Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 68 (2004) 621–629
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

The flavones luteolin and apigenin inhibit in vitro antigen-specific proliferation and interferon-gamma production by murine and human autoimmune T cells

Richard Verbeek[a], Arianne C. Plomp[a], Eric. A.F. van Tol[b], Johannes M. van Noort[a,*]

[a]*Division of Biomedical Research, TNO Prevention and Health, P.O. Box 2215, 2301 CE Leiden, The Netherlands*
[b]*Numico Research BV, Bosrandweg 20, 6704 PH Wageningen, The Netherlands*

Received 23 January 2004; accepted 10 May 2004

Abstract

Plant-derived flavonoids are inhibitors of various intracellular processes, notably phosphorylation pathways, and potential inhibitors of cellular autoimmunity. In this study, the inhibiting effects of various flavonoids on antigen-specific proliferation and interferon-gamma (IFN-γ) production by human and murine autoreactive T cells were evaluated in vitro. T-cell responses were evaluated for the human autoantigen alpha B-crystallin, a candidate autoantigen in multiple sclerosis, and for the murine encephalitogen proteolipid protein peptide PLP (139–151).

The flavones apigenin and luteolin were found to be strong inhibitors of both murine and human T-cell responses while fisitin, quercitin, morin and hesperitin, members of the subclasses of flavonoles and flavanones, were ineffective. Antigen-specific IFN-γ production was reduced more effectively by flavones than T-cell proliferation, suggesting that the intracellular pathway for IFN-γ production in T cells is particularly sensitive to flavone inhibition.

These results indicate that flavones but not flavanoles or flavanones are effective inhibitors of the potentially pathogenic function of autoreactive T cells. The effects of flavones were the same for human and murine autoreactive T cells, stressing the usefulness of animal models of autoimmunity for further studies on the effects of flavonones on autoimmune diseases.

Keywords: Flavonoids; T cells; Autoimmune; Multiple sclerosis; Apigenin; Luteolin

1. Introduction

Flavonoids are naturally occurring polyphenolic compounds that are widely found in the plant kingdom. As intrinsic components of fruit, vegetables and beverages such as wine and tea [1] many of the 4000 different flavonoids known to date are part of a regular diet. Flavonoids are composed of two aromatic rings linked through three carbon atoms that form an oxygenated heterocycle. Variations on the basic structure of flavonoids yield different classes of flavonoids [2]. These structural variations may explain the observed differences in the bioactivity of these related compounds. Three of the major subclasses are flavones, flavonoles and flavanones, respectively. The first two are the most commonly occurring flavonoids in plants, while flavanones are especially abundant in citrus fruits [1,2]. The relatively strong dietary representation of these three classes of flavonoids renders them particularly relevant to human health [3,4]. Several epidemiological studies on the effects of flavonoids suggest positive effects on human health. The low incidence of cardiovascular disease in the Mediterranean population, for example, correlates with a high intake of flavonoid-rich food [5]. Similarly positive effects of flavonoids have been reported on coronary disease [6,7] and cancer [8–10]. Biological mechanisms underlying such effects, however, remain to be established.

Flavonoids are best known for their anti-oxidant activities [11], yet they have many more biological effects. Many different enzymes involved in intracellular signaling can be affected by flavonoids [12]. Especially the effects of

Abbreviations: PLP, proteolipid protein; IFN, interferon; IL, interleukin; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis

* Corresponding author. Tel.: +31 715181541; fax: +31 715181901.
E-mail address: JM.vanNoort@pg.tno.nl (J.M. van Noort).

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.05.012

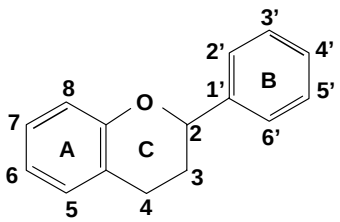
flavonoids on protein kinases are of great interest since they directly influence immune functions in the host. Effects on protein tyrosine kinases such as the CD4-associated $p54^{lck}$ and $p59^{fyn}$, for example, influence T-cell activation, growth and differentiation [12–17].

So far, in vitro effects of flavonoids on T-cell functions have only been studied using aspecific activation of naïve T cells by mitogens as a test system. Depending of the subclasses and structure, flavonoids suppressed concanavalin A-induced and, less effectively, LPS-induced leukocyte proliferation [18,19]. Moreover, T cells stimulated by PMA together with anti-CD28 were suppressed in their proliferation and interleukin (IL)-2 production by flavonoids [20]. Yet, in human disease and especially in autoimmune disease, pivotal control is exerted by memory or memory/effector T cells that become activated in an antigen-specific manner. It is well established that intracellular signaling in antigen-stimulated memory T cells differs from lectin-stimulated naïve T cells in levels and patterns of phosphorylation, kinetics and requirement for costimulatory signals and antigen dosages [13,21–23]. The impact of flavonoids on memory T cells relevant to human autoimmune disease, therefore, remains to be established.

