Propolis and Some of its Constituents Down-Regulate DNA Synthesis and Inflammatory Cytokine Production but Induce TGF-β1 Production of Human Immune Cells

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Propolis, the resinous product collected by honey bees from plants, is used as folk medicine since ancient time. Recently, immunoregulatory and *anti*-inflammatory properties of propolis have been published. The detailed mechanisms of actions of propolis and its components on immune cells, however, are still unknown. Therefore, we studied the effects of different propolis extracts, of the flavonoids hesperidin and quercetin as well as of caffeic acid phenethyl ester (CAPE) on basic human immune cell functions. In detail, we measured the effects on DNA synthesis and production of different types of cytokines, namely IL-1β, IL-12, IL-2, IL-4, IL-10 and TGF-β1, of mitogen-activated peripheral blood mononuclear cells (PBMC) as well as of purified T lymphocytes.
Our data clearly show that propolis as well as its constituents studied are capable of dose-dependently suppressing phythemagglutinin (PHA)-induced DNA synthesis of PBMC and T cells. Moreover, cytokines produced by monocytes/macrophages (IL-1β, IL-12), by Th1 type (IL-2) as well as Th2 type (IL-4) lymphocytes were found to be also suppressed, whereas the production of TGF-β1 by T regulatory cells was ascertained to be increased. These data convincingly demonstrate that propolis has a direct regulatory effect on basic functional properties of immune cells which may be mediated by the Erk2 MAP-kinase signal pathway. Thus, the bee product propolis can be considered as a powerful natural *anti*-inflammatory medicine influencing different types of immune-responses probably *via* immunoregulatory T cells.

Key words: Propolis, Immune Cells, Cytokines

Introduction

Propolis is the generic name for the resinous product of complex composition collected by honey bees from various plant sources (reviews cf. Burdock, 1998; Banskota *et al.*, 2001). Chemical analyses revealed that propolis contains more than 300 constituents among them phenolic compounds, including flavonoids as major components (Burdock, 1998; Hegazy *et al.*, 2000; Banskota *et al.*, 1998). In addition to flavonoids, propolis contains cinnamic acid derivatives such as caffeic acid (3.4-hydroxycinnamic acid) and its esters and also sesquiterpenes, quinones and coumarins (Burdock, 1998; Hegazy *et al.*, 2000; Banskota *et al.*, 1998).

Propolis has been used as a folk medicine since antiquity, mainly as an antibiotic (Burdock, 1998; Hegazy *et al.*, 2000), *anti*-cancer agent (Burdock, 1998; Banskota *et al.*, 2001) and wound healing promoter (Bretz *et al.*, 1998; Vennat *et al.*, 1998).

In addition to these biological effects, *anti*-inflammatory and immunomodulatory properties of propolis and its constituents have been shown recently by a number of investigators. Most of these biological effects were attributed to flavonoids and caffeic acid derivatives. Immunomodulatory (Brätter *et al.*, 1999) as well as *anti*-inflammatory effects (Burdock, 1998; Banskota *et al.*, 2001; Fitzpatrick *et al.*, 2001; Ledon *et al.*, 1997; Menezes *et al.*, 1999; Park and Kahng, 1999; Volpert and Elstner, 1993; Ivanovska *et al.*, 1995; Krol *et al.*, 1996; Mirzoeva and Calder, 1996; Sud'ina *et al.*, 1993; Natarjan *et al.*, 1996; Song *et al.*, 2002; Guardia *et al.*, 2001) have been demonstrated *in vitro* and *in vivo*. This holds true for the specific, *i.e.* adaptive immune

response as well as for the non-specific response (Brätter *et al.*, 1999; Fitzpatrick *et al.*, 2001; Ledon *et al.*, 1997; Menezes *et al.*, 1999; Park and Kahng, 1999; Ivanovska *et al.*, 1995; Krol *et al.*, 1996; Mirzoeva and Calder, 1996; Sud'ina *et al.*, 1993; Natarajan *et al.*, 1996; Song *et al.*, 2002) where *anti*-oxidative (Song *et al.*, 2002), *anti*-complement actions (Ivanovska *et al.*, 1995) as well as inhibitory effects on granulocyte functions (Krol *et al.*, 1996) and the eicosanoid synthesis (Mirzoeva and Calder, 1996; Sud'ina *et al.*, 1993) have been described.

Caffeic acid phenethyl ester (CAPE) has been shown to be a potent inhibitor of activation of nuclear transcription factor NF-$\varkappa$B in the human myelo-monocytic cell line U 937 (Natarajan *et al.*, 1996). The detailed molecular and cellular basis of the action of propolis on immune cells, however, are still unknown, as yet.

