Eur J Med Res (2005) 10: 325-331

IN VITRO STUDY ON THE IMMUNOLOGICAL EFFECT OF BROMELAIN AND TRYPSIN ON MONONUCLEAR CELLS FROM HUMANS

H. Barth[1,3], A. Guseo[2], R. Klein[1]

[1]Department of Internal Medicine II, University of Tübingen, Germany
[2]St. George Hospital of County Fejér, Department of Neurology, Skékesfehérvár, Hungary
[3]Present address: Department of Internal Medicine II, University of Freiburg, Germany

Abstract
Background: Proteolytic enzymes such as bromelain and trypsin are used as an adjuvant therapeutic approach in the treatment of chronic inflammatory, malignant and autoimmune diseases. *In vitro* studies have shown that proteolytic enzymes modulate surface adhesion molecules on T-cells. In this study we analysed the influence of bromelain and trypsin on the cytokine production and proliferation of peripheral blood mononuclear cells (PBMC) from patients with a classical cellular mediated autoimmune disorder, namely encephalomyelitis disseminata (ED) as well as from healthy controls.
Methods: PBMC from patients with ED (n = 12) and healthy controls (n = 12) were cultured for seven days at 37 °C under three different conditions: without antigen, with bromelain and with trypsin (final concentrations 10-1000μg/ml). Proliferation was determined by ^{3}H-thymidine incorporation. Secretion of cytokines into the supernatant was measured by a double sandwich ELISA. Intracellular cytokines were determined by flow cytometric analysis.
Results: PBMC from patients with ED and healthy controls showed a significantly increased proliferative response to bromelain (ED: 14053 ± 7585 cpm with bromelain vs. spontaneous proliferation of 430 ± 255 cpm; healthy controls: 10689 ± 4607 cpm vs. 327 ± 193) but not to trypsin. Bromelain induced in all 24 individuals a significant increase of the macrophage/monocyte associated cytokines interleukin (IL)-6, granulocyte-macrophage-colony stimulating factor (GM-CSF), tumour necrosis factor alpha (TNFα) ($p<0.01$) as well as of the type 1 cytokine gamma-interferon (IFNγ). In contrast, the type 2 cytokines IL-4 and IL-5 were not induced. Flow cytometric analysis revealed a significant increase of IFNγ-producing CD4+ T-cells. There were no differences in cytokine production between ED patients and healthy controls.
Conclusion: These results indicate that bromelain – but not trypsin – activates macrophages/monocytes and type 1 cells independently from the underlying disease and may stimulate, therefore, the innate as well as the adaptive immune system.

Key words: proteolytic enzymes, bromelain, trypsin, T-cells, macrophages/monocytes, cytokines

INTRODUCTION

Proteolytic enzymes such as bromelain and trypsin have been recommended as adjuvant therapeutic approaches in the treatment of viral and malignant diseases [1, 2]. Furthermore, there is evidence that in patients with rheumatic and other inflammatory diseases oral therapy with proteolytic enzymes produces certain analgesic and antiinflammatory effects [3-7]. However, the results obtained in different studies are often inconsistent [3]. Nevertheless, the immunomodulatory potency of proteolytic enzymes at least *in vitro* is nowadays without doubt. Thus, it was shown that they modulate surface adhesion molecules on T-cells, macrophages and natural killer (NK)- cells and induce the secretion of IL-1β, IL-6 and TNFα by peripheral blood mononuclear cells (PBMC) [8-14]. Bromelain can also inhibit the T-cell signal transduction by blocking the Raf-1/extracellular regulated kinase (ERK)-2 pathway [15]. Beneficial effects of orally administered proteolytic enzymes in experimental allergic encephalomyelitis (EAE) suggested the application of these substances in the therapy of T-cell mediated autoimmune diseases [16], and, indeed, in patients with diabetes type 1 an inhibition of T-helper type 1 cells has been described during intake of bromelain [17]. Type 1 and type 2 TH cells are defined by their cytokine production and exhibit distinct functional properties. Thus, type 1 TH cells secrete interferon-gamma (IFNγ) and tumour necrosis factor-beta (TNFβ) and are involved in macrophage activation and delayed-type hypersensitivity (DTH) reactions. In contrast, type 2 TH cells produce interleukin (IL)-4, IL-5 and IL-13 and mediate antibody responses, including IgE production [18]. These T helper cell subsets represent polarized forms of the specific immune response and counteract each other. They are closely associated with resistance or susceptibility to bacterial infections but also appear to be involved in the induction and regulation of autoimmunity [19, 20]. One could, therefore, speculate that the beneficial effect of oral enzymes in the EAE model or diabetes mellitus type 1 may be due to their immunomodulatory properties on the different TH subsets.

