 1.4% agarose gel. DNA size marker standards (S, λ phage DNA digested by *Hin*dIII) was electrophoresed with the genomic DNA. Similar results were obtained in three independent experiments.

and DNA fragmentation of rat eosinophils and neutrophils are mediated via the glucocorticoid receptor.

4. Discussion

Upon inflammation of tissues, numerous leukocytes infiltrate into the inflammatory site. The functions of infiltrated leukocytes are enhanced more than those of peripheral blood leukocytes; for example, production of superoxide (Watson et al., 1997) and nitric oxide (Miles et al., 1995) in rat neutrophils, and production of tumor necrosis factor-α (Costa et al., 1993) and granulocyte–macrophage colony-stimulating factor (Sullivan and Broide, 1996) in human eosinophils. It is also reported that the survival rate of peripheral blood neutrophils and that of extravasated neutrophils differ (Tsuchida et al., 1995; Watson et al., 1997). Extravasated neutrophils survive longer than peripheral blood neutrophils because apoptosis of neutrophils is inhibited by stimulation with the adhesion molecule, CD11b (Coxon et al., 1996; Watson et al., 1997) and CD11a (Watson et al., 1997). Meagher et al. (1996) have reported that glucocorticoids have opposing effects on the survival of human peripheral blood eosinophils and neutrophils because they enhance eosinophil apoptosis but decrease neutrophil apoptosis. In the present study, we investigated whether the survival of extravasated eosinophils and neutrophils is similarly affected by glucocorticoids.

It was demonstrated that dexamethasone inhibited the survival of rat eosinophils extravasated into the peritoneal cavity (Figs. 1 and 3a) and enhanced survival of extravasated neutrophils (Figs. 2 and 3b) incubated in the absence of survival-enhancing factors. These findings are consistent with those obtained when human peripheral blood eosinophils (Meagher et al., 1996) and neutrophils (Cox, 1995; Liles et al., 1995; Meagher et al., 1996) were incubated in the absence of the survival-enhancing factors. The EC_{50} of dexamethasone for the prolongation of neutrophil survival and the inhibition of eosinophil survival was calculated to be 6.4×10^{-10} M and 1.5×10^{-8} M, respectively. Neutrophils responded to dexamethasone at much lower concentrations than did eosinophils (Figs. 1 and 2), indicating that glucocorticoids at concentrations that inhibit eosinophil survival prolong neutrophil survival. It was suggested that the effects of glucocorticoids on the survival of rat eosinophils and neutrophils are mediated via the glucocorticoid receptor because mifepristone (Chobert et al., 1983), a glucocorticoid receptor antagonist, inhibits the effect of glucocorticoids, as it does in human eosinophils and neutrophils (Cox, 1995; Meagher et al., 1996).

It was also suggested that glucocorticoids induce apoptosis of eosinophils extravasated into the peritoneal cavity of rats (Figs. 1 and 3a). It is reported that glucocorticoids induce eosinophil apoptosis in the presence of survival-enhancing cytokines such as interleukin-3, interleukin-5 and granulocyte–macrophage colony-stimulating factor (Lamas et al., 1991; Wallen et al., 1991; Her et al., 1991; Hallsworth et al., 1992) and in the absence of these cytokines (Meagher et al., 1996). In contrast, higher concentrations of these cytokines inhibit the effect of glucocorticoids on eosinophil survival (Lamas et al., 1991; Wallen et al., 1991; Her et al., 1991). However, it has been reported that glucocorticoids activate apoptosis-inducing proteases such as caspase-1 (interleukin-1β-converting enzyme) in lymphoblastic leukemia cells (Geley et al., 1997) and T cells (Moreno et al., 1996) and caspase-3 (CPP-32, Yama, apopain) (Miyashita et al., 1997) in a B cell line. Helmberg et al. (1995) have demonstrated that glucocorticoids have an apoptotic effect on Jurkat cells by inhibiting AP-1 transcriptional activity and not by inducing the genes correlated with apoptosis, using transcriptional activation-

deficient glucocorticoid receptor mutants. The same mechanism might be involved in the induction of apoptosis by glucocorticoids of rat eosinophils extravasated from capillary vessels.

