

Differences in the anti-inflammatory effects of theophylline and pentoxifylline: important for the development of asthma therapy?

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Antiasthma drugs are now being re-evaluated for their anti-inflammatory effects. Theophylline is an immunomodulator; however, weak effects and the narrow therapeutic window make it a controversial drug. We compared the immunomodulatory potencies of theophylline with those of the xanthines pentoxifylline (POF) and A802715. Using a whole-blood, cell-culture system, we studied the effects on the release of tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), and interleukin-6 (IL-6) in six healthy subjects, and, in granulocyte suspensions, the effects on the release of reactive oxygen species (ROS). We also studied the influence of a 14-day treatment with theophylline or POF on the release of the cytokines named above in 14 asthmatics. We found that equimolar concentrations of A802715 most effectively inhibit ROS generation, followed by POF; the effects of theophylline were weakest. A802715-inhibited release of TNF- α was four times as potent as that of theophylline, and POF two times as potent. Inhibition of IFN- γ by A802715 was three times as potent, and by POF two times. Neither drug influenced IL-6 release. After a 14-day treatment of asthmatics, POF proved to inhibit TNF- α release more effectively (by 44.3%) than theophylline (7.5%). It is concluded that study of xanthine derivatives in asthmatics might help the development of asthma therapy. POF seems to be an especially promising candidate.

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Asthma is increasingly considered an inflammatory immune disease. As a consequence, antiasthma drugs are now being re-evaluated for their immunomodulatory potencies. Although some researchers have suggested that it is obsolete, theophylline is still a mainstay of treatment. It exerts anti-inflammatory effects, particularly in the late asthmatic response (1).

The major disadvantages in prescribing theophylline for asthma are the narrow therapeutic window and the relatively weak effects compared with other antiasthma drugs (1). In view of the need for immunomodulation, several related methylxanthines and phosphodiesterase inhibitors are under investigation. Among them are substances with effects distinctly more pronounced *in vitro* and in animal models, such as A802715 (7-propyl-1-(5-hydroxy-5-methylhexyl)-3-methylxanthine) or HWA 138 (2, 3). However, these have not yet been

developed into clinical drugs. A xanthine derivative already used as a clinical drug is pentoxifylline (POF), which has long been administered for therapy of peripheral vascular disease (4). Like theophylline, POF is a methylxanthine that exerts, at a cellular level, nonspecific inhibition of phosphodiesterases, antagonism of the adenosine receptor, and inhibition of mediators such as tumor necrosis factor- α (TNF- α) (4). Unlike theophylline, it is tolerated relatively well over a wide range of dosage. We and others have shown that POF exerts distinct anti-inflammatory properties, especially in diseases mediated by the proinflammatory cytokine TNF- α (5, 6), as well as in diseases mediated by granulocytes; e.g., leucocytoclastic vasculitis (4) and adult respiratory distress syndrome (ARDS) (4). Both physiopathologic mechanisms play important roles in asthma since

granulocyte activation with release of reactive oxygen species (ROS) and TNF- α release contribute to the early and late asthmatic response (7, 8). In the interest of the development of new antiasthma drugs and development of xanthine derivatives, we determined the relative effectiveness of theophylline, A802715, and POF to inhibit release of the proinflammatory mediators TNF- α , interferon-gamma (IFN- γ), and interleukin (IL)-6 and ROS generation. Furthermore, we report the effect of 14-day treatment of asthmatic subjects with theophylline or POF with regard to *ex vivo* inhibition of the secretion of the above-named cytokines. Comparing the effectiveness of the two xanthines, we present evidence that, in the interest of development of new antiasthma treatments, it seems reasonable to intensify investigation of theophylline-related xanthine derivatives.

Material and methods

General

The experiments were approved by the local ethics committee of the University of Lübeck, Germany. All patients and volunteers gave informed written consent before entering the study. The anti-inflammatory capacity of the drugs A802715, theophylline, and POF was investigated *in vitro* in short-term cultures of whole blood of healthy donors (referred to as "*in vitro* experiments"). Moreover, the effect of a 14-day oral treatment with theophylline or POF in asthmatics was analyzed in the same experimental setting (referred to as "*ex vivo* experiments").

