

Short courses of high-dose inhaled budesonide and serum IgG subclass levels in healthy volunteers

Jan Van Schoor, MD,^a John H. Toogood, MD,^b and
Romain A. Pauwels, PhD^a Ghent, Belgium and London, Ontario, Canada

Background: The incidence of systemic side effects of inhaled budesonide increases at doses exceeding 2000 µg/day.

Objective: This study was carried out to investigate whether high-dose inhaled budesonide affects serum IgG subclass concentrations in healthy adult volunteers.

Methods: Two groups of 10 subjects each inhaled 2.4 mg of budesonide per day in a double-blind, crossover study of morning (8:00 AM and noon, group A) and diurnal (8:00 AM and 8:00 PM, group B) dosing schedules for 4 weeks each, separated by a 2-week washout period. The budesonide was inhaled through a pressurized metered-dose inhaler, mounted on a 750 ml Nebuhaler (ASTRA Pharmaceuticals, Lund, Sweden). The IgG subclass levels were determined at baseline and every 2 weeks until the end of the study period (10 weeks).

Results: There were no statistically significant changes in the serum IgG subclass concentrations over the 10-week study period in group A, group B, or groups A and B combined.

Conclusion: Inhalation of budesonide, 2.4 mg/day, through a large-volume spacer for repeated 1-month periods does not influence serum IgG subclass concentrations in healthy adults, suggesting that budesonide does not cause systemic humoral immunosuppression when given at therapeutic doses. (*J ALLERGY CLIN IMMUNOL* 1996;97:113-8.)

Key words: IgG subclasses, inhaled glucocorticosteroids, budesonide, asthma

It is now recognized that suppression of the inflammatory cascade should be the cornerstone of management in bronchial asthma. Inhaled glucocorticosteroids are well established in the long-term treatment of adult and childhood asthma, with an increasing focus on their role as first-line therapy in all but mild, intermittent asthma.^{1,2} The value of inhaled glucocorticosteroid therapy in patients with chronic obstructive pulmonary disease (COPD) is less well established; the results of short-term trials suggest beneficial effects in at least a subgroup of such patients, but results of

Abbreviations used

BDP: Beclomethasone dipropionate
COPD: Chronic obstructive pulmonary disease
PBS: Phosphate-buffered saline

controlled trials are needed for definitive conclusions.^{3,4}

The broad array of side effects of systemic administration of glucocorticosteroids is well known; suppression of host defense mechanisms against infections is one of the clinically more important ones.⁵ Inhaled glucocorticosteroids have a high ratio of local antiinflammatory to systemic activity but are not completely devoid of systemic side effects, especially when administered at higher doses. Measurable effects on parameters of hypothalamo-pituitary-adrenal axis function, bone metabolism, androgen production, skin thickness, and, in children, linear growth have been reported. At present, the clinical importance of these

From ^athe Department of Respiratory Diseases, University Hospital, Ghent, Belgium; and ^bthe Department of Medicine, University of Western Ontario, London, Ontario, Canada.

Received for publication Jan. 4, 1995; revised Apr. 12, 1995; accepted for publication Apr. 13, 1995.

Reprint requests: Jan Van Schoor, MD, Department of Respiratory Diseases, University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium.

Copyright © 1996 by Mosby-Year Book, Inc.
0091-6749/96 \$5.00 + 0 1/1/65637

changes for the long term is not clearly established and requires further investigation.⁶