In the present paper we studied the effect of propolis and some of its constituents on basic functions of mitogen-activated immune cells of human peripheral blood, namely on cell growth and DNA synthesis, resp., and the production of cytokines *in vitro*. In detail monocyte/macrophage derived interleukin-1β (IL-1β) and interleukin-12 (IL-12), the Th1 type cytokine interleukin-2 (IL-2), the Th2 type interleukin-4 (IL-4), and the immunosuppressive cytokines interleukin 10 (IL-10) and transforming growth factor-β1 (TGF-β1) were ascertained in supernatants of cell cultures of peripheral blood mononuclear cells (PBMC) and purified T lymphocytes.

Our results show that propolis, CAPE, quercetin, hesperidin, and other flavonoids strongly inhibit DNA synthesis and inflammatory cytokine production of Th1 as well Th2 type T cells. On the other hand, production of TGF-β1 which is capable of mediating inhibitory *i.e.* immunosuppressive actions on effector functions (Shull *et al.*, 1992) was found to be increased.

These findings convincingly demonstrate that the bee product propolis has a direct regulatory action on basic immune cell functions. Moreover, they confirm that propolis can be considered as a suitable alternative natural *anti*-inflammatory medicine.

Material and Methods

Materials

Quercetin, hesperidin, and CAPE were purchased from Sigma (Deisenhofen, Germany), phythemagglutinin (PHA) from Sigma or Virotech (Rüsselsheim, Germany), pokeweed mitogen PWM) was from Serva (Heidelberg, Germany). Substances were used at concentrations indicated in the figure legends.

Extract preparation

Propolis (Poland, 1997) was purchased from Honig-Mehler (Neichen, Germany). Fifty grams of the material were extracted by intensive stirring in a mixture of 150 ml MilliQ-water and 250 ml chloroform at room temperature for 4 h. Phases were allowed to separate (30 min) and the aqeous phase was carefully collected (fraction **P1**, 8.1 mg/ml dry mass). The remaining chloroform phase was extracted as described above by 450 ml 100 % (v/v) ethanol, yielding the **P2** fraction (27.8 mg/ml). Alternatively, fifty grams of propolis were extracted directly by 400 ml 50 % (v/v) ethanol (**P3**, 45.6 mg/ml) or 400 ml 100 % (v/v) ethanol (**P4**, 67.4 mg/ml). All fractions were cleared by filtration through folded filters No. 597 (Schleicher & Schüll, Dassel, Germany). Finally, the extracts were subjected to sterile filtration using disposable filtration units PV050/2 (Schleicher & Schüll). Sterile extracts were kept at 4 °C and used within 72 h in the assays.

Cells

Peripheral blood mononuclear cells were isolated from the heparinized venous blood of healthy donors by density gradient centrifugation over Ficoll-Paque gradients (Pharmacia LKB, Sweden), as described by Boyum (1968). Monocytes and B lymphocytes were depleted by passage through nylon wool columns (Polysciences, Warrington, PA). PBMC or T lymphocytes (90–95 % T cells) were suspended in serum-free AIM-V medium (Life Technologies, Germany).

Proliferation assay/DNA synthesis

PBMC and purified T lymphocytes (10^5 cells/100 μl) were stimulated in serum-free AIM-V medium with PHA (1 μg/ml; Virotech) in the pres-

ence of the propolis extracts, flavonoids or CAPE, respectively. After 90 h the cultures were pulsed for an additional 6 h with 3H-methyl-thymidine (0.2 μCi per well; Amersham, Braunschweig, Germany). Cells were harvested onto glass fibre filters and the incorporated radioactivity was measured by scintillation counting (Reinhold *et al.*, 1997).

Cytokine induction

PBMC and T cells were induced to produce cytokines, as IL-2, IL-4, IL-10, IL-12, and TGF-β1, by stimulation with PWM as described previously (Reinhold *et al.*, 1997). Briefly, 10^6 cells/ml were incubated in serum-free AIM-V medium with 2 μg/ml PWM and different concentrations of propolis extracts or propolis constituents. After 48 h the cell culture supernatants were harvested for cytokine determination and stored at – 70 °C. Amounts of TGF-β1 were determined after 4 h of cell culture. IL-1β-mRNA and Erk2-mRNA were induced by 1 μg/ml PHA-L (Boehringer, Mannheim, Germany) in PBMC cultured in complete Iscove's modified medium supplemented with 10 % fetal calf serum (Gibco BRL, Eggenstein, Germany) and 60 units/ml penicillin for 24 h.

