

## Short communication

# Effects of *in vitro* treatment with fluticasone propionate on natural killer and lymphokine-induced killer activity in asthmatic and healthy individuals

**Background:** Topical corticosteroids are beneficial in the treatment of allergic respiratory disorders; they exert effects on a number of cells involved in allergic inflammatory reactions. On the other hand, major histocompatibility complex (MHC)-unrestricted cytotoxicity (i.e., natural killer [NK] cell activity) may play a role in the inflammatory allergic reaction. The objective was to gain insight into the mechanisms of the therapeutic effects of fluticasone propionate (FP), an inhaled corticosteroid used in asthma and rhinitis therapy. Therefore, we evaluated the NK and lymphokine-activated killer (LAK) activity of effector cells *in vitro* treated or not with FP.

**Methods:** Evaluations were made on peripheral blood mononuclear cells (PBMNCs), obtained from healthy volunteers ( $n=10$ ) and from asthmatic atopic subjects ( $n=10$ ) with allergy to *Parietaria*.

**Results:** Asthmatic patients had significantly increased NK activity ( $P=0.0008$ ), and interleukin (IL)-2- ( $P=0.0005$ ) and interferon (IFN)- $\alpha$ -induced LAK activities ( $P=0.0005$ ). In both groups, FP  $10^{-7}$  M significantly reduced NK activity ( $P<0.0001$ ), IL-2-induced LAK activity ( $P<0.0001$ ), and IFN- $\alpha$ -induced LAK activity ( $P<0.0001$ ). Similar results were obtained with FP  $10^{-8}$  M.

**Conclusions:** Since MHC-unrestricted cytotoxicity has been implicated in the development of allergen-induced eosinophilic airway inflammation, inhibition of NK and LAK activity by FP may contribute to the steroid therapeutic effect in asthma.

G. Di Lorenzo<sup>1</sup>, M. Esposito Pellitteri<sup>1</sup>, A. Drago<sup>1</sup>, P. Di Blasi<sup>3</sup>, G. Candore<sup>2</sup>, C. Balistreri<sup>2</sup>, F. Listi<sup>2</sup>, C. Caruso<sup>2</sup>

<sup>1</sup>Istituto di Medicina Interna e Geriatria and

<sup>2</sup>Dipartimento di Biopatologia e Metodologie

Biomediche, Università degli Studi di Palermo,

Palermo; <sup>3</sup>Direzione Medica Glaxo Wellcome, Verona, Italy

Key words: asthma; fluticasone propionate; human allergy; natural killer cell activity.

Dr Gabriele Di Lorenzo

Istituto di Medicina Interna e Geriatria

Via del Vespro 141

90127 Palermo

Italy

Accepted for publication 13 November 2000

Natural killer (NK) cells are recognized as an important component in immune responses to tumors and a number of pathogens (1, 2). NK cells rapidly produce interferon (IFN)- $\gamma$  (3). They also produce several immunoregulatory mediators including those involved in the pathogenesis of allergic diseases (1–4). Recent data show that NK cells determine the development of allergic eosinophilic airway disease in mice (3). This observation is compatible with the enhanced NK cell activity observed in human asthma (5–7). NK cells constitutively express IL-2 receptors (8) and respond to the type-1 cytokine IL-2 by enhanced antitumor and antiviral cytotoxic activities, cytokine secretion, and regulation of other immunocytes (9). The major histocompatibility complex (MHC)-unrestricted cytotoxicity of NK cells is believed to contribute at least partly to the activity of lymphokine-activated killer (LAK) cells, which are cytokine-dependent cytotoxic effector cells capable of lysing a broad range of target cells, including those typically resistant to NK cells (10).

Corticosteroids are potent anti-inflammatory agents widely used in the management of asthma and other allergic diseases (11, 12). Corticosteroid administration produces multiple immunomodulatory effects, including downregulation of cytokine production by CD4 T cells (12–16) and NK activity impairment (17–19).