The literature data on the effects of glucocorticosteroid administration on serum IgG and IgG subclass levels in human beings are limited. The results of small, open, prospective studies indicate that short courses of systemic glucocorticosteroids induce suppression of serum total IgG in healthy volunteers⁷ and in young patients with asthma^{8,9}; this effect is detectable 1 to 2 weeks after the therapy has been initiated and may last for several weeks after discontinuation. These findings are supported by results from cross-sectional studies, performed in groups of patients with asthma,^{10,11} aspirin-induced asthma,¹² and COPD¹³; systemic glucocorticosteroids appear to suppress serum levels of multiple IgG subclasses in a nonselective fashion. To our knowledge, only one report, published as an abstract, specifically addressed the issue of a possible influence of inhaled glucocorticosteroids on serum IgG levels. In an open, prospective trial of 10 asthmatic patients, DeCotiis and Settignano¹⁴ found that an inhaled dose of 400 µg beclomethasone dipropionate (BDP) per day for 4 weeks, significantly decreased serum total IgG levels in the sera of these subjects after treatment, when compared with their pretreatment values. Finally, circumstantial evidence is found in the report by O'Keeffe et al.,¹⁵ who found significantly lowered total serum IgG levels in 27 patients with COPD receiving glucocorticosteroid therapy when compared with 31 patients with COPD not taking glucocorticosteroids. In this group of 27 patients, 25 were receiving inhaled glucocorticosteroids (type and daily dose not specified), and the remaining two were receiving low-dose oral glucocorticosteroids (5 mg of prednisolone per day).¹⁵

Viral respiratory tract infections are a major cause of exacerbations of asthma,¹⁶ whereas bacterial respiratory tract infections are mainly implicated in exacerbations of COPD, other than asthma.¹⁷ IgG subclass deficiencies have been associated with an increased susceptibility to respiratory tract infections, of both viral and bacterial nature. The association of IgG₂ deficiency with recurrent sinopulmonary infections caused by encapsulated bacteria, such as pneumococci, is clinically the best established one.¹⁸ Suppression of serum IgG subclass levels by glucocorticosteroid therapy therefore represents a phenomenon with potential clinical importance.

In view of these data, we thought that relevant information on possible systemic immune effects of inhaled glucocorticosteroids would be obtained by

measuring the effects of 4-week courses of 2.4 mg of inhaled budesonide per day on serum IgG subclass concentrations.

METHODS

The serum samples that were analyzed in this study originate from a trial performed by Toogood et al.,¹⁹ who studied the effect of inhaled glucocorticosteroids on bone metabolism. The study protocol was approved by the Institutional Review Board on Human Research at the University of Western Ontario. In summary, of the original study population of 48 nonsmoking healthy subjects, who all had a normal pulmonary function and had not used inhaled or oral glucocorticosteroids previously, those 20 subjects who were randomly assigned to receive the high dose of budesonide, 2.4 mg/day were selected for this study. The trial had a balanced cross-over design, stratified by sex. The budesonide was inhaled twice daily, that is, at 8:00 AM and noon (AM schedule) or 8:00 AM and 8:00 PM (AM-PM schedule). This was done to determine whether altering the dose schedule had a different effect on serum osteocalcin and cortisol levels, two parameters that show cyclic diurnal variations. The 10 subjects in group A (mean age, 32.70 years; SD, 10.45 years) started with the AM schedule, whereas the 10 in group B (mean age, 28.10 years; SD, 2.60 years) started with the AM-PM schedule. After 4 weeks of treatment, followed by a 2-week washout period, each subject crossed over to the alternative dosing schedule for an additional 4 weeks. The budesonide was inhaled in a standard fashion from a conventional metered-dose inhaler mounted on a 750 ml Nebuhaler spacer (ASTRA Pharmaceuticals, Lund, Sweden). Inhalation commenced at residual volume, proceeded at 25 L/min, and was followed by 10 seconds of breath-holding and a postinspiratory mouth rinse with expectoration. Patients were instructed in the use of a device that measures inspiratory flow and monitored to ensure that they maintained the same technique during all phases of the study. This was done to minimize inter- and intrasubject variability in the delivery of the drug to the lungs and its subsequent systemic absorption. Six serum samples were taken from each subject: at baseline and at 2, 4, 6, 8, and 10 weeks; the washout period was between weeks 4 and 6. Samples were stored at -20°C and assayed in batches.

Polystyrene flat-bottomed 96-well microtiter plates (Nunc-Immuno Plate, MaxiSorp F96) were purchased from Nunc (Roskilde, Denmark). The wells of a given plate were coated with mouse, anti-human IgG subclass monoclonal antibodies against one subclass, dissolved in phosphate-buffered saline (PBS). The monoclonal antibodies used—HP6188 (anti-IgG₁), HP6014 (anti-IgG₂), HP6095 (anti-IgG₃), and HP6196 (anti-IgG₄)—have been previously characterized^{20,21} and were obtained at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. The plates were incubated overnight at 4°C and

washed four times with PBS-Tween in a Denley WW015 Wellwash 5000 stacking microplate washer (Denley Instruments Ltd., West Sussex, U.K.); after this, the wells were saturated with bovine serum albumin 1% in PBS and incubated for 1 hour at 25° C, after which they were washed four times with PBS-Tween.

