

Suppression of the immune system by oral glucocorticoid therapy in bronchial asthma

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The effect of systemic glucocorticoid therapy on immune parameters was studied in patients with bronchial asthma. Patients were divided into two groups: 1) those receiving oral glucocorticoid; 2) control patients who did not receive systemic glucocorticoid treatment. The glucocorticoid dose varied between 5 and 70 mg per day. Patients had been taking oral therapy for at least 1 year. Glucocorticoid treatment was associated with an increased frequency of respiratory tract infections. Therefore, we need to define immune parameters which may predict an increased risk of infections. In this study, we analyzed several surface markers on lymphocytes and monocytes by flow cytometry. A significant reduction of the ratio of peripheral blood CD4⁺ to CD8⁺ T cells was associated with the administration of oral glucocorticoids. Furthermore, the expression of the HLA-DR molecule on monocytes was reduced in patients with systemic glucocorticoid therapy compared to control patients. Moreover, the capacity to elaborate cytokines by peripheral blood mononuclear cells upon stimulation was greatly reduced after exposure to glucocorticoids *in vivo* and *in vitro*. In addition, the humoral immune response was affected, because reduced IgG, IgM, and IgA levels were observed in patients receiving oral glucocorticoids. These results indicate that systemic glucocorticoid treatment in patients with bronchial asthma is associated with cellular and humoral immunosuppression which results in an increased risk of bacterial and viral infections.

Key words: asthma; cytofluorometric analysis; cytokines; glucocorticoids; immune monitoring; immunosuppression; inflammation.

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Bronchial asthma is now viewed as a disease with an important inflammatory component. Glucocorticoids (GC) have anti-inflammatory effects on the airways and are of proven therapeutic benefit in this chronic lung disease. There are inhaled GC preparations available that are highly active topically. However, severe cases of chronic asthmatic inflammation remain difficult to treat and some patients depend on systemic GC in high doses in order to maintain adequate pulmonary function. The mechanism of action of GC as anti-inflammatory agents is still uncertain.

GC are not only anti-inflammatory but also immunosuppressive agents. Therefore, they are widely used to treat patients with autoimmune diseases or to protect from rejection of transplanted organs. The effects of GC on cells are mediated through the binding of steroid molecules to specific receptor proteins located in the cyto-

plasm of target cells. Steroid-receptor complexes translocate to the nucleus of the cell, where they interact with GC-responsive elements to modulate gene expression. T cells and their cytokines have been implicated in the pathogenesis of asthma (1–12) and other inflammatory lung diseases. Recently, it has been shown that GC downregulate the expression of cytokine genes in T cells (2–4, 6, 8, 13–17), an action which might explain their immunosuppressive effects and their therapeutic potential.

However, the use of immunosuppressive agents may lead to downregulation of the immune system. This may be followed by a higher incidence of respiratory infections, in turn, leading to additional inflammation of the airways (18). Therefore, suppression of the normal immune defense is a possible side-effect of systemic GC treatment in bronchial asthma. In this study, we observed an increased

incidence of infectious complications in patients receiving GC. Thus, it is of interest to identify immune parameters which are suitable to monitor patients receiving systemic GC therapy. In this paper, we describe the immunologic changes observed in patients with bronchial asthma receiving systemic GC therapy. Some of these immune markers may predict increased susceptibility to infection.

Material and methods

Patients

A group of 74 patients with chronic bronchial asthma was recruited from the Davos-Wolfgang High-Altitude Clinic in a retrospective study. All patients met the American Thoracic Society definition of asthma (19). Seventeen patients were diagnosed as having allergic asthma, as they had a positive history of a bronchoconstrictive response after allergen exposure, a positive wheal and flare reaction to skin prick tests, a total serum IgE level of >100 kU/l, as well as increased specific IgE against house-dust mite (*Dermatophagoides pteronyssinus*), cat allergen, grass pollen, or birch pollen. The remaining 57 patients were diagnosed as having nonallergic asthma. These patients had a history of postinfectious onset of asthma, no history of allergen-induced bronchospasm, and negative skin prick test reactions to a standard set of allergens. In the analysis, we compared the patients who received systemically 5–100 mg prednisone per day for at least 1 year (GC patients, $n=55$) with those who received no systemic GC (control [C] patients, $n=19$, most of them receiving inhaled GC). Patients were asked about the frequency of infectious complications of the respiratory tract needed to be treated by antibiotics in the most recent years. The information about infectious complications was obtained from medical records. On the basis of this information, the patients who received systemic GC were subdivided into two groups: 1) fewer than three, and 2) three or more respiratory tract infections per year. In addition, a group of 20 healthy control subjects with no history of allergy or lung disease was studied. Informed consent was obtained from the patients, and the study was approved by the Medical Ethics Committee of Davos.

Collection of blood

For flow cytometry, EDTA-anticoagulated blood (10 ml) was collected from the patients between 7:30 and 9:00 a.m. (before the morning prednisone dose). Standard, hospital-approved protocols for immunologic monitoring were followed. For Ig

assays, 5 ml blood was taken at the same time and allowed to clot at room temperature. The serum was then withdrawn and stored at -20°C until analysis. Heparinized blood (20 ml) was collected for purification of peripheral blood mononuclear cells (PBMC).