Volunteers and patients

A total of 20 subjects were included in the study. Six healthy volunteers (three men, three women, aged 27.83 (22–37) years (mean, range) served as donors for the *in vitro* experiments. They were healthy members of the staff of the Research Center Borstel. None of them had taken any medication or drug before blood sampling.

Fourteen subjects with mild asthma allergies, aged 43 (22–68) years (mean, range), were included in the "*ex vivo*" investigations (six women, eight men). They were allergic to grass ($n=6$) or birch ($n=8$) pollen allergen. The investigation was done outside the periods of individual pollen-induced symptoms, and lung-function tests were normal ($Fev_1 > 85\%$ predicted) without any antiasthma treatment. Patients were given the xanthines POF or theophylline orally for 14 days (POF: 25 mg/kg body weight/day in two doses p.o. [i.e., 2×800 mg in most patients]), Trental, (Hoechst, Germany), or theophylline (Euphyllin,

Byk Gulden, Germany, a slow-release formulation; dosage was adjusted to serum concentrations); for reasons related to drug approval, we were unable to study A802715 in patients. Blood was investigated before and after treatment. Blood sampling was done at 1 p.m. in every patient to neutralize variations in diurnal cytokine concentrations. Serum concentrations of drugs were found to be within 5–10 mg/ml in theophylline-treated patients. In POF-treated patients, both POF and its metabolite 1-(5-hydroxyhexyl)-3,7-di-methylxanthine were measured by Dr Rentschler GmbH, Laupheim, Germany, by gas liquid chromatography, as previously described (9). None of the asthmatic patients received additional corticosteroids or non-steroidal anti-inflammatory drugs.

Short-term, whole-blood, cell-culture assay

A whole-blood (WB), short-term, cell-culture system, as previously described (10), was used to investigate cytokine release in both the "*in vitro*" and the "*ex vivo*" experiments. Briefly, 5 ml of heparinized (20 units/ml) WB diluted 1:4 with RPMI medium (supplemented with 1% penicillin-streptomycin, Gibco BRL plus 1% L-glutamine 200 mM, Gibco BRL plus 10% FCS, Gibco BRL) was placed into 24-well microtiter plates (Costar, 0.5 ml/well) and incubated for 24 h (37°C, 5% CO₂) in the presence of 50 ng/ml bacterial lipopolysaccharide (LPS) (from *Salmonella friedland*; generous gift of Dr Helmut Brade, Borstel, Germany) and phytohemagglutinin (PHA) (HA 16, *Phaseolus* spp., Murex Diagnostics Ltd). In the "*in vitro*" experiments, four concentrations of the respective drug were added per experiment to determine the dose dependency of effects and the 50% inhibitory concentration (IC₅₀) (A802715 [1-(5-hydroxy-5-methylhexyl)-3-methyl-7-propylxanthine], Hoechst, kindly provided by Hoechst AG, Werk Kalle-Albert, Wiesbaden, Germany, supplied in powder form, dissolved in ultrafiltered RPMI medium by vigorous vortexing; POF, Hoechst, Germany, supplied in pyrogen-free saline; theophylline, Euphyllin [Byk Gulden, Germany], supplied in pyrogen-free saline). In "*ex vivo*" experiments using patient blood before and after POF or theophylline treatment, cell-culture experiments were done exactly as in volunteers except that no further drug (i.e., theophylline or POF) was added. The viability of cells in culture experiments was determined with trypan blue or MTT staining. MTT (dimethylethimazol-diphenyltetrazolium bromide, Sigma, dissolved in PBS at 5 mg/ml) is converted by the mitochondria of living cells to the blue-colored substance formazan, and the amount of formazan produced is proportional to the number of living cells present

(11, 12). After 4 h of incubation, the optical density as a measure of viability was determined in a micro-ELISA reader (MR580, Dynatech Laboratories, Inc., USA) at a wavelength of 550 nm. Viability exceeded 93% in every experiment.