Serum samples were kept frozen at -20° C until analysis. The serum was subsequently diluted in Tris-HCl buffer; for each IgG subclass, three dilutions were inoculated onto the plate (for IgG₁: 1:40,000, 1:70,000, and 1:100,000; and for IgG₂, IgG₃, and IgG₄: 1:500, 1:1000, and 1:2000). A pooled human serum sample was included as a control for monitoring reproducibility; it was diluted in an identical fashion. A "blank" (consisting solely of Tris-HCl) for detection of background reaction was also included. Calibration curves were generated by making a series of eight dilutions of the Netherlands Red Cross Blood Transfusion Service human standard serum in Tris-HCl; for IgG₁, dilutions ranged from 1:10000 to 1:640,000, and for IgG₂, IgG₃, and IgG₄, dilutions ranged from 1:100 to 1:8000. All reactions were performed in duplicate on the plates. After the inoculation into the respective wells, the plates were incubated for 2 hours at 37° C, after which they were washed four times with PBS-Tween. Horseradish peroxidase-conjugated mouse, anti-human monoclonal antibodies (anti-IgG₁: HP6188, anti-IgG₂: HP6014, anti-IgG₃: HP6095, and anti-IgG₄: HP6196)^{20, 21} in Tris-HCl were added to the wells and incubated for 2 hours at 25° C, after which they were washed four times with PBS-Tween. The color reaction was developed by adding substrate, consisting of o-phenylenediamine in citrate-phosphate buffer/H₂O₂; it was stopped by adding 2 mol/L H₂SO₄ 1:10 in H₂O. The color reaction was subsequently read by measuring the absorption at 492 nm with a Titertek Multiskan MCC filter-photometer plate reader (Flow Laboratories Inc., McLean, Va.). Standard dose-response curves were constructed from averaged absorbance values and concentrations that had been entered into a curve-fitting four-parameter logistic program, available in a Titertek software package, version 2.0A (Flow Laboratories Ltd., Rickmansworth, U.K.). The concentration of each analyte in each sample was obtained by interpolating the averaged absorbance value from the standard curve and multiplying the result by the dilution factor. Only values from the linear portions of the curves were considered valid. The intraassay coefficients of variation were 6.1% for IgG₁, 12.9% for IgG₂, 5.9% for IgG₃, and 16.5% for IgG₄; the interassay coefficients of variation were 14% for IgG₁, 11.3% for IgG₂, 11.0% for IgG₃, and 15.4% for IgG₄. The sum of the serum IgG subclass concentrations came within 15% of the total IgG serum level, determined by nephelometry.

The IgG subclass levels per group were compared by using the Friedman analysis of variance test. The baseline IgG subclass levels of group A and group B were compared by means of the Mann-Whitney U test. Prob-

TABLE I. Friedman test *p* values over the complete study period (baseline to week 10)

	IgG ₁	IgG ₂	IgG ₃	IgG ₄
Group A	0.103	0.136	0.626	0.147
Group B	0.687	0.549	0.900	0.894
Groups A + B	0.883	0.894	0.659	0.293

ability (*p*) values below 0.05 were considered to be statistically significant.

RESULTS

Table I shows that there were no statistically significant changes in the serum levels of any of the four IgG subclasses of both groups over the 10-week study period. The evolution of the serum IgG subclass concentrations is represented in Figs. 1 and 2. Because the serum levels of all four subclasses of both groups failed to show a trend in either direction over the study period, we did not perform additional statistical analyses on the data of the separate treatment periods.