Determination of cytokine production

The concentrations of IL-2, IL-4, IL-10, and IL-12 in the cell culture supernatants were determined with commercially available "sandwich" ELISAs (R&D systems, Minneapolis, MN).

Active TGF-β1 was measured with an ELISA as described by Danielpour (1993) using a mouse monoclonal *anti*-TGF-β1,-β2,-β3 antibody and a chicken *anti*-TGF-β1 antibody (R&D Systems). This assay is sensitive to 50 pg of TGF-β1 per ml. Samples were tested after transient acidification (Reinhold *et al.*, 1997).

Cell viability

Cell viability was measured using a modified MTT assay (Reinhold *et al.*, 1993). The tetrazolium salt MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) (Serva, Heidelberg, Germany) was dissolved in AIM-V medium at 5 mg/ml. 25 μl of this stock solution were added to 100 μl of cell culture medium, and the plates were incubated at 37 °C for 4 h. Following centrifugation of the plates at 600 × *g* for 10 min, the medium was removed by aspiration. To each well 100 μl acidified isopropyl alcohol/dimethylformamide solution (0.04 M HCl in isopropanol/dimethylformamide 1:1) were added. The formazane concentration was measured with an ELISA reader (570 nm wavelength filter).

RNA isolation

Cells were harvested by centrifugation (500 × *g*, 4 °C, 10 min) and washed once in phosphate-buffered saline (PBS). Total RNA was prepared by using the RNeasy Kit provided by Qiagen (Hilden, Germany) following the recommended protocol. The resulting RNA was quantified spectrophotometrically using a GeneQuant (Pharmacia, Freiburg, Germany), aliquoted, and stored ethanol-precipitated at – 80 °C until further use.

Reverse transcription/PCR

In each case, 1 μg of total RNA were transcribed in a final volume of 20 μl by 20 units of AMV reverse transcriptase (Promega, Mannheim, Germany) in the supplied buffer with the addition of 0.5 mmol/l desoxynucleoside triphosphates (dNTP), 10 mmol/l random hexanucleotides (Boehringer, Mannheim, Germany) and 50 units of placenta RNase inhibitor (Ambion, Austin, USA) during a 1 h incubation at 37 °C. The enzymes were inactivated by a 10 min incubation at 65 °C, and the reaction mixture was kept frozen at – 70 °C until enzymatic amplification.

Quantitative PCR for IL-1β and Erk-2 was performed using the iCycler (real-time PCR device, Bio-Rad, Munich, Germany). All samples were analyzed in triplicate. A 30 μl reaction mixture consisted of 15 μl HotStarTaq Master Mix (Qiagen, Hilden, Germany), 1.2 μl of the RT-reaction, 0.3 μl SYBR-Green I (1:10.000) (Molecular Probes, Eugene, USA), and 0.5 μmol/L of the specific primers for IL-1β (Stratagene, Heidelberg, Germany) or Erk-2 (upstream: 5′-CATCGCCGAAGCACCATTCAAG downstream: 5′-GATAAGCCAAGACGGGCTGGAG). Initial denaturation and activation of Taq-polymerase at 95 °C for 15 min was followed by 40 cycles with denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec, and elongation at 72 °C for 30 sec. 18S mRNA amounts were determined using the RT

primer pair available from Ambion (Austin, USA) and used to normalize cDNA contents. The fluorescence intensity of the double-strand specific SYBR-Green I, reflecting the amount of actually formed PCR-product, was read real-time at the end of each elongation step. Then specific initial template mRNA amounts were calculated by determining the time point at which the linear increase of sample PCR product started, relative to the corresponding points of a standard curve obtained by serially dilution of known copy numbers of the corresponding cloned PCR fragments. Data are given as arbitrary units normalized to 18S-RNA amounts.

Results

DNA synthesis of T cells and PBMC

DNA synthesis and increased cell growth of specific immune cells are preconditions of an effective adaptive immune response *in vivo.* Mononuclear cells (PBMC) and purified T lymphocytes from human peripheral blood were stimulated with PHA in presence and absence of various concentrations of different propolis extracts or its constituents CAPE, hesperidin, quercetin, and other flavonoids for 72 h. DNA synthesis was measured after labelling with 3H-thymidine. As shown in Fig. 1, DNA synthesis of PHA stimulated PBMC and T cells was suppressed by the different ethanol/chloroform extracts of propolis (**P2–P4**) in a concentration-dependent manner. Similar results were found for the aqueous propolis extract **P1**, however, only at higher concentrations (data not shown). It should be mentioned that the viability of cells was not changed in presence of propolis during that time. Inhibitory effects were also obtained for some of the propolis constituents like CAPE, quercetin, and hesperidin (Fig. 2). The strongest effects at a concentration of 10 μM were ascertained in presence of quercetin and CAPE.