Fluticasone propionate (FP), a highly lipophilic topical corticosteroid (Glaxo Wellcome, UK), has been shown to be safe and effective in the treatment of asthma and of both seasonal and perennial rhinitis (11, 20–23). When taken by the inhaled route, FP exerts a significant beneficial effect on asthma. Moreover, in clinical studies, FP had a higher therapeutic score than other inhaled corticosteroids (22, 23). To date, there are no studies of the effect of FP on MHC-unrestricted cytotoxicity. To gain insight into the mechanisms of the therapeutic effects of FP, we evaluated the NK and LAK activity of effector cells *in vitro*, treated or not with FP.

## Material and methods

### Subjects

After informed consent was obtained, peripheral blood was sampled from healthy laboratory staff ( $n=10$ ) and from asthmatic atopic subjects ( $n=10$ ) with allergy to *Parietaria*. The severity of asthma had been evaluated by the International Consensus Criteria (24). The subjects had a history of more than 2 years of symptoms consistent with asthma, and, by these criteria, they were classified as having persistent moderate asthma. They had a methacholine PC<sub>20</sub> less than 16 mg/ml (25). Skin prick tests were performed and evaluated, exactly as previously described (25, 26).

### FP

Pure FP was obtained from Glaxo Wellcome (Verona, Italy) and dissolved in anhydrous ethanol (95%) at a concentration of  $10^{-3}$  M. It was then diluted at a concentration of  $10^{-7}$  or  $10^{-8}$  M in complete medium, i.e., RPMI-1640 (Gibco, Grand Island, NY, USA), supplemented with a 10% heat-inactivated pool of AB sera (AB Pool, Flow Laboratories, Irvine, UK), 1% glutamine (Flow Laboratories), 80 µg gentamycin, 100 U/ml streptomycin, and 1% HEPES buffer (Boehringer Mannheim, Germany). Control medium was prepared with the same dilution of ethanol.

### Cell lines

The human erythromyeloid leukemia-derived cell line K-562 was maintained in complete medium, i.e., RPMI 1640 medium (Gibco, Grand Island, NY, USA), supplemented with 10% heat-inactivated fetal calf serum (Flow Laboratories), gentamycin, streptomycin, and HEPES buffer (Boehringer Mannheim, Germany), at 37°C with 5% CO<sub>2</sub>. Cultures were fed biweekly to maintain constant exponential cell growth (27).

### Effector cell preparation

Peripheral blood mononuclear cells (PBMNC) obtained from venous blood drawn into heparinized-coated Vacutainer tubes (Becton Dickinson, Mountain View, CA, USA) by centrifugation on Ficoll-Hypaque (Nycomed As Diagnostica, Oslo, Norway) were washed twice and resuspended in complete medium at appropriate concentrations for the NK cell cytotoxicity assay (27).

To assess LAK activity, PBMNC ( $5 \times 10^6$ /ml) were incubated v/v with 500 UI/ml of IFN- $\alpha$  (28) (Wellcome Italy Spa, Pomezia, Rome, Italy) or with 100 UI/ml of recombinant interleukin-2 (Proleukin, Chiron) (27) for 5 days, with and without FP  $10^{-7}$  or  $10^{-8}$  M.

### Cytotoxicity assay

NK and LAK activities were assessed by a <sup>51</sup>Cr release assay with the K-562 line (33, 34). Briefly,  $1 \times 10^6$  target cells were incubated with 100 µCi of <sup>51</sup>Cr (specific activity, 5 mCi/mmol; Amersham Pharmacia Biotech, Buckinghamshire, UK) for 2 h in medium at 37°C, 5% CO<sub>2</sub>. After labeling, the cells were washed three times in complete medium and counted in Trypan blue to assess viability, which was always greater than 95%.