The baseline IgG subclass levels of groups A and B were not significantly different for any of the subclasses: *p* = 0.151 for IgG₁, *p* = 0.705 for IgG₂, *p* = 0.344 for IgG₃, and *p* = 0.647 for IgG₄. Differences in the dosing schedule did not result in statistically significant changes in IgG subclass serum levels between the two groups (results not shown). The results from both groups were therefore pooled and the IgG subclass concentrations of the combined population of 20 subjects were compared in the same fashion as each group separately. Again, no statistically significant changes in the serum levels of any of the subclasses could be demonstrated (Table I).

DISCUSSION

Because we did not detect any influence of high-dose budesonide on serum IgG subclass concentrations, it appears unlikely that budesonide causes clinically relevant systemic humoral immunosuppression at therapeutic doses. Perhaps our study groups were too small to detect more discrete IgG subclass changes, but the clinical significance of such changes, if any, would be very questionable. The budesonide dose used was, however, large enough to adversely affect parameters of adrenocortical function and bone metabolism in the study groups, as reported by Toogood et al.,¹⁹ indicating that systemic absorption did occur. It could also be argued that the study period was not long enough to detect IgG subclass changes. The

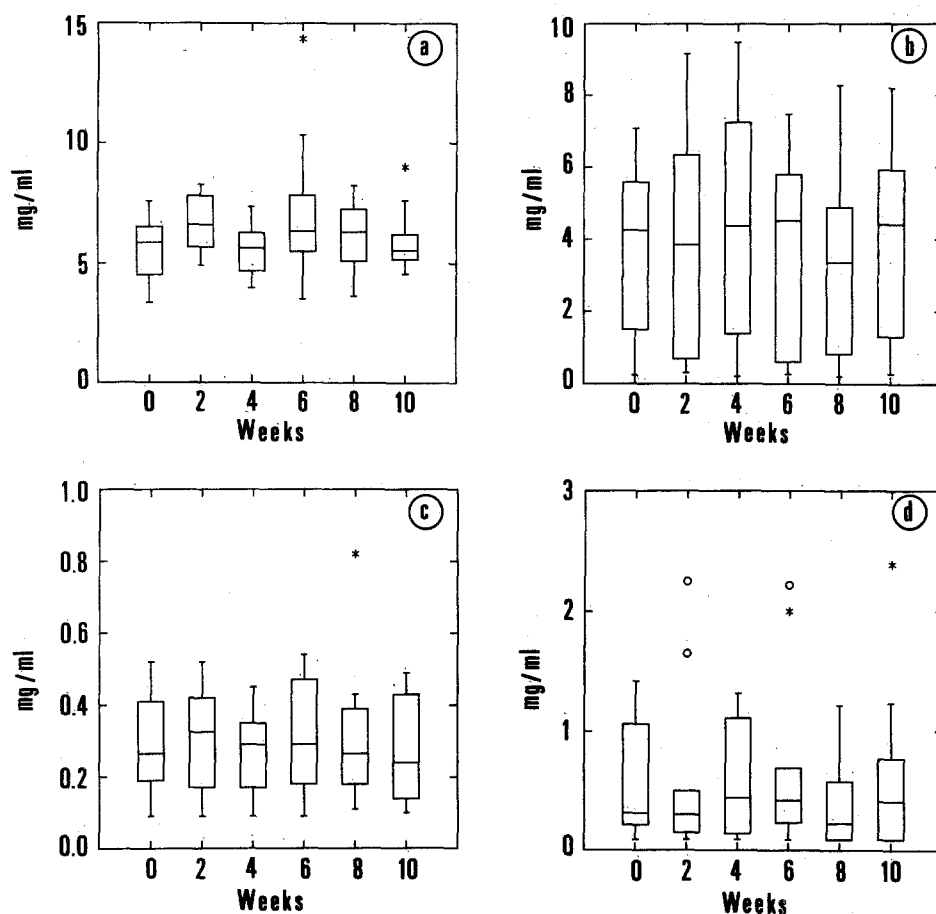


FIG. 1. Box and whisker plots showing evolution of serum (a) IgG₁, (b) IgG₂, (c) IgG₃, and (d) IgG₄ levels in group A. Asterisks and open circles represent outside values and far outside values, respectively.

serum half-lives of the IgG subclasses are approximately 7 days for IgG₃ and 21 days for the other three subclasses, respectively.¹⁸ Because the study duration greatly exceeds these intervals, we think this 10-week observation period is sufficiently long to detect possible changes in IgG subclass levels, taking into account their half-lives. To the best of our knowledge, no studies have specifically investigated the potential effects of prolonged administration of budesonide on serum IgG and IgG subclass levels.