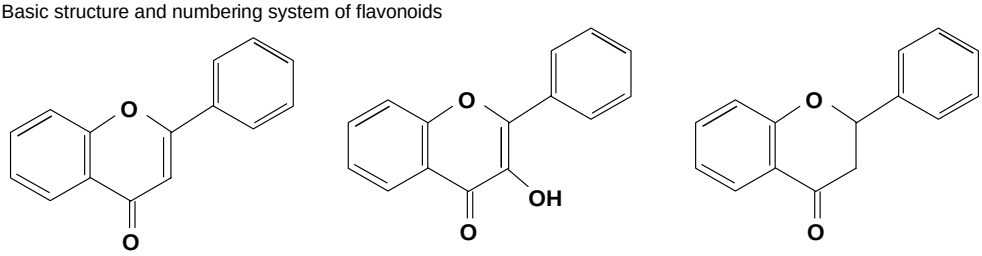
The goal of the present study was to evaluate the in vitro effect of flavonoids on T-cell functions that are considered relevant to autoimmune disease and in particular to multiple sclerosis (MS). MS is a T-cell mediated demyelinating disease of the central nervous system in which neurological dysfunction is most likely caused by autoimmune T-cell responses to central nervous system myelin antigens [24–26]. Both in MS and its animal model counterpart, experimental autoimmune encephalomyelitis (EAE), putative auto-antigens have been identified. Previous research indicates that for human T cells, the myelin-associated protein alpha B-crystallin is a dominant target antigen [27,28]. The secretion of interferon-gamma (IFN-γ) by T cells in response to antigen contributes to the development of disease [29].

EAE is a widely used animal model for MS. By immunizing susceptible strains of mice or rats with appropriate myelin-derived proteins or peptides, autoimmune inflammation in the central nervous system can be induced leading to clinical and pathological features very similar to those observed in MS-patients. Since, in contrast to humans, laboratory animals are generally tolerant for alpha B-crystallin, this antigen cannot be used to induce experimental disease [30,31]. Peptides derived from other myelin proteins are therefore frequently used to induce experimental disease. In SJL mice, one of the most potent experimental antigens to trigger EAE is the peptide fragment 139–151 of proteolipid protein (PLP). Also in this model the production of IFN-γ by antigen-specific autoreactive T cells is considered crucial for the development of disease [32,33].

Human memory T cells against alpha B-crystallin and murine memory T cells against the PLP peptide 139–151 are suitable objects of study and they allow a direct comparison between the effects of flavonoids on autoimmune memory T cells from either humans or mice. In order to examine the impact of flavonoids on these T cells, we selected six different flavonoids belonging to the flavones, flavonoles or flavanones (Fig. 1). They included the



Basic structure and numbering system of flavonoids



flavones							flavonols							flavanones				
substitutes	5	6	7	8	3'	4'		5	7	2'	3'	4'	5'		5	7	3'	4'
luteolin	OH	*	OH	*	OH	OH	quercitin	OH	OH	*	OH	OH	*	hesperitin	OH	OH	OH	OCH_3
apigenin	OH	*	OH	*	*	OH	fisitin	*	OH	*	OH	OH	*					
							morin	OH	OH	OH	*	OH	*					

Fig. 1. Structural characteristics of different flavonoid classes. The figure depicts the basic structure of flavonoids, indicating ring lettering and atom numbering. In the bottom part, structural features are illustrated of the three groups of flavonoids studied here. Flavonoid structures adopted from Middleton and Kandaswami [12].

flavones luteolin and apigenin, the flavonoles fisetin, quercitin and morin, and the flavonone hesperitin.

2. Materials and methods

2.1. Flavonoids

Morin and fisetin were obtained from Indofine (Belle Mead, NJ). Apigenin, luteolin, quercitin and hesperitin were kindly provided by Kaden Biochemicals (Hamburg, Germany).

2.2. Animals

Female SJL/J mice were bred under specific pathogen-free conditions at the facilities of TNO Prevention and Health (Leiden, The Netherlands). All mice were used between ages 8 and 12 weeks.

2.3. Autoreactive human memory lymphocytes

Human peripheral blood monocytes (PBMC) were isolated from whole blood obtained from healthy donors by Histopaque 1077 (Sigma Diagnostics, St. Louis, MO) density gradient centrifugation. Human lymphocytes specific for alpha B-crystallin were obtained by culturing the isolated PBMC in RPMI-1640 (Life Technologies, Paisley, Scotland) in a concentration of 2×10^6 cells per ml supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM glutamax and 5% normal human serum in the presence of 10 μg/ml recombinant human alpha B-crystallin. On days 5 and 8, 10% Lymphocult-T (Biotest Seralc, Zaventem, Belgium) was added to the culture medium and served as source of IL-2 to stimulate T cells to proliferate. At day 10, alpha B-crystallin specific T cells were collected and re-seeded in culture medium at 1×10^5 cells per well in the presence of 2×10^5 autologous irradiated (30 Gy) PBMC, 10 μg/ml human alpha B-crystallin and varying concentrations of flavonoids. After 72 h of culture 100 μl of supernatant was removed for cytokine analysis and T cells were cultured for another 18 h in the presence of 20 kBq [^{3}H]thymidine. The [^{3}H]thymidine incorporation was measured by a betaplate counter (Perkin-Elmer, Turku, Finland).

PBMC were collected from five to seven healthy donors. All experiments were performed in triplicate with T cells from each donor.

2.4. Autoreactive murine memory lymphocytes

SJL mice were immunized s.c. in the flank with 40 μg PLP (139–151) emulsified in complete Freund's adjuvant containing 1 mg/ml *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Detroit, MI). After 10 days, spleen as well as axilliary and inguinal lymph nodes were isolated and spleen cells as well as lymph node cells were seeded at 1×10^5 cells per well in the presence of 2×10^5 syngeneic irradiated (30 Gy) splenocytes from naive mice in 200 μl culture medium supplemented with 100 U/ml penicillin, 100 μg/ml steptomycin, 50 μM 2-mercapthoetanol, 10 mM glutamax and 5% v/v FCS (BioWhittaker, Verviers, Belgium). Also, 10 μg/ml PLP (139–151) and varying concentrations of flavonoids were added at the beginning of the culture. After 72 h of culture 100 μl of supernatant was removed for cytokine analyses and the cells were cultured for another 18 h in the presence of 20 kBq [^{3}H]thymidine. Incorporation of [^{3}H]thymidine was measured by a betaplate counter.