Both NK (with and without FP  $10^{-7}$  or  $10^{-8}$  M) and LAK cytotoxicity tests were performed in standard V-shaped microplates (Nunc, Kamstrup, Denmark) in a total volume of 200 µl. All tests were performed in triplicate with 5000 target cells/well and with effector-to-target ratios decreasing from 100:1 to 6.25:1. Plates were centrifuged for 1 min at 100 g before incubation for 4 h at 37°C in a humidified incubator with 5% CO<sub>2</sub>. After incubation, the plates were centrifuged for 10 min at 400 g. Then 100 µl of supernatant from each well was harvested, and the radioactivity was assessed with a  $\gamma$  counter. Spontaneous and maximum releases were determined in wells containing radiolabeled cells and complete medium or 1%

Triton X-100, respectively. The percentage of cytotoxicity was calculated as follows (cpm: counts/min):

$$\% \text{ cytotoxicity} =$$

$$\frac{\text{mean experimental cpm} - \text{mean spontaneous cpm}}{\text{mean maximum cpm} - \text{mean spontaneous cpm}} \times 100$$

Results are presented as lytic units (LU), defined as the number of effector cells required to lyse 20% of target cells ( $\text{LU}_{20} \times 10^{-6}$ ), and were calculated with the exponential fit equation described by Pross & Lotzova (10). With this method, all the experimental data can be reduced to a single numerical parameter that is directly proportional to the lytic activity, thus resulting in a more accurate measurement of cytotoxicity activity.

### Statistical analysis

Final data, expressed as means  $\pm$  SD, were derived with StatDirect software (Tidestone Technologies, Inc, UK) on a computer. Analysis of variance was used to compare inter- and intragroup differences in  $\text{LU}_{20} \times 10^{-6}$ . A *P* value of less than 0.05 was considered significant.

## Results

As shown in Fig. 1, NK activity was significantly increased in asthmatic patients ( $15.27 \pm 0.7$  vs  $14.4 \pm 0.8$   $\text{LU}_{20} \times 10^{-6}$ ;  $P=0.0008$ ); this is consistent with earlier findings (5). In both groups of subjects, treatment of the cells with FP  $10^{-7}$  M significantly reduced NK activity ( $15.27 \pm 0.7$  vs  $11.8 \pm 0.7$  and  $14.4 \pm 0.8$  vs  $10.3 \pm 0.6$   $\text{LU}_{20} \times 10^{-6}$ ) ( $P<0.0001$ ). Similar results were obtained with FP  $10^{-8}$  M ( $15.27 \pm 0.7$  vs  $12.3 \pm 0.6$  and  $14.4 \pm 0.8$  vs  $12.3 \pm 0.8$   $\text{LU}_{20} \times 10^{-6}$ ) ( $P<0.0001$ ).

Recombinant IL-2-induced LAK activity (Fig. 2) was also increased in asthmatic patients ( $23.5 \pm 1.0$  vs  $22.2 \pm 0.8$   $\text{LU}_{20} \times 10^{-6}$ ,  $P=0.0005$ ). In both groups of subjects, treatment of the cells with FP  $10^{-7}$  M significantly reduced LAK activity ( $23.5 \pm 1.0$  vs  $6.74 \pm 0.8$  and  $22.2 \pm 0.8$  vs  $4.95 \pm 0.4$ ,  $\text{LU}_{20} \times 10^{-6}$ ) ( $P<0.0001$ ). Similar results were obtained with FP  $10^{-8}$  M ( $23.5 \pm 1.0$  vs  $7.40 \pm 0.9$  and  $22.2 \pm 0.8$  vs  $5.65 \pm 0.5$ ,  $\text{LU}_{20} \times 10^{-6}$ ) ( $P<0.0001$ ).