Aim of our study was, therefore, to analyse *in vitro* the influence of the proteolytic enzymes bromelain

and trypsin on proliferation and cytokine production by PBMC from healthy individuals and patients with the type 1 TH cell mediated autoimmune disease encephalomyelitis disseminata (ED). It became evident that bromelain, but not trypsin, exhibited an immunomodulatory effect resulting in enhanced cell proliferation activity and secretion of TH type 1 related cytokines by PBMC.

Patients and Methods

Subjects

Twelve healthy blood donors (2 female, 10 male; mean age: 41 years, range: 26-60 years) and twelve patients with encephalomyelitis disseminata (ED) exhibiting a relapsing-remitting course were included into the study (9 female, 3 male; mean age: 32 years, range 27-47 years). ED patients were diagnosed by established diagnostic criteria [21] and neurological examinations were carried out by one of the authors (A. Guseo). The patients had an EDSS (Expanded Disability Status Scale) ranging from 1 to 3. None of the subjects has received polyenzyme preparations or had any prophylactic medical treatment.

All patients and healthy blood donors had given their informed consent to participate in the study, according to the 1975 Helsinki Committee ethical guidelines.

Antibodies

Purified anti-human IL-2, IL-4, IL-5, IL-6, GM-CSF, IFN-γ, and TNFα monoclonal antibodies (mAb) and the respective biotinylated anti-human mAb as well as R-phycoerythrin (PE)-conjugated anti-human IL-4, IL-5, IFN-γ were purchased from Pharmingen (San Diego, CA, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-human CD4 and CD3 mAb, R-Phycoerythrin (PE)-conjugated anti-human CD8 and CD19 mAb were obtained from Immunotech (Marseille, France).

Antigens

Bromelain and trypsin were kindly provided by MUCOS, Geretsried, Germany (Ch. numbers 968301 and 0970298, respectively). Identity and purity had been proven by electrophoresis, isoelectric focusion, and measuring of the absorption spectrum. Analysis of microbiological purity revealed no contamination with E. coli, Salmonellae, Pseudomonas aeruginosa, Staphylococcus aureus, an amount of candida less than 100kBE/g, and total germ number of less than 10.000kBE/g. Endotoxins could not be detected.

Cell Culture

PBMC were isolated from heparinized blood by centrifugation through Ficoll-Hypaque. The cells were washed two times in Hanks' salt solution and resuspended in 1 million cells/ml in RPMI 1640 medium supplemented with gentamycin and 25% autologous serum. PBMC were cultured without antigen, with bromelain as well as with trypsin in four concentrations ranging from 10 to 1000μg/ml for 7 days at 37°C, 5% CO_2 in a humidified atmosphere.

Lymphocyte Proliferation Assay

For proliferation assays PBMC ($1x10^6$/well) were seeded into 96-well cell culture plates and stimulated with bromelain and trypsin in parallel. During the last 18h, the cultures were pulsed with ^{3}H-thymidine (0.74 Mbq/ml, 20μl/well) and then harvested onto glass-fiber filters. The incorporated radioactivity was measured by liquid scintillation spectroscopy using a β-counter and given as counts per minute (cpm). All cultures were performed in fourfold.

Measurement of Cytokines by ELISA

For cytokine production 5×10^5 PBMC/ml were cultured with bromelain and trypsin in parallel in duplicates (concentrations ranging from 1000μg/ml) in 24-well plates and were maintained as indicated above. Culture supernatants were collected at day 7 and kept frozen at –20 °C until quantitative cytokine determination [22].