Analysis of leukocytes and lymphocyte subsets

Blood leukocyte and differential counts were determined by automatic blood count analysis (Technicon H1, Technicon, Tarrytown, NY, USA). Specific binding of monoclonal antibodies (mAb) was analyzed by direct immunofluorescence, by standard methods recommended by Becton-Dickinson Monoclonal Center (Mountain View, CA, USA), with a flow cytometer (Epics Profile, Coulter Corp., Hialeah, FL, USA). Briefly, 50 μl of whole blood was incubated in the presence of saturating concentrations of fluorescein- or phycoerythrin-conjugated mAb on ice in the dark for 30 min. Erythrocytes were lysed by adding 3 ml lysing solution (155 mM NH_4Cl , 10 mM KHCO_3 , 0.1 mM EDTA, pH 7.3) for 3–5 min. Leukocytes were washed twice with phosphate-buffered saline containing 2% fetal calf serum (FCS) and 0.1% sodium azide. Cytofluorometric analysis was performed with scatter gates on the lymphocyte and monocyte fractions with laser excitation at 488 nm. The number of immunofluorescence-positive cells was determined for 10 000 analyzed cells. Specific binding of mAbs was controlled by subtraction of isotype-matched murine Ig. mAbs against CD3 (T cells), CD4 (T helper/inducer), CD8 (T suppressor/cytotoxic), CD14 (monocytes), CD16 (NK cells), CD19 (B cells), CD25 (interleukin [IL]-2 receptor α -chain), and HLA-DR were purchased from Immunotech (Marseille, France) and from Dakopatts A/S (Glostrup, Denmark).

Cytokine production by PBMC

To determine the *in vitro* effect on cytokine production of PBMC, cells were isolated from blood, as previously described (7, 11, 20). PBMC were cultured in complete culture medium (RPMI 1640 supplemented with 10% FCS; Life Technologies, Inc., Basel, Switzerland) in the presence of the indicated concentrations of prednisone or dexamethasone (both from Ciba Geigy, Basel, Switzerland) at 10^5 cells/200 μl and stimulated with 0.5 $\mu\text{g}/\text{ml}$ anti-CD2 mAb 4B2, 0.5 $\mu\text{g}/\text{ml}$ anti-CD2 mAb 6G4, and 1 $\mu\text{g}/\text{ml}$ anti-CD28 mAb (all from The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). After incubation for 72 h at 37°C in 5% CO_2 in a fully humidified atmosphere, the supernatants were harvested and frozen at -80°C until analysis.

For determination of the *in vivo* effect of GC, three patients with severe bronchial asthma and peripheral blood eosinophilia of >10% who had not received systemic GC for at least 1 month before were treated with 25 mg of prednisone (Merck, Darmstadt, Germany) orally twice a day for 7 days. There were no alterations in their usual medications during the 1-week course of prednisone. PBMC were isolated before and after the treatment with prednisone. PBMC were cultured at 10^6 cells/100 μ l in complete culture medium alone and in the presence of 10 μ g/ml phytohemagglutinin (PHA) (Boehringer Mannheim, Rotkreuz, Switzerland). Supernatants were harvested after 24 h and frozen at -80°C until analysis.

IL-4 and interferon-gamma (IFN- γ) were measured in the culture supernatants by ELISA, as described previously (20). The sensitivity of the assays was 35 pg/ml for IL-4 and 20 pg/ml for IFN- γ , according to recombinant standards. IL-5 was analyzed with a commercial ELISA kit (R & D Systems, Bühlmann, Basel, Switzerland) according to the manufacturer's instructions. The lower detection limit was 5 pg/ml.

Statistical analysis

Statistical analysis was performed with the unpaired Student's *t*-test. Differences associated with probability values of $P < 0.05$ were considered to be significant. Correlation coefficients and statistical significance were determined by Pearson's linear regression analysis.

Results

GC treatment is associated with increased incidence of infectious complications

Patient characteristics are summarized in Table 1. GC and C patients were similar except for treatment with GC. Most of the GC patients demonstrated an increased risk of respiratory tract infections (Fig. 1, Table 1). This increased susceptibility to infections implies that these patients developed drug-associated oversuppression of the immune system.

GC treatment is associated with increased total numbers of leukocytes and with decreased relative numbers of lymphocytes and eosinophils

It has been previously reported that patients receiving prednisone may develop neutrophilia (18). To avoid this, we collected blood for analysis before the morning prednisone dose. Nevertheless, we observed significantly higher absolute leukocyte

Table 1. Patient characteristics

Parameter	GC patients			C patients
	Total	Infections/year		
		<3	≥ 3	
Total number of subjects	55	14	41	19
Age (years)*	48.7	49.7	48.3	48.5
Sex (M/F)	24/31	7/7	17/24	6/13
FEV ₁ (%)*	67.9	79.9	64.5	82.2
Duration of disease (years)*	21.1	20.7	21.9	20.9
Atopy (positive/negative)	12/43	4/10	8/33	5/14
Total daily steroid dose (mg)*	21.6	19.8	22.2	<1.0 (inh.)

* Mean value of patients. inh.: inhalative.

