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6048 *Current Pharmaceutical Design*, 2014, *20*, 6048-6057

Activated Immune System and Inflammation in Healthy Ageing: Relevance for Tryptophan and Neopterin Metabolism

Lucile Capuron[1], Simon Geisler[2], Katharina Kurz[3], Friedrich Leblhuber[4], Barbara Sperner-Unterweger[5] and Dietmar Fuchs[2*]

[1]*Laboratory of Nutrition and Integrative Neurobiology, NutriNeuro, UMR INRA 1286, University Victor Segalen Bordeaux 2, Bordeaux, Cedex, France;* [2]*Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Center for Chemistry and Biochemistry, Innsbruck, Austria;* [3]*Department of Internal Medicine, Medical University, Innsbruck, Austria;* [4]*Neurologic Clinic Wagner-Jauregg, Linz, Austria;* [5]*Clinic for Biological Psychiatry, Department of Psychiatry and Psychotherapy, Medical University, Innsbruck, Austria*

Abstract: Immune activation not only accompanies inflammation in various disorders including infections, autoimmune syndromes and cancer, but it also represents a characteristic feature of ageing. Immune deviations which are most widely expressed in the elderly include increased neopterin production and tryptophan breakdown. These biochemical events result from the activation of the immune system and are preferentially triggered by pro-inflammatory stimuli, such as the Th1-type cytokine interferon-γ. They seem to play a role in the development of several age-related disorders and might be involved in the pathogenesis of common symptoms, including neurobehavioral disorders (e.g., cognitive and mood disturbances), anemia, cachexia, weight-loss but also immunodeficiency. Concentrations of the biomarkers neopterin and Kyn/Trp were found to be predictive of overall disease specific mortality in coronary artery disease, infections and various types of cancer. Immune activation and inflammation are also accompanied by high output of reactive oxygen species and thereby may lead to the development of oxidative stress and contribute to the vitamin deficiency which is often observed in the elderly. Accordingly, increases in neopterin were found to correlate with a substantial decline in key vitamins, including folate and vitamin-B_6, -B_{12}, -C, -D and -E.

Keywords: Aging, neopterin, tryptophan breakdown, inflammation, antioxidants, vitamins.

INTRODUCTION

Older age is associated with an increased frequency of chronic diseases such as cardiovascular and neurodegenerative disorders, including dementias, but also autoimmune syndromes like arthritis. Malignancies, cataract, osteoporosis, and type 2 diabetes are conditions that are also highly prevalent in the elderly. Aspects of immune dysregulation and immunodeficiency may underlie these effects, and one hallmark of the ageing process is the decrease in immunocompetence. This phenomenon can be involved in the pathogenesis of various age-related disorders [1, 2], which may account for the increased incidence of infections and cancer, autoimmune diseases, cardiovascular and neurodegenerative disorders in the elderly. Aside from immunodeficiency, signs of an activated immune system have been also demonstrated in older-aged persons. Consistent with this notion, several studies have reported increased concentrations of neopterin in older age [3-10]. In addition, an association between increased neopterin concentrations and enhanced tryptophan breakdown, as indicated by the kynurenine to tryptophan ratio (Kyn/Trp), has been documented in older aged persons [8, 10, 11]. Interestingly, these alterations were found to predict survival in nonagenarians [8, 11]. The view that chronic inflammation developing with older age may play a particular role on life expectancy in the healthy elderly is further underlined by recent studies in rats that demonstrated that immunosuppression with mTOR inhibitor rapamycin was able to significantly prolong life-span [12]. Similar observations have been reported in animals fed with stilben resveratrol [12]. Aside from that, both the compounds were found earlier to suppress neopterin production and tryptophan breakdown in human peripheral blood mononuclear cells (PBMC) *in vitro* [14, 15].

*Address correspondence to this author at the Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Center for Chemistry and Biochemistry, Innsbruck, Austria; Tel: ++43 512 9003 70350;
Fax: ++43 512 9003 73330; E-mail: dietmar.fuchs@i-med.ac.at

In this article, the relevance of the association of inflammation and immune activation for the development of chronic diseases will be discussed. In attention, the parallel induction of neopterin formation and tryptophan breakdown in human PBMC by Th1-type cytokine interferon-γ (IFN-γ) as well as its clinical correlates will be presented. Particular attention will be given to clinical conditions which are observed with higher frequencies in elderly individuals, such as cardiovascular and neurodegenerative disorders. The associations of chronic immune activation, oxidative stress and vitamin depletion with the development of moderate hyperhomocysteinemia as well as disturbed activity of phenylalanine hydroxylase (PAH) will be described. Finally, possibilities to interfere with the chronic inflammatory status in healthy elderly will be highlighted.

IMMUNOBIOLOGICAL BACKGROUND OF NEOPTERIN PRODUCTION AND INDOLEAMINE 2,3-DIOXYGENASE (IDO) ACTIVITY

When human monocyte-derived macrophages and dendritic cells are stimulated with the Th1-type cytokine IFN-γ, cells produce and release increased amounts of pteridine derivative neopterin [16, 17]. Accordingly, increased neopterin concentrations are indicative of cellular immune activation and are well documented in various clinical conditions such as viral infections, malignancies and autoimmune pathologies like rheumatoid arthritis. Neopterin concentrations reflect the disease activity and moreover, they were shown to predict the outcome of patients with cardiovascular diseases, HIV-1 infection and various types of cancer [18-20].