Murine T cells of four different mice were pooled. All experiments were performed in triplicate with at least three different pools of T cells derived from different groups of mice. The use of animals for this study was approved by an animal welfare committee.

2.5. Cytokine analysis

The levels of interleukin-4 and interferon-γ in culture supernatants were determined using commercially available ELISA kits (PharMingen, San Diego, CA). Cytokine production in culture supernatant of lymph node-derived T cells was tested for each individual experiment with T cells stimulated with different flavonoids. With cultured murine spleen and human T cells, the cytokine concentration was determined in pooled culture supernatant for each experimental condition.

2.6. Viability analysis by flowcytometry

To determine levels of cell death and/or apoptosis in T-cell cultures, T cells were harvested at the end of the cultures and stained with specific antibodies against annexin V (Genzyme, Cambridge, MA) to assess apoptosis, or with propidium iodine to determine the percentage of dead cells by flowcytometry using a Facscalibur (Becton Dickson, Mountain View, CA).

2.7. Statistical methods

Statistical analysis was done using SPSS statistical package. One way ANOVA was used followed by a post hoc LSD test for multiple comparisons. In all cases a P-value of <0.05 was considered significant.

3. Results

3.1. Viability of lymphoid cells cultured in the presence of varying doses of flavonoids

Since previous reports indicate that flavonoids may induce apoptosis and in this way, could aspecifically affect

levels of antigen-specific responses in cultured T cells [34], we first examined the influence of flavonoids on T-cell apoptosis and death. After 72 h of culturing T cells in the presence of various concentrations of flavonoids, apoptosis was monitored by flowcytometric analysis for the expression of annexin V. Cell death was assessed by measuring permeability for propidium iodide. The evaluation was performed at the end of a 72-h culture period, the maximum assay time used in our studies, thus reflecting the maximum effect of flavonoids on T-cell viability. The results revealed that the concentration range of up to 35 μM for all flavonoids tested was appropriate to study effects on T-cell functions without compromising cell vitality (Fig. 2).

3.2. Effects of flavonoids on antigen-specific T-cell proliferation

A marked difference was observed between the abilities of the three main groups of flavonoids to change the responses by T cells, as illustrated in Fig. 3. Proliferative responses of murine lymph node-derived T cells to PLP (139–151) were markedly reduced in the presence of even low concentrations of luteolin and apigenin (Fig. 3a). At 35 μM of flavones, this response was reduced by well over 50%. In contrast, the flavonoles and flavanones had no clear effect on T-cell proliferation (Fig. 3b and c). Only quercitin was found to have some inhibiting effects, however only at the highest dose tested.

When the effects of flavonoids were measured on antigen-specific responses by murine spleen-derived T cells, none of the flavonoids had any inhibitory activity (Fig. 3d–f). The difference between the effects of the flavonoids on either lymph node-derived T cells and spleen-derived T cells of immunized mice possibly relates to a difference in the cellular composition of either lymphoid organ, with memory T cells being more abundant in lymph nodes. Conceivably, the presence of significant numbers of B cells and macrophages in splenocyte cultures reduces the amounts of flavonoids available to T cells in the same culture. Also the percentage of T cells in the spleen capable of reacting to PLP (139–151) stimulation is lower than in lymph node-derived T cells since lymph node-derived T cells react much more vigorously to PLP (139–151) stimulation in absence of flavonoids.

Using PBMC from healthy donors, human T cells against the myelin protein alpha B-crystallin were similarly examined for their antigen-specific proliferative response in the presence of increasing concentrations of flavonoids. Previously, we have demonstrated that PBMC-derived T cells responsive to alpha B-crystallin express high levels of CD45 RO, classifying them as memory T cells [28]. Similar to our studies in mice, the flavones apigenin and luteolin markedly inhibited antigen-specific responses in a dose-dependent manner. In the presence of 35 μM apigenin, responses were reduced with about 16%, and with luteolin inhibition reached levels of about 23% (Fig. 3g). The flavonoles morin and fistin did not exert such inhibitory effects. Only the flavonole quercitin and the flavanone hesperitin were found to lead to some inhibition but only at the highest dose tested (Fig. 3h and i). Overall, therefore, the effects of flavonoids on antigen-specific responses by human T cells very closely parallel those on murine lymph node-derived T-cell responses.

3.3. Effects of flavonoids on antigen-induced IFN-γ release

For the effector function of T cells, antigen-induced cytokine production is at least as important as their proliferative response. In MS and EAE, production of IFN-γ by autoantigen-specific T-cells is of prime importance for the development of clinical disease. Murine T cells against PLP (139–151) as well as human T cells specific for alpha B-crystallin are known to produce much more IFN-γ in response to their antigen than IL-4 or IL-10 [28,36–38]. In agreement with these data, no significant levels of IL-4 or IL-10 could be detected in any of our T-cell cultures after addition of the appropriate autoantigen (data not shown). In contrast, IFN-γ production in response to the autoantigen was substantial for both human and murine T cells.