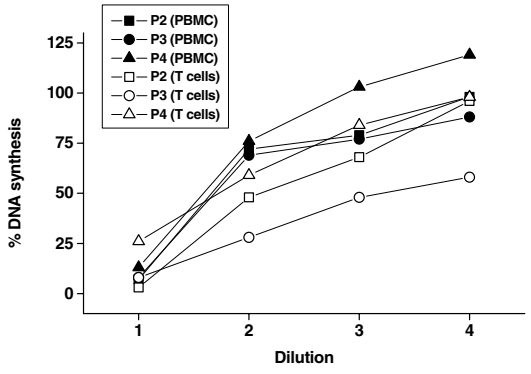

Fig. 1. Propolis extracts suppresses the DNA synthesis of human PBMC and purified T cells. PBMC or T cells were stimulated by PHA in presence of the different alcohol/chloroform extracts of propolis **P2**, **P3** and **P4** at different concentrations. DNA synthesis was ascertained by 3H-methyl-thymidine incorporation after 96 h. The mean values were obtained in 4 independent experiments (SD < 20%). The final dilutions of the original propolis extracts in the cell culture were: **1** = 1:10,000, **2** = 1:100,000, **3** = 1:1,000,000, **4** = 1:10,000,000.

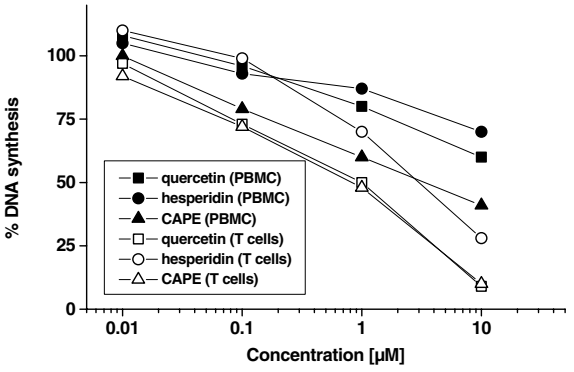

Fig. 2. Concentration-dependent suppression of DNA synthesis of human PBMC and purified T cells by quercetin, hesperidin and CAPE. PBMC or T cells were stimulated by PHA in presence of different concentrations of quercetin, hesperidin and CAPE, resp. DNA synthesis was ascertained by 3H-methyl-thymidine incorporation after 96 h. The mean values were obtained in 3 independent experiments(SD < 20 %).

Cytokine production/release

Important functional properties of immune cells are their capability to synthesize and secrete soluble polypeptide factors referred to as cytokines. Most cytokines are secreted and then bind to specific receptors on the surface of target cells. Upon binding they act to regulate growth and/or differentiation and to optimize the immune and inflammatory response. Special cytokine patterns do at least in part reflect different functions of individual cell types of the immune and hematopoietic system. Proinflammatory cytokines like IL-1β and IL-12 are produced mainly by dendritic cells, monocytes and macrophages. The inflammatory cytokine IL-2 is secreted mainly by Th1 type T lym-

phocytes whereas IL-4 is regarded as typical for Th2 cells (Romagnani, 1994).

TGF-β1 is capable of mediating inhibitory activity on effector functions of leucocytes engaged in the immune response (Shull *et al.*, 1992). Together with IL-10, TGF-β1 is produced mainly by T-regulatory cells and mediates the inhibition of primary naive T cells *in vitro* (Levings and Roncarolo, 2000; Sakaguchi, 2000).

Given these key roles of cytokines in regulating functions of cells of the immune system, there has been some interest in the effect of propolis and its constituents in modulating the production of different types of cytokines by PBMC and T cells.

Mononuclear cells or T cells from human peripheral blood were stimulated by PHA or (PWM) in presence or absence of propolis extracts, quercetin, hesperidin and other flavonoids or CAPE for a given time. Supernatants and cells were harvested separately and the cytokine concentrations were measured in the supernatants. In case of IL-1β and the MAP kinase Erk2, mRNA amounts were ascertained in cells.

IL-2, IL-4, IL-10, IL-12

PWM-stimulated PBMC and T cells were cultured for 48 h in presence of different propolis extracts (**P1–P4**), flavonoids or CAPE.