Briefly, wells of a 96-well microtiter plate were coated overnight at 4 °C with 1.75μg/ml anti-human cytokine monoclonal antibody (IL-2, IL-4, IL-5, IL-6, GM-CSF, IFN-γ, TNFα) in hydrogen bicarbonate buffer, pH 9.6, and blocked for 1h at room temperature with phosphate buffered saline (PBS 60mM, pH 4,7) containing 0.5% bovine serum albumin (BSA). Culture supernatants were used undiluted and incubated for 2h at 37 °C; 1.25μg/ml biotinylated anti-human cytokine antibodies (IL-2, IL-4, IL-5, IL-6, GM-CSF, IFN-γ, TNFα) were added for 2h at 37°C. After another incubation for 1h at room temperature with avidin-peroxidase (2.5μg/ml), substrate solution (0.5 mg ortho-phenylene-diamine/ml citrate buffer [pH 5.0] and 0.01% H_2O_2) was added. The reaction was stopped with 25% sulphuric acid and optical density was measured at 450nm in a microtiter plate reader. Results were related to a standard curve obtained with cytokine standards (Pharmingen, San Diego, CA, USA) in different concentrations for each cytokine.

Flow Cytometry and Intracellular Cytokine Analysis

For cell staining and intracellular cytokine analysis, PBMC were isolated and cultured for seven days as described above. PBMC were incubated with 0.25μg FITC anti-human CD3 and 0.25μg PE anti-human CD19 or 0.25μg FITC anti-human CD4 and 0.25μg PE anti-human CD8 for 30 min at 4 °C. Cells were washed twice with cold phosphate-buffered saline (PBS; centrifugation at 500xg for 10min, 4 °C) and resuspended in 400μl PBS for final flow cytometric analysis.

For intracellular cytokine analysis, on day seven cells were restimulated with PMA (50ng/ml) and ionomycin (1 μg/ml) in the presence of Brefeldin A (10μg/ml) for 6 hours [22]. Cells were washed twice in

PBS containing 2% fetal calf serum (FCS) and stained with 0.25μg PE anti-human CD3 for 30 minutes. Cells were then washed with PBS/2% FCS and fixed in PBS containing 4% paraformaldehyde for 20 minutes. After two additional washes in PBS/2% FCS containing 0.2% saponin (permeabilization buffer) cells were resuspended in 100μl permeabilization buffer and stained with 0.2 μg PE anti-human IFN-γ, 0.1 μg PE anti-human IL-4 or 0.1 μg PE anti-human IL-5 antibodies. After 30 minutes cells were washed two times in permeabilization buffer and resuspended in PBS/2% FCS for analysis.

Cells were analysed by two-colour flow cytometry using a Becton Dickinson FACScan flow cytometer. The lymphocyte population was gated using forward and side scatter parameters to exclude debris and dead cells. The data were analysed using Lysis II software (Becton Dickinson). Quadrants were set based upon isotype controls for each antibody.

Statistics

The Mann-Whitney-test was used to compare data between groups. Differences were considered significant for $p < 0.05$.

Results

Proliferative Activity of PBMC after Stimulation with Bromelain or Trypsin

Incubation of PBMC with bromelain lead to a significantly increased proliferative response when compared to the unstimulated cell cultures ($p<0.001$). The proliferation activity was dose dependent and strongest proliferation was obtained at bromelain concentrations of 10 and 100μg/ml. There was no significant difference in proliferation activity between healthy controls and ED patients (Fig. 1a).

In contrast, trypsin was not able to induce a marked proliferation of PBMC in both study groups (Fig. 1b).

Cytokine Secretion by PBMC after Stimulation with Bromelain or Trypsin

Bromelain at concentrations of 10 or 100μg/ml induced a significant secretion of the macrophage/monocyte-associated cytokines IL-6, GM-CSF and TNFα as well as the production of the type 1 related cytokine IFNγ by PBMC from healthy controls and ED patients ($p<0.01$). The production of IL-2 and the type 2 cytokines IL-4 and IL-5 was not markedly enhanced by bromelain. Differences in cytokine levels between healthy controls and ED patients were not observed (Fig. 2).

There were no significant differences in cytokine production when PBMC were incubated with trypsin as compared to the unstimulated cultures (data not shown).

Expression of CD3/CD19 and CD4/CD8 on Non-stimulated and Bromelain-stimulated PBMC and Determination of Intracellular Cytokines in CD3 Positive T Cells.