IFN- $\alpha$ -induced LAK activity (Fig. 3) was increased in asthmatic patients too ( $20.59 \pm 1.0$  vs  $19.28 \pm 0.9$   $\text{LU}_{20} \times 10^{-6}$ ;  $P=0.0005$ ). In the two groups, the treatment of the cells with FP  $10^{-7}$  M significantly reduced IFN- $\alpha$ -induced LAK activity ( $20.59 \pm 1.0$  vs  $10.6 \pm 0.9$  and  $22.2 \pm 0.8$  vs  $8.74 \pm 0.6$   $\text{LU}_{20} \times 10^{-6}$ ;  $P<0.0001$ ). The treatment of the cells with FP  $10^{-8}$  M also reduced IFN- $\alpha$ -induced LAK activity ( $20.59 \pm 1.0$  vs  $11.59 \pm 0.9$  and  $22.2 \pm 0.8$  vs  $9.69 \pm 0.6$   $\text{LU}_{20} \times 10^{-6}$ ;  $P<0.0001$ ).

## Discussion

Topical corticosteroids have proven efficacy in the treatment of allergic respiratory disorders and have been shown to inhibit both the immediate and late response to allergen provocation. Topical cortico-

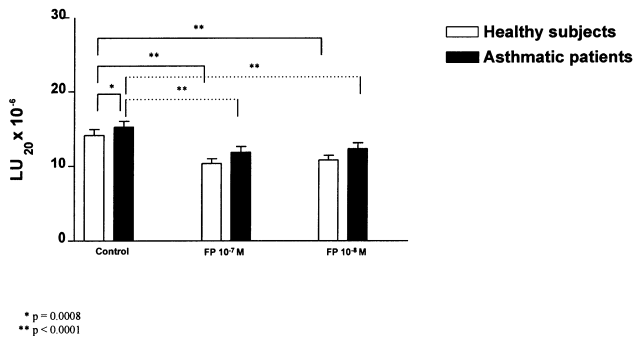


Figure 1. NK activity in healthy ( $n=10$ ) and asthmatic ( $n=10$ ) individuals. Cells were untreated or treated with either FP  $10^{-7}$  or FP  $10^{-8}$  M. Results are expressed as mean  $\pm$  SD of individual values measured as  $LU_{20} \times 10^{-6}$ . NK activity was significantly increased in asthmatic subjects ( $P=0.0008$ ). In both groups of subjects, the treatment of cells with FP  $10^{-7}$  M significantly reduced NK ( $P<0.0001$ ). Similar results were obtained with FP  $10^{-8}$  M ( $P<0.0001$ ).

steroids exert effects on a number of cells involved in the allergic inflammatory reaction, a fact which may explain their potent effect in clinical allergy studies. Steroids may exert direct inhibitory actions on several inflammatory cells implicated in pulmonary and airway diseases (11, 29). They have a direct inhibitory effect on mediator release from eosinophils, but are poorly effective in preventing secretion of reactive oxygen species and eosinophil basic proteins (20, 30). Steroids inhibit the permissive action of cytokines such as interleukin (IL)-5 on eosinophil survival (31, 32), and this contributes to the reduction in airway eosinophils seen with steroid therapy. Steroids markedly reduce the numbers of dendritic cells in nasal mucosa (33). Chronic steroid treatment is associated with a marked reduction in mucosal mast-cell number (34), and is very effective in inhibiting activation of lymphocyte cells and in blocking the release of cytokines that are likely to play an important role in the recruitment and survival of inflammatory cells involved in asthmatic inflammation (35–39).

Several of the effects exerted by FP may well account for these results, notably the ability to induce eosinophil apoptosis and to reduce type-2 cytokine production (6, unpublished observations). Finally, the present study and earlier reports have demonstrated that *in vitro* treatment with steroids significantly decreases NK and LAK activities (17–19, 40–43). Since NK cells have been implicated in the development of allergen-induced eosinophilic airway inflammation (6), NK and LAK inhibition may contribute to its therapeutic effect in asthma. In fact, NK cells play a critical role in immunization and the subsequent development of allergen-induced airway inflammation (3). These new data suggest the possibility of a pathogenic role for the increased MHC-unrestricted cytotoxicity observed in asthma (5–7) and suggest that NK cell activity may be one of the factors governing the development of allergic eosinophilic airway disease.

