

Effects of “systemic” budesonide concentrations on *in vitro* allergen-induced activation of blood mononuclear cells isolated from asthmatic patients

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Blood levels of inhaled corticosteroids are significantly lower than those measured in the lung, but their concentration could still have anti-inflammatory effects. To determine whether budesonide, at concentrations similar to those obtained in blood after drug inhalation (10^{-9} M), could downregulate the allergen-induced activation of mononuclear cells, we studied 21 atopic patients, sensitized to *Dermatophagoides pteronyssinus* (*Der p*). On blood mononuclear cells, isolated from these patients, incubated with *Der p* allergen extract and with or without budesonide, we evaluated: 1) the proliferative response of T cells; 2) the expression of two surface activation markers, the HLA-DR antigens and the interleukin (IL)-2 receptors; and 3) the release of cytokines known to modulate the allergic processes. Allergen-induced T-cell proliferation was associated with increased HLA-DR antigen and IL-2 receptor expression ($P < 0.001$), and with increased release of IL-2, interferon-gamma (IFN- γ), IL-1 β , tumor necrosis factor-alpha (TNF- α), and granulocyte/macrophage colony-stimulating factor (GM-CSF). The addition of budesonide at the beginning of the cell cultures induced a dose-dependent inhibition of T-cell proliferation, still significant ($P < 0.05$) at the lowest concentrations tested (10^{-9} and 10^{-10} M). A significant inhibitory effect on T-cell proliferation was also present when budesonide (10^{-9} M) was added to the cell cultures 3 or 5 days after the beginning of the cell cultures. In addition, budesonide 10^{-9} M significantly decreased the expression of IL-2 receptors ($P < 0.05$), but not of HLA-DR antigens, and significantly reduced the release of IL-1 β and GM-CSF ($P < 0.05$), but not of IL-2, IFN- γ , and TNF- α . Thus, the blood concentrations of budesonide, after drug inhalation, may exert some anti-inflammatory effect, downregulating both “local” (through the bronchial circulation) and “systemic” allergen-specific immune reactions.

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Glucocorticosteroids are mainly employed in the treatment of allergic disorders, including bronchial asthma (6, 10). One of the major targets of corticosteroids are mononuclear cells, i.e., macrophages/monocytes and T cells, which are thought to play a key role in the pathogenesis of allergic processes (24, 30, 36). In line with their clinical effectiveness, corticosteroids are able to downregulate the release of various cytokines by activated monocytes/macrophages and T cells (18, 20, 27) and to inhibit *in vitro* the proliferative response of T cells to mitogens and

antigens (20). Since the systemic administration of corticosteroids is often associated with a wide variety of side-effects, topical forms of glucocorticosteroids have been synthesized, in order to decrease the high frequency of these complications (9). In bronchial asthma, inhaled steroids, such as budesonide, have been shown to be clinically effective and to have a high topical to systemic ratio (32). However, although the blood concentrations of budesonide are approximately 10 times lower than those measured in the lung (32, 35), these could still be potentially

effective in downregulating blood mononuclear cell activation. In this context, it has been demonstrated that inhalation of just one clinically relevant dose (500 µg) of budesonide results in a blood concentration of 10^{-9} M for several hours (32), and that budesonide at concentrations equal to or higher than 10^{-10} M is effective in inhibiting *in vitro* the release of granulocyte/macrophage colony-stimulating factor (GM-CSF) by human blood monocytes activated with lipopolysaccharide (LPS) (19). Since activation of circulating mononuclear cells has been described in patients with asthma (7, 8, 13, 34), a slight "systemic downregulating effect" of inhaled corticosteroid could result in better control of the allergic inflammation, without producing clinically relevant adverse effects on host defense mechanisms. With this background, the present study was designed to evaluate the effects of budesonide, at concentrations of the drug similar to the blood levels obtained in delivering the drug by inhalation, on allergen-induced blood mononuclear cell activation. Blood mononuclear cells, isolated from atopic asthmatic patients sensitized to *Dermatophagoides pteronyssinus* (*Der p*), were stimulated *in vitro* with purified allergen extract, and the inhibitory effects of budesonide were tested on T-cell proliferation, on the expression of surface activation markers (HLA-DR antigens and interleukin (IL)-2 receptor), and on the release of cytokines (IL-2, IL-4, interferon-gamma (IFN-γ), IL-1β, GM-CSF, and tumor necrosis factor alpha (TNF-α)).

Material and methods

Study population

Twenty-one atopic patients (23 ± 2 years of age, nine women and 12 men) entered this study. The inclusion criteria were as follows: 1) perennial asthma, with or without rhinitis, for the last 2 years or longer; 2) allergic sensitization to *Der p* and no reaction to birch-pollen extract, as demonstrated by skin prick test and radioallergosorbent test (RAST); 3) no symptoms at the time of the study; 4) no respiratory infections in the previous 4 weeks; and 5) forced expiratory volume in 1 s (FEV₁) values higher than 80% of predicted values. None of the patients were currently receiving antiasthmatic treatment other than β₂-stimulants on an "as necessary" basis. All subjects were informed of the nature and the aim of the study and gave written consent.