Our findings differ from those of DeCotiis and Settippa,¹⁴ who reported significantly decreased serum total IgG levels after 4 weeks of inhaled BDP at a dose of 400 µg/day in asthmatic patients. Because total IgG is merely the summation of the four IgG subclass levels,¹⁸ an IgG suppression automatically implies a suppression of one or more IgG subclasses. There are, however, several differences between the two studies, which should be emphasized. First, DeCotiis and Settippa¹⁴ stud-

ied asthmatic patients, whereas we studied healthy volunteers. Second, in our study the budesonide was inhaled from a spacer, the use of which was not mentioned in the other study. However, Toogood et al.²² previously demonstrated that the inhalation technique that was used in our study (see Methods) increased the systemic absorption of the inhaled budesonide in a group of asthmatic adults, compared with inhalation from a metered-dose inhaler; differences in inhalation technique are therefore unlikely to account for the absence of IgG subclass suppression in our study. Finally, the type of inhaled corticosteroid used could, in theory, also explain the differing results; we used budesonide, whereas DeCotiis and Settippa¹⁴ used BDP. In general, at higher doses, BDP is known to cause systemic side effects more easily than budesonide.²³ A low dose of 400 µg of BDP per day in adults, however, is unlikely to cause systemic adverse effects in the average patient.⁶ It is therefore somewhat surprising that this dose

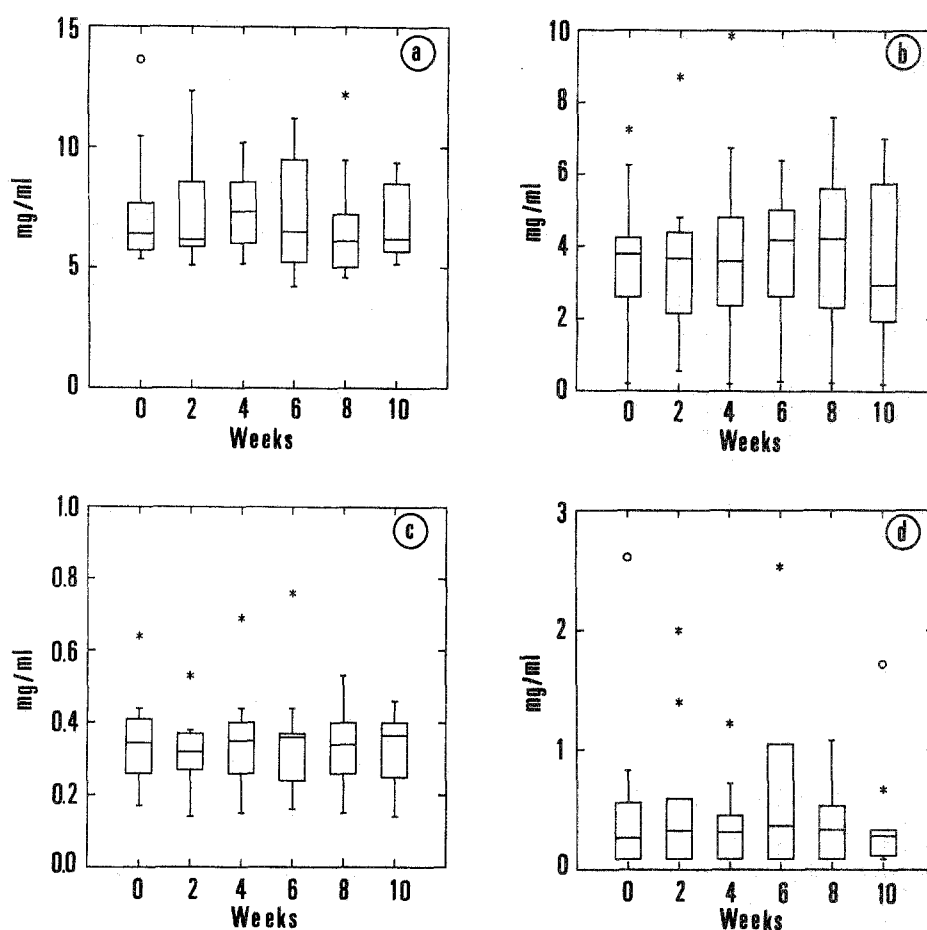


FIG. 2. Box and whisker plots showing evolution of serum (a) IgG₁, (b) IgG₂, (c) IgG₃, and (d) IgG₄ levels in group B. Asterisks and open circles represent outside values and far outside values, respectively.