Determination of TNF- α , IFN- γ , and IL-6 concentrations

In cell-culture supernatants, TNF- α was determined by the enzyme-linked immunosorbent assay (ELISA) technique (Cytoscreen TM Human TNF- α ELISA, Laboserv, Giessen, Germany). The assay was done according to the manufacturer's protocol. Extinctions of the samples were read in an ELISA reader (MR 5000 Microplate Reader, Dynatech Laboratories) at a wavelength of 450 nm (reference wavelength 630 nm) and compared with extinctions of an TNF- α standard enclosed (WHO reference). Concentrations were expressed as pg/ml. No significant cross-reactivity has been reported by the manufacturer for tumor necrosis factor-beta (TNF- β), IL-1, IL-2, IFN- γ , or IFN- β . The lower detection limit of the assay is set at <1 pg/ml. To measure IFN- γ , the same method of detection was used as with TNF- α (CytoscreenTM Human IFN- γ) (ELISA, Laboserv, Giessen, Germany). Calculation of cytokine concentrations was done by comparison with a recombinant IFN- γ standard (WHO reference) and expression as pg/ml. The lower detection limit is <4 pg/ml, and cross-reactivity has not been reported by the manufacturer for IFN- α and IFN- β .

IL-6 activity was measured with the IL-6-dependent mouse hybridoma cell line B13.29, clone B9 (13). Serial dilutions of the heat-inactivated samples were incubated with the hybridoma cells for 48 h before performing the MTT assay. IL-6 activity was calculated by probit analysis from serial dilutions and expressed in ng/ml against an internal standard of human recombinant IL-6 (Boehringer, Mannheim, Germany).

Measurement of reactive oxygen species (ROS)

ROS generation was measured, as previously described (14), by detection of luminol-enhanced chemiluminescence *ex vivo* in highly purified peripheral blood neutrophil (PBN) cultures. After density gradient centrifugation over Ficoll-paque (research grade, Pharmacia, Uppsala, Sweden), the granulocytes were obtained by sedimentation through polyvinylalcohol (Merck-Schuchardt, München, Germany); the remaining erythrocytes were eliminated by hypotonic lysis in aqua dest. Cells were resuspended in DMEM, and the cell count was adjusted to 1.66×10^6 cells/ml before measurement of chemiluminescence. Stimulation of the cells was

done with TNF- α (human, recombinant, 10 μ g/ml, Boehringer Mannheim Biochemica, Germany) or *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP, Sigma, Deisenhofen, Germany). Cell suspensions were preincubated in an incubator for 45 min to allow the cells to reach a constant temperature of 37°C; then luminol was added to enhance light reactions, and the suspensions were placed in a six-channel luminometer (Berthold Biolumat LB9505, Wildbad, Germany). The cell suspensions were then supplemented with the drug to be investigated (A802715, POF, or theophylline; 800–100 μ M), followed by the respective stimulant after 30 min (TNF- α at 100 ng/ml; FMLP at 100 μ M). The dosage of xanthines was chosen on the basis of calculations on relevant serum concentrations of POF: since, after oral administration, we found POF trough serum concentrations of approx. 500 ng/ml, and there are several metabolites with five- to eightfold higher serum concentrations and even more distinct efficacy, we considered POF at 100 μ M relevant *in vivo*. Chemiluminescence was detected, and the area under curve was calculated with computer support (Program Berthold, Wildbad, Germany). For control purposes, FMLP- or TNF- α -stimulated cell suspensions were also examined without addition of xanthines.

Statistics

Unless stated differently, data are expressed as means $\pm$ SEM, and comparisons between values were made with the rank sum test according to Wilcoxon, Mann and Whitney, or the rank correlation test according to Spearman. $P < 0.05$ was considered significant.

Results

Influence of xanthines on in vitro release of TNF- α , IFN- γ , and IL-6

Drugs for investigation (A802715, POF, and theophylline) were added to PHA/LPS-stimulated whole-blood cell cultures of six healthy donors. Any drug investigated was able dose-dependently to inhibit the release of TNF- α and IFN- γ *in vitro*; the 50% inhibitory concentrations are depicted in Tables 1 and 2. Release of both cytokines was most effectively inhibited by A802715, followed by POF and theophylline. The inhibition of TNF- α release exerted by POF was approx. two times and by A802715 approx. four times as potent as that exerted by theophylline; inhibition of IFN- γ by POF was approx. two times and by A802715 three times more effective. For IL-6 release, no influence of either drug was to be observed in these experiments (not shown).