Collection of blood mononuclear cells

Heparinized blood samples were diluted 2:3 with Hanks' balanced salt solution (HBSS) without calcium and magnesium 1×, with 0.35 g/l sodium bicarbonate (Flow Laboratories; Irvine, UK). To isolate peripheral blood (PB) mononuclear cells, blood

samples were layered on top of Ficoll lymphocyte separation medium (Flow Laboratories) and then centrifuged for 20 min at 1500 g. Mononuclear cell layers were then washed, and the cells were counted and resuspended at the desired cell density in complete medium (RPMI-1640, supplemented with 2 g/l NaHCO₃ and 50 IU/ml penicillin plus 50 µg/ml streptomycin (Flow Laboratories), 2 mM L-glutamine (Flow Laboratories), nonessential aminoacids (Flow Laboratories), and 5 mM 2-mercaptoethanol (Sigma Chemical Company; St Louis, MO, USA), containing 10% autologous serum). At the beginning and at the end of the cell cultures, cell viability by trypan blue dye exclusion test (Flow Laboratories) was measured in all experimental conditions: cell viability was always found to be > 90% (3).

D. pteronyssinus allergen extract

A purified *Der p* powder preparation (Bayropharm; Milan, Italy) whose *Der p* I content was nearly 30% of the dry weight of the powder, as quantified by ELISA with *Der p* I specific monoclonal antibody (mAb) (Lofarma; Milan, Italy), was used (5). We chose to use an allergen extract containing multiple allergenic determinants, as identified by polyacrylamide gel electrophoresis, instead of a more purified allergen extract containing a single determinant because the former extract probably reproduces more closely "natural" exposure (5). A nonrelevant allergen was used (purified birch extract) (Pharmacia; Uppsala, Sweden) as control. The *Lymulus* amoebocyte gelation test (PBI International; Milan, Italy) was used to assess the presence of bacterial endotoxin in the allergen extracts (5). Briefly, the assay was performed, upon reconstitution of the allergen extracts at 1 mg/ml in culture medium, according to the manufacturer's instructions and yielded, respectively, 0.01 and 0.02 endotoxin units/ml, levels not different from those found in the medium alone (data not shown). Preliminary experiments on T-cell proliferation and cytokine release established that the allergen concentrations best inducing blood mononuclear cell activation were 10 µg/ml for *Der p* allergen extract and 15 µg/ml for purified birch extract (not shown).

T-cell proliferation assay

PB mononuclear cells collected from the 21 patients were incubated (2×10^5 cells/well) in 96-well plates (Costar Corporation; Cambridge, MA, USA) at 37°C in 5% CO₂, with or without *Der p* allergen extract (10 µg/ml) or purified birch extract (15 µg/ml), added at the beginning of the cell culture (day 0). To test the ability of budesonide (Astra Simes;

Milan, Italy) (Bud) to inhibit T-cell proliferative response to the allergen, different Bud concentrations (from 10^{-10} to 10^{-7} M) were added at day 0 of incubation to cell cultures obtained from all patients. In preliminary sets of experiments, we established that PB mononuclear cells require 5–7 days before proliferation and cytokine secretion peak in response to allergen stimulation (data not shown). Therefore, at day 6, ^3H -labeled thymidine (Amersham; Amersham, UK) was added ($0.5 \mu\text{Ci}/\text{well}$), and the cells were collected 18 h later by a Skatron cell harvester. The ^3H -labeled thymidine incorporation in cell cultures, evaluated by a beta-counter, was used as a T-cell proliferation index (31). In addition, to evaluate the possible effects of corticosteroids on the late phases of the allergen-induced T-cell activation, Bud (10^{-9} and 10^{-8} M) was also added 3 or 5 days after the beginning of the cell cultures (10 patients). Unstimulated control cultures included mononuclear cell cultures in plain complete medium (CTR). Each experimental condition was set up in triplicate.

Evaluation of surface antigen expression on lymphocytes

The surface expression of HLA-DR and interleukin-2 receptor (IL-2r) was evaluated by specific monoclonal antibody staining and flow-cytometry analysis (3) in cell cultures of the 21 patients. Thirty-six hours after stimulation with allergens, the PB mononuclear cells were washed in essential medium MEM (Flow Laboratories) and resuspended in a staining medium containing 1% fetal calf serum and 0.2% sodium azide in phosphate-buffered saline (Flow Laboratories). PB mononuclear cell suspensions were placed in round-bottom, 96-well, microtiter plates (Costar Corporation) and incubated for 60 min at 4°C in 50 μl of the staining medium with fluorescein isothiocyanate (FITC)-conjugated mAbs to human HLA-DR or IL-2r (CD25) (Technogenetics; Milan, Italy). A mAb against human platelets, PTF 19 (31), was used as a negative control preparation. The cells were then washed three times with staining medium and analyzed by single-color immunofluorescence flow cytometry (FACS Scan, Becton Dickinson Immunocytometry Systems; Mountain View, CA, USA).