In order to further analyse the immunomodulatory properties of bromelain we investigated the expression of the surface molecules CD3/CD19 and CD4/CD8 on PBMC from three healthy controls in response to 10 or 100μg bromelain/ml. Bromelain lead to an increased expression of CD3 (mean of the three controls: 77 ± 2.7%) when compared to the unstimulated cultures (70 ± 3.9%). Analyses of CD4 and CD8 revealed an enhanced expression for the CD4 marker (CD4: 51 ± 3.6% vs. unstimulated cultures: 38 ± 5.7%; CD8: 25 ± 2.8% vs. unstimulated cultures: 28 ± 2%). In Figure 3 an example for one healthy controls is shown.

Measurement of intracellular cytokines produced by CD3 positive T cells of the three healthy individuals after incubation of PBMC with bromelain and restimulation with PMA and ionomycin revealed an enhanced production for the type 1 cytokine IFNγ when

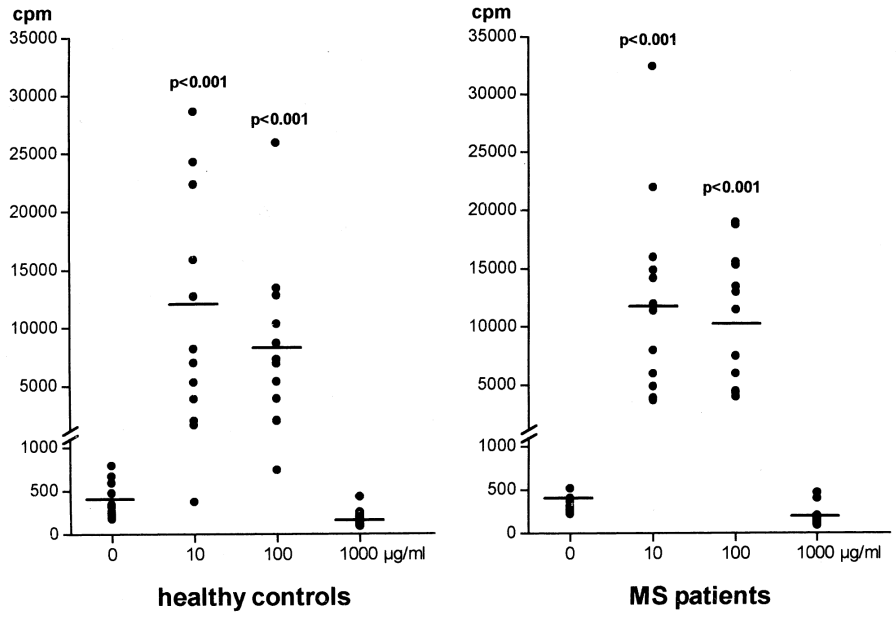

Fig.1. Proliferative activity of PBMC from healthy individuals (n = 12) and patients with encephalomyelitis disseminata (= multiple sclerosis, MS) (n = 12) in response to different bromelain concentrations (10-1000 μg/ml) as compared to unstimulated PBMC (cpm = counts per minute). Bars represent geometric means.