would lead to serum IgG suppression. A systemic humoral immunosuppressive action of BDP at low doses could be a specific feature of BDP, but this has not been investigated until now. This is not the case for budesonide, as the results of this study indicate.

In summary, until now no firm data have indicated that inhaled budesonide elicits systemic humoral immunosuppression, when given at high doses for relatively short periods as in this study. The effects of inhalation of larger doses, administered over longer periods, clearly need more investigation. The apparent lack of systemic humoral immunosuppression, however, does not automatically rule out a local humoral immunosuppression at the level of the airways themselves, because inhaled glucocorticosteroids have a high topical activity. The airways act as the first line of defense against infections caused by inhaled viruses and bacteria, and IgG plays an important role in the lower airway defense mechanisms.²⁴ In addition,

cell-mediated immune mechanisms are potentially more vulnerable to inhaled glucocorticosteroid use than are IgG-mediated mechanisms, and several reports in the literature have indeed implicated inhaled BDP as a major contributing factor in the development of pulmonary tuberculosis in patients with asthma^{25,26} or in the development of disseminated aspergillosis in a patient with allergic bronchopulmonary aspergillosis and bilateral fungal balls.²⁷ In this study, the incidence of upper and lower respiratory tract infections was not recorded. Until now, however, no studies have specifically addressed the possible effects of inhaled glucocorticosteroids on lower airway defense mechanisms in general and on airway IgG and its subclasses in particular. Studies exploring this topic will enable us to make definitive conclusions regarding the possible immunosuppressive actions of these drugs. In the immunocompetent patient the incidence, severity, and duration of viral or bacterial respiratory tract infections do not appear to be

increased by inhaled BDP treatment, given in doses of up to 1600 $\mu\text{g/day}$.²⁸⁻³⁰

We thank Mrs. Danny Michiels, for help in developing the ELISA assay and for assisting in the determinations of the IgG subclass levels, and Mrs. Christiane Vandeven, for preparing the manuscript.