Evaluation of the release of different cytokines by mononuclear cells

The production of different cytokines known to be involved in the inflammatory and immunomediated processes that characterize the allergic reaction (24, 29, 30, 36) was studied with PB mononuclear cells obtained from seven patients, as previously described (3). Briefly, the release of GM-CSF, IL-1 β ,

TNF- α , IL-2, IL-4, and IFN- γ was evaluated in supernatants of cultures containing 5×10^5 blood mononuclear cells/well. Cultures of PB mononuclear cells, isolated from the same donor, were set up in complete medium with or without *Der p* allergen extract ($10 \mu\text{g}/\text{ml}$), with or without Bud (10^{-9} and 10^{-8} M). *Der p* allergen extract and Bud were added, as appropriate, at the beginning of the cell cultures. The culture supernatants were harvested at day 6 of incubation and stored at -80°C , and the different cytokine concentrations were subsequently measured by the enzyme-amplified sensitivity method EASIA (Medgenix; Brussels, Belgium, and Amersham; Amersham, UK) (3).

Statistical analysis

All data were expressed as arithmetic mean $\pm$ standard error of the mean. Comparison between values was done with Student's *t*-test. Analysis of variance (ANOVA) was used to compare differences in the potency of various concentrations of the drug tested. The mean values of each single experimental condition were said to be significantly different when the probability of the event was below 5% (i.e., $P < 0.05$).

Results

Effects of budesonide on allergen-induced T-cell proliferative response

An allergen-specific proliferation of PB T cells was observed in all patients. ^3H -labeled thymidine incorporation was higher in cultures stimulated with *Der p*

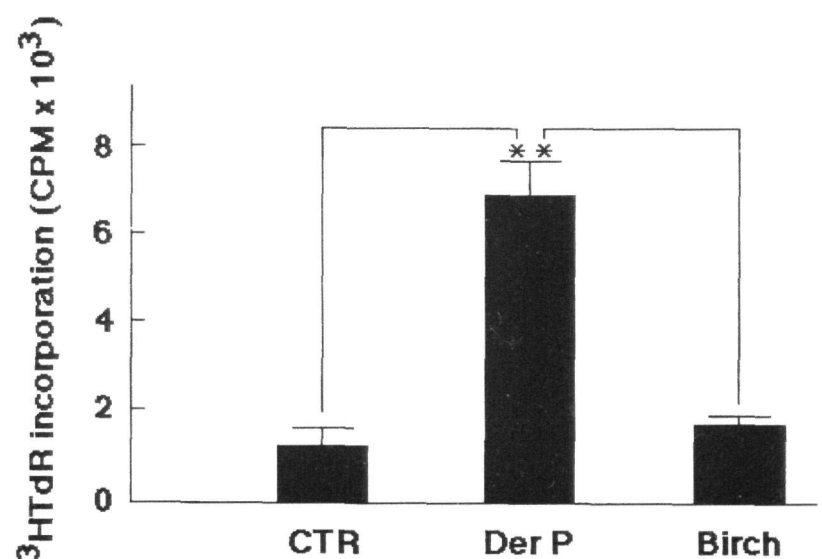


Fig. 1. Results of proliferative response to *D. pteronyssinus* (*Der p*) allergen extract of peripheral blood (PB) mononuclear cells isolated from atopic patients sensitized to *Der p*. Proliferation of T cells, measured as ^3H -labeled thymidine incorporation, is shown on ordinate and different culture conditions on abscissa. Cells were cultured for 7 days with *Der p* allergen extract or with non-relevant allergen (purified birch-allergen extract (birch)). Data represent mean $\pm$ standard error of mean of results obtained in 21 individuals. ** $P < 0.01$.