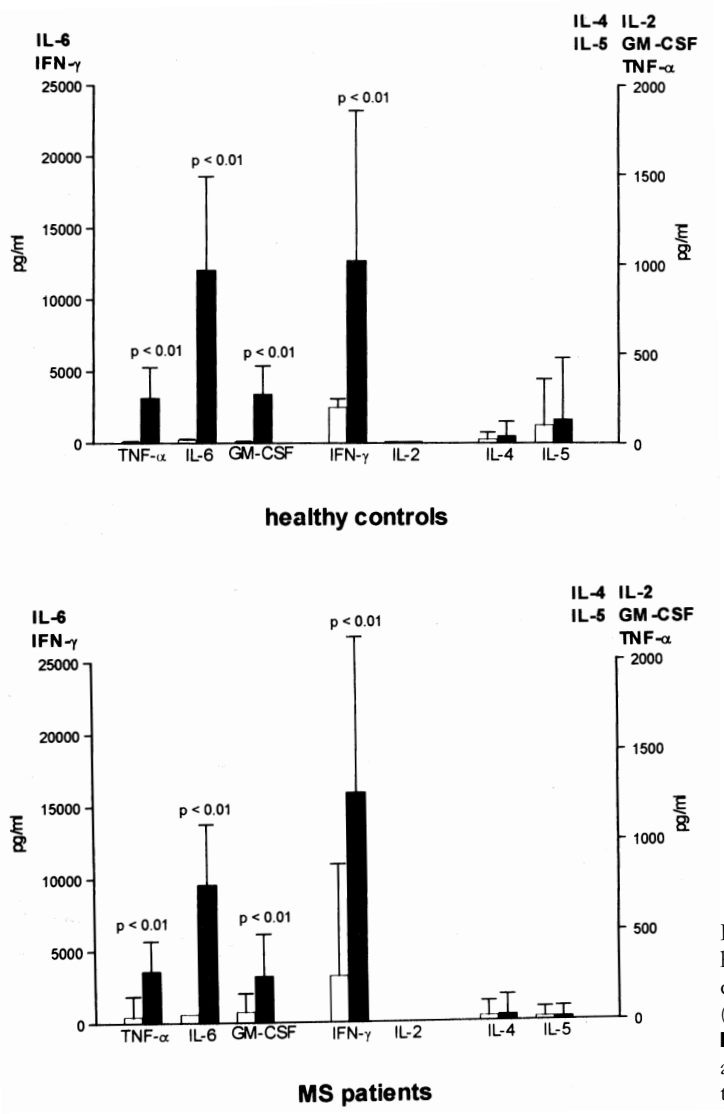

Fig. 2. Cytokine levels in supernatants of PBMC from healthy individuals (n = 12) and patients with encephalomyelitis disseminata (= multiple sclerosis, MS) (n = 12) stimulated with bromelain (10 or 100 µg/ml; ■) as compared to non-stimulated cultures (□) . Results are expressed as pg/ml. Mean (bars) and standard deviation are given.

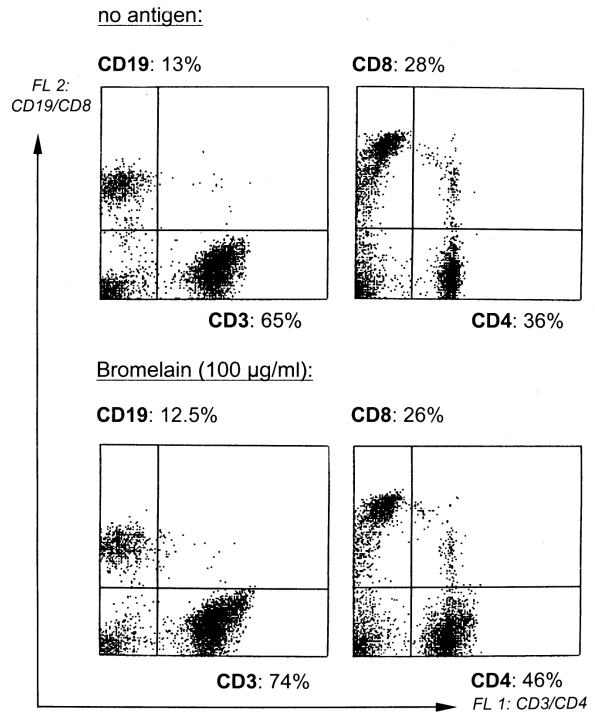

Fig. 3. Two-color flow cytometric analysis of non-stimulated PBMC and PBMC stimulated with bromelain (100µg/ml). Data are given for one healthy control as an example. The percentages of CD3, CD19, CD4, and CD8 positive cells were determined. Quadrants were set based upon isotype controls for each antibody.