IFN-γ is produced and released during pro-inflammatory immune response from type 1 T-helper cells. This cytokine strongly induces the enzyme GTP cyclohydrolase-I (GCH, EC 3.5.4.16) [16] (Fig. **1**) that is required for antimicrobial effects of IFN-γ, but also for the biosynthesis of the monoamines serotonin, noradrenaline (norepinephrine) and adrenaline (epinephrine). GCH initiates the conversion of GTP to 5,6,7,8-tetrahydrobiopterin (BH_4), which is the cofactor of the aromatic amino acid monooxygenases phenyla-

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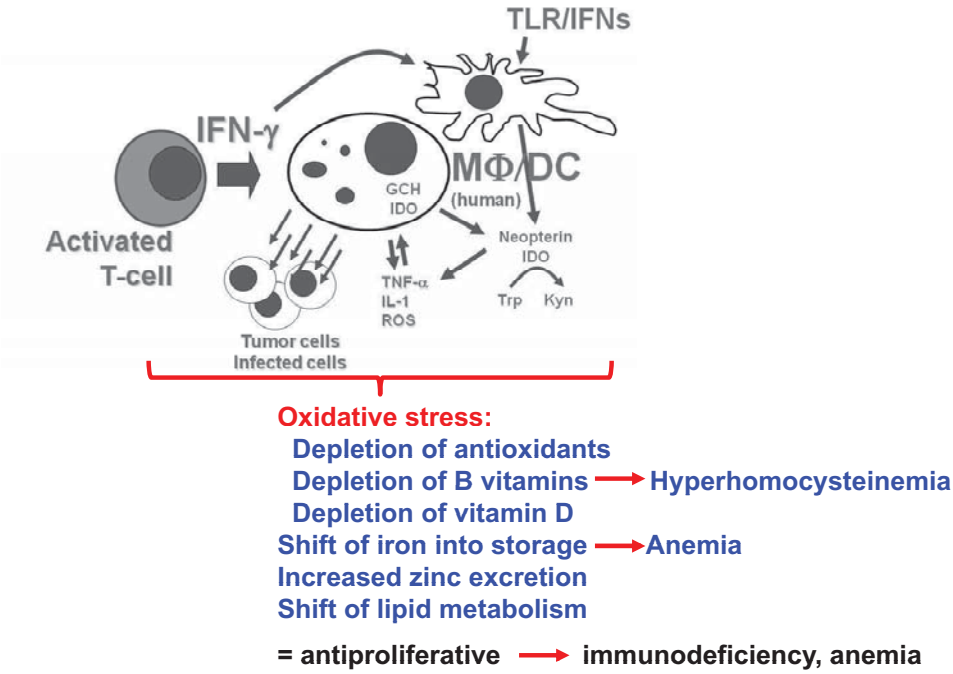

Fig. (1). During innate and adaptive immune responses, Th1-type cytokine interferon-γ (IFN-γ) is released from activated T-cells and induces the production of neopterin by GTP-cyclohydrolase I (GCH) and tryptophan breakdown by indoleamine 2,3-dioxygenase (IDO) in human monocyte-derived macrophages (MΦ) and dendritic cells (DC). Both the pathways can also be stimulated by other interferons and lipopolysaccharide via toll-like receptor (TLR) and are paralleled by the formation of other pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) and by high output of reactive oxygen species (ROS). All these biochemical events are devoted to arrest unwanted proliferation of infected and malignant tumor cells. Large-scale production of ROS may wipe out antioxidant pools including several vitamins and detoxifying enzymes, thus causing oxidative stress. Other antiproliferative activities include the shift of iron from circulation into storage, restriction of Zn by enhanced excretion and shift of lipid metabolism. The development of weight loss/cachexia, immunodeficiency and anemia are side effects of this immune system response.

lanine 4-hydroxylase (EC 1.14.13.39; PAH), tyrosine 5-hydroxylase (EC 1.14.16.2), tryptophan 5-hydroxylase (EC 1.14.16.4) and nitric oxide (NO) synthases (EC 1.14.13.39) [21] and glycerylether monoxygenase (GEMO) [22]. Most of these enzymes play an important role in the biosynthesis of neurotransmitters, and only the conversion of arginine to nitric oxide ($NO^{\cdot}$) by NO-synthases seems relevant for immunological purposes [23]. Through the activation of GCH, the cytokine IFN-γ enhances BH_4 availability for inducible $NO^{\cdot}$ synthase (NOS) and gaseous $NO^{\cdot}$ which combines with superoxide anion ($O_2^{\cdot-}$) to toxic peroxynitrite ($ONOO^-$). However, human monocyte derived-macrophages and dendritic cells are deficient in pyruvoyl tetrahydropterin synthase, which is responsible for the conversion of intermediate 7,8-dihydroneopterin triphosphate to sepiapterin and BH_4 [21]. Therefore once stimulated with IFN-γ, human monocytes/macrophages produce neopterin at the expense of BH_4. Only the stimulated human and primate monocyte-derived cells produce and release relevant amounts of neopterin whereas other human cells and cells from other species produce BH_4 [24] (Fig. **2**).