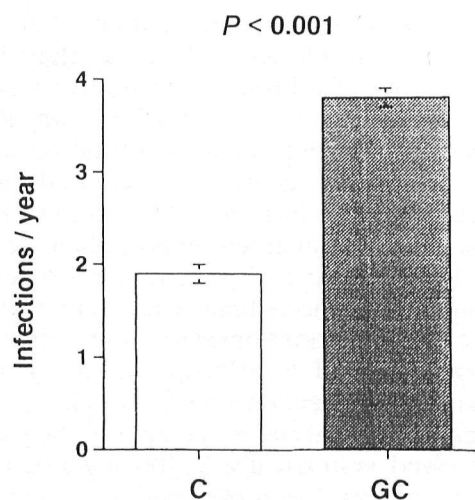


Fig. 1. Susceptibility to infections of respiratory tract of patients who received systemic glucocorticoids (GC) and of control patients who did not receive systemic glucocorticoids (C). Presented as means $\pm$ SEM.

numbers and lower relative lymphocyte numbers in GC than C patients (Fig. 2A and C). This effect might be due to the known mobilization of neutrophils (Fig. 2B) in the bone marrow by GC. In addition, decreased relative (Fig. 2D) and absolute (data not shown) eosinophil numbers were found in the peripheral blood of GC patients. This indicates decreased expression of eosinophil survival factors by T cells due to the GC treatment (2, 4, 8, 9, 12, 16, 21, 22). Absolute and relative leukocyte numbers did not significantly differ between C patients and healthy control subjects (data not shown).

Interestingly, we observed in GC patients who had more than three respiratory tract infections per year a more profound reduction of the relative numbers of peripheral blood lymphocytes and eosinophils than in GC patients with less than three

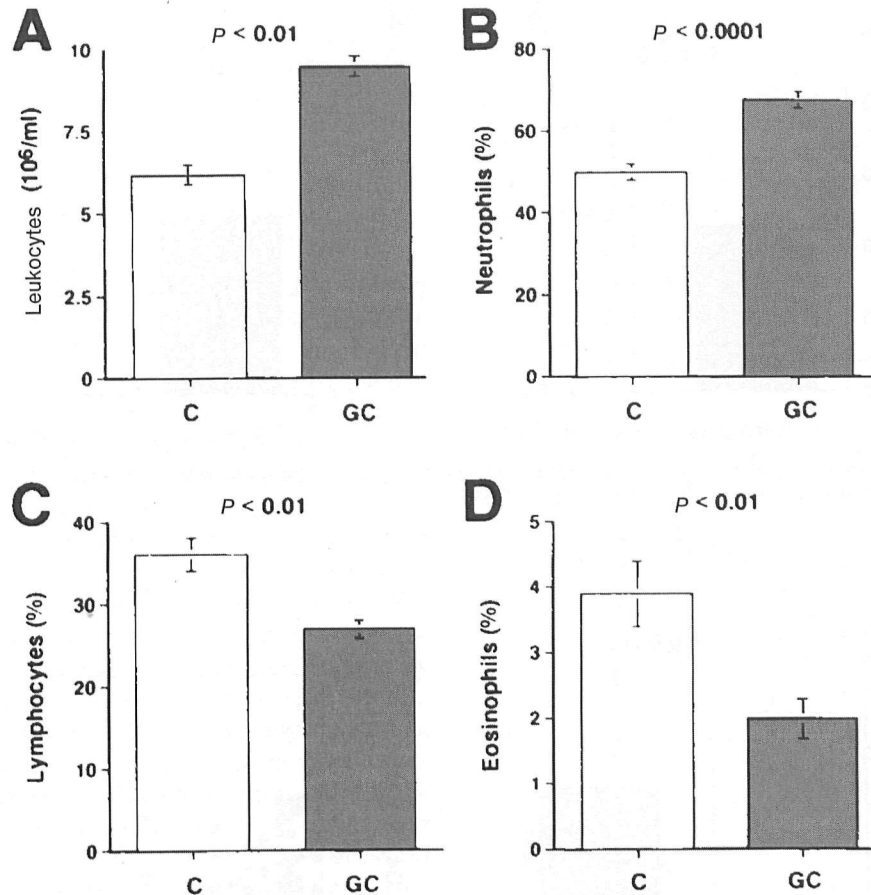


Fig. 2. Absolute leukocyte (A), relative neutrophil (B), relative lymphocyte (C), and relative eosinophil (D) numbers of patients who received systemic glucocorticoids (GC) and of control patients who did not receive systemic glucocorticoids (C). Presented as means $\pm$ SEM. No differences were observed between C patients and healthy control subjects (data not shown).

infections (Fig. 3). These data suggest that low T-cell numbers and their inability to produce sufficient amounts of cytokines may be responsible for the immunosuppression observed in these patients.

GC treatment is associated with a decreased ratio of peripheral CD4⁺ to CD8⁺ T cells

Beside reduction of cytokine expression in T cells, GC inhibit the proliferative response of T cells *in vitro* (3), suggesting that T cells represent one primary target of GC. Therefore, we analyzed T-cell subpopulations, natural killer (NK) cells, and T-cell activation markers in GC and C patients. GC patients showed a significantly lower ratio of CD4⁺ to CD8⁺ peripheral blood lymphocytes than C patients (Fig. 4A, left panel). This effect may depend on the dose of GC taken by the patients, as we found a significant negative correlation between the CD4/CD8 ratio and the daily prednisone dose (Fig. 4A, right panel). In addition, the CD4/CD8 ratio appeared to be further downregulated in GC patients with three or more respiratory

tract infections per year due to a further reduction of CD4⁺ T cells (Fig. 4B). Moreover, we observed significant correlations between the relative numbers of both T-cell subpopulations and the daily prednisone dose (data not shown). The CD4/CD8 ratio did not differ significantly between C patients and healthy control subjects (data not shown).