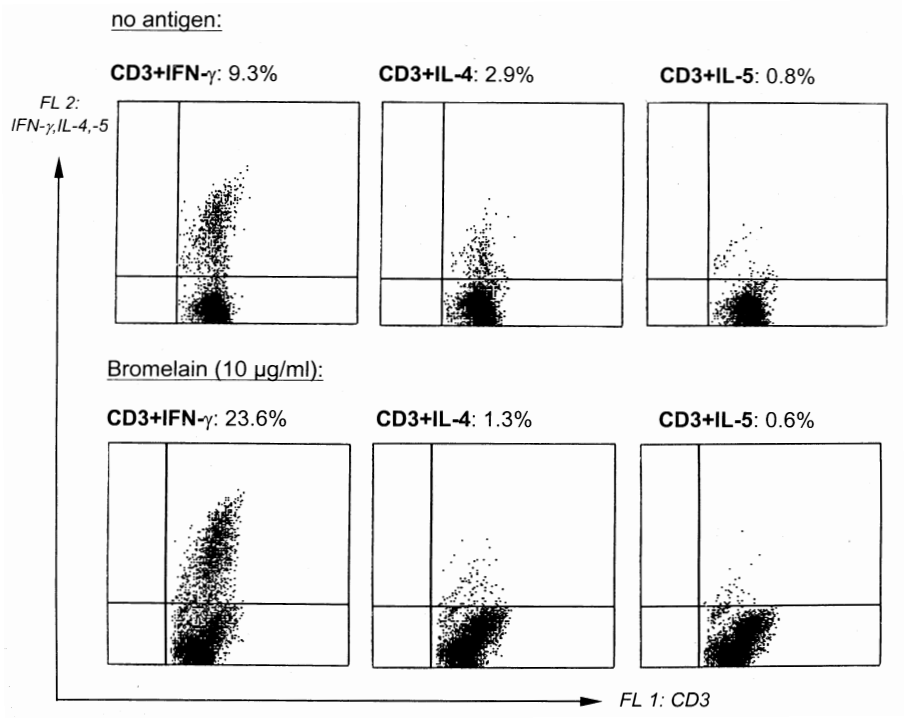

Fig. 4. Analysis of intracellular cytokines (IFN-γ, IL-4, IL-5) produced by CD3 positive cells of PBMC stimulated with bromelain (10µg/ml) as compared to non-stimulated cell cultures from one healthy control. The percentages of CD3 positive cells producing only IFN-γ, IL-4 and IL-5 were determined. Quadrants were set based upon isotype controls for each antibody.

compared to the cell cultures without bromelain (mean of the three controls: 19.5 ± 3.5% vs. 9.7 ± 2.1%). The production of the type 2 cytokines IL-4 and IL-5 was not influenced. In Figure 4 data are given for one healthy control as an example. Again, trypsin had no effect in these experiments.

Discussion

In this study it is shown that bromelain – but not trypsin – induced the secretion of the macrophage/monocyte derived cytokines IL-6, GM-CSF, TNFα and IFN-γ – the latter being produced also by T helper type 1 cells –, while the production of the type 2 cytokines IL-4 and IL-5 was not affected, indicating an involvement of cells of the innate immune system. The enhancement of CD3 and CD4 positive T-cells after incubation with bromelain and the increase of CD3 positive T cells producing the type 1 cytokine IFN-γ by FACS analysis is, however, a strong argument that also type 1 T cells are activated. The immunomodulatory effect of bromelain was similar in patients with ED and healthy controls indicating that it had no specific stimulatory effect on autoreactive T-cells involved in the immunopathogenesis of ED.

There are several reports analysing the influence of proteolytic enzymes on immunocompetent cells in the literature. Brakebusch et al. [23] analysed their effect on granulocyte and macrophage function in patients with a disorder of the humoral immunosystem X-linked agammaglobulinemia. They found an acceleration of phagocytosis, respiratory burst and killing of Candida albicans which was – similarly to our experiments on the T-cell level – related to bromelain but not trypsin. Hale et al. [8] showed that treatment of T cells with bromelain for one hour selectively removed CD44, CD45RA, E2/MIC2, CD6, CD7, CD8, and Leu 8/LAM1 but left intact CD2, CD3, and CD28 T-cell surface molecules. They also found that bromelain markedly enhanced CD2-mediated T cell activation. It is, however, still unclear whether T cell activation by bromelain occurs directly via the cleavage of surface molecules or indirectly via antigen presenting cells. In this respect the observation by Mynott et al. [15] is of interest showing that an inhibitory action of bromelain is not simply caused by degradation of certain surface molecules. Thus, T-cell activation by PMA plus ionophore, two membrane-permeable agonists whose action is independent from extracellular receptors, was blocked by bromelain. Furthermore, bromelain inhibited cytokine mRNA accumulation induced by PMA plus ionophore but not that of T cell receptor induced cytokine production. In the last years the biological actions of proteases could be attributed to the activation of a new sub-family of G protein-coupled receptors, termed proteinase-activated receptors (PARs) [24]. After stimulation, PARs couple to various G proteins and activate signal transduction pathways resulting in the rapid transcription of genes that are involved in inflammation. PARs can, therefore, modulate the secretion of cytokines, inflammatory mediators or neuropeptides. Modulation of PAR function could be, therefore, a new aspect for the treatment of inflammatory or immune diseases, and bromelain could be one candidate as such a therapeutic substance.