In parallel to the production of neopterin, IFN-γ enhances the expression of indoleamine 2,3-dioxygenase (IDO) (Fig. **1**). IDO activity and tryptophan breakdown can be detected not only in human monocytic cells [24, 25] but also in other human cells and cells from other species [17, 27, 28] upon treatment with inflammatory stimuli. IDO degrades tryptophan to form N-formyl-kynurenine which rapidly decomposes to kynurenine [28, 29], and the ratio of kynurenine to its substrate tryptophan (Kyn/Trp) provides a useful estimate of IDO activity and endogenously formed IFN-γ *in vitro* [30]. Therefore, in clinical conditions which go along with immune activation, Kyn/Trp together with concentrations of neopterin reflect the degree of Th1-type immune activation [29, 31]. Like liver cells, human monocytes/macrophages were demonstrated to further convert kynurenine to the down-stream product quinolinic acid [32] and elevated serum and cerebrospinal fluid quinolinic acid concentrations have been documented in patients suffering from diseases which are characterized also by elevated neopterin and Kyn/Trp concentrations [33, 34]. The tryptophan metabolic changes were found to parallel the disease course of HIV-1 infection or cancer as it is known for neopterin concentrations, and also to predict outcome, e.g., a low serum tryptophan concentration being the strongest predictor of death in patients with malignant melanoma [35].

In sum, increases in neopterin and Kyn/Trp may reflect a sort of "*Chronic Immune Activation Syndrome*" [30] characterized by the activation of macrophages within the Th1-type immune response. Pro-inflammatory cytokines like IFN-γ seem to play a major role triggering directly neopterin production and tryptophan degradation. The stimulation of GCH and IDO by IFN-γ is further up-regulated by other pro-inflammatory stimuli, such as TNF-α and lipopolysaccharide [36]. However, alterations of Kyn/Trp are not always due to altered IDO activity. When there is no correlation between Kyn/Trp and the concentrations of an immune activation marker like neopterin, the abnormal Kyn/Trp may relate to an enhanced activity of tryptophan 2,3-dioyxgenase (TDO), an isoenzyme of IDO which is not induced by pro-inflammatory cytokine but is rather up-regulated by tryptophan itself and corticosteroids [28, 37].

To date, the monitoring of Kyn/Trp is not yet available via immunoassays and therefore chromatographic methods like high pressure liquid chromatography (HPLC) with ultraviolet and fluorescence monitoring [38] or combined liquid chromatography mass spectrometry (LC-MS) are successfully applied [39]. As observed *in vitro*, a close association between increases in neopterin concentrations and in Kyn/Trp has been shown also *in vivo* [29, 35]. Still, there is a limitation when comparing results obtained in humans and primates to other animal systems like rats and mice. It is well established that NO is a strong inhibitor of the expression of IDO gene and of IDO function [40]. Although iNOS and IDO are activated by pro-inflammatory cytokines at the same time, NO released from iNOS might down-regulate tryptophan conversion to kynurenine (Fig. **2**). Consequently, IDO activity in animal models is expected to be much lower as compared to human conditions.

ASSOCIATION BETWEEN IMMUNE ACTIVATION AND DISTURBED PHENYLALANINE METABOLISM

Phenylalanine is an essential aromatic amino acid that is required for protein biosynthesis and also substrate for PAH. The phenylalanine to tyrosine ratio (Phe/Tyr) serves as an estimate of the turnover rate of PAH [41]. The product tyrosine is another important proteinogenic amino acid and precursor of the biosynthesis of DOPA and the catecholamines dopamine, epinephrine (adrenaline) and norepinephrine (noradrenaline). For the two enzymatic hydroxylation steps of phenylalanine to tyrosine and to L-dihydroxyphenylalanine (L-DOPA), the cofactor BH_4 -a reduced form of biopterin- is required as a hydrogen donor. Thereby from BH_4, quinonoid 7,8-dihydrobiopterin (BH_2) is formed, which subsequently needs to be re-recycled by dihydropteridine reductase [21, 42]. L-DOPA is an intermediate product in the biosynthetic pathway of the amines dopamine, adrenaline and noradrenaline [41].

More than two decades ago, elevated serum phenylalanine concentrations have been described in various inflammatory conditions including cancer, burns, trauma, sepsis and HIV-1 infection [43-45]. Phenylalanine levels were usually expressed either as absolute concentrations or as percentage of all amino acids. From the available literature, it is quite obvious that the pattern of diseases known to be associated with elevated phenylalanine levels is greatly overlapping with the conditions associated with elevated neopterin levels, although the percentage changes of phenylalanine levels is much lower than that of neopterin. Still there is the impression that any concurrent increase of neopterin and phenylalanine levels might involve a link due to the underlying shared biochemistry. More recently this issue was investigated in more detail and in addition to absolute phenylalanine concentrations Phe/Tyr was included in the new analyses. Interestingly, associations were observed between the altered phenylalanine metabolism and immune activation markers like neopterin and soluble TNF-receptors [46-48]. Finally, in patients with malignant melanoma [49] and hepatitis C virus infection [50, 51], a significant increase in phenylalanine concentrations and Phe/Tyr was reported after treatment with interferon-α/ribavirin. These observations supported the view that inflammation and immune activation may disturb proper function of PAH, leading ultimately to alterations in the metabolism of neurotransmitters known to play a role in neuropsychiatry. Consistent with this notion, alterations in the metabolism of phenylalanine were found to correlate with general behavioural and neurovegetative symptoms (e.g., sleep alterations, sickness, digestive and motor symptoms) in healthy elderly individuals whereas alterations in tryptophan metabolism were predominantly associated with depressive symptoms [52]. In patients with HCV infection treated with IFN-α, those with disturbed phenylalanine metabolism were more likely to develop fatigue [51]. Thus, impaired PAH activity and increased tryptophan breakdown due to activated IDO, may poten-