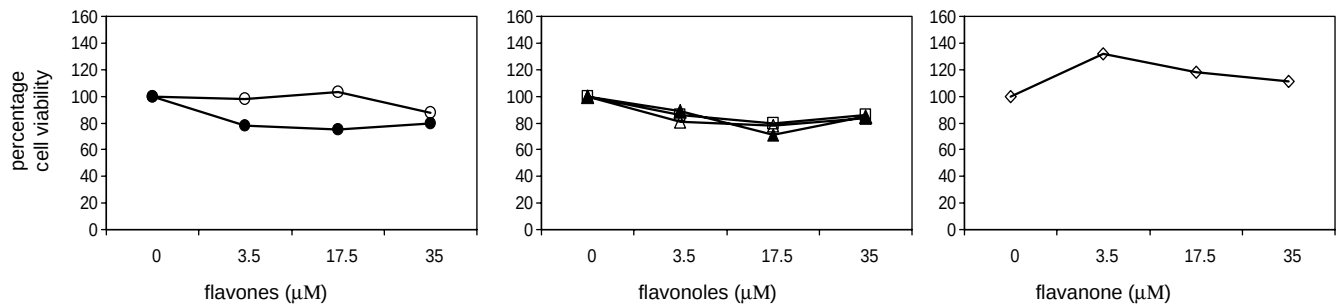


Fig. 2. The effect of flavonoids on T-cell viability and apoptosis. In order to select a range of flavonoid concentrations that do not affect cell viability, levels of apoptosis and cell permeability were evaluated after culturing lymphocytes in the presence of flavonoids. After 72 h of culture, apoptosis was evaluated by flowcytometric analysis of annexin V expression, and cell permeability was assessed by propidium iodide staining. Viable cells are expressed as percentage living cells in the control. Depicted are the effects of luteolin (●), apigenin (○), fisetin (▲), morin (□), quercitin (△), hespertin (◇).

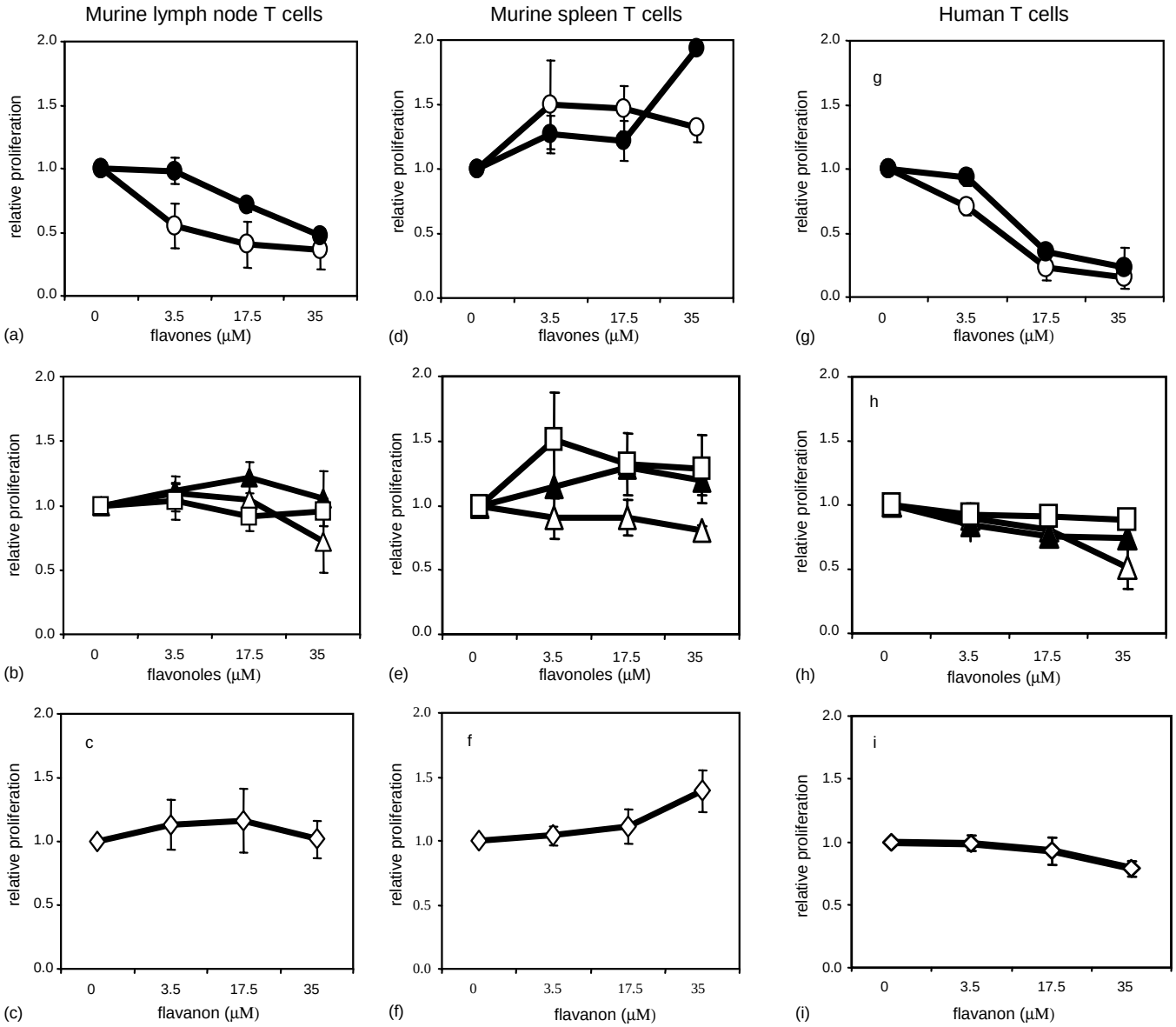


Fig. 3. The effects of flavonoids on antigen-triggered proliferation of murine and human T cells. Mice were immunized with PLP (139–151) and after 10 days, lymph node- and spleen-derived T cells were isolated and cultured in the presence of 10 μg/ml antigen and the indicated dose of flavonoids. Human T cells were obtained by priming human peripheral blood T cells with 10 μg/ml recombinant human alpha B-crystallin. After 10 days of culture, responsive T cells were harvested and reseeded in the presence of antigen along with varying concentrations of flavonoids. Proliferation of both murine and human T cells was measured after 3 days and expressed as the percentage of proliferation observed in control cultures in the absence of any flavonoids. Background responses of T-cell proliferation in absence of antigen were negligible (<400 counts per minute (cpm)) in comparison with the control response. Control responses are around 12,000 cpm for lymph node-derived T cells and 3000 cpm for spleen cells-derived T cells. The background levels of proliferation and the control response for human T cells are comparable with spleen-derived T cells. Depicted are the effects of luteolin (●), apigenin (○), fisetin (▲), morin (□), quercitin (△), hespertin (◇). Apigenin and luteolin both reduce the proliferation significantly ($P < 0.05$) in both murine lymph node-derived T cells (a) and human T cells (g).