Table 1. *In vitro* inhibition of TNF- α release by xanthines A802715, POF, and theophylline as determined by ELISA after culturing of whole-blood samples of six healthy volunteers. Cells were stimulated by addition of LPS/PHA and coincubated with dilutions of respective drug in order to construct dose-effect curve and to determine 50% inhibitory concentration (IC_{50}). Experiments were done with and without LPS/PHA stimulation and with or without addition of drugs to obtain appropriate controls

Drug	IC_{50} [μ M] (mean $\pm$ SD)	Potency of inhibition at IC_{50} as compared with theophylline
A802715	42.2 $\pm$ 12.9	3.8
POF	76.0 $\pm$ 17.6	2.1
Theophylline	163.2 $\pm$ 58.2	1

Table 2. *In vitro* inhibition of IFN- γ release by drugs. Experiments were done exactly as described in legend to Table 1 except for prolonged incubation period of 48 h

Drug	IC_{50} [μ M] (mean $\pm$ SD)	Potency of inhibition at IC_{50} as compared with theophylline
A802715	124.8 $\pm$ 44.6	3.2
POF	173.9 $\pm$ 30.8	2.3
Theophylline	404.7 $\pm$ 90.6	1

Influence of xanthines on *in vitro* generation of ROS

Highly purified granulocyte suspensions of selected healthy donors (two per experiment) were investigated in duplicate. We found an inhibition of ROS generation by each of the drugs, independently of the stimulus (TNF- α or FMLP); representative data using FMLP as stimulant are shown in Fig. 1. Expressed on an equimolar basis, inhibition achieved after stimulation with FMLP was most effective by A802715 (maximum inhibition of 98%), and by POF (maximum inhibition: 94%). Theophylline-induced inhibition was at a maximum of 49%. Effects after stimulation with TNF- α were almost identical.

Influence of POF and theophylline on *ex vivo* release of cytokines

Patients were treated with theophylline or POF for 14 days. Serum concentrations of theophylline were 7.0 $\pm$ 0.64 μ g/ml. Concentrations of POF were 509.7 $\pm$ 120.7 ng/ml. Concentrations of the POF metabolite BL 194, which is known to be even more effective than POF (2), were 1156.8 $\pm$ 314.2 ng/ml. In every patient, treatment with POF resulted in a distinct inhibition of TNF- α release; Fig. 2 gives data as means + SEM ($P < 0.017$). As also shown in Fig. 2, IFN- γ and IL-6 release was not statistically significantly influenced by POF. On the other hand, treatment with theophylline resulted in a significant inhibition of IFN- γ in every patient investigated ($P < 0.027$), whereas only insignificant influence was exerted on TNF- α and IL-6 release.

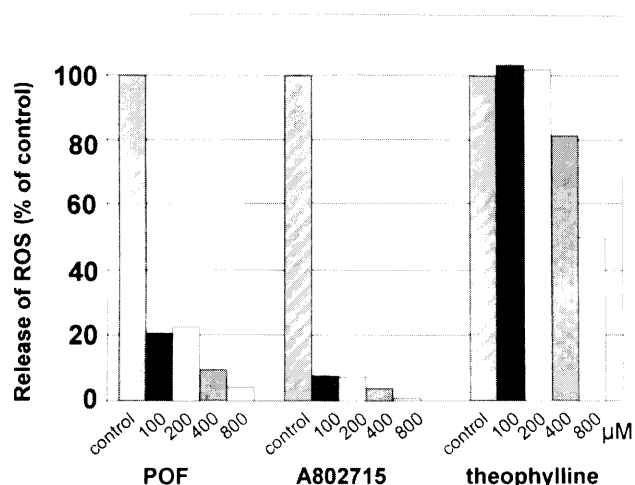


Fig. 1. Influence of xanthine derivatives on release of ROS as determined by chemiluminescence. Granulocyte suspensions from healthy volunteers were stimulated by addition of 100 μ M *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP) in presence of 100–800 μ M POF, theophylline, or A802715. Chemiluminescence was determined and expressed as percent inhibition of control experiments without addition of xanthines. Each bar represents means of experiments done in two subjects.