It has been demonstrated that neutrophilia and lymphopenia occur on the day that GC is administered. We found no evidence that such lymphopenia is due to a specific T-cell subpopulation within such a short course of GC therapy (data not shown). Thus, the decreased CD4/CD8 ratio observed in our GC patients was due to a GC mechanism which was not detectable within the first 24 h.

Beside the T-cell subpopulations, we determined the relative amounts of NK cells within the lymphocyte gate during flow-cytometric analysis. NK cell numbers from both groups were in the normal range and did not significantly differ from healthy control subjects (data not shown).

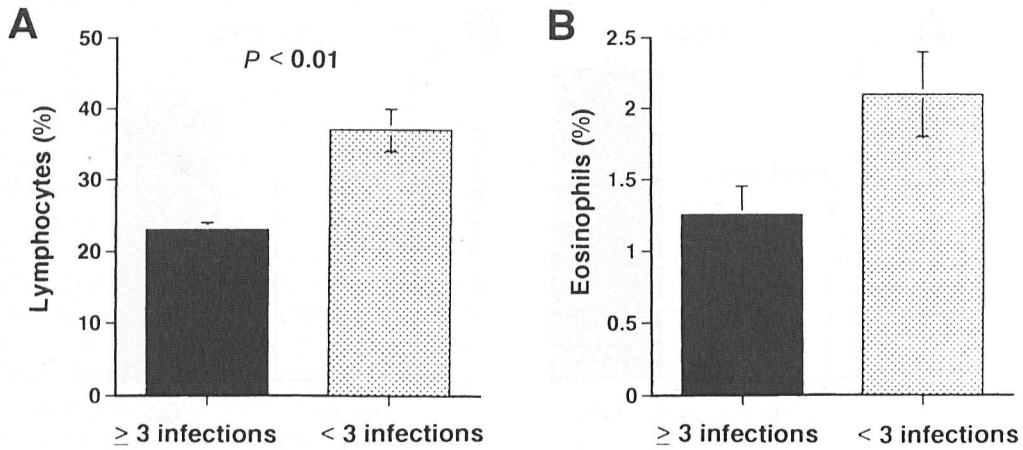


Fig. 3. Relative lymphocyte (A) and relative eosinophil (B) numbers of GC patients with fewer than three and at least three respiratory tract infections per year. Presented as means $\pm$ SEM.