In contrast, glucocorticoids markedly inhibit the spontaneous apoptosis of rat neutrophils extravasated to the peritoneal cavity (Fig. 5b), indicating that glucocorticoids affect the survival of rat eosinophils and neutrophils by differential modulation of their apoptosis. Glucocorticoids prolonged neutrophil survival at very low concentrations (Fig. 2b and Fig. 3b), suggesting that, at physiological concentrations, they act as an endogenous neutrophil survival factor as well as granulocyte colony-stimulating factor and granulocyte–macrophage colony-stimulating factor (Colotta et al., 1992). Dexamethasone, a synthetic glucocorticoid, inhibits not only spontaneous apoptosis but also Fas/Fas ligand-induced apoptosis of human peripheral blood neutrophils (Liles et al., 1996). Meagher et al. (1996) demonstrated that cortisol, an endogenous glucocorticoid, also prolongs the survival of human peripheral blood neutrophils. Recently, it has been reported that the effect of glucocorticoids on the prolongation of neutrophil survival needs new gene expression and protein synthesis (Cox and Austin, 1997). However, the precise mechanism by which glucocorticoids differentially affect the survival of eosinophils and neutrophils remains to be elucidated.

In conclusion, glucocorticoids have contradictory effects on the survival of rat eosinophils and neutrophils that have infiltrated into the peritoneal cavity. Glucocorticoids inhibited the survival of eosinophils but prolonged that of neutrophils by modulating apoptosis via the glucocorticoid receptor. These findings indicate that eosinophils and neutrophils extravasated to inflammatory sites from capillary vessels respond to glucocorticoids like peripheral blood eosinophils and neutrophils. This report is the first to describe the effects of glucocorticoids on the survival and apoptosis of extravasated eosinophils and neutrophils.

Acknowledgements

We are grateful to Dr. Régine Sitruk-Ware, Laboratoires Exelgyn, France for providing us with mifepristone. This work was supported in part by a Grant-in-Aid for Encouragement of Young Scientists (to T.N., 10771276) from the Ministry of Education, Science, Sports and Culture of Japan.

References

Afford, S.C., Pongracz, J., Stockley, R.A., Crocker, J., Burnett, D., 1992. The induction by human interleukin-6 of apoptosis in the promonocytic cell line U937 and human neutrophils. J. Biol. Chem. 267, 21612–21616.

Alam, R., Forsythe, P., Stafford, S., Fukuda, Y., 1994. Transforming growth factor β abrogates the effects of hematopoietins on eosinophils and induces their apoptosis. J. Exp. Med. 179, 1041–1045.

Borregaard, N., Cowland, J.B., 1997. Granules of the human neutrophilic polymorphonuclear leukocyte. Blood 89, 3503–3521.

Chobert, M.N., Barouki, R., Finidori, J., Aggerbeck, M., Hanoune, J., Philibert, D., Deraedt, R., 1983. Antiglucocorticoid properties of RU38486 in a differentiated hepatoma cell line. Biochem. Pharmacol. 32, 3481–3483.

Colotta, F., Re, F., Polentarutti, N., Sozzani, S., Mantovani, A., 1992. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. Blood 80, 2012–2020.

Costa, J.J., Matossian, K., Resnick, M.B., Beil, W.J., Wong, D.T.W., Gordon, J.R., Dvorak, A.M., Weller, P.F., Galli, S.J., 1993. Human eosinophils can express the cytokines tumor necrosis factor-α and macrophage inflammatory protein-1α. J. Clin. Invest. 91, 2673–2684.

Cox, G., 1995. Glucocorticoid treatment inhibits apoptosis in human neutrophils: separation of survival and activation outcomes. J. Immunol. 154, 4719–4725.

Cox, G., Austin, R.C., 1997. Dexamethasone-induced suppression of apoptosis in human neutrophils requires continuous stimulation of new protein synthesis. J. Leukocyte Biol. 61, 224–230.

Coxon, A., Rieu, P., Barkalow, F.J., Askari, S., Sharpe, A.H., von Andrian, U.H., Arnaout, M.A., Mayadas, T.N., 1996. A novel role for the β2 integrin CD11b/CD18 in neutrophil apoptosis: a homeostatic mechanism in inflammation. Immunity 5, 653–666.

Druilhe, A., Cai, Z., Hailé, S., Chouaib, S., Pretolani, M., 1996. Fas-mediated apoptosis in cultured human eosinophils. Blood 87, 2822–2830.