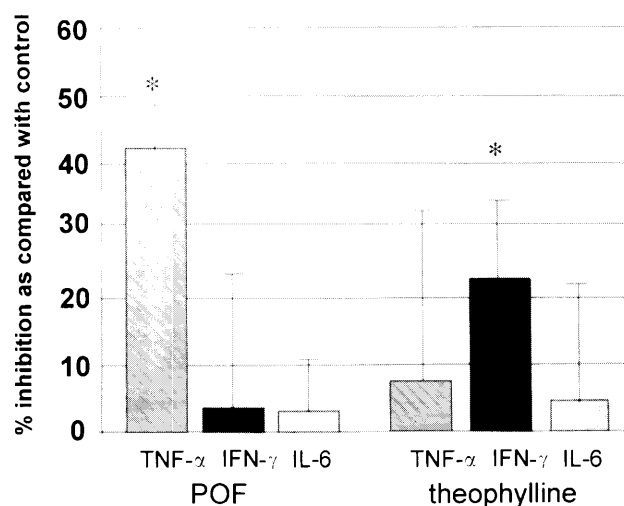


Fig. 2. Lipopolysaccharide/phytohemagglutinin (LPS/PHA)-stimulated TNF- α release in short-term culture of whole blood of asthmatic patients. Total of 14 subjects were given 14-day treatment of either POF ($n=8$) or theophylline ($n=6$). Before and after treatment, blood was sampled at 1 p.m., and cytokines TNF- α and IFN- γ were determined by ELISA. Additionally, IL-6 was measured by bioassay. Data are given in mean (bars) + SEM (error bars). *Significant differences as compared with control. TNF- α after POF treatment: $P < 0.017$; IFN- γ after theophylline treatment: $P < 0.027$.

Discussion

There is growing evidence that inflammatory mechanisms are key players in the physiopathology of asthma. Modulation of its intercellular regulation as well as its intracellular signal transduction

is increasingly considered important in therapy; therefore, inhaled steroids are first-line remedies in asthma. Theophylline has anti-inflammatory capacity, although it is minor compared to that of steroids (15). The objective of this study was *ex vivo* to characterize further the anti-inflammatory effects of theophylline and of related compounds. We found an oral 14-day theophylline treatment of asthmatic patients to inhibit the release of IFN- γ , whereas POF treatment inhibited the release of TNF- α (Fig. 2). When the drug of interest was added *in vitro* in cell-culture experiments, release of both cytokines and ROS was inhibited by both compounds, while POF had a distinctly higher potency than theophylline. The differences between xanthines in effects and potencies might be put to clinical use in future.

The effects of the drugs on lymphocytes, granulocytes, and monocytes were studied, since all of these cell types are involved in the physiopathology of severe asthmatic reactions. This occurs independently of the cause of asthma, whether exogenous allergic or intrinsic. We studied the effects on the TH1 lymphokine IFN- γ and the TH2 lymphokine IL-6. However, determination of the TH2 lymphokine IL-4, although most important in asthma with eosinophil activation, was not included, because the absence of influence of xanthines on its release has already been demonstrated by other authors (16). Neutrophils are also probably important mediators of the asthmatic reaction; we investigated their effects on the generation of ROS in granulocytes as a parameter of activation. In asthma, ROS generation seemed to be upregulated; ROS contribute to inflammation and cell damage in the bronchi (17). There is growing evidence that TNF- α , in particular, is an early mediator in the physiopathology of asthma. Mast-cell and alveolar macrophage-derived TNF- α is upregulated in the sputa of patients with acute asthma attacks (18). Further evidence that TNF- α and other proinflammatory monokines are of central importance derives from findings of elevated serum concentrations of soluble TNF receptor (sTNF-R) and IL-1 receptor antagonist (IL-1RA) in asthma (19).

Except for IL-6, all of the parameters investigated were inhibited by all xanthines investigated. The mechanisms of action are probably 1) non-selective inhibition of phosphodiesterases and thus elevation of intracellular cAMP, and 2) induction of adenylate cyclase with the same, synergistic result (20). The *in vivo* relevance of these mechanisms to anti-inflammatory action has been demonstrated, at least for POF in several clinical settings (5, 6, 21).

Xanthine derivatives show quantitative differences in the ability to increase cAMP *in vitro* and

subsequently to inhibit TNF- α release in mononuclear cells. Semmler et al. found that the experimental drug A802715, an analog of POF (3), was approx. 2½ times more effective than POF in TNF- α inhibition; POF was approx. four times more effective than theophylline (2). This can be confirmed by our data, as shown in Tables 1 and 2. To this, our data add that the anti-inflammatory potency of the different xanthines differs in the experiments using volunteer blood (considered "*in vitro*" experiments) quantitatively with regard to IFN- γ release (Table 2). On the other hand, after 14-day treatment of patients with theophylline or POF (referred to as "*ex vivo*" experiments), we saw qualitative differences, as shown in Fig. 2.