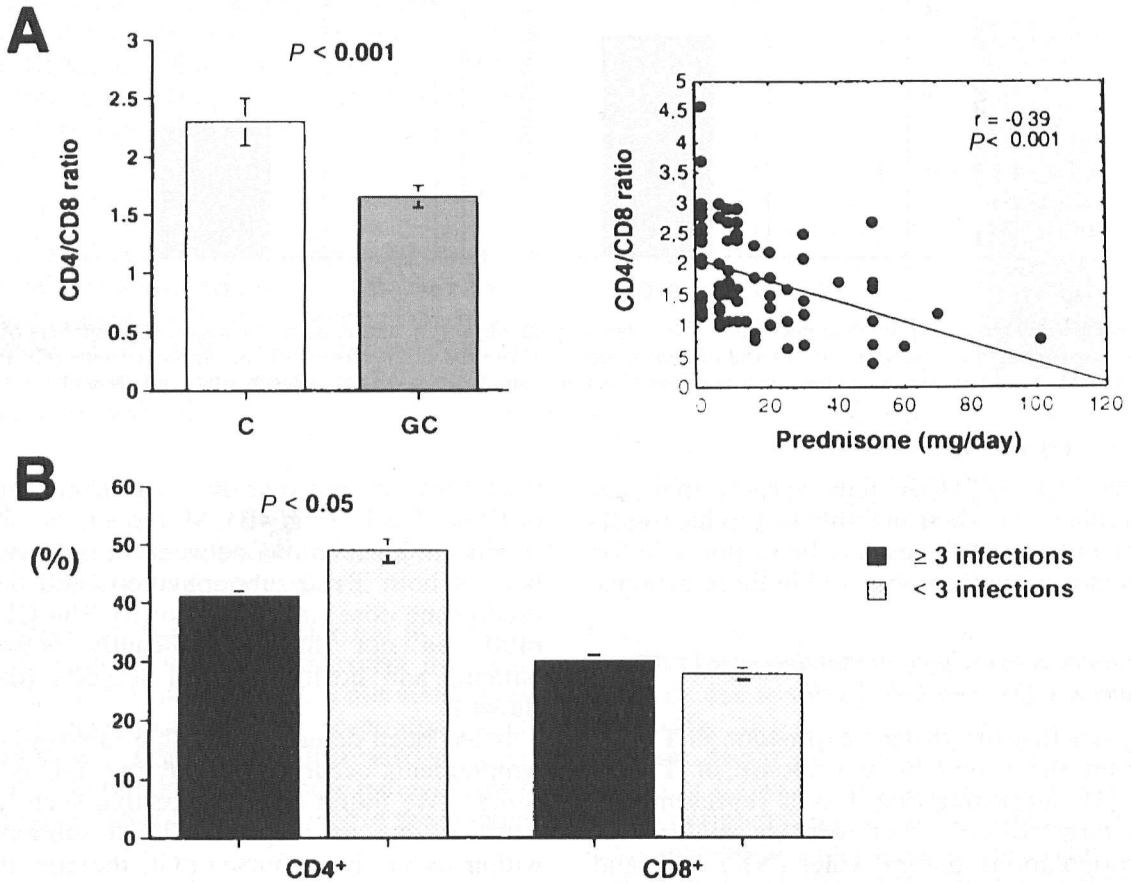


Fig. 4. Relationship between numbers of CD4⁺ and CD8⁺ T cells and glucocorticoid treatment. A) Left panel: ratio of CD4⁺ to CD8⁺ peripheral blood T cells in patients who received systemic glucocorticoids (GC) and in control patients who did not receive systemic glucocorticoids (C). Presented as means $\pm$ SEM. No differences were observed between C patients and healthy control subjects (data not shown). Right panel: relationship between CD4/CD8 ratio of peripheral T cells and prednisone dose. B) Relative numbers of CD4⁺ and CD8⁺ peripheral blood T cells of GC patients with fewer than three and at least three respiratory tract infections per year. Presented as means $\pm$ SEM.

In previous studies, we described increased T-cell activation in bronchial asthma, as indicated by

an increase in the surface expression of CD25 and the HLA-DR molecule (7, 10–12). Therefore, we

analyzed the *in vivo* activation state of CD4⁺ and CD8⁺ cells in the two groups of patients. GC patients and C patients did not significantly differ in their activation state of both T-cell subpopulations (data not shown).

GC treatment is associated with decreased monocyte HLA-DR molecule expression

GC treatment has been shown to modulate IFN- γ gene expression in asthma (2, 6, 8, 13, 23). The association between IFN- γ gene expression and decreased HLA-DR⁺ monocytes has been clearly demonstrated in mice with disrupted IFN- γ genes (24). Furthermore, immunosuppressive therapy, including GC, is accompanied by decreased expression of HLA-DR on monocytes in patients after kidney transplantation (25). IFN- γ therapy restores HLA-DR molecule expression in patients with reduced monocyte surface expression (26). We examined HLA-DR expression on monocytes in the two groups of patients. We found significantly decreased monocyte HLA-DR expression in GC compared to C patients (Fig. 5A, upper panel). Moreover, we observed a significant negative correlation between HLA-DR expression on monocytes and the daily prednisone dose (Fig. 5A, lower panel). In addition, monocyte HLA-DR expression appeared to be further downregulated in GC patients with three or more respiratory tract infections per year, although the difference from the group of GC patients with less than three infections was not statistically significant (Fig. 5B). The monocyte HLA-DR expression did not differ between C patients and healthy control subjects (data not shown). These data suggest downregulation of the IFN- γ gene due to systemic GC treatment in patients with bronchial asthma.

GC treatment is associated with decreased IFN- γ , IL-4, and IL-5 expression in vitro and in vivo

To determine the effect of GC on cytokine gene expression, we exposed PBMC from atopic patients to different concentrations of prednisone and dexamethasone stimulated with anti-CD2 and anti-CD28 mAbs. As shown in Fig. 6, both GC inhibited IFN- γ , IL-4, and IL-5 production of stimulated PBMC in a dose-dependent manner. It should be noted that these inhibiting concentrations are equivalent to pharmacologic doses. In addition, we observed with both prednisone and dexamethasone an inhibition of anti-CD2- and anti-CD28-induced PBMC proliferation (data not shown), confirming previously published work (3).

We next investigated the effect of a 1-week course of prednisone treatment on cytokine gene expres-