Edamatsu, T., Xiao, Y.Q., Tanabe, J., Mue, S., Ohuchi, K., 1997. Induction of neutrophil chemotactic factor production by staurosporine in rat peritoneal neutrophils. Br. J. Pharmacol. 121, 1651–1658.

Geley, S., Hartmann, B.L., Kapelari, K., Egle, A., Villunger, A., Heidacher, D., Greil, R., Auer, B., Kofler, R., 1997. The interleukin-1β-converting enzyme inhibitor *CrmA* prevents Apo1/Fas- but not glucocorticoid-induced poly(ADP-ribose) polymerase cleavage and apoptosis in lymphoblastic leukemia cells. FEBS Lett. 402, 36–40.

Hallsworth, M.P., Litchfield, T.M., Lee, T.H., 1992. Glucocorticoids inhibit granulocyte–macrophage colony-stimulating factor-1 and interleukin-5 enhanced in vitro survival of human eosinophils. Immunology 75, 382–385.

Helmberg, A., Auphan, N., Caelles, C., Karin, M., 1995. Glucocorticoid-induced apoptosis of human leukemic cells is caused by the repressive function of the glucocorticoid receptor. EMBO J. 14, 452–460.

Her, E., Frazer, J., Austen, K.F., Owen, W.F. Jr., 1991. Eosinophil hematopoietins antagonize the programmed cell death of eosinophils: cytokine and glucocorticoid effects on eosinophils maintained by endothelial cell-conditioned medium. J. Clin. Invest. 88, 1982–1987.

Iwai, K., Miyawaki, T., Takizawa, T., Konno, A., Ohta, K., Yachie, A., Seki, H., Taniguchi, N., 1994. Differential expression of *bcl-2* and susceptibility to anti-Fas-mediated cell death in peripheral blood lymphocytes, monocytes, and neutrophils. Blood 84, 1201–1208.

Kawabori, S., Soda, K., Perdue, M.H., Bienenstock, J., 1991. The dynamics of intestinal eosinophil depletion in rats treated with dexamethasone. Lab. Invest. 64, 224–233.

Lamas, A.M., Leon, O.G., Schleimer, R.P., 1991. Glucocorticoids inhibit eosinophil responses to granulocyte–macrophage colony-stimulating factor. J. Immunol. 147, 254–259.

Lee, A., Whyte, M.K.B., Haslett, C., 1993. Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. J. Leukocyte Biol. 54, 283–288.

Liles, W.C., Dale, D.C., Klebanoff, S.J., 1995. Glucocorticoids inhibit apoptosis of human neutrophils. Blood 86, 3181–3188.

Liles, W.C., Kiener, P.A., Ledbetter, J.A., Aruffo, A., Klebanoff, S.J., 1996. Differential expression of Fas (CD95) and Fas ligand on normal human phagocytes: implications for the regulation of apoptosis in neutrophils. J. Exp. Med. 184, 429–440.

Lopez, A.F., Williamson, D.J., Gamble, J.R., Begley, C.G., Harlan, J.M., Klebanoff, S.J., Waltersdorph, A., Wong, G., Clark, S.C., Vadas,

M.A., 1986. Recombinant human granulocyte–macrophage colony-stimulating factor stimulates in vitro mature human neutrophil and eosinophil function, surface receptor expression, and survival. J. Clin. Invest. 78, 1220–1228.

Matsumoto, K., Schleimer, R.P., Saito, H., Iikura, Y., Bochner, B.S., 1995. Induction of apoptosis in human eosinophils by anti-Fas antibody treatment in vitro. Blood 86, 1437–1443.

Meagher, L.C., Cousin, J.M., Seckl, J.R., Haslett, C., 1996. Opposing effects of glucocorticoids on the rate of apoptosis in neutrophilic and eosinophilic granulocytes. J. Immunol. 156, 4422–4428.

Miles, A.M., Owens, M.W., Milligan, S., Johnson, G.G., Fields, J.Z., Ing, T.S., Kottapalli, V., Keshavarzian, A., Grisham, M.B., 1995. Nitric oxide synthase in circulating vs. extravasated polymorphonuclear leukocytes. J. Leukocyte Biol. 58, 616–622.

Miyashita, T., Mami, U., Inoue, T., Reed, J.C., Yamada, M., 1997. *Bcl-2* relieves the *trans*-repressive function of the glucocorticoid receptor and inhibits the activation of CPP32-like cysteine proteases. Biochem. Biophys. Res. Commun. 233, 781–787.