In all cases a strong suppression of IL-12, IL-10, IL-4 and IL-2 release by PBMC was measured with the propolis extracts (Fig. 3A). Similar results were obtained with T lymphocytes under identical conditions (Fig. 3B). Significant immunosuppressive effects on the release of these cytokines were also observed with quercetin, hesperidin and CAPE in mononuclear cells and T lymphocytes, respectively (Fig. 4A and 4B).

TGF-β1

TGF-β1 was measured in supernatants of PWM-stimulated PBMC or T cells, resp., after 4 h of culture (Reinhold *et al.*, 1997). In contrast to all other cytokines, propolis extracts as well as flavonoids and CAPE induced an increased secretion of TGF-β1 (Fig. 5A and 5B). This holds true for

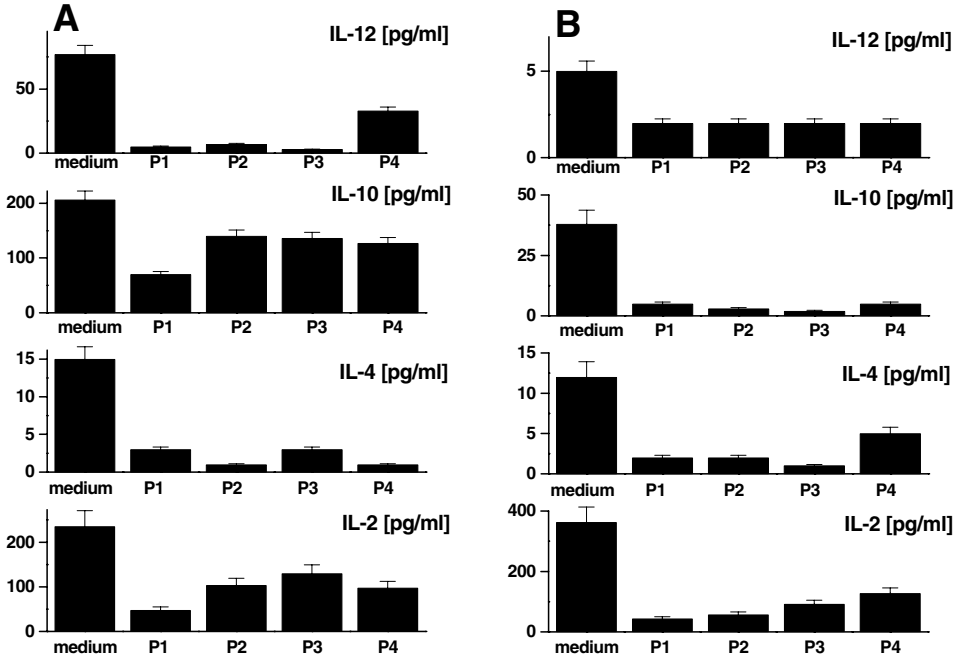

Fig. 3. Propolis extracts suppresses the production/secretion of the inflammatory cytokines IL-12, IL-10, IL-4 and IL-2 in human PBMC and T cells. Mononuclear cells (Fig. 3A) or T lymphocytes (Fig. 3B) were stimulated by PWM in presence of the different propolis extracts. The concentrations of the cytokines in the cell culture supernatants were measured after 48 h of cell culture. The mean values were obtained in 3 independent experiments. The final dilutions of the original propolis extracts in the cell culture were: **P1** = 1:100, **P2**, **P3**, **P4** = 1:10,000.