Interestingly, the effect of flavonoids on antigen-induced IFN-γ production was found to closely parallel the effects on proliferative responses, but they appeared to be more pronounced. Even at a concentration of apigenin as low as 3.5 μM, IFN-γ production by murine lymph node-derived T cells was found to be reduced by about 40%. IFN-γ production was completely blocked at 35 μM luteolin and reduced by 65% with apigenin (Fig. 4a). Also two flavonoles, viz. fisetin and quercitin, exerted clearly detectable inhibitory effects up to about 50% at doses of 35 μM (Fig. 4b). Morin and hespertin remained ineffective in influencing IFN-γ production at all doses tested.

In line with the more pronounced impact of flavonoids on IFN-γ production as compared with proliferative responses, also spleen-derived T cells were sensitive in their antigen-induced IFN-γ production to several flavonoids (Fig. 4d–f). Flavones reduced this production with about 50% at the highest doses tested. Similar effects were found for the flavonole quercitin, which completely blocked IFN-γ production, and for fisetin. The flavonole morin and the flavanone

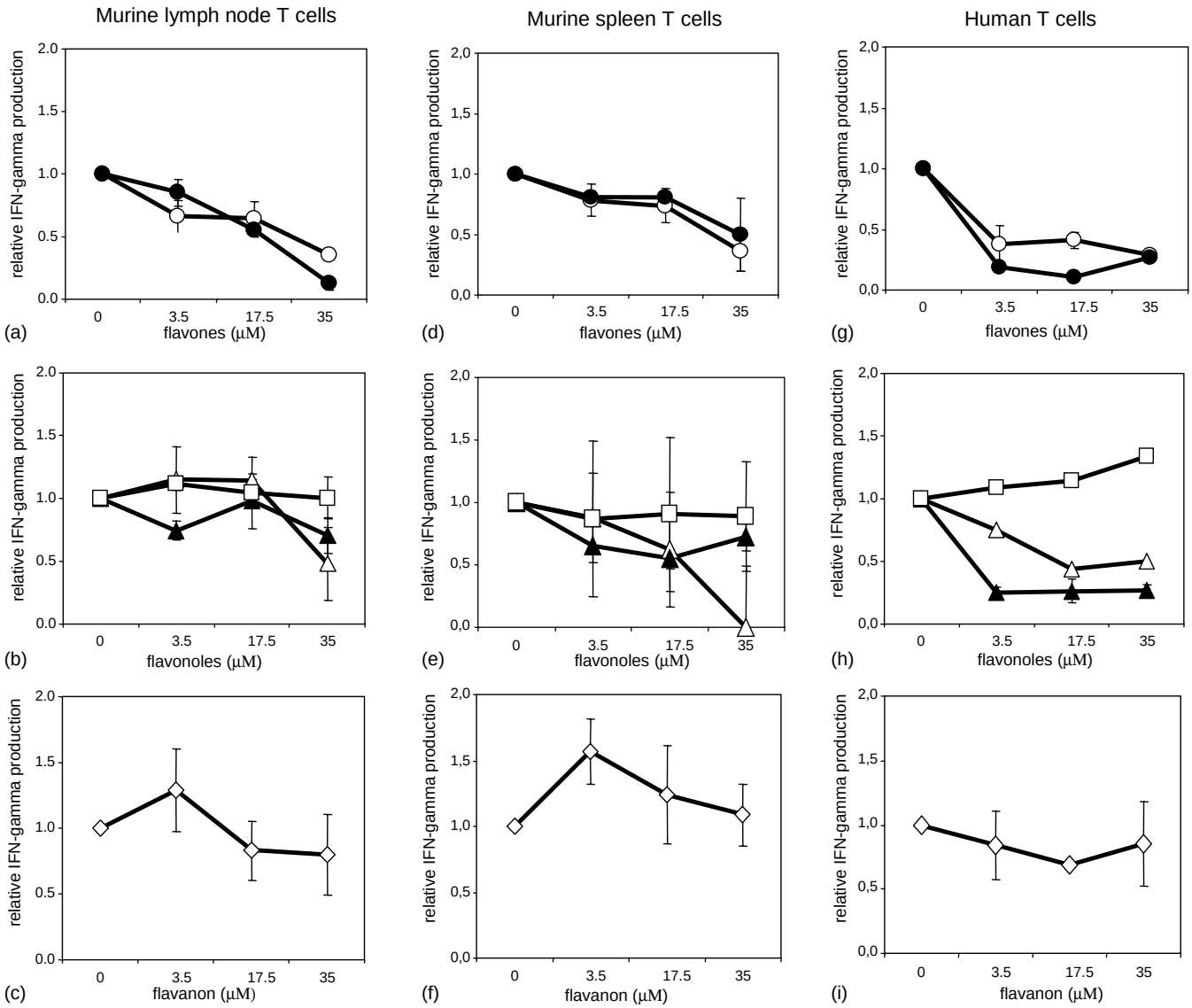


Fig. 4. The effects of flavonoids on antigen-triggered IFN-γ production by murine and human T cells. Mice were immunized with PLP (139–151) and after 10 days, lymph node- and spleen-derived T cells were isolated and cultured in the presence of 10 μg/ml antigen and the indicated dose of flavonoids. Human T cells were obtained by priming human peripheral blood T cells with 10 μg/ml recombinant human alpha B-crystallin. After 10 days of culture, responsive T cells were harvested and reseeded in the presence of antigen along with varying concentrations of flavonoids. Culture supernatants of both murine and human T cells were harvested 3 days following antigen stimulation, and the amounts of IFN-γ in the supernatants were determined by ELISA. These amounts are expressed as the percentage of IFN-γ found in supernatants of control cultures in the absence of any flavonoids. Background production of IFN-γ by T cells cultures in absence of antigen are negligible (<10 pg/ml). IFN-γ production by control cultures of T cells is around 2200 pg/ml for lymph node-derived T cells and 5000 pg/ml for spleen cells-derived T cells. Background levels of IFN-γ production and the IFN-γ production by control cultures of human T cells are comparable with lymph node-derived T cells. Depicted are the effects of luteolin (●), apigenin (○), fisetin (▲), morin (□), quercitin (△), hespertin (◇). Both apigenin and luteolin reduce the IFN-γ production significantly ($P < 0.05$) in murine-derived lymph node T cells (a). Quercitin reduce significantly the IFN-γ production in murine-derived spleen cells (e) and apigenin, luteolin, fisitin reduce significantly the IFN-γ production in human T cells (g–h).

hesperitin remained ineffective in inhibiting antigen-induced IFN-γ production in murine spleen-derived T cells.

Similarly, in human T cells, antigen-induced IFN-γ production was more strongly affected by flavonoids than the proliferative responses (Fig. 3g–i). In this case it was found that already at low concentrations flavones reduced IFN-γ production by well over 50%. Where flavonoles fisetin and quercitin were found to inhibit the IFN-γ production, the other flavonole tested, viz. morin, remained ineffective. The flavanone hesperitin only slightly reduced IFN-γ production. Again, therefore, the effects of the different flavonoids on human antigen-specific T cells were essentially the same as compared to murine T cells.

4. Discussion

In this study, we have established the effects of flavonoids on antigen-specific responses of both murine and human autoimmune T cells. Essentially different

intracellular pathways control antigen-induced responses in T cells as compared to mitogen-activated naive T cells, involving different phosphorylation cascades and transcription factors [39,40]. Since T cells are crucial for the development of human autoimmune disease, a focus on this type of cell provides relevant information on the possible therapeutic application of flavonoids in human autoimmune disease. In our study, we have focused on T cells reactive with autoantigens in mice and humans that have a documented relevance to autoimmune demyelination.