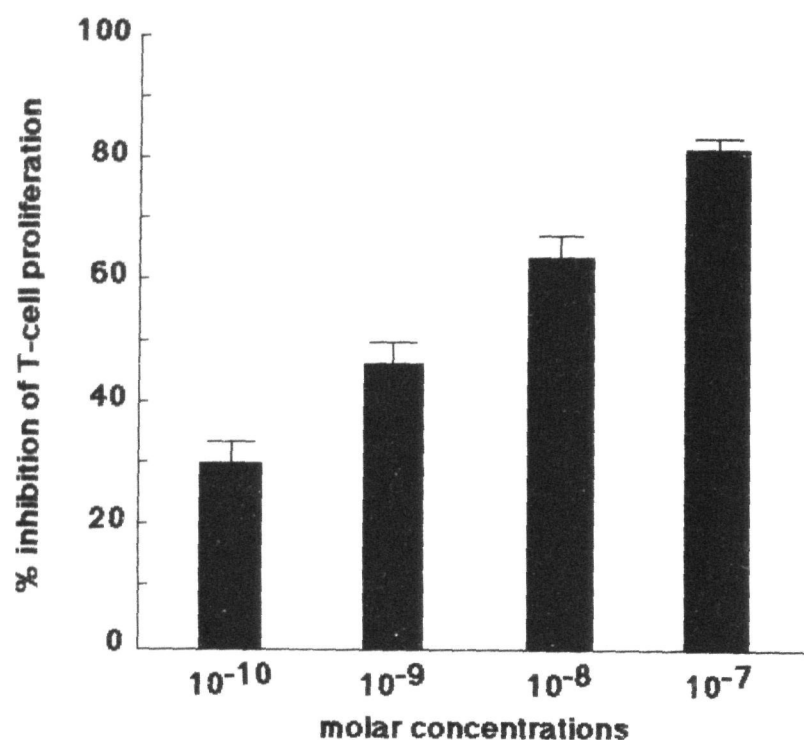


Fig. 2. Evaluation of effects of different concentrations (10^{-10} , 10^{-9} , 10^{-8} , and 10^{-7} M) of budesonide (Bud), added at beginning of cell culture (day 0), on T-cell proliferation to *Der p* allergen extract. Percentage of inhibition of T-cell proliferation is shown on ordinate, and different concentrations of drug are shown on abscissa. Data represent mean $\pm$ standard error of mean of results obtained in 21 individuals with atopy, sensitized to *Der p*.

allergen extract (6725.2 ± 1162.8 cpm) than in CTR cultures (1267.5 ± 252.7 cpm) or in cultures stimulated with a nonrelevant allergen (birch-allergen extract) (1760.8 ± 126.2 cpm) ($P < 0.01$, each comparison) (Fig. 1). In the *Der p* allergen extract-stimulated cultures, the addition of Bud (from 10^{-10} to 10^{-7} M), at the beginning of the cell cultures (day 0), induced

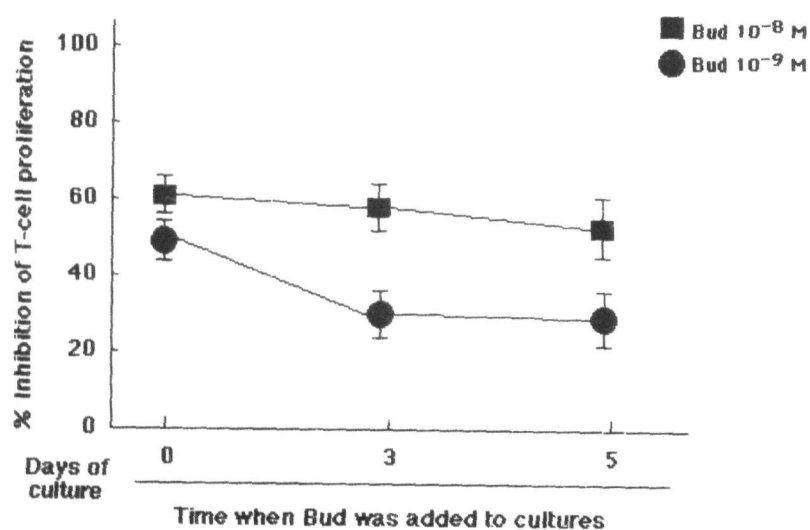


Fig. 3. Evaluation of effects of different concentrations of Bud 10^{-8} M (■) and 10^{-9} M (●), on T-cell proliferation to *Der p* allergen extract. Different concentrations of Bud were added at beginning of cell culture (day 0) or 3 or 5 days after beginning of cell culture. Percentage of inhibition of T-cell proliferation, evaluated as ^3H -labeled thymidine incorporation, is shown on ordinate, and days of incubation in presence of different concentrations of Bud are shown on abscissa. Data, expressed as mean $\pm$ standard error of mean, represent results of evaluations of 10 individuals with atopy, sensitized to *Der p*. * $P < 0.05$; ** $P < 0.01$.

a dose-dependent inhibition of the ^3H -labeled thymidine incorporation, statistically significant also at the lowest concentration (10^{-10} M) tested ($P < 0.05$). To evaluate possible effects of corticosteroids on the late phases of the allergen-induced T-cell activation, we compared the addition of Bud to the cell cultures at days 0, 3, and 5. In the three culture conditions (days 0, 3, and 5), Bud 10^{-8} M was similarly effective in inhibiting ^3H -labeled thymidine incorporation ($P < 0.01$, each comparison), while the inhibitory