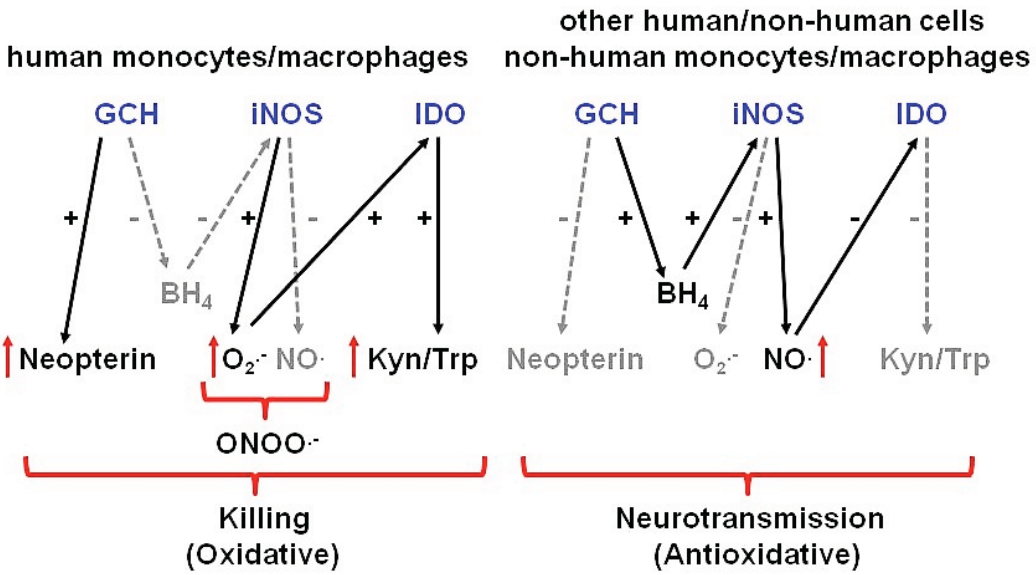

Fig. (2). Human and non human monocyte-derived cells (MΦ) and dendritic cells (DC) as well as other human cells and cells from other species differ in the regulation and interaction of interferon-γ-(IFN-γ)-induced biochemical reactions: IFN-γ stimulates GTP-cyclohydrolase I (GCH) which in human MΦ and DC leads to the production of neopterin at the expense of 5,6,7,8-tetrahydrobiopterin (BH_4), the necessary cofactor of cytokine-inducible nitric oxide ($NO^{\cdot}$) synthase (iNOS), whereas other human cells and cells from other species produce almost exclusively BH_4. Therefore, high output of $NO^{\cdot}$ is one hallmark of these cells, but human MΦ and DC do not form $NO^{\cdot}$, instead iNOS produces superoxide anion ($O_2^{\cdot-}$) which combines with NO produced at the same time to form highly toxic peroxynitrite (ONO_2^-). $NO^{\cdot}$ accumulating in other cells than human MΦ and DC slow down the activity of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) in these other cells whereas IDO is very active in human MΦ and DC. As a consequence, IFN-γ in human MΦ and DC is a strong positive regulator of cell-toxic killing mechanisms whereas in other cells and cells from other species it supports and protects biosynthesis of neurotransmitters.

tially contribute to neuropsychiatric abnormalities in patients suffering from chronic immune activation pathologies or undergoing cytokine treatment [42, 53].