The main result of our study is that the flavones luteolin and apigenin but not the flavonoles or flavanones tested are potent inhibitors of antigen-triggered proliferative responses by autoreactive T cells. Also the IFN-γ secretion is strongly inhibited by flavones and to a lesser degree by flavonoles. The same response profile was observed for murine and human T cells, underpinning the usefulness of murine studies for evaluating possible applications of flavonoids in human disease. At doses of flavonoids that do not detectably affect cellular viability in culture, IFN-γ production was more strongly affected than proliferative responses for both murine and human T cells, suggesting that IFN-γ production relies on signaling cascades that are relatively sensitive to flavonoid inhibition. Yet, the flavonoids that provide the strongest inhibition of IFN-γ production are the same that inhibit proliferation as well. Of obvious relevance is the question how the flavonoid concentrations applied in our study compare to those that can be found in biological fluids such as plasma or serum following normal intake of flavonoids. Unfortunately, current bioavailability studies on flavonoids are limited and far from conclusive. Flavonoids absorption in the gastrointestinal tract is influenced by several factors such flavonoid structure, level and type of flavonoid glycosylation, dietary source, and the nature of the gut microflora [41]. When flavonoids are ingested as part of a regular diet, serum concentrations of the original flavonoid rarely exceed 1 μM [42–44]. When ingested as pure compounds, serum levels can exceed this concentration [45–47]. A major confounding factor in bioavailability studies, however, is the formation of biologically active metabolites derived from flavonoids during absorption as well as by the action of the gut microflora. Exactly which metabolites may be formed from each flavonoid is not fully known. Consequently, it is not fully known which biologically active metabolites and how much of these may be present to complement the function of the original unmodified flavonoid in biological fluids. It has been repeatedly found that the total antioxidant capacity of serum after flavonoid intake, for example, can vastly exceed the levels that would be expected based solely on the amount of the original flavonoid detectable in it [43,48]. Serum levels of about 1 μM of flavonoids after normal dietary intake should therefore be considered as a minimum level, with an unknown range of similarly active metabolites possibly complementing this level.

Several flavonoids including apigenin, luteolin, fisetin, hesperitin and quercitin have previously been examined for their ability to reduce mitogen-induced proliferative responses in naive T cells [18,35]. These studies have clarified inhibitory effects of not only apigenin but also quercitin, fisetin and hesperitin on proliferation of cytotoxic T cells and their killing of targets. Quercitin is also known to arrest human leukemic T cells in cell cycle [16]. In the present study we focused on autoantigen-specific T cells demonstrating significant inhibitory effects only by the flavones luteolin and apigenin. The fact that a more restricted selection of flavonoids appears to be effective in inhibiting T cells is in line with the notion that these cells are more readily triggered than naive cells, requiring lower levels of costimulation and less antigen [21,22]. Also, intracellular signaling pathways linked to receptor triggering in T cells are different from naive T cells. They involve less overall phosphorylation and an almost complete lack of tyrosine kinase ZAP-70 phosphorylation, reduced SLP-76 expression and decreased calcium flux [13,23,39,40]. These differences in phosphorylation patterns are of particular relevance given the well-known ability of flavonoids to inhibit protein phosphorylation and hence, effector functions of immune cells including macrophages, mast cells and lymphocytes. The differential effects of flavonoids on T cells as compared to naive T cells may well be associated with this difference in phosphorylation signalling steps during activation.