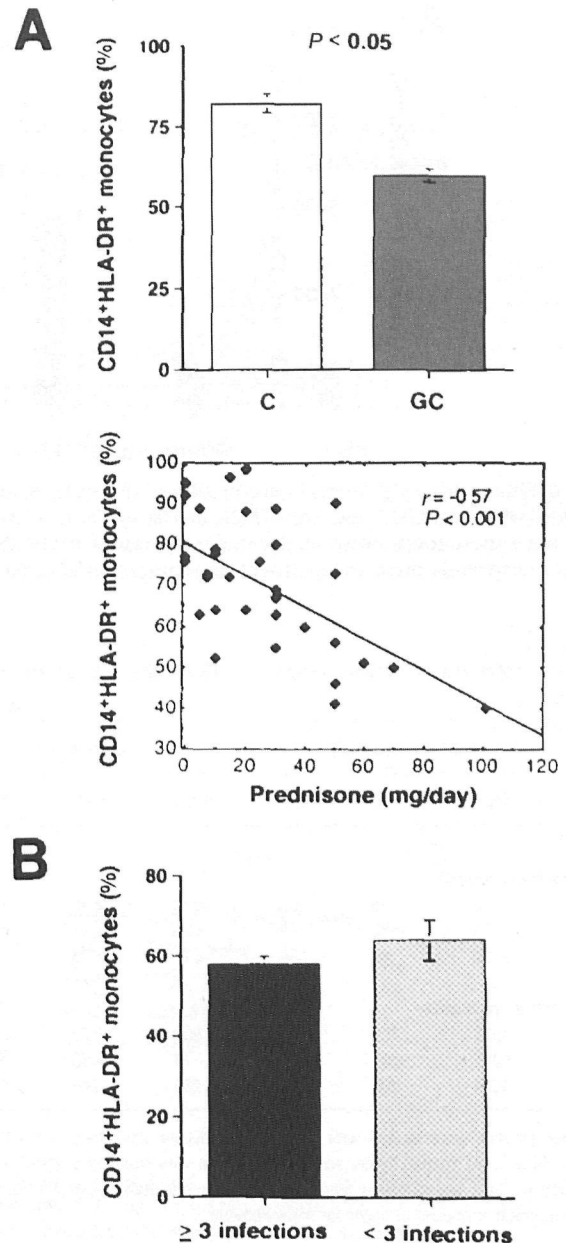


Fig. 5. Relationship between numbers of HLA-DR⁺ monocytes and glucocorticoid treatment. A) Upper panel: expression of HLA-DR molecule on peripheral blood CD14⁺ monocytes of patients who received systemic glucocorticoids (GC) and of control patients who did not receive systemic glucocorticoids (C). Presented as means $\pm$ SEM. No differences were observed between C patients and healthy control subjects. Lower panel: relationship between HLA-DR molecule expression on monocytes and prednisone dose. B) Expression of HLA-DR molecule on peripheral blood CD14⁺ monocytes of GC patients with fewer than three and at least three respiratory tract infections per year. Presented as means $\pm$ SEM.

sion by PBMC in three patients with severe bronchial asthma. Treatment with prednisone was associated with downregulation of PHA-induced IFN- γ , IL-4, and IL-5 production (Table 2). Spontaneous

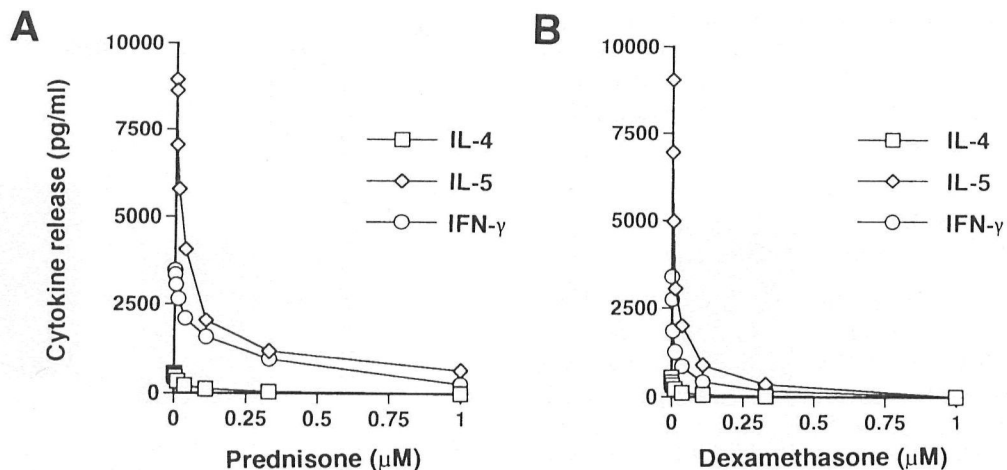


Fig. 6. Glucocorticoids suppress production of IFN- γ , IL-4, and IL-5 in PBMC *in vitro*. To induce cytokine production, we stimulated PBMC with anti-CD2 and anti-CD28 mAbs for 72 h in presence of indicated concentrations of prednisone or dexamethasone. Culture supernatants were analyzed for cytokines by ELISA. Results are expressed as means of duplicate measurements. This figure represents three independent experiments that yielded similar results.

Table 2. Suppression of cytokine production in PBMC after oral glucocorticoid therapy*

	Before treatment			After treatment		
	IFN- γ	IL-4	IL-5	IFN- γ	IL-4	IL-5
pg/ml						
Spontaneous secretion						
1.	<20	<35	<5	<20	<35	<5
2.	<20	<35	<5	<20	<35	<5
3.	<20	40	56	<20	<35	<5
PHA-induced production						
1.	1696	151	110	893	<35	35
2.	1345	<35	32	73	<35	<5
3.	1674	826	618	938	246	381

* Three patients were treated with 25 mg of prednisone orally twice daily for 7 days. PBMC were isolated before and after treatment with prednisone. PBMC were cultured for 24 h, and cytokines were measured in supernatants by ELISA. Results are expressed as means of duplicate measurements.

secretion of these cytokines by cultured PBMC was detectable in only one of three patients. In this patient, the spontaneous secretion of IFN- γ , IL-4, and IL-5 was also reduced after oral GC therapy (Table 2). These results suggest that GC suppress the expression of cytokine genes *in vitro* and *in vivo*.