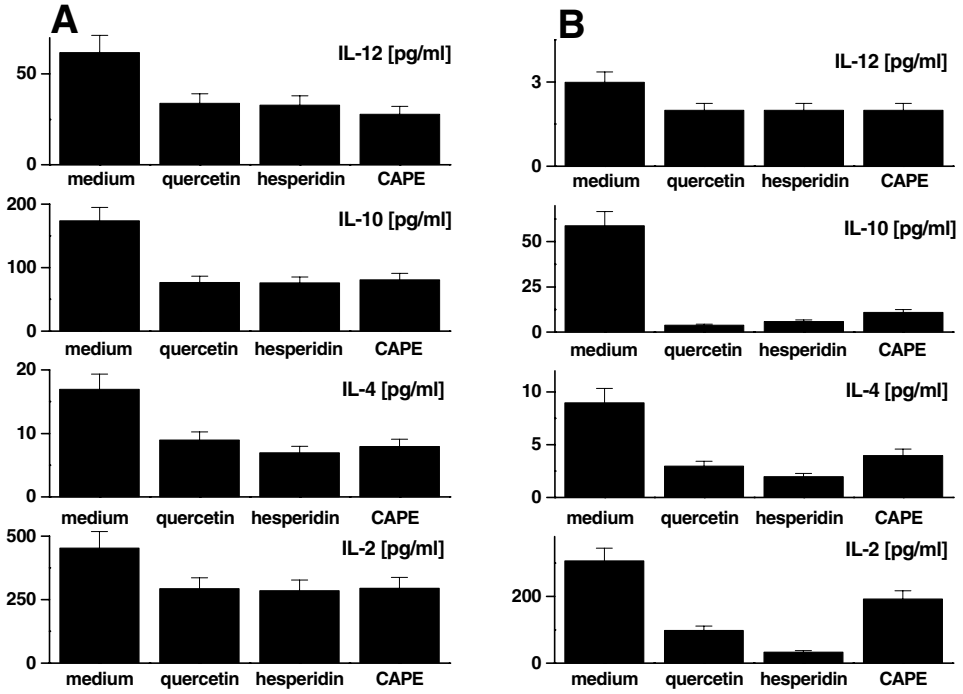

Fig. 4. Suppression of the production/secretion of the cytokines IL-12, IL-10, IL-4, and IL-2 in human T lymphocytes and PBMC. PBMC (Fig. 4A) or T lymphocytes Fig. 4B) were stimulated by PWM in presence of quercetin (20 μM), hesperidin (20 μM) or CAPE (10 μM), resp. The concentrations of the cytokines in the cell culture supernatant were measured after 48 h. The mean values were obtained in 3 independent experiments.

PWM-induced T cells and at a higher degree also for PWM-stimulated PBMC.

Interleukin-1β

As shown in Fig. 6B, a strong inhibition of mRNA expression of IL-1β in PHA-stimulated PBMC were observed for hesperidin and quercetin. No such effect could be demonstrated for CAPE. In case of the inhibition of IL-1β mRNA expression by hesperidin an inhibition constant (IC-50) of 4.5×10^{-6} M could be calculated (data not illustrated).

Erk-2 mRNA

To get a first information on possible molecular mechanisms responsible for the negative regulation of cellular growth by propolis and its components, we studied one of the MAP kinase signal pathways involved in growth promoting mechanisms. We measured the induction of mRNA expression of the extracellular-signal-regulated kinase (Erk-2), which is capable of regulating several transcription factors which in turn control the regulation of critical genes of lymphocytes including that of IL-2 (Hardy and Chaudhri, 1997).

In our experiments Erk-2 mRNA amounts were found to be increased in response to PHA stimulation, whereas in presence of hesperidin, quercetin, and CAPE the expression of the MAP kinase Erk-2 was strongly suppressed in PHA-stimulated PBMC down to 60 % in case of CAPE and hesperidin, and to 44 % in case of quercetin (Fig. 6A). Cyclosporin A, a strong immunosuppressive agent used for comparison, provoked an inhibition of Erk-2 expression down to 15 % (data not shown).

Discussion

Data presented in this work provide evidence for a direct functional effect of propolis and some of its constituents such as CAPE and different flavonoids on human immune cells. Our results con-

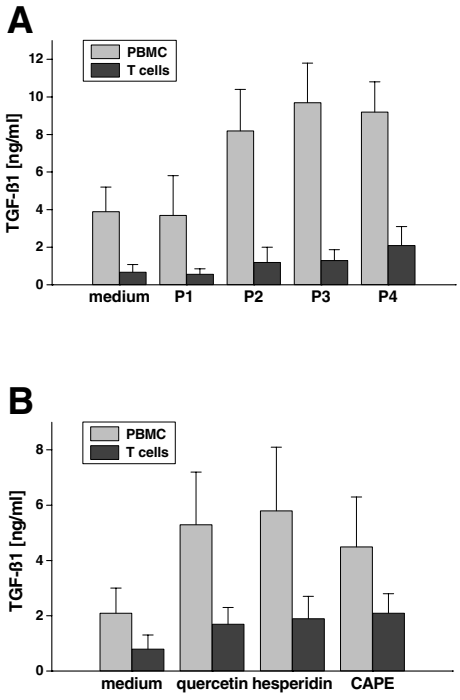

Fig. 5. Propolis extracts, quercetin, hesperidin and CAPE induces the production/secretion of the immunosuppressive cytokine TGF-β1 in human PBBMC and T lymphocytes. PBMC and purified T cells were stimulated with PWM in presence of the different propolis extracts (A) as well as quercetin (20 μM), hesperidin (20 μM) or CAPE (10 μM), resp. (B) for 4 h. The concentration of active TGF-β1 was measured after transient acidification of the cell culture supernatants. The mean values were obtained in 3 independent experiments. The final dilutions of the original propolis extracts in the cell culture were: **P1** = 1:100, **P2**, **P3**, **P4** = 1:10,000.