ASSOCIATION BETWEEN IMMUNE ACTIVATION AND MODERATE HYPERHOMOCYSTEINAEMIA

The non-proteinogenic amino acid homocysteine is an intermediate in the methionine metabolism and it accumulates during deficiency in B vitamins folate, B_{12}, and/or B_6 [54, 55]. Moderate hyperhomocysteinemia is a well established coronary risk factor [56] which develops when dietary supply with B-vitamins is inadequate. Moderate hyperhomocysteinemia was originally observed in patients with cardiovascular diseases, leading to the hypothesis that homocysteine represents a potent risk factor for the pathogenesis of the disease [57]. Folate and B_{12} deficiency was in the primary focus to contribute to hyperhomocysteinemia although lowered concentrations of vitamin B_6 (pyridoxal-5'-phosphate) were also well established in patients with cardiovascular risk [58]. From the beginning, B vitamin status was considered as a critical factor determining plasma/serum concentrations of homocysteine, but it was not generally acknowledged as a causal factor in coronary risk mediated by elevated homocysteine. Other, unknown factors were thought to be involved and immune activation/inflammation was considered to play a major role [59]. Further studies extended the spectrum of conditions which go along with hyperhomocysteinemia and immune activation to various other inflammatory conditions including autoimmune rheumatoid arthritis and neurodegenerative disorders [60, 61]. From the usually observed correlation between homocysteine and neopterin concentrations and the often existing coincidence with lower concentrations of B-vitamins, it was concluded that inflammation and immune activation may cause an increased demand of B-vitamins during inflammatory diseases [60, 62]. This conclusion is in line with the increased production of homocysteine by stimulated peripheral blood mononuclear cells [63]. This finding suggests that stimulation of the immune system may contribute to moderate hyperhomocysteinemia during certain diseases. Indeed, in patients with sepsis post-trauma, an increase in homocysteine concentrations is common and was found to preferentially take place in patients who died in the course of sepsis although the supply with B-vitamins was identical in all patients [64]. It appears therefore plausible that the immune activation which underlies the elevated neopterin production and reflects macrophage activation is associated with the development of oxidative stress when reactive oxygen species (ROS) released during the immune response wipe out antioxidant defense systems and oxidative stress is emerging. Notably, IFN-γ is the most potent stimulus not only for neopterin production and tryptophan breakdown by IDO but also the strongest trigger of ROS formation by the activated macrophages [65].

Multiple trauma and sepsis are often accompanied by strong inflammatory responses and patients receive standardized enteral nutrition after the end of hypodynamic shock. In those patients, homocysteine levels were increased at follow up and these increases were significantly related to survival; total homocysteine being higher in non-survivors than in survivors [64]. Homocysteine correlated with Kyn/Trp and to some extent with neopterin concentrations. Because of standardized enteral nutrition, differences in vitamin supply were unlikely to underlie the development of hyperhomocysteinemia in patients but could rather be associated with a stronger pro-inflammatory response. A similar relationship was observed with respect to increases in homocysteine and phenylalanine concentrations and Phe/Tyr in the same patients [46], because biochemical abnormalities were preferentially observed in the group of non-survivors and correlated with concentrations of neopterin and Kyn/Trp.

Vitamin-B supplementation is able to slow-down homocysteine concentrations in patients presenting with moderate hyperhomocysteinemia. Thereby, folate and vitamin B_{12} appear to be more efficient than vitamin B_6 to do so [66-68], but at the same time there is no influence on the degree of immune activation as indicated by unchanged neopterin concentrations. This is the case in patients with coronary artery disease and various forms of dementia [69, 70]. Usually deficiency in folate and vitamin B_{12} are considered to be of greatest relevance for the disturbed metabolism of homocysteine in patients with CAD or dementia and in the elderly, although there is also vast data on diminished vitamin B_6 concentrations in the same populations of patients [71]. The decline of vitamin B_6 was found to be related to the immune activation status of patients [72-77] and Ulvik *et al*. [74] concluded from their findings that the acute phase and activated cellular immunity are associated with increased cellular uptake and catabolism of vitamin B_6. In line with that the oxidation-sensitive aldehyde, functional group of vitamin B_6 can be easily destroyed under conditions of oxidative stress [59, 60]. Aside from that, vitamin B_6 is not only involved in the conversion of homocysteine, but is also required for several catabolic enzymes down-stream of kynurenine [37, 74].

In conclusion, moderate hyperhomocysteinemia is associated with low B vitamin status which is however not necessarily due to primary vitamin deficiency. It is more likely that the overwhelming production of ROS during clinical conditions associated with inflammation and immune activation may increase the demand for oxidation-labile vitamins like folate, B_{12} and also B_6 [60]. This relationship can explain the coincidence of vitamin deficiency and increased concentrations of immune activation markers apparent in several inflammatory conditions like cardiovascular and neurodegenerative diseases [61, 72, 77]. Interestingly, 25-hydroxy and 1,25-dihydroxy vitamin D are also candidates to be depleted during chronic inflammation [78].

ON THE DETERMINATION OF NEOPTERIN AND KYN/TRP CONCENTRATIONS AND POTENTIAL PITFALLS OF HPLC

The easiest way to measure neopterin concentrations is through commercially available immunosassays such as ELISA or RIA [79,80]. HPLC is also feasible, but any acidification of serum, plasma or CSF specimens during the protein precipitation step prior to HPLC by, e.g., hypochlorous acid ($HClO_4$), partially oxidizes 7,8-dihydroneopterin to neopterin and thus contributes to higher neopterin levels than found by the direct measurement with immunoassays [81]. The alternative measurement of -total- neopterin concentrations cannot be recommended because then sample collection and storage require more stringent precautions for 7,8-dihydroneopterin than neopterin alone since 7,8-dihydroneopterin is destroyed upon exposure to air oxygen and thus concentrations of -total- neopterin are more variable [82]. Usually such stringent conditions for sample collection and storage may only be kept in research studies but not in an outpatient setting. Only the precipitation of protein from serum, plasma or CSF specimens by methanol or acetonitrile can be thus recommended when HPLC measurements of neopterin concentrations are in the focus because these reagents do not influence the neopterin content of the specimens [81]. In studies in which serum/plasma neopterin concentrations were measured with immunoassays or liquid chromatography, still significant correlations were reported between native and total neopterin concentrations after oxidation [82]. Also relationships between total neopterin concentrations and Kyn/Trp have been observed in patients with cardiovascular disease [39]. So it seems that, although the absolute concentrations may differ, total-neopterin could still be a useful marker of inflammatory status if samples are well sampled, stored and handled.