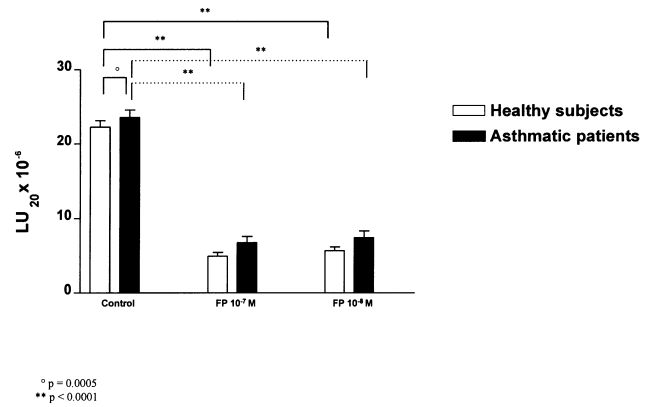


Figure 2. IL-2 induced LAK activity in healthy ( $n=10$ ) and asthmatic ( $n=10$ ) individuals. Cells were untreated or treated with either FP  $10^{-7}$  or FP  $10^{-8}$  M. Results are expressed as mean  $\pm$  SD of individual values measured as  $LU_{20} \times 10^{-6}$  of target cells. IL-2-induced LAK activity was increased in asthmatic patients ( $P=0.0005$ ). Treatment of cells with FP  $10^{-7}$  M significantly reduced LAK activity in both groups of subjects ( $P<0.0001$ ). Similar results were obtained with FP  $10^{-8}$  M ( $P<0.0001$ ).

The mechanism by which glucocorticoids inhibit the action of MHC-unrestricted cells has been extensively investigated. Therapeutically administered glucocorticoids depress the NK and LAK activity of human peripheral blood, and direct suppression of MHC-unrestricted cell activity has been demonstrated *in vitro* after incubation of PBMCs with glucocorticoids. Although the mechanisms whereby glucocorticoids act on MHC-unrestricted activity are little understood, complex relationships have been thought to occur with interferons and IL-2, which are endogenous inducers of MHC-unrestricted activity (18, 19, 40–43). Our results were obtained with mononuclear cells, and not isolated effector cells;

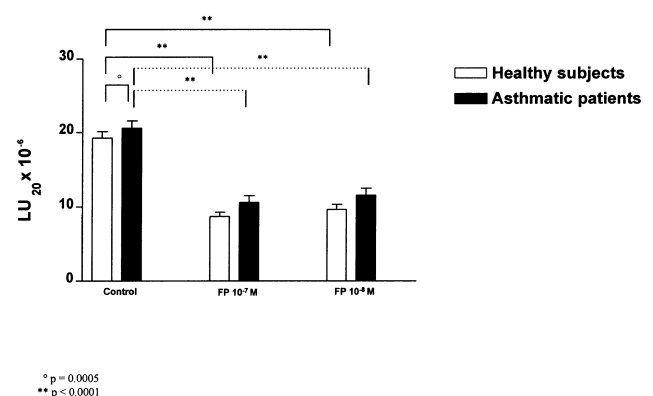


Figure 3. IFN- $\alpha$ -induced LAK activity in healthy ( $n=10$ ) and asthmatic ( $n=10$ ) individuals. Cells were untreated or treated with either FP  $10^{-7}$  or FP  $10^{-8}$  M. Results are expressed as mean  $\pm$  SD of individual values measured as  $LU_{20} \times 10^{-6}$  of target cells. IFN- $\alpha$ -induced LAK activity was increased in asthmatic patients ( $P=0.0005$ ). Treatment of cells with FP  $10^{-7}$  M significantly reduced the LAK activity in both groups ( $P<0.0001$ ). Similar results were obtained with FP  $10^{-8}$  M ( $P<0.0001$ ).