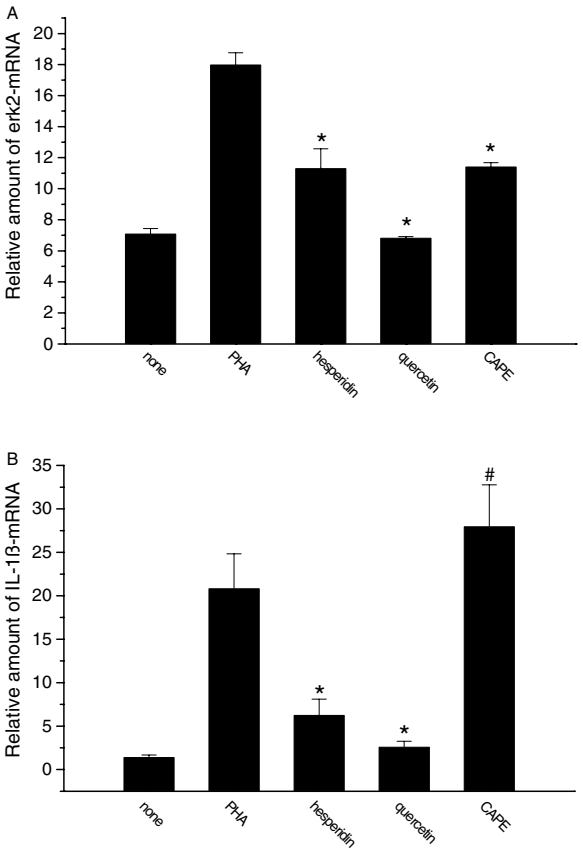

Fig. 6. Effects of the activation-dependent increase of Erk2-mRNA (A) and IL-1β-mRNA (B) by propolis constituents. 24 h after activation of PBMC by 1 μg/ml PHA there was a significant increase of Erk2-mRNA as well as IL-1β-mRNA contents when compared to the unstimulated control. This induction of Erk-2- mRNA and IL-1β-mRNA expression was markedly suppressed by the simultaneous addition of hesperidin or quercetin (1 μmol/l). Using CAPE (1 μmol/l), Erk-2-mRNA expression was also found to be suppressed whereas IL-1β-mRNA was slightly increased. Data are given as mean ± SD of 4 (A) and 3 (B) independent experiments. * $p < 0.01$, # $p < 0.05$.

vincingly show that propolis extracts are capable of strongly suppressing DNA synthesis of PBMC and purified T cells from human peripheral blood in a concentration-dependent manner.

These effects at least in part are mediated by some of its constituents, namely CAPE and the flavonoids quercetin and hesperidin. The largest fraction of constituents isolated from propolis are flavonoids which represent 10–29 % of the whole mass (Greeneway *et al.*, 1990). Caffeic acid derivatives are also frequently occuring substances in propolis, representing up to 20 % of the propolis mass (Hegazi *et al.*, 2000; Banskota *et al.*, 1998).

In our study the efficiency of propolis compounds in changing DNA synthesis and proliferation, respectively, were found to be gradually different. CAPE was ascertained to be the most potent suppressor at all, whereas quercetin was shown to be the most immunosuppressive substance for T cells and PBMC among the flavonoids tested.

Considering the fact that more than 300 different substances (Hegazi *et al.*, 2000; Banskota *et al.*, 1998; Burdock, 1998) including more than a dozen of flavonoids have been identified as constituents of propolis, one can speculate that the

effect of propolis on the cell cycle has to be considered as a resultant of various negative and, probably, also positive effectors.

Antiproliferative effects of propolis, CAPE, and of flavonoids have been shown also for other than immune cells, mainly transformed cell lines or tumor cells (reviews *cf.* Burdock, 1998; Peterson and Dwyer, 1998). This clearly suggests that propolis, CAPE and some flavonoids act *via* a common growth regulating mechanism which finally leads to a cell cycle arrest.