The HPLC measurement of Kyn/Trp also requires a protein precipitations step, which is a very stable method in human specimens [38]. However, in specimens from other species, the potentially higher concentrations of nitrite due to activated iNOS and

$NO^{\cdot}$ production can destroy kynurenine when in the so-called Sandmeyer type reaction the amino group of aromatic or heterocyclic amine derivatives quickly reacts with nitrite to form an aryl diazonium salt that can decompose rapidly. This can lead to substantial lowering of the measured kynurenine concentrations in specimens obtained from rats or mice [83].

INCREASED NEOPTERIN, KYN/TRP, PHE/TYR AND HOMOCYSTEINE IN THE ELDERLY

More than two decades ago, increases in serum neopterin concentrations have been described in older age [3], and this observation was confirmed in several studies thereafter [4-10]. Moreover, even in nonagenarians, a prognostic value was observed that higher blood neopterin concentrations were associated with shorter residual life span [8].

Comparing three small groups of different age ranges [10], concentrations of neopterin were found to be highest in a group aged >71 years, whereas in the middle aged group (61 -71 years) and the youngest individuals <61 years, serum neopterin concentrations were almost equally low (Fig. **3**). Alterations of serum kynurenine concentrations were quite similar to changes observed for neopterin, but these did not reach the level of significance. Only the steady decline of tryptophan concentrations between the 3 age groups and the mirror increase of tryptophan breakdown (Kyn/Trp) were significant ($p < 0.05$), with tryptophan levels being lowest while Kyn/Trp being highest in the oldest individuals (Fig. **3**). Homocysteine concentrations were also increased with older age and this was associated with a decline of folate concentrations but not of vitamin B_{12}. Likewise, age correlated positively with neopterin ($rs = 0.529$), Kyn/Trp ($rs = 0.509$) and homocysteine concentrations ($rs = 0.570$, all $p < 0.01$) and inversely with tryptophan ($rs = -0.377$, $p < 0.05$). Positive associations were also observed between neopterin and Kyn/Trp ($rs = 0.576$) and homocysteine concentrations ($rs = 0.648$), the latter also correlating with Kyn/Trp ($rs = 0.694$, all $p < 0.01$). Gender, had no influence on the findings although the strengths of correlations were generally somewhat lower in females than in males [10]. Phenylalanine concentrations and Phe/Tyr were not determined in this study, but a more recent analyses in 284 individuals aged >65 years revealed a significant relationship between Phe/Tyr and Kyn/Trp ($rs = 0.172$, $p < 0.01$) but the association with neopterin concentrations was not significant [52]. However, in patients suffering from cardiovascular disease, significant associations between concentrations of immune activation markers including neopterin and higher phenylalanine and Phe/Tyr concentrations have been reported recently [84]. Data pointed to deficiency of BH_4 which may underlie the impaired conversion of phenylalanine by PAH and could also relate to an impaired production of nitric oxide and increased blood pressure in those patients.

Data from available studies support the view that healthy ageing is associated with immune activation as reflected by the increase of neopterin and Kyn/Trp concentrations, together with impaired PAH activity and/or insufficient supply with B-vitamins [42, 59, 60]. Oxidative stress can easily contribute to these effects, by impairing PAH function through the oxidation of BH_4 and/or degradation of B-vitamins like methyltetrahydrofolate (MTHF). Because IFN-γis the most important inducer of neopterin production and of tryptophan breakdown by IDO, it also triggers the high output production of ROS [65]. In such situations, high concentrations of ROS are released, which may easily overwhelm the detoxifying mechanism of cells allowing thus the development of oxidative stress that, with time, may wipe out antioxidant pools and thus indirectly contribute to depletion of oxidation-labile vitamins including vitamin C and E and the B vitamins [60, 85]. The important impact of inflammation on moderate hyperhomocysteinemia has been un-