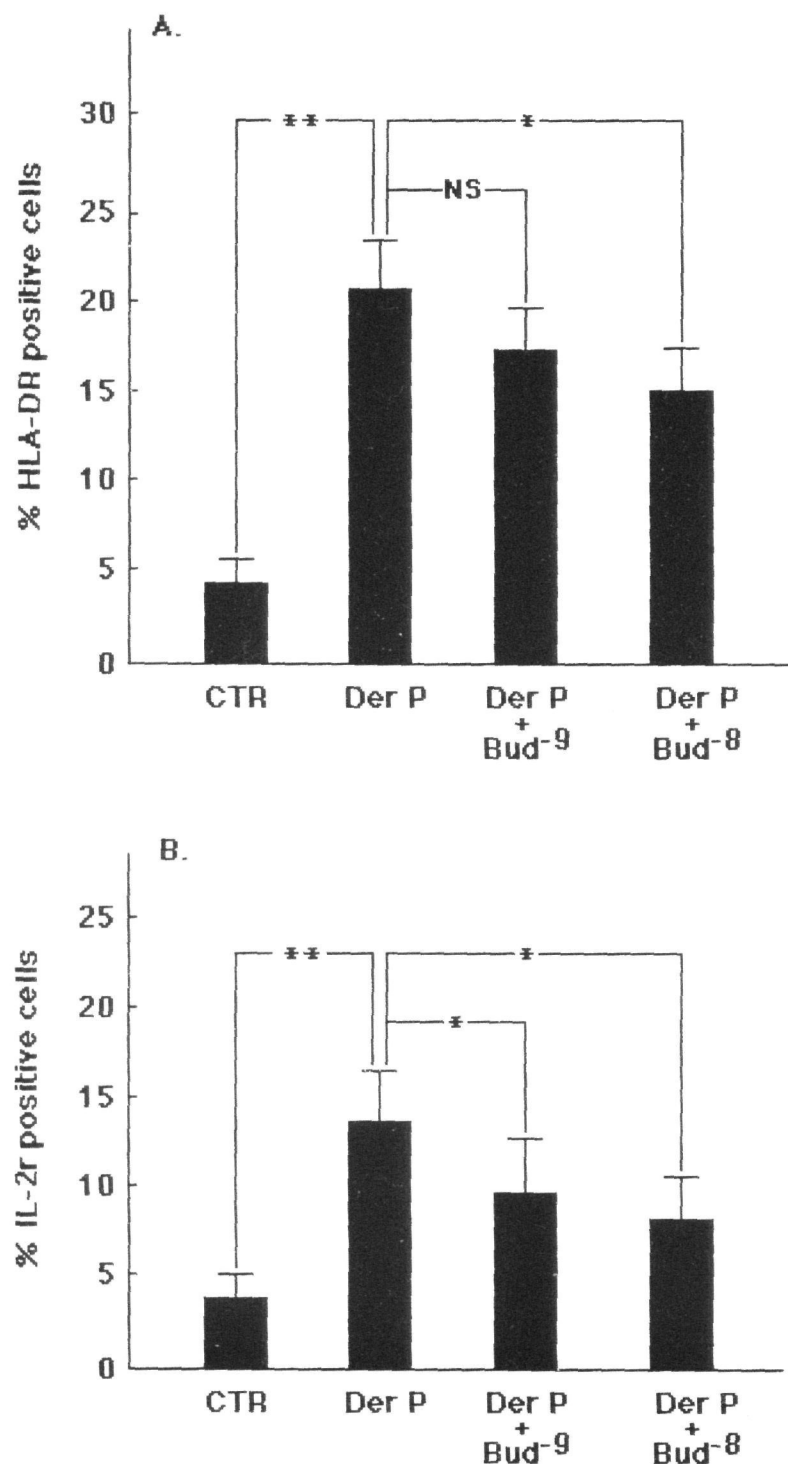


Fig. 4. Evaluation of effects of Bud (10^{-8} and 10^{-9} M) on surface expression of HLA-DR and IL-2r on blood lymphocytes by specific antibodies staining and flow-cytometry analysis. PB mononuclear cells were cultured for 36 h with medium alone, control cultures (CTR), or *Der p* allergen extract. Percentages of HLA-DR-positive (A) and IL-2r-positive (B) cells are shown on ordinate, and different culture conditions on abscissa. Data, expressed as mean $\pm$ standard error of mean, represent results of evaluation of 21 individuals with atopy, sensitized to *Der p*. * $P < 0.05$; ** $P < 0.01$; NS = not significant.