therefore, we cannot exclude the possibility that FP inhibition depends on the effect induced on other PBMNC. It is interesting to observe that the *in vitro* FP effects were not reversed by IL-2 or by IFN- $\alpha$ . Although additional work is needed to elucidate the mechanisms involved, we suggest that FP inhibition of cytokines involved in the regulation of MHC-unrestricted activity (1–3, 9–11, 13–16) is responsible for the observed reduction of NK and LAK activities.

In conclusion, our results show that NK and LAK activities were increased in asthmatic patients with respect to healthy subjects, confirming previous data concerning NK activity (5–7). The biologic significance of this increase may be questionable; however, NK cells have been shown to produce IL-5 and this secretion is also regulated by IL-4, IL-10, and typical type-2 cytokines, as well as by IL-12 (44). Interestingly, in

both groups, *in vitro* treatment of the cells with FP significantly depressed NK and LAK activity. Since MHC-unrestricted effector cells have been implicated in the development of allergen-induced eosinophilic airway inflammation, the steroid-induced NK and LAK inhibition may contribute to its therapeutic effect in asthma.

## Acknowledgments

This study was supported by grants from MURST (60%) to Gabriele Di Lorenzo and Calogero Caruso and Glaxo Wellcome Italia SpA. We thank Antonella Pirazzoli and Renato Testi, Glaxo Wellcome Italia SpA, for the criticism and the suggestions. We are also indebted to Professor Sergio Bonini for his help in discussing this study.