The differences between the compounds in TNF- α release probably derive from the variations in the ability to cause cAMP elevation discussed above; in contrast to our "*in vitro*" findings, however, POF could not significantly reduce IFN- γ concentrations "*ex vivo*". The literature offers no explanation of this effect; obviously, mechanisms other than modulation of cAMP are predominant in the regulation of IFN- γ . For further clarification, IL-6, which is clearly not controlled by cAMP (22), was investigated and consistently found to be uninfluenced by either drug.

For methodological reasons, neutrophil function, as determined by ROS generation, was investigated solely in the "*in vitro*" setting. Since, in order to obtain pure granulocyte suspensions, multiple isolation and purification steps are necessary, we considered the cells to be extremely artificially altered and irrelevant for the *in vivo* situation. On the contrary, the whole-blood-cell culture assay otherwise used in our experiments probably best reflects the *in vivo* situation, since cells remain in the compartment blood without further isolation steps (10), and the composition of cells reflecting the asthmatic inflammation is preserved. In accordance with published data indicating modulating effects of POF on normal and diseased human neutrophil function (23–25), we here additionally demonstrate that either compound investigated distinctly inhibited ROS formation by granulocytes independently of the stimulus (Fig. 1). Again POF was more effective than theophylline.

New insights into the immunomodulatory properties of xanthines have already led to a re-evaluation of these substances in asthma, and emphasis on, beside bronchodilation, their anti-inflammatory effects (26, 27). The differences found in this comparative study might help differentiate therapeutic aspects further and may turn out to be important in controlling the inflammation of chronic asthma. In other studies, POF has proven to be an efficient immunomodulator in

several *in vitro* and clinical settings. The inhibition of TNF- α by POF was recently demonstrated in mast cells (6) and in alveolar macrophages (28), both of which are the natural sources of TNF- α in the lung. Furthermore, in former studies, we showed synergistic inhibitory effects with corticosteroids concerning TNF- α and IFN- γ *in vitro* (22) and response to corticosteroid therapy in man in sarcoidosis (5). Xanthines with a higher relative potency than theophylline might be able to reduce the need for corticosteroids in asthmatics, as has been suggested of theophylline (29) but never convincingly demonstrated. At the same time, the occurrence of adverse effects might be reduced since POF, as compared to theophylline, is well tolerated. Therefore, it seems highly reasonable to continue study of POF efficacy in asthmatics and, given proven effects, to develop compounds with equally minor adverse effects and even greater anti-inflammatory potency.