In addition to exhibiting growth regulating activity on lymphocytes, cytokine production and/or release was also found to be strongly influenced by propolis extracts and substances within this bee product. These effects were not restricted to one special type of cytokines or cells, resp., that are responsible for its production and/or release. Significant changes in cytokine production and/or release were ascertained for proinflammatory cytokines like IL-1β and IL-12, for the Th1 type cytokine IL-2, for the Th2 type cytokine IL-4 (Romagnani, 1994) as well as the immunoregulatory T cell-derived cytokine TGF-β1 which in contrast to the other cytokines was found to be increased in response to propolis or its constituents.

IL-12 synthesized by dendritic cells and macrophages is thought to drive differentiation of T cells toward the Th1 type cells. This is in line with our finding of a strongly decreased IL-2 production which is known to be characteristic for Th1 cells. On the other side, production of IL-4 is known to be a strong indicator for differentiation of T precusors to Th2 type cells. Therefore our data suggest that both Th1 as well Th2 type lymphocytes were targeted by propolis and its components. These results support the conclusion that Th1-dependent inflammatory processes and autoimmune diseases as well as Th2-(IL-4-)dependent allergic diseases as asthma bronchiale could be potentially treated by propolis and/or its constituents.

This view is further supported by our finding that the production of TGF-β1 is increased in the presence of propolis, CAPE, or the flavonoids used here. TGF-β1 can be produced by various cell types. The most prominent cells for immunoregulation are the immunoregulatory T cells which are characterized by their capability to synthesize and secrete latent TGF-β1 and IL-10 (Sakaguchi, 2000).

Targeted disruption of the mouse transforming growth factor-β1 gene (Shull *et al.*, 1992) or the interleukin-10 gene (Rennick *et al.*, 1997) results in multifocal inflammatory diseases (Shull *et al.*, 1992; Rennick *et al.* 1997). This clearly suggests that besides other functions both cytokines have strong immunosuppressive functions which may explain both growth suppression and the decreased production of the other cytokines, even of IL-10. In our experiments, IL-10 was found to be suppressed in T lymphocytes whereas in PBMC its production was only weakly influenced indicating different sources of IL-10 in PBMC and T cell fractions.

Recently, Natarajan *et al.*, 1996 reported that CAPE is a potent and specific inhibitor of activation of nuclear transcription factor NF-$\varkappa$B. Activation of NF-$\varkappa$B is induced by mitogen and cytokines. The most probable explanation for the CAPE effect is the inhibition of DNA binding of NF-$\varkappa$B by CAPE which leading to suppression of induction of gene expression *e.g.* of various inflammatory cytokines.

Changes in cell growth and cytokine production should be associated with changes in signal transduction pathways. To get a first information on this point, we studied the effect of propolis and its constituents on the changes of Erk-2 mRNA in PBMC. Erk-2 belongs to one of the three major groups of mitogen-activated protein kinases which is extracellularly regulated *via* MEK 1/2 and which is playing an important role in T cell receptor-CD3-mediated T cell activation (Hardy and Chaudhri, 1997).

In our study we found a strongly decreased Erk-2 expression when PHA-activated PBMC were exposed to quercetin, hesperidin, or CAPE, respectively. As expected, strong Erk-2 suppression was also ascertained in response to cyclosporin A, an immunosuppressive drug. These data clearly suggest that one way of signalling triggered by propolis components is mediated by the MAP kinase Erk-2. Again, the potential role of propolis components as immunoregulating drug is underscored.

This finding does not exclude that additional signal pathways and transcription factors targeted by caffeic acid derivatives or plant flavonoids are responsible for the growth regulating and immunomodulating activities of propolis.

Remarkably, most of the effects resulting from the application of propolis as a complex mixture were found to equal those provoked by the different substances studied here, namely the group of flavonoids like quercetin and hesperidin, as well as CAPE. This is suggesting that the main effects of propolis on the immune functions are really due, at least in part, to these particular compounds. The question arises whether the special composition of propolis with respect to the quality and quantity of these substances does define its particular biological action *in vivo*.

In conclusion, our data provide a rational basis for the beneficial usage of propolis as folk medicine since ancient time. Recently, *anti*-inflammatory properties have been decluced from its *anti*-oxidative, radical scavening, anticomplement activities, and its inhibitory actions on the eicosanoid production and granulocyte function. In this study we have provided evidence that propolis and a selected number of its constituents have a direct regulatory effect on basic immune cell functions, particularly of lymphocytes. Propolis and its constituents were shown to be capable of regulating lymphocyte growth *via* the Erk-2 signal pathway, suppressing proinflammatory and Th1 as well Th2 derived cytokines, and inducing the regulatory T lymphocyte derived TGF-β1. These data confirm that propolis or its constituents can be considered as a suitable powerful natural *anti*-inflammatory medicine.

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