Structural features of luteolin and apigenin, the most potent inhibitors of T cells, include the presence of a double bond in the C ring between C-2 and C-3 together with an absence of a hydroxyl group at C-3 (Fig. 1). Flavonoles, bearing a hydroxyl group at the C-3 position are known to inhibit the functional activation of naive T cells, but are apparently much less active in inhibiting T cells. Interestingly, the structural features that typify the most potent flavonoid inhibitors of T cells in our study are different from the structural features of the more potent flavonoid inhibitors of for example, xanthine oxidase [49,50], multi-drug transporter [51], cytochrome P450 [52] or HIV integrase functions [53]. This confirms that detailed structural features endow flavonoids with a distinct level of selectivity in their biological effects. Based on such selectivities, other flavonoids may be identified with similar or perhaps even more potent activities against T cells that may help further examine the possibility to utilize flavonoids as immune-suppressive agents in the treatment of autoimmune disorders. Our data suggest that animal model approaches in this respect are valid in the apparent absence of species-specific effects.

References

[1] Hertog MG, Hollman PC, Katan MB, Kromhout D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. Nutr Cancer 1993;20:21–9.

[2] Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 1998;56:317–33.
[3] Hollman PC, Katan MB. Dietary flavonoids: intake, health effects and bioavailability. Food Chem Toxicol 1999;37:937–42.
[4] Hollman PC, Katan MB. Health effects and bioavailability of dietary flavonols. Free Radic Res 1999;31(Suppl):S75–80.
[5] Formica JV, Regelson W. Review of the biology of quercetin and related bioflavonoids. Food Chem Toxicol 1995;33:1061–80.
[6] Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993;342:1007–11.
[7] Knekt P, Jarvinen R, Reunanen A, Maatela J. Flavonoid intake and coronary mortality in Finland: a cohort study. Br Med J 1996;312: 478–81.
[8] Wenzel U, Kuntz S, Brendel MD, Daniel H. Dietary flavone is a potent apoptosis inducer in human colon carcinoma cells. Cancer Res 2000;60:3823–31.
[9] Knekt P, Jarvinen R, Seppanen R, Hellovaara M, Teppo L, Pukkala E, et al. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am J Epidemiol 1997;146:223–30.
[10] Wang HK. The therapeutic potential of flavonoids. Exp Opin Invest Drugs 2000;9:2103–19.
[11] Huk I, Brovkovych V, Nanobash VJ, Weigel G, Neumayer C, Partyka L, et al. Bioflavonoid quercetin scavenges superoxide and increases nitric oxide concentration in ischaemia-reperfusion injury: an experimental study. Br J Surg 1998;85:1080–5.
[12] Middleton EJ, Kandaswami C. Effects of flavonoids on immune and inflammatory cell functions. Biochem Pharmacol 1992;43:1167–79.
[13] Farber DL. T cell memory: heterogeneity and mechanisms. Clin Immunol 2000;95:173–81.
[14] Robak J, Gryglewski RJ. Bioactivity of flavonoids. Pol J Pharmacol 1996;48:555–64.
[15] Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA. Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 2001;74: 418–25.
[16] Yoshida M, Yamamoto M, Nikaido T. Quercetin arrests human leukemic T-cells in late G1 phase of the cell cycle. Cancer Res 1992;52:6676–81.
[17] Schwartz A, Sutton SL, Middleton EJ. Quercetin inhibition of the induction and function of cytotoxic T lymphocytes. Immunopharmacology 1982;4:125–38.
[18] Lee SJ, Choi JH, Son KH, Chang HW, Kang SS, Kim HP. Suppression of mouse lymphocyte proliferation in vitro by naturally-occurring biflavonoids. Life Sci 1995;57:551–8.
[19] Namgoong SY, Son KH, Chang HW, Kang SS, Kim HP. Effects of naturally occurring flavonoids on mitogen-induced lymphocyte proliferation and mixed lymphocyte culture. Life Sci 1994;54:313–20.
[20] Atluru S, Atluru D. Evidence that genistein, a protein-tyrosine kinase inhibitor, inhibits CD28 monoclonal-antibody-stimulated human T cell proliferation. Transplantation 1991;51:448–50.
[21] Jenkins MK, Khoruts A, Ingulli E, Mueller DL, McSorley SJ, Reinhardt RL, et al. In vivo activation of antigen-specific CD4 T cells. Annu Rev Immunol 2001;19:23–45.
[22] Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science 2000;290:92–7.
[23] Hussain SF, Anderson CF, Farber DL. Differential SLP-76 expression and TCR-mediated signaling in effector and memory CD4 T cells. J Immunol 2002;168:1557–65.
[24] Noseworthy JH. Progress in determining the causes and treatment of multiple sclerosis. Nature 1999;399(6738 Suppl):A40–7.
[25] Wingerchuk DM, Lucchinetti CF, Noseworthy JH. Multiple sclerosis: current pathophysiological concepts. Lab Invest 2001;81:263–81.
[26] Wekerle H. Immune pathogenesis of multiple sclerosis. Brain autoimmune reactivity and its control by neuronal function. Mult Scler 1998;4:136–7.
[27] van Noort JM, van Sechel AC, Bajramovic JJ, el Ouagmiri M, Polman CH, Lassmann H, et al. The small heat-shock protein alpha B-crystallin as candidate autoantigen in multiple sclerosis. Nature 1995;375: 798–801.
[28] van Noort JM, Bajramovic JJ, Plomp AC, van Stipdonk MJ. Mistaken self, a novel model that links microbial infections with myelin-directed autoimmunity in multiple sclerosis. J Neuroimmunol 2000; 105:46–57.
[29] Panitch HS, Bever CTJ. Clinical trials of interferons in multiple sclerosis. What have we learned? J Neuroimmunol 1993;46: 155–64.
[30] van Stipdonk MJ, Willems AA, Plomp AC, van Noort JM, Boog CJ. Tolerance controls encephalitogenicity of alpha B-crystallin in the Lewis rat. J Neuroimmunol 2000;103:103–11.
[31] van Stipdonk MJ, Willems AA, Verbeek R, Boog CJ, van Noort JM. T- and B-cell nonresponsiveness to self-alpha B-crystallin in SJL mice prevents the induction of experimental allergic encephalomyelitis. Cell Immunol 2000;204:128–34.
[32] Swanborg RH. Experimental autoimmune encephalomyelitis in the rat: lessons in T-cell immunology and autoreactivity. Immunol Rev 2001;184:129–35.
[33] Renno T, Lin JY, Piccirillo C, Antel J, Owens T. Cytokine production by cells in cerebrospinal fluid during experimental allergic encephalomyelitis in SJL/J mice. J Neuroimmunol 1994;49:1–7.
[34] Lee WR, Shen SC, Lin HY, Hou WC, Yang LL, Chen YC. Wogonin and fisetin induce apoptosis in human promyeloleukemic cells, accompanied by a decrease of reactive oxygen species, and activation of caspase 3 and Ca(2+)-dependent endonuclease. Biochem Pharmacol 2002;63:225–36.
[35] Schwartz A, Middleton EJ. Comparison of the effects of quercetin with those of other flavonoids on the generation and effector function of cytotoxic T lymphocytes. Immunopharmacology 1984;7: 115–26.
[36] Polak T, Schlaf G, Scholl U, Krome-Cesar C, Mader M, Felgenhauer K, et al. Characterization of the human T cell response against the neuronal protein synapsin in patients with multiple sclerosis. J Neuroimmunol 2001;115:176–81.
[37] Rohowsky-Kochan C, Molinaro D, Cook SD. Cytokine secretion profile of myelin basic protein-specific T cells in multiple sclerosis. Mult Scler 2000;6:69–77.
[38] Di Rosa F, Francesconi A, Di Virgilio A, Finocchi L, Santilio I, Barnaba V. Lack of Th2 cytokine increase during spontaneous remission of experimental allergic encephalomyelitis. Eur J Immunol 1998; 28:3893–903.
[39] Hall SR, Heffernan BM, Thompson NT, Rowan WC. CD4+ CD45RA+ and CD4+ CD45RO+ T cells differ in their TCR-associated signaling responses. Eur J Immunol 1999;29:2098–106.
[40] Dubey C, Croft M, Swain SL. Naive and effector CD4 T cells differ in their requirements for T cell receptor versus costimulatory signals. J Immunol 1996;157:3280–9.
[41] Erlund I, Kosonen T, Alfthan G, Maenpaa J, Perttunen K, Kenraali J, et al. Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. Eur J Clin Pharmacol 2000;56: 545–53.
[42] Nielsen SE, Young JF, Daneshvar B, Lauridsen ST, Knuthsen P, Sandstrom B, et al. Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion. Br J Nutr 1999;81:447–55.
[43] Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J Nutr 2000;130:2073S–85S.
[44] Ameer B, Weintraub RA, Johnson JV, Yost RA, Rouseff RL. Flavanone absorption after naringin, hesperidin, and citrus administration. Clin Pharmacol Ther 1996;60:34–40.
[45] Hollman PC, Bijsman MN, van Gameren Y, Cnossen EP, de Vries JH, Katan MB. The sugar moiety is a major determinant of the absorption