## References

1. BIRON CA. Activation and function of natural killer cell responses during viral infections. *Curr Opin Immunol* 1997;**9**:24–34.
2. SCHARTON-KERSTEN TM, SHER A. Role of natural killer cells in innate resistance to protozoan infections. *Curr Opin Immunol* 1997;**9**:44–51.
3. KORSGREN M, PERSSON CG, SUNDLER F, et al. Natural killer cells determine development of allergen-induced eosinophilic airway inflammation in mice. *J Exp Med* 1999;**189**:553–562.
4. WARREN HS, KINNEAR BF, PHILLIPS JB, LANIER LL. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. *J Immunol* 1995;**154**:5144–5152.
5. TIMONEN T, STENIUS-AARNIALA B. Natural killer cell activity in asthma. *Clin Exp Immunol* 1985;**59**:85–90.
6. JIRA M, ANTOSOVA E, VONDRA V, STREJCEK J, MAZAKOVA H, PRAZAKOVA J. Natural killer and interleukin-2 induced cytotoxicity in asthmatics. I. Effect of acute antigen-specific challenge. *Allergy* 1988;**43**:294–298.
7. KREJSEK J, KRAL B, VOKURKOVA D, et al. Decreased peripheral blood  $\gamma\delta$  T cells in patients with bronchial asthma. *Allergy* 1998;**53**:73–77.
8. CARUSO C, CANDORE G, CIGNA D, COLUCCI AT, MODICA MA. Biological significance of soluble IL-2 receptor. *Mediators Inflamm* 1993;**2**:3–21.
9. TRINCHIERI G. Biology of natural killer cells. *Adv Immunol* 1989;**47**:187–376.
10. PROSS HF, LOTZOV E. Role of natural killer cells in cancer. *Nat Immun* 1993;**12**:279–292.
11. BARNES PJ, PEDERSEN S, BUSSE WW. Efficacy and safety of inhaled corticosteroids. *Am J Respir Crit Care Med* 1998;**157** Suppl 3:S1–S53.
12. SCHLEIMER RP. An overview of glucocorticoid anti-inflammatory actions. *Eur J Clin Pharmacol* 1993;**45** Suppl 1:3–7.
13. ARYA SK, WONG-STAAAL F, GALLO RC. Dexamethasone-mediated inhibition of human T cell growth factor and  $\gamma$ -interferon messenger RNA. *J Immunol* 1984;**133**:273–276.
14. WANG Y, CAMPBELL HD, YOUNG IG. Sex hormones and dexamethasone modulate interleukin-5 gene expression in T lymphocytes. *J Steroid Biochem Mol Biol* 1993;**44**:203–210.
15. SNIJDEWINT FGM, KAPSENBERG ML, WAUBEN-PENRIS PJJ, BOS JD. Corticosteroids class-dependently inhibit *in vitro* Th1- and Th2-type cytokine production. *Immunopharmacology* 1995;**29**:93–101.
16. BRINKMANN V, KRISTOFIC C. Regulation by corticosteroids of Th1 and Th2 cytokine production in human CD4<sup>+</sup> effector T cells generated from CD45RO<sup>–</sup> and CD45RO<sup>+</sup> subsets. *J Immunol* 1995;**155**:3322–3328.
17. VAN IERSEL AJ, MIEREMET-OOMS MA, VAN DER ZON JM, et al. Suppression of intestinal mucosal natural killer cells by corticosteroids. *Aliment Pharmacol Ther* 1997;**11**:347–353.
18. VAN IERSEL GJ, MIEREMET-OOMS MA, VAN DER ZON AM, et al. Effect of cortisol and ACTH on corticosteroid-suppressed peripheral blood natural killer cells from healthy volunteers and patients with Crohn's disease. *Immunopharmacology* 1996;**34**:97–104.
19. VAN IERSEL GJ, VAN DER SLUYS VEER A, VERSPAGET HW, GRIFFIOEN G, VAN HOGEZAND RA, LAMERS CB. Contribution of plasma cortisol to corticosteroid-suppressed peripheral blood natural killer cell activity in Crohn's disease. *Immunopharmacology* 1995;**29**:11–17.
20. DI LORENZO G, GERVASI F, DRAGO A, et al. Comparison of the effects of fluticasone propionate, aqueous nasal spray and levocabastine on inflammatory cells in nasal lavage and clinical activity during the pollen season in seasonal rhinitis. *Clin Exp Allergy* 1999;**29**:1367–1377.
21. RATNER P, PAULL B, FINDLAY S, et al. Fluticasone propionate given once daily is as effective for seasonal allergic rhinitis as beclomethasone dipropionate given twice daily. *J Allergy Clin Immunol* 1992;**90**(3 part 1):285–291.
22. FULLER R, JOHNSON M, BYE A. Fluticasone propionate – an update on preclinical and clinical experience. *Respir Med* 1995, **89** Suppl A:3–18.
23. BARNES NC, HALLETT C, HARRIS TAJ. Clinical experience with fluticasone propionate in asthma: a meta-analysis of efficacy and systematic activity compared with budesonide and beclomethasone dipropionate at half the microgram dose or less. *Respir Med* 1998;**92**:95–104.
24. Expert Panel Report 2. Guidelines for the diagnosis and management of asthma. National Heart, Lung and Blood Institute, National Institutes of Health. Bethesda, Maryland, USA, Publication no. 97–4051, July 1997.