The activation of TH1-cells and macrophages by bromelain as presented in this *in vitro* study is somewhat contrary to the observation that it may be *in vivo* helpful in TH1/type1 mediated autoimmune diseases such as EAE or diabetes by shifting the pathogenic type 1 pattern to the protective type 2 reaction [16, 17]. However, it has been recently shown that also a TH type 1 response can activate control mechanisms required to suppress autoimmunity, and the recent success of pilot studies using the bacterial vaccine BCG in therapy of ED patients supports this hypothe-

sis [25]. Furthermore, it is an often made experience with different immunomodulatory substances that *in vitro* results do not correlate with reactions observed *in vivo* [26, 27], and also with respect to bromelain our preliminary *in vivo* data show that in ED patients treated with this substance for twelve months rather the type 2 reactivity is induced *in vivo* as shown by an increased IL-5 production by PBMC from these patients (manuscript in preparation). Considering the high doses in which bromelain is administered orally (about 540 mg/day) it is not unlikely that it stimulates the gut associated immune system hereby activating type 2 TH or regulatory T cells [28-30], and, indeed, it was shown that – at least in mice – bromelain enzymes remain intact and functionally active within the gastrointestinal tract [31].

Considering the well tolerability and lack of side effects of proteolytic enzymes, the until now presented *in vivo* and *in vitro* effects of proteolytic enzymes on immunocompetent cells justify, therefore, further *in vivo* studies analysing immunological reactions during enzyme treatment in relation to clinical effects.

Acknowledgements: We would like to thank MUCOS Pharma GmbH & Co., Geretsried, Germany for financial support.
H. Barth was supported by the fortüne-project (No. 509), University of Tübingen