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References

- Barnes PJ, Pauwels RA. Theophylline in the management of asthma: time for reappraisal? *Eur Respir J* 1994;7:579-91.
- Semmler J, Gebert U, Eisenhut T, et al. Xanthine derivatives: comparison between suppression of tumour necrosis factor- α production and inhibition of cAMP phosphodiesterase activity. *Immunology* 1993;78:520-5.
- Hoffmann H, Weis M, Frank G, Birg A, Schönharting MM, Jochum M. Amelioration of endotoxin-induced acute lung injury in pigs by HWA 138 and A802715: new analogs of pentoxifylline. *Shock* 1995;4:166-70.
- Samlaska MCP, Winfield EA. Pentoxifylline. *J Am Acad Dermatol* 1994;30:603-21.
- Zabel P, Entzian P, Dalhoff K, Schlaak M. Pentoxifylline in treatment of sarcoidosis. *Am J Respir Crit Care Med* 1997;155:1665-9.
- Zabel P, Greinert U, Entzian P, Schlaak M. Effects of pentoxifylline on circulating cytokines (TNF- α and IL-6) in severe pulmonary tuberculosis. In: Fiers W, Buurman WA, editors. *Tumor necrosis factor: molecular and cellular biology and clinical relevance*. Basel: Karger, 1993:178-81.
- Barnes PJ. Reactive oxygen species and airway inflammation. *Free Radic Biol Med* 1990;9:235-43.
- Barnes PJ. Cytokines as mediators of chronic asthma. *Am J Respir Crit Care Med* 1994;150:S42-9.
- Bryce TA, Burrows JL. Determination of oxpentifylline and a metabolite, 1-(5'-hydroxyhexyl)-3-7-dimethylxanthine, by gas-liquid chromatography using a nitrogen-selective detector. *J Chromatogr* 1980;181:355-61.
- De-Groote D, Zangerle PF, Gevaert Y, et al. Direct stimulation of cytokines (IL-1b, TNF- α , IL-6, IL-2, IFN- γ and GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. *Cytokine* 1992;4:239-48.
- Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J Immunol Methods* 1986;89:271-7.
- Hansen MB, Nielsen SE, Berg K. Reexamination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *J Immunol Methods* 1989;119:203-10.
- Aarden LA, De Groot ER, Schaap OL, Lansdorp PM. Production of hybridoma growth factor by human monocytes. *Eur J Immunol* 1987;17:1411-16.
- Grage-Griebenow E, Lorenzen D, Fetting R, Flad H-D, Ernst M. Phenotypical and functional characterization of Fc γ receptor I (CD 64)-negative monocytes, a minor human monocyte subpopulation with high accessory and antiviral activity. *Eur J Immunol* 1993;23:3126-35.
- Tinkelman D. Theophylline therapy for children with asthma. *Eur Respir J* 1996;6:79-83.
- Rott O, Cash E, Fleischer B. Phosphodiesterase inhibitor pentoxifylline, a selective suppressor of T helper type 1-but not type 2-associated lymphokine production, prevents induction of experimental autoimmune encephalomyelitis in Lewis rats. *Eur J Immunol* 1993;23:1745-51.
- Crystal R. Oxidants and respiratory tract epithelial injury: pathogenesis and strategies for therapeutic intervention. *Am J Med* 1991;91:3C-39S,3C-44S.
- Shah A, Church MK, Holgate ST. Tumor necrosis factor alpha: a potential mediator of asthma. *Clin Exp Allergy* 1995;25:1038-44.
- Yoshida S, Hashimoto S, Nakayama T, Kobayashi T, Koizumi A, Horie T. Elevation of serum soluble tumour necrosis factor (TNF) receptor and IL-1 receptor antagonist levels in bronchial asthma. *Exp Immunol* 1996;106:73-8.
- Matzky R, Darius H, Schrör K. The release of prostacyclin (PGI₂) by pentoxifylline from human vascular tissue. *Drug Res* 1982;32:1315-18.
- Zabel P, Wolter DT, Schönharting MM, Schade UF. Oxpentifylline in endotoxaemia. *Lancet* 1989;2:1474-7.
- Funk JO, Ernst M, Schlaak M, Zabel P. Pentoxifylline exerts synergistic immunomodulatory effects in combination with dexamethasone or cyclosporine. *Int J Immunopharmacol* 1995;17:1007-16.
- McDonald RJ. Pentoxifylline reduces injury to isolated lungs perfused with human neutrophils. *Am Rev Respir Dis* 1991;144:1347-50.
- Zheng H, Crowley JJ, Chan JC, et al. Attenuation of tumor necrosis factor-induced endothelial cell cytotoxicity and neutrophil chemiluminescence. *Am Rev Respir Dis* 1990;142:1073-8.
- Boogaerts MA, Malbrain S, Meeus P, van-Hove L, Verhoef GEG. *In vitro* modulation of normal and diseased human neutrophil function by pentoxifylline. *Blut* 1990;61:60-5.
- Kidney J, Dominguez M, Taylor PM, Rose M, Chung KF, Barnes PJ. Immunomodulation by theophylline in asthma. *Am J Respir Crit Care Med* 1995;151:1907-14.
- Ward AJM, McKenniff M, Evans JM, Page CP, Costello JF. Theophylline – an immunomodulatory role in asthma? *Am Rev Respir Dis* 1993;147:518-23.
- Marques LJ, Poulakis N, Zheng L, Guzman J, Wang YM, Costabel U. Pentoxifylline inhibits TNF- α production by human alveolar macrophages. *Eur Respir J* 1996;8:339s.
- Nassif EG, Weinberger M, Thompson R, Huntley W. The value of maintenance theophylline in steroid-dependent asthma. *N Engl J Med* 1981;304:71-5.