GC treatment is associated with decreased Ig levels

Although GC are known to suppress the humoral immune response in experimental animals, there have been conflicting data on the influence of GC on Ig levels in asthmatic patients (27–29). We observed in GC patients significantly decreased

gamma globulin (data not shown) and Ig concentrations in serum compared to C patients (Fig. 7A). Moreover, gamma globulin and Ig concentrations appeared to be further downregulated in GC patients with three or more respiratory tract infections per year compared to the group with fewer than three infections (Fig. 7B). C patients and healthy control subjects did not show significant differences (data not shown). The most prominent effect was observed for IgG. In addition, we found a significant negative correlation between the daily GC dose and the IgG concentration in serum ($r = -0.26$; $P < 0.05$), confirming previously published results (28). The hypogammaglobulinemic effect of GC was not due to decreased B-cell numbers as assessed by flow-cytometric analysis with an antihuman CD19 mAb (data not shown), but instead it was caused by reduced Ig production or secretion by B cells.

Short-term effects of high-dose GC treatment on immune parameters

To determine whether the observed changes in some of the investigated surface protein parameters are suitable for immune monitoring of patients with bronchial asthma receiving systemic GC, we tested one representative patient receiving large amounts of GC. As shown in Fig. 8, after increase of the GC dose from 40 to 80 mg per day, we observed a profound decrease in the number of CD4⁺ T cells. In contrast, an increase of GC dose was associated with only a slight decrease in CD8⁺ T-cell numbers. In addition to the reduction of T-cell numbers, HLA-DR⁺ expression on monocytes also markedly decreased after higher

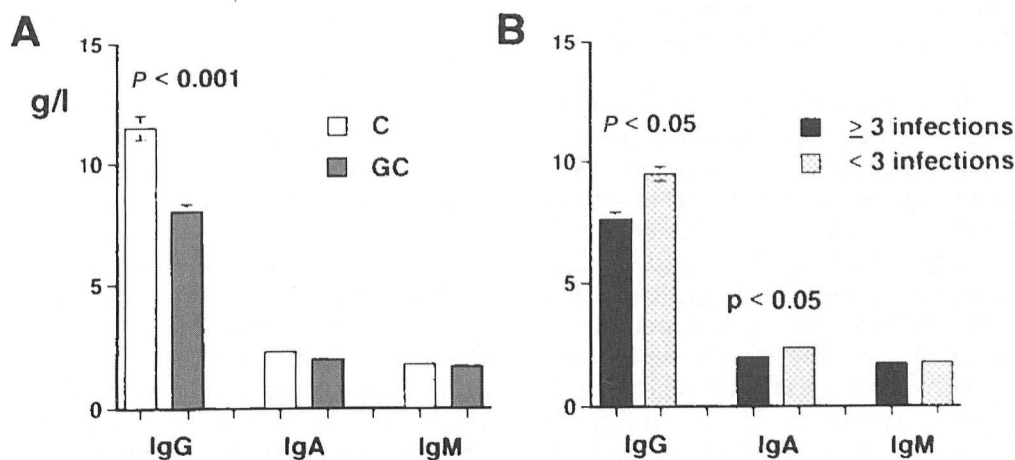


Fig. 7. Association between Ig concentrations in serum and glucocorticoid treatment. A) Concentrations of Ig in serum of patients who received systemic glucocorticoids (GC) and of control patients who did not receive systemic glucocorticoids (C). Presented as means $\pm$ SEM. No differences were observed between C patients and healthy control subjects. B) Concentrations of Ig in serum of GC patients with fewer than three and at least three respiratory tract infections per year. Presented as means $\pm$ SEM.

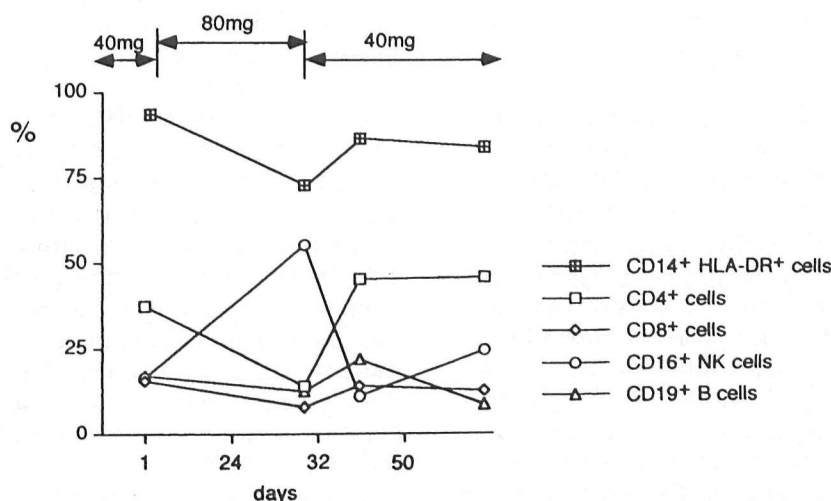


Fig. 8. Effect of high-dose glucocorticoid therapy on lymphocytic and monocytic surface markers in one patient with bronchial asthma. Increase of the GC dose from 40 to 80 mg was followed by profound decrease of CD4⁺ T cells and HLA-DR expression on monocytes. Changes of immune parameters were reversible after reduction of GC dose (indicated at top).

GC application. After reduction of the GC dose, all immune parameters returned to normal levels. These results suggest that an immune monitoring of GC patients by cytofluorometric analysis of T cells and monocytes may be useful in the management of patients with chronic bronchial asthma receiving high-dose GC therapy to prevent oversuppression of the immune system.