REFERENCES

1. Kokron O, Micksche M, Cerni C, Titscher R, Wybra H. Proteolytic enzymes in the treatment of malignant pleural effusions and solid metastases. Chemotherapy 1976; 8: 517-521
2. Taussig SJ, Batkin S. Bromelain, the enzyme complex of pineapple (Ananas comosus) and its clinical application. J Ethnopharmacol 1988; 22: 191-203
3. Leipner J, Iten F, Saller R. Therapy with proteolytic enzymes in rheumatic disorders. Bio-Drugs 2001; 15: 779-789
4. Tilwe GH, Beria S, Turakhia NH, Daftary GV, Schiess W. Efficacy and tolerability of oral enzyme therapy as compared to diclofenac in active osteoarthritis of knee joint: an open randomized controlled clinical trial. J Assoc Physicians India 2001; 49: 617-621
5. Maurer HR. Bromelain: biochemistry, pharmacology and medical use. Cell Mol Life Sci 2001; 58: 1234-1245
6. Shahid SK, Turakhia NH, Kundra M, Shanberg P, Daftary GV, Schiess W. Efficacy and safety of phlogenzym, a protease formulation, in sepsis in children. J Assoc Physicians India 2002; 50: 527-551
7. Desser L, Holomanova D, Zavadova E, Pavelka K, Mohr T, Herbacek I. Oral therapy with proteolytic enzymes decreases excessive TGF-beta levels in human blood. Cancer Chemother Pharmacol 2001; 47: S10-S15
8. Hale LP, Haynes BF. Bromelain treatment of human T cells removes CD44, CD45RA, E2/MIC2, CD6, CD7, CD8 and leu8/LAM 1 surface molecules and markedly enhances CD2-mediated T cell activation. J Immunol 1992; 149: 3809-3816
9. Lehmann PV. Immunomodulation by proteolytic enzymes. Nephrol Dial Transplant 1996; 11: 953-955
10. Desser L, Rehberger A, Krokon E, Paukovits W. Cytokine synthesis in human peripheral blood mononuclear cells after oral administration of polyenzyme preparations. Oncology 1993; 50: 403-407
11. Desser L, Rehberger A, Paukovits W. Proteolytic enzymes and amylse induce cytokine production in human peripheral blood mononuclear cells *in vitro*. Cancer Biotherapy 1994; 9: 253-263
12. Eckert K, Grabowski E, Stange R, Schneider U, Eschmann K, Maurer HR. Effects of oral bromelain administration on the impaired immunocytotoxicity of mononuclear cells from mammary tumor patients. Oncol Rep 1999; 6: 1191-1199
13. Engwerda CR, Andrew D, Murphy M, Mynott TL. Bromelain activates murine macrophages and natural killer cells *in vitro*. Cell Immunol 2001; 25: 5-10
14. Engwerda CR, Andrew D, Ladhams A, Mynott TL. Bromelain modulates T cell and B cell immune responses *in vitro* and *in vivo*. Cell Immunol 2001; 25: 66-75
15. Mynott TL, Ladhams A, Scarmato P, Engwerda CR. Bromelain, from pineapple stems, proteolytically blocks activation of extracellular regulated kinase-2 in T-cells. J Immunol 1999; 163: 2568-2575
16. Targoni OS, Tary-Lehmann M, Lehmann PV. Prevention of murine EAE by oral hydrolytic enzyme treatment. J Autoimmun 1999; 12: 191-198
17. Roep BO, van den Engel NK, van Halteren AG, Duinkerken G, Martin S. Modulation of autoimmunity to beta-cell antigens by proteases. Diabetologia 2002; 45: 686-692
18. Mosmann TR, Coffmann RL. TH1 and ZH2 cells: different patterns of lymphokine secretion lead to different functional properties. Ann Rev Immunol 1989; 7: 145-173
19. Romagnani S, Parronchi P, D'Elios NN, Romagnani P, Annunziato F, Piccinni MP, Manetti R, Sampgnara S, Mavilia C, De Carli M, Maggi E, Del Prete GF. An update on human Th1 and Th2 cells. Int Arch Allergy Immunol 1997; 113: 153-156
20. Romagnani S. The Th1/Th2 paradigm. Immunol Today 1997; 18: 263-266
21. Poser CM, Paty DW, Scheinberg L, McDonald WI, Davies FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtolle WW. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983; 13: 227-231
22. Barth H, Berg PA, Klein R. Methods for the *in vitro* determination of an individual disposition towards TH1- or TH2-reactivity by the application of appropriate stimulatory antigens. Clin Exp Immunol 2003; 134: 78-85
23. Brakebusch M, Wintergerst U, Petropoulou T, Notheis G, Husfeld L, Belohradsky BH, Adam D. Bromelain is an accelerator of phagocytosis, respiratory burst and killing of Candida albicans by human granulocytes and monocytes. Eur J Med Res 2001; 6: 193-200
24. Steinhoff M, Buddenkotte J, Shpacovitch V, Rattenholl A, Moormann C, Vergnolle N, Luger TA, Hollenberg MD. Proteinase-activated receptors: transducers of proteinase-mediated signalling in inflammation and immune response. Endocr Rev 2004; Oct 12 (Epub ahead of print)
25. Ristory G, Buzzi MG, Sabatini U, Giugni E, Bastianello S, Viselli F, Buttinelli C, Ruggieri G, Colonesse C, Pozzili C, Salvetti M. Use of bacille Calmette-Guérin (BCG) in multiple sclerosis. Neurology 1999; 53: 1588-1589
26. Stein GM, Berg PA. Mistletoe extract-induced effects of immunocompetent cells: *in vitro* studies.l Anti-Cancer Drugs 1997; 8: S39-S42
27. Stein GM, Henn W, von Laue HB, Berg PA. Modulation of the cellular and humoral immune responses of tumor patients by mistletoe therapy. Eur J Med Res 1998; 3: 194-202
28. Groux H, Powrie F. Regulatory T cells and inflammatory bowel disease. Immunol Today 1999; 20: 442-446

29. Weiner HL, Friedmann A, Miller A, Khoury SJ, Al-Sabbagh A, Santos L, Sayegh M, Nussenblatt RB, Trentham DE, Hafler DA. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. Annu Rev Immunol 1994; 12: 809-837
30. Weiner HL. Oral tolerance : immune mechanisms and treatment of autoimmune diseases. Immunol Today 1997; 18: 335-342
31. Hale LP. Proteolytic activity and immunogenicity of oral bromelain within the gastrointestinal tract of mice. Int Immunopharmacol 2004; 4: 255-264

Received: February 4, 2005 / Accepted: June 16, 2005

Address for correspondence:
Reinhild Klein MD
Associate Professor of Clinical Immunology
Department of Internal Medicine II
University of Tübingen
Otfried-Müller-Str. 10
D-72076 Tübingen, Germany
Tel.: +49 7071-29 84479
Fax: +49 7071-29 2760
e-mail: reinhild.Klein@med.uni-tuebingen.de