Table 1. Levels of cytokines measured in supernatants of peripheral blood mononuclear cells of atopic patients, sensitized to *Der p*, after 6-day incubation with medium alone (control) or with *Der p* allergen extract

Cytokine	Control	6-day culture	<i>Der p</i>
IL-2 (IU/ml)	0.2±0.1		3.5±0.7***
IL-4 (pg/ml)	<30 pg/ml		<30 pg/ml
IFN-γ (IU/ml)	0.8±0.3		26.2±1.7***
GM-CSF (pg/ml)	43.9±25.2		655.3±150.7***
IL-1β (pg/ml)	242.5±63.3		2.356±356.4***
TNF-α (pg/ml)	105.7±1.9		733.5±103.6**

*** $P < 0.001$, as compared to control values.

** $P < 0.01$, as compared to control values.

activity of Bud 10^{-9} M at day 0 was similar to that of Bud 10^{-8} M ($P < 0.05$) and was significantly reduced at days 3 and 5, as compared to day 0 ($P < 0.05$, each comparison) (Fig. 3).

Effects of budesonide on allergen-induced HLA-DR and IL-2r expression by blood lymphocytes

Less than 1% of the blood lymphocytes were recognized by the control PTF 19 mAb (not shown). The CTR cultures showed low basal expression of HLA-DR antigens and of IL-2r (Fig. 4), while the incubation with *Der p* allergen extract for 36 h significantly enhanced the expression of the two surface molecules ($P < 0.01$). When added to the cell cultures at the same time as *Der p* allergen extract, Bud was able significantly to induce a dose-dependent downregulation of the expression of both HLA-DR antigens and IL-2r on PB mononuclear cells. However, the inhibitory effect on HLA-DR antigen was statistically significant in the presence of Bud 10^{-8} M ($P < 0.05$), but not of Bud 10^{-9} M ($P > 0.05$), while IL-2r expression was significantly reduced by both Bud 10^{-8} and 10^{-9} M ($P < 0.05$, each comparison).