25. DI LORENZO G, MANSUETO P, MELLUSO M, et al. Nonspecific airway hyperresponsiveness in monosensitive Sicilian patients with allergic rhinitis. Its relation to total serum IgE levels and blood eosinophils during and out of pollen season. *Clin Exp Allergy* 1997;**27**:1052–1059.
26. European Academy of Allergology and Clinical Immunology. Position paper. Skin tests used in type I allergy testing. *Allergy* 1989;**44** Suppl 10:1–59.
27. CARUSO C, CANDORE G, COLUCCI AT, et al. Natural killer and lymphokine-activated killer activity in HLA-B8,DR3-positive subjects. *Hum Immunol* 1993;**38**:226–230.
28. KRISHNARAJ R. Senescence and cytokines modulate the NK cell expression. *Mech Ageing Dev* 1997;**96**:89–101.
29. ADCOCK IM, GILBEY T, GELDER CM, CHUNG KF, BARNES PJ. Glucocorticoid receptor localization in normal human lung and asthmatic lung. *Am J Respir Crit Care Med* 1996;**154**:771–782.
30. KITA H, ABU-GHAZALEH R, SANDERSON CJ, GLEICH GJ. Effect of steroids on immunoglobulin-induced eosinophil degranulation. *J Allergy Clin Immunol* 1991;**87**:70–77.
31. LAMAS AM, LEON OG, SCHLEIMER RP. Glucocorticoids inhibit eosinophil responses to granulocyte-macrophage colony-stimulating factor. *J Immunol* 1991;**147**:254–259.
32. WALLEN N, KITA H, WEILLER D, GLEICH GJ. Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J Immunol* 1991;**147**:3490–3495.
33. HOLM AF, FOKKENS WJ, GODTHELP T, MULDER WG, VROOM TM, RINJNTJES E. Effect of 3 months' nasal steroid therapy on nasal T cells and Langerhans cells in patients suffering from allergic rhinitis. *Allergy* 1995;**50**:204–209.
34. DJUKANOVIC R, WILSON JW, BRITTEN YM, et al. Effect of an inhaled corticosteroid on airway inflammation and symptoms of asthma. *Am Rev Respir Dis* 1992;**145**:674–699.
35. CORRIGAN CJ, KAY AB. CD4 lymphocyte activation in acute severe asthma. Relationship to disease severity and atopic status. *Am Rev Respir Dis* 1990;**141**:970–977.
36. VIRCHOW JC Jr, OEHLING A, BOER L, et al. Pulmonary function, activated T cells, peripheral blood eosinophilia, and serum activity for eosinophil survival *in vitro*: a longitudinal study in bronchial asthma. *J Allergy Clin Immunol* 1994;**94**:240–249.
37. NELSON DJ, MCWILLIAM AS, HAINING S, HOLT PG. Modulation of airway intraepithelial dendritic cells following exposure to steroids. *Am J Respir Crit Care Med* 1995;**151**:475–481.
38. DI LORENZO G, MANSUETO P, MELLUSO M, et al. Serum levels of soluble IL-2R, CD4 and CD8 in bronchial asthma. *Mediators Inflamm* 1995;**4**:270–272.
39. SHI HZ, SUN JJ, PAN HL, LU JQ, ZHANG JL, JIANG JD. Alterations of T-lymphocyte subsets, soluble IL-2 receptor, and IgE in peripheral blood of children with acute asthma attacks. *J Allergy Clin Immunol* 1999;**103**:388–394.
40. MCVICAR DW, MERCHANT RE, MERCHANT LH, YOUNG HF. Corticosteroids inhibit the generation of lymphokine-activated killer activity *in vitro*. *Cancer Immunol Immunother* 1989;**29**:211–218.
41. GATTI G, CAVALLO R, SARTORI ML, et al. Inhibition by cortisol of human natural killer (NK) cell activity. *J Steroid Biochem* 1987;**26**:49–58.
42. PARILLO JE, FAUCI AS. Comparison of the effector cells in human spontaneous cellular cytotoxicity and antibody-dependent cellular cytotoxicity: differential sensitivity of effector cells *in vivo* and *in vitro* to corticosteroids. *Scand J Immunol* 1981;**13**:573–579.
43. ONSRUD M, THORSBY E. Influence of *in vivo* hydrocortisone on some human blood lymphocyte subpopulations. I. Effect on natural killer cell activity. *Scand J Immunol* 1981;**13**:573–579.
44. WARREN HS, KINNEAR BF, PHILLIPS JH, LANIER LL. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10 and IL-12. *J Immunol* 1995;**154**:5144–5152.