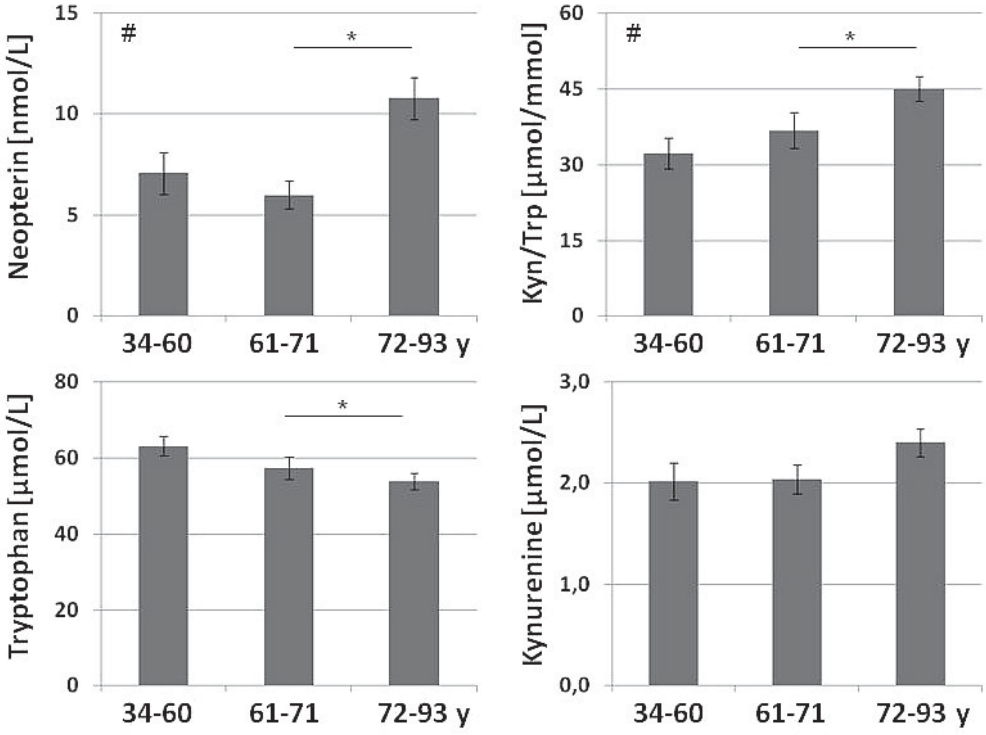

Fig. (3). Concentrations of neopterin (upper left), tryptophan (lower left) and kynurenine (lower right) and the kynurenine to tryptophan ratio (Kyn/Trp; upper right) in healthy individuals of 3 age-categories (group 1, n = 13; group 2, n = 15; group 3, n = 15; adapted from ref. [9]); graphs show mean values ± S.E.M., #p <0.05 Kruskal Wallis test for all age groups, *p <0.05 Mann Whitney test.

derlined by the recent observation that an increase of homocysteine concentrations is developing in patients post trauma or with sepsis with poor prognosis but not in survivors [64]. Both groups of patients received identical diet by standardized enteral nutrition. Thus different supply with B-vitamins cannot explain this result. Rather oxidative stress due to the stronger inflammatory reaction in patients with poor outcome may destroy oxidation labile compounds including B-vitamins and as a consequence total homocysteine concentrations would rise. A similar relationship might exist in older age, when higher degree of immune activation is associated with B-vitamins deficiency and increased total homocysteine levels [10].

Interestingly, a correlation between neopterin and homocysteine concentrations was found also in patients with neurodegenerative disorders [86, 87] and in healthy controls [88]. *In vitro*, activation of PBMC was also found to be associated with the accumulation of total homocysteine [63], and this is again most probably due to the enhanced demand for B-vitamins by proliferating cells. Altogether, these data suggest that immune activation increases the demand for B-vitamins with ageing [10].

Renal impairment is well known to retard excretion of blood neopterin and also kynurenine or homocysteine. Therefore, aging, immune activation and inflammation may influence kidney function, and as a consequence highly increased neopterin and Kyn/Trp concentrations are observed in dialysis patients [89-92]. Thus, impaired kidney function needs to be taken into account under such conditions, for instance by calculating the ratio to creatinine or cystatin or the clearance rate [93].

NEOPTERIN, KYN/TRP, PHE/TYR AND HOMOCYSTEINE AND SPECIFIC SYMPTOMS IN OLDER AGED INDIVIDUALS

The increased concentrations of neopterin and Kyn/Trp and of other markers of immune response and inflammation, like interleukin-6 and C-reactive protein (CRP), may appear as being in contrast with the decreased immunocompetence developing in the elderly. Nevertheless, increased neopterin production as well as increased tryptophan breakdown can be clearly referred to biochemical pathways preferentially induced by the Th1-type cytokine IFN-γ, as IFN-γ activates GCH, the key enzyme of pteridine biosynthesis, and IDO which converts tryptophan to kynurenine (Fig. **1**). The association found between Kyn/Trp and neopterin concentrations further confirms a relationship between tryptophan breakdown and immune activation, and thus, the likely involvement of activated IDO. Data indicate that endogenous formation of IFN-γ increases with older age, consistent with the enhanced expression of IFN-γ and TNF-α in T-cells found in elderly individuals [94]. Moreover, the higher neopterin concentrations in the elderly population were found to be associated with the decline of $CD28^{+}CD45RA^{+}$ T-cells and with lower tetanus toxoid antibody concentrations [95]. This latter observation compares favorably with the fact that activation of Th1-type immune response, via the activation of IDO is able to impair T-cell responsiveness [96, 97]. Thus, the chronically activated immune system and tryptophan depletion could relate to the development of immunodeficiency which can be observed in elderly persons.

The essential amino acid tryptophan is required for the synthesis of proteins and thus is necessary for the proliferation and growth of cells. Accordingly, cytokine-induced IDO activity is an effective antimicrobial and antitumoral mechanism [27]. IDO not only plays a key role in the host defense against a variety of infectious pathogens [28], but studies have shown that it also down-regulates the immune response of the host, by slowing down T-cell proliferation very efficiently [98,99], suppressing effector T-cell function, rendering T-cells more sensitive to apoptosis and promoting the differentiation of regulatory T cells [100, 101]. These and other studies have established a crucial role of IDO in tolerance induction and thus, IDO has been identified as a promising target for therapeutic purpose, notably for the treatment of chronic inflammatory diseases.