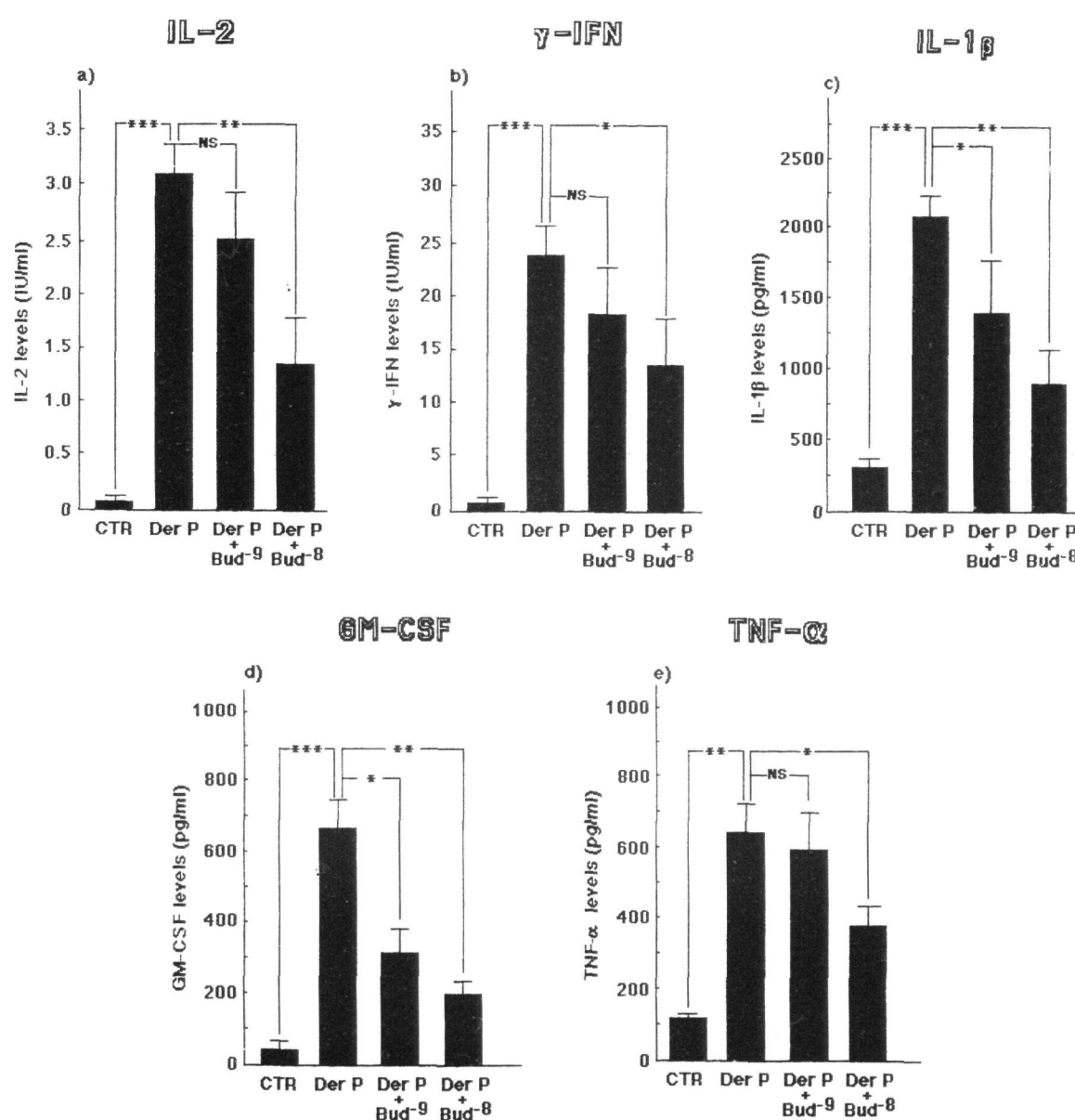


Fig. 5. Inhibitory effect of Bud (10^{-9} and 10^{-8} M) on release of cytokines: a) IL-2; b) IFN-γ; c) IL-1β; d) GM-CSF; and e) TNF-α. PB mononuclear cells were cultured for 6 days with medium alone, CTR cultures, or *Der p* allergen extract. Levels of various cytokines released in supernatants of different cell cultures are shown on ordinate, and various culture conditions on abscissa. Data, expressed as mean ± standard error of mean, represent results of evaluation of seven individuals with atopy, sensitized to *Der p*. * $P < 0.05$; ** $P < 0.01$; NS = not significant.

Effects of budesonide on allergen-induced cytokine release by blood mononuclear cells

Supernatants of PB mononuclear cell cultures, stimulated with *Der p* allergen extract and incubated with or without Bud (10^{-9} and 10^{-8} M), were tested for the presence of cytokines. As compared to CTR cultures, the *Der p* allergen extract-stimulated PB mononuclear cell supernatants showed significantly higher levels of IL-2 ($P < 0.01$), IFN- γ ($P < 0.01$), GM-CSF ($P < 0.01$), IL-1 β ($P < 0.01$), and TNF- α ($P < 0.05$) (Table 1). In contrast, IL-4 was detectable in the cell culture supernatants of only two patients, the concentrations being below the sensitivity of the assay (30 pg/ml) in the other five cultures. Bud, added to the cell cultures at the same time as *Der p* allergen extract, was able significantly to downregulate, in a dose-dependent fashion, the secretion of the cytokines by PB mononuclear cells (Fig. 5). Budesonide 10^{-8} M, but not 10^{-9} M, significantly inhibited the secretion of IFN- γ ($P < 0.05$), IL-2 ($P < 0.01$), and TNF- α ($P < 0.05$), while the IL-1 β and GM-CSF levels were statistically decreased in the presence of both Bud 10^{-9} M ($P < 0.05$) and Bud 10^{-8} M ($P < 0.01$) (Fig. 5).

Discussion

By evaluating blood mononuclear cells of patients sensitized to *Der p*, we have demonstrated that: 1) allergen-induced T-cell proliferation is associated with increased expression of two surface activation molecules, HLA-DR antigens and IL-2r and with increased release of cytokines such as IL-2, IFN- γ , IL-1 β , TNF- α , and GM-CSF; 2) Bud, at a concentration similar to that present in blood after the inhalation of a single 500- μ g dose (32), is able to induce a significant inhibition of allergen-induced T-cell proliferation and of IL-1 β and GM-CSF release; 3) a significant inhibitory effect on T-cell proliferation is also present when Bud is added to the cell cultures 3 or 5 days after the beginning of the allergen stimulation, demonstrating that the drug acts also on the late phase of allergen-induced T-cell activation.

Many of the known mononuclear cell activators can be classified into two groups: nonspecific and specific. While nonspecific activators, such as mitogens, stimulate a sizeable proportion of monocytes and lymphocytes of each individual, specific activators, such as antigens and allergens, are able to stimulate most of the monocytes directly via their immunoglobulin receptors (13), but the antigen- or the allergen-specific T cells only via their surface specific membrane T-cell antigen receptor(s) and coordinately expressed molecules (16). In patients with allergic asthma, increased spontaneous production