Discussion

The long-term systemic use of GC is associated with substantial and often irreversible adverse side-effects. This involves the risk of suppressing the endogenous pituitary-adrenal function and causing

iatrogenic Cushing's syndrome (30). Other possible side-effects of systemic GC therapy include cataract formation, interference with bone metabolism, decreased linear growth in children, purpura, and thinning of the skin. Furthermore, GC therapy in man has been reported to be accompanied by impaired delayed-hypersensitivity reactions and an increased frequency of granulomatous infections (31, 32). In addition, the association of GC therapy or other immunosuppressive treatments in bronchial asthma with Kaposi's sarcoma and infectious diseases has been recently observed (18, 33–36). In this study, we confirmed these data and found an increased susceptibility to infections in patients receiving GC therapy. Accordingly, oversuppression

of the immune system must be considered an important adverse effect of GC therapy in bronchial asthma.

The present study was undertaken to determine to what extent GC therapy might influence immune markers in peripheral blood. The association of GC treatment with hypogammaglobulinemia in asthma (28, 29) and normal adult male volunteers (37) has been previously reported. We found a dose-dependent effect of systemic GC on IgG levels. The association between low IgM or IgA concentrations in serum and GC therapy was also significant; however, it was not as strong as the association with low levels of IgG. Moreover, Ig concentrations were found to be further reduced in GC patients with three or more respiratory tract infections per year compared to the GC patients with no increased frequency of infections. This suggests that low Ig concentrations do indeed account for an increased infectious risk in these patients. In addition, reduced Ig concentrations were not due to decreased B-cell numbers. This implies a more functional B-cell defect as the cause of hypogammaglobulinemia in most cases.

Beside the suppression of the humoral immune response, there is evidence of a suppression of cellular immunity. For example, GC decrease cytokine expression *in vivo* (2, 4, 6, 8, 15) as well as inhibit T-cell proliferation *in vitro* (3). GC also induce and regulate T-cell apoptosis (38). Recently, it has been shown that activated mature T cells are sensitive to GC-induced apoptosis *in vitro* (39, 40). In agreement with earlier studies in the rat (41), we found a decreased CD4/CD8 ratio of peripheral blood T cells in GC compared to C patients, mainly resulting from CD4⁺ T-cell decrease. This suggests that differential regulation of T-cell apoptosis by GC might also occur *in vivo*.

Asthma has been associated with an increase in the number of activated T cells (1–12). It is likely that activated T cells are targets of GC in inducing apoptosis, as GC therapy leads to a decrease in T-cell activation (4, 7). We found no significant differences in T-cell activation in GC and C patients. As GC patients had a greater severity of lung disease than C patients, we believe that the relatively low T-cell activation in this group is an effect of GC treatment. In asthma, the CD4⁺ T-cell subpopulation seems to be activated selectively (1, 4, 5) suggesting that these cells might be the primary target of GC in inducing apoptosis, and thus explaining the reduced CD4/CD8 ratio observed in GC patients in this study.

Our finding of decreased IFN- γ production by PBMC after GC treatments agrees with previously published work (13, 23). In contrast, other groups observed that GC therapy in bronchial asthma is

associated with increased numbers of bronchoalveolar lavage or bronchial biopsy cells expressing mRNA for IFN- γ , as assessed by *in situ* hybridization (2, 6, 8). It is possible that differences in methods caused this discrepancy. For example, *in situ* hybridization is a semiquantitative technique, whereas we have measured cytokine production by ELISA. In addition, mRNA expression may not always lead to cytokine production.

Downregulation of IFN- γ gene expression by GC is further suggested by our data on monocyte HLA-DR expression. HLA-DR expression on monocytes seems to be regulated by IFN- γ . Decreased HLA-DR expression on monocytes has been observed in several secondary immunodeficiencies (25). We observed decreased HLA-DR molecule expression on monocytes of GC patients, suggesting that IFN- γ gene expression was oversuppressed at least in a subgroup of these patients.

To complement our studies on cytokine expression, we also measured the *in vitro* and the *in vivo* effects of GC on IL-4 and IL-5 production of purified PBMC. In agreement with previous studies (2, 4, 6, 8, 14–17), we observed reduced IL-4 and IL-5 production associated with GC treatment. These data suggest that GC downregulate Th1 and Th2 immune responses and that GC may display their anti-inflammatory effects by reducing the production of cytokines. However, they also indicate that oversuppression of cytokine genes may lead to insufficient B-cell help by T cells, explaining the hypogammaglobulinemia observed in most patients receiving systemic GC treatment. Recent data suggest that GC reduce the expression of cytokine genes by inhibition of the transcription factor NF- κ B (42, 43).

In conclusion, our results provide evidence of GC-induced oversuppression of the immune system in a group of patients with bronchial asthma. As shown in this study, these patients may be at increased risk of bacterial and viral infection and even of the development of certain tumors. This risk should be considered since there is a strong correlation between episodes of bronchitis and exacerbation of asthma. Thus, systemic GC may control asthma, but they also may cause additional inflammation of the airways. Therefore, immune markers such as the CD4/CD8 ratio, HLA-DR expression on monocytes, and IgG levels in serum should be monitored if long-term systemic GC treatment is needed. However, in accordance with European guidelines on the treatment of asthma, it is advisable to minimize the long-term use of systemic GC as much as clinically possible. Our data as well as recently published work (44) suggest that inhaled GC do not alter immune functions.

Acknowledgments

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