Moreno, M.B., Memon, S.A., Zacharchuk, C.M., 1996. Apoptosis signaling pathways in normal T cells: differential activity of *Bcl-2* and IL-1β-converting enzyme family protease inhibitors on glucocorticoid- and Fas-mediated cytotoxicity. J. Immunol. 157, 3845–3849.

Owen Jr., W.F., Rothenberg, M.E., Silberstein, D.S., Gasson, J.C., Stevens, R.L., Austen, K.F., Soberman, R.J., 1987. Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. J. Exp. Med. 166, 129–141.

Rothenberg, M.E., Owen Jr., W.F., Silberstein, D.S., Woods, J., Sobermann, R.J., Austen, K.F., Stevens, R.L., 1988. Human eosinophils have prolonged survival, enhanced functional properties, and become hypodense when exposed to human interleukin-3. J. Clin. Invest. 81, 1986–1992.

Rothenberg, M.E., Petersen, J., Stevens, R.L., Silberstein, D.S., McKenzie, D.T., Austen, K.F., Owen Jr., W.F., 1989. IL-5-dependent conversion of normodense human eosinophils to the hypodense phenotype uses 3T3 fibroblasts for enhanced viability, accelerated hypodensity, and sustained antibody-dependent cytotoxicity. J. Immunol. 143, 2311–2316.

Sanderson, C.J., 1992. Interleukin-5, eosinophils, and disease. Blood 79, 3101–3109.

Savill, J.S., Wyllie, A.H., Henson, J.E., Walport, M.J., Henson, P.M., Haslett, C., 1989. Macrophage phagocytosis of aging neutrophils in inflammation: programmed cell death in the neutrophil leads to its recognition by macrophages. J. Clin. Invest. 83, 865–875.

Schleimer, R.P., 1990. Effects of glucocorticoids on inflammatory cells relevant to their therapeutic applications in asthma. Am. Rev. Respir. Dis. 141, S59–S69.

Stern, M., Meagher, L., Savill, J., Haslett, C., 1992. Apoptosis in human eosinophils. Programmed cell death in the eosinophil leads to phagocytosis by macrophages and is modulated by IL-5. J. Immunol. 148, 3543–3549.

Sullivan, S., Broide, D.H., 1996. Compartmentalization of eosinophil granulocyte–macrophage colony-stimulating factor expression in patients with asthma. J. Allergy Clin. Immunol. 97, 966–976.

Takeda, Y., Watanabe, H., Yonehara, S., Yamashita, T., Saito, S., Sendo, F., 1993. Rapid acceleration of neutrophil apoptosis by tumor necrosis factor-α. Int. Immunol. 5, 691–694.

Tsuchida, H., Takeda, Y., Takei, H., Shinzawa, H., Takahashi, T., Sendo, F., 1995. In vivo regulation of rat neutrophil apoptosis occurring spontaneously or induced with TNF-α or cycloheximide. J. Immunol. 154, 2403–2412.

Tsuyuki, S., Bertrand, C., Erard, F., Trifilieff, A., Tsuyuki, J., Wesp, M., Anderson, G.P., Coyle, A.J., 1995. Activation of the Fas receptor on lung eosinophils leads to apoptosis and the resolution of eosinophilic inflammation of the airways. J. Clin. Invest. 96, 2924–2931.

Valerius, T., Repp, R., Kalden, J.R., Platzer, E., 1990. Effects of IFN on human eosinophils in comparison with other cytokines: a novel class of eosinophil activators with delayed onset of action. J. Immunol. 145, 2950–2958.

Wallen, N., Kita, H., Weiler, D., Gleich, G.J., 1991. Glucocorticoids inhibit cytokine-mediated eosinophil survival. J. Immunol. 147, 3490–3495.

Watson, R.W.G., Rotstein, O.D., Nathens, A.B., Parodo, J., Marshall, J.C., 1997. Neutrophil apoptosis is modulated by endothelial transmigration and adhesion molecule engagement. J. Immunol. 158, 945–953.

Woolley, K.L., Gibson, P.G., Carty, K., Wilson, A.J., Twaddell, S.H., Woolley, M.J., 1996. Eosinophil apoptosis and the resolution of airway inflammation in asthma. Am. J. Respir. Crit. Care Med. 154, 237–243.