of cytokines by blood monocytes has been described (13) together with increased frequency of allergen-specific blood T cells (5). In addition, during acute exacerbation of the disease, increased expression of surface proteins associated with lymphocyte activation (HLA-DR, IL-2r, and "very late activation antigen" (VLA-1)) have been reported on blood CD4-positive T cells (8). These findings support the concept of a subclinical "systemic" activation of the immune system in allergic asthma. Consistent with the *in vivo* observations, we have demonstrated *in vitro* that in atopic asthma the T-cell proliferative response to the allergen is associated with increased expression of HLA-DR molecules and IL-2r on lymphocytes and with the release of various cytokines thought to be involved in the pathogenesis of asthma (29). A marked increase in the secretion of IFN- γ , IL-1 β , GM-CSF, and, to a lower level, of IL-2 and TNF- α , was observed in each culture in the presence of *Der p* allergen extract, while IL-4 was detected only in the PB mononuclear cell cultures of two patients. The levels of a secretory protein in a cell culture supernatant is dependent on multiple factors such as: 1) rate of synthesis and secretion of the protein, 2) frequency of the cell population(s) releasing the protein, and 3) catabolic activity for that protein of the "target" cells (3, 5, 12, 31). In addition, activated monocytes and T cells can also bind and internalize cytokines. Therefore, although it is difficult, on the basis of *in vitro* data, to speculate on the possible different ability of a given stimulus to induce preferential secretion of a cytokine by mononuclear cells, it is interesting to note that *Der p* allergen extract was very active in stimulating the release of cytokines involved in the antigen-presentation processes (IFN- γ , GM-CSF, and IL-1 β) and in the proliferation of T cells (IL-2), but was not very effective in inducing the release of detectable levels of IL-4, the main stimulatory cytokine of IgE production (30). Possible explanations of the detection of low levels of IL-4 are as follows: 1) only a very small proportion of blood T cells is able, after stimulation, to synthesize this cytokine (1); 2) detectable amounts of IL-4 are produced only by allergen-specific T-cell clones (25); 3) the activation of T-cell subsets producing IL-4 is inhibited by the presence of IFN- γ (11, 12). In the experimental condition reported in the present study, blood mononuclear cells are used and not T-cell lines or clones, and we found that high levels of IFN- γ are produced. Corticosteroids may be effective in the treatment of asthma by inhibiting several steps of the inflammatory process through increasing or decreasing transcription of genes related to cytokines, peptides and peptidases, lipocortins, cell surface receptors, and endonucleases (4, 18, 20, 23, 28). In this context, a most critical aspect of corticosteroid action is the inhibition of the tran-

scription of various cytokines relevant to the pathogenesis of asthma, including IL-1 β , IL-4, TNF- α , and GM-CSF (14). However, for some cytokines, such as IL-1 and GM-CSF, increased breakdown of mRNA has also been demonstrated (17, 33). A post-transcriptional regulatory mechanism could explain the different effects of Bud on cytokine release observed in the present study; i.e., the drug is more effective, at the lower concentration tested, in suppressing IL-1 and GM-CSF than IL-2, TNF- α , or IFN- γ release. Interestingly, in a recent report, Linden & Brattsand, using LPS-activated blood mononuclear cells isolated from healthy donors, have demonstrated that the secretion of GM-CSF is more sensitive to Bud than the secretion of IL-1 β or IL-6 (19). Since it was also shown in the same study that even relatively high doses of Bud were not sufficient for a complete inhibition of IL-1 β and IL-6 secretion, the authors hypothesized that the relatively selective downregulation of GM-CSF could explain why the important antiasthmatic effect of the drug is not associated with impairment of the host defense mechanisms (19).

Using a different system, closer to the clinical situation (i.e., blood mononuclear cells from atopic asthmatic patients stimulated with the allergen), we confirmed that Bud, at concentrations similar to the systemic blood levels obtained in clinical practice (10^{-9} M), is effective in inducing a significant inhibition of allergen-induced T-cell proliferation and of IL-1 β and GM-CSF release. On the contrary, in the same culture conditions, Bud 10^{-9} M significantly downregulated neither the expression of surface activation molecules (HLA-DR) nor the secretion of IL-2, TNF- α , and IFN- γ . Although steroid receptors appear to be similar in the different cells belonging to the same or different tissues, cellular responses to glucocorticosteroids are dependent on cell types and functions (21). Since there is direct evidence that different subpopulations of T cells (and possibly of monocytes) release different cytokines (1), it is reasonable to postulate a different sensitivity to glucocorticosteroids of different cell subsets committed to release different cytokines. An alternative explanation lies in the demonstration that the same cell, in the same conditions, can show a different sensitivity in different steroid-modulated functions (15, 21, 22, 26). Glucocorticosteroids can influence not only protein translation but also post-transcriptional processes of protein synthesis (e.g., glycosylation, cleavage, and compartmentalization) (29). Therefore, in addition to protein release or expression on cell surface, studies designed to evaluate these phenomena at the specific mRNA levels in monocytes and T cells will clarify the kinetics of allergen-induced mononuclear cell activation and modulation of this activation by glucocorticoster-

oids. Regardless of the specific mechanisms involved, our *in vitro* data support the hypothesis that blood levels of Bud, after inhalation, may exert some anti-inflammatory effect in downregulating both "local" (through the bronchial circulation) and "systemic" allergen-specific immune reactions. Further studies designed to determine the possible *in vivo* significance of these observations are needed in order to ascertain whether some systemic activity of inhaled corticosteroids should be further reduced or enhanced.

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