REFERENCES

1. International Consensus Report on Diagnosis and Treatment of Asthma. *Eur Respir J* 1992;5:601-41.
2. Guidelines on the management of asthma. *Thorax* 1993;48(suppl):S1-24.
3. Wedzicha JA. Inhaled corticosteroids in COPD: awaiting controlled trials. *Thorax* 1993;48:305-7.
4. Pauwels RA, Löfdahl CG, Pride NB, Postma DS, Laitinen LA, Ohlsson SV. European Respiratory Society study on chronic obstructive pulmonary disease (EUROSCOP): hypothesis and design. *Eur Respir J* 1992;5:1254-61.
5. Truhan AP, Razzaque Ahmed A. Corticosteroids: a review with emphasis on complications of prolonged systemic therapy. *Ann Allergy* 1989;62:375-90.
6. Dukes MNG, Holgate ST, Pauwels RA. Report of a workshop assessing the risks and benefits of inhaled anti-inflammatory treatment for asthma. *Eur Respir J* 1994;7:830-2.
7. Butler WT, Rossen RD. Effects of corticosteroids on immunity in man. I. Decreased serum IgG concentration caused by 3 or 5 days of high doses of methylprednisolone. *J Clin Invest* 1973;52:2629-40.
8. Posey WC, Nelson HS, Branch B, Pearlman DS. The effects of acute corticosteroid therapy for asthma on serum immunoglobulin levels. *J ALLERGY CLIN IMMUNOL* 1978;62:340-8.
9. Settipane GA, Pudupakkam RK, McGowan JH. Corticosteroid effect on immunoglobulins. *J ALLERGY CLIN IMMUNOL* 1978;62:162-6.
10. Klaustermeyer WR, Wong SC, Schoettler JJ, Gianos ME, Heiner DC. Quantitative immunoglobulins and IgG subclasses in patients with corticosteroid-dependent reversible airway obstruction. *Ann Allergy* 1989;63:327-30.
11. Hamilos DL, Young RM, Peter JB, Agopian MS, Iklé DN, Barka N. Hypogammaglobulinemia in asthmatic patients. *Ann Allergy* 1992;68:472-81.
12. Szczeklik A, Schmitz-Schumann M, Nizankowska E, Milewski M, Roehlig F, Virchow C. Altered distribution of IgG subclasses in aspirin-induced asthma: high IgG₄, low IgG₁. *Clin Exp Allergy* 1992;22:283-7.
13. Klaustermeyer WB, Gianos ME, Kurohara ML, Dao HT, Heiner DC. IgG subclass deficiency associated with corticosteroids in obstructive lung disease. *Chest* 1992;102:1137-42.
14. DeCotiis BA, Settipane GA. The effect of inhaled beclomethasone on serum immunoglobulins. *J ALLERGY CLIN IMMUNOL* 1980;65:218-9.
15. O'Keeffe S, Gzel A, Drury R, Cullina M, Greally J, Finnegan P. Immunoglobulin G subclasses and spirometry in patients with chronic obstructive pulmonary disease. *Eur Respir J* 1991;4:932-6.
16. Pattermore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms. I. Epidemiology. *Clin Exp Allergy* 1992;22:325-36.
17. Murphy TF, Sethi S. Bacterial infection in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1992;146:1067-83.
18. Jefferis R, Kumararatne DS. Selective IgG subclass deficiency: quantification and clinical relevance. *Clin Exp Immunol* 1990;81:357-67.
19. Toogood JH, Jennings B, Hodsman AB, Baskerville J, Fraher LJ. Effects of dose and dosing schedule of inhaled budesonide on bone turnover. *J ALLERGY CLIN IMMUNOL* 1991;88:572-80.
20. Jefferis R, Reimer CB, Skvaril F, et al. Evaluation of monoclonal antibodies having specificity for human IgG sub-classes: results of an IUIS/WHO collaborative study. *Immunol Lett* 1985;10:223-52.
21. Jefferis R, Reimer CB, Skvaril F, et al. Evaluation of monoclonal antibodies having specificity for human IgG subclasses: results of the 2nd IUIS/WHO collaborative study. *Immunol Lett* 1992;31:143-68.
22. Toogood JH, Baskerville J, Jennings B, Lefcoe NM, Johanson SA. Use of spacers to facilitate inhaled corticosteroid treatment of asthma. *Am Rev Respir Dis* 1984;129:723-9.
23. Brogden RN, McTavish D. Budesonide: an updated review of its pharmacological properties, and therapeutic efficacy in asthma and rhinitis. *Drugs* 1992;44:375-407.
24. Reynolds HY. Immunoglobulin G and its function in the human respiratory tract. *Mayo Clin Proc* 1988;63:161-74.
25. Horton DJ, Spector SL. Clinical pulmonary tuberculosis in an asthmatic patient using a steroid aerosol. *Chest* 1977;71:540-2.
26. Shaikh WA. Pulmonary tuberculosis in patients treated with inhaled beclomethasone. *Allergy* 1992;47:327-30.
27. Anderson CJ, Craig S, Bardana EJ. Allergic bronchopulmonary aspergillosis and bilateral fungal balls terminating in disseminated aspergillosis. *J ALLERGY CLIN IMMUNOL* 1980;65:140-4.
28. Tarlo S, Broder I, Spence L. A prospective study of respiratory infection in adult asthmatics and their normal spouses. *Clin Allergy* 1979;9:293-301.
29. Toogood JH, Jennings B, Greenway RW, Chuang L. Candidiasis and dysphonia complicating beclomethasone treatment of asthma. *J ALLERGY CLIN IMMUNOL* 1980;65:145-53.
30. Frank A, Dash CH. Inhaled beclomethasone dipropionate in acute infections of the respiratory tract. *Respiration* 1985;48:122-6.