of dietary flavonoid glycosides in man. Free Radic Res 1999; 31(6):569–73.

[46] Graefe EU, Wittig J, Mueller S, Riethling AK, Uehleke B, Drewelow B, et al. Pharmacokinetics and bioavailability of quercetin glycosides in humans. J Clin Pharmacol 2001;41:492–9.

[47] Manach C, Morand C, Texier O, Favier ML, Agullo G, Demigne C, et al. Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. J Nutr 1995;125:1911–22.

[48] Rice-Evans C. Flavonoid antioxidants. Curr Med Chem 2001;8: 797–807.

[49] Cos P, Ying L, Calomme M, Hu JP, Cimanga K, Van Poel B, et al. Structure–activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. J Nat Prod 1998;61:71–6.

[50] van Hoorn DE, Nijveldt RJ, van Leeuwen PA, Hofman Z, M'Rabet L, De Bont DB, et al. Accurate prediction of xanthine oxidase inhibition based on the structure of flavonoids. Eur J Pharmacol 2002;451: 111–8.

[51] Di Pietro A, Conseil GJM, Dayan G, Baubichon-Cortay H, Trompier D, Steinfels E, et al. Modulation by flavonoids of cell multidrug resistance mediated by P-glycoprotein and related ABC transporters. Cell Mol Life Sci 2002;59:307–22.

[52] Williamson G, Faulkner K, Plumb GW. Glucosinolates and phenolics as antioxidants from plant foods. Eur J Cancer Prev 1998;7:17–21.

[53] Fesen MR, Pommier Y, Leteurtre F, Hiroguchi S, Yung J, Kohn KW. Inhibition of HIV-1 integrase by flavones, caffeic acid phenethyl ester (CAPE) and related compounds. Biochem Pharmacol 1994;48(3): 595–608.