Pro-inflammatory cytokines like IFN-γ, pteridine metabolism and activated IDO may also play a role in the pathogenesis of symptoms like anemia [102, 103], weight loss, cachexia [104,105] and frailty [5, 106] that are also quite common in elderly individuals. These symptoms may relate to underlying diseases which are associated with increased neopterin and Kyn/Trp concentrations like cancer [35, 107], autoimmune syndromes such as rheumatoid arthritis and systemic lupus erythematosus [29, 108-110], all representing clinical conditions which are often associated with older age. They may already be under development in the elderly individuals but not yet diagnosed. Elevated neopterin, Kyn/Trp and quinolinic acid concentrations are also quite common in patients with neurodegenerative diseases like dementias and Parkinson's disease [33, 112, 113]. A large number of studies has been performed in patients suffering from coronary artery disease and the elevated neopterin concentrations and the accelerated tryptophan breakdown have been already documented by several groups as significant predictors of shortened survival [114-121]. In most of these clinical conditions, increased Phe/Tyr seems to be also widely distributed [42, 84, 122] but the number of studies is still much more limited. However, one may speculate that at least some of the neurobehavioral symptoms including depression, cognitive deficit and fatigue may relate to the biochemical alterations caused by chronic immune activation and inflammation.

THERAPEUTIC OPTIONS TO TREAT SYMPTOMS WHICH ARE ASSOCIATED WITH TH1-TYPE IMMUNE ACTIVATION AND INFLAMMATION IN THE ELDERLY

Th1-type immune activation and inflammation in the elderly are indicated by increased neopterin concentrations and accelerated tryptophan breakdown by IDO. This association may suggest that treatments able to counteract Th1-type immune response are promising to halt or even reverse ageing-related processes which are among the down-stream biochemical pathways induced by IFN-γ. Activation of the Th1-type immune response leads to a decline in serum/plasma tryptophan concentrations, which corresponds to the rapid depletion of tryptophan in supernatants of stimulated PBMC. This relationship explains why during diseases with a background of immune activation and inflammation, a decline of tryptophan is common [31]. Nutritional status may also promote chronic inflammation related to the aging processes, as nutrients that are known to be deficient in the elderly (e.g., omega 3-fatty acids, other antioxidants) exert potent immunomodulatory actions [123, 124]. Moreover, the finding that immune activation (e.g., increased neopterin concentrations) in patients with coronary artery disease was significantly associated with lower concentrations of several antioxidant vitamins and compounds like lutein [85] strengthens the point that inflammatory processes during ageing may be responsible for the increased demand for antioxidant vitamins. This conclusion is further supported by the concurrent increase of neopterin and homocysteine concentrations with increasing age [10], which indicates that also demand for B-vitamins is increasing in the elderly.

In vitro, we and others have shown in freshly isolated human peripheral blood mononuclear cells that anti-inflammatory and antioxidant compounds (e.g., aspirin, vitamins C, E and trihydroxystilbene resveratrol) and also plant extracts and beverages like wine, beer, cacao and green and black tea exert significant suppressive effects on the Th1-type immune activation cascade [125-130]. The observed effects may rely on the interaction of antioxidant compounds with pro-inflammatory cascades involving important signal transduction elements such as nuclear factor-κB (NF-κB) [128, 130]. Interestingly, specific inhibition of tryptophan-kynurenine metabolism in Drosophila melanogaster was associated with extended life span [131].

The intake of a vitamin-rich diet can help to overcome, at least partially, mood lowering and cognitive disturbances [132, 133]. This diet may contribute to improve tryptophan and thus serotonin availability. The suppression of cytokine-induced IDO activity could relate to this beneficial aspect of healthy food. A large variety of food compounds with antioxidant properties like vitamins C and E but also beverages like cacao, wine and beer or anti-inflammatory drugs like aspirin or statins were found to suppress neopterin production and tryptophan breakdown in stimulated human PBMC [127, 134]. Among these compounds are also the life-prolonging compounds like rapamycin [12] and resveratrol [13, 135]. However, unlike cell-toxic immunosuppressants such as rapamycin, antioxidant rich diet and compounds can be easily recommended as a strategy to slow-down the aging process and the biochemical consequences of inflammation. Recent data on the positive influence of Mediterranean diet on the concentrations of inflammatory parameters underlines this conclusion [136]. Mediterranean diet, rich in fresh fruit and vegetables, nuts, and olive oil and red wine corresponds to high levels of antioxidants which may be responsible for the decline of oxidative stress markers and blood pressure. However, levels of C-reactive protein, triglycerides and myeloperoxidase remained unchanged. Nevertheless, manipulating tryptophan breakdown by interfering with its underling immunological pathway namely Th1-type immune activation is promising not only to improve the neuropsychiatric presentation of elderly individuals but also to enhance immune function and to reduce the risk of weight loss and cachexia.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

Declared none.

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Received: October 2, 2013 **Accepted: March 12, 2014**

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