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Neopterin suppresses the activity of tryptophan-degrading enzyme indoleamine 2,3-dioxygenase in human peripheral blood mononuclear cells

Abstract: *In vitro*, large amounts of neopterin are released from human monocyte-derived macrophages and dendritic cells primarily upon stimulation with Th1-type cytokine interferon-γ (IFN-γ). IFN-γ also induces the enzyme indoleamine 2,3-dioxygenase (IDO), which degrades tryptophan (TRP) to form kynurenine (KYN). IDO-mediated TRP catabolism is very effective in suppressing the proliferation of T lymphocytes as well as of pathogens *in vitro* and *in vivo*. In this study, we investigated whether exogenously added neopterin may influence IDO activity in resting and in stimulated peripheral blood mononuclear cells (PBMC). PBMC were isolated from healthy donors, and neopterin was added in a concentration range from 0.01 to 50 μmol/L. After 30 min, PBMC were stimulated or not with 10 μg/mL of mitogen phytohemagglutinin (PHA). After 48 h, culture supernatants were collected, KYN and TRP concentrations were measured by high-performance liquid chromatography, and the ratio of KYN vs. TRP was calculated as an estimate of IDO activity. Spontaneous as well as PHA-induced TRP breakdown was suppressed by exogenously added neopterin in a dose-dependent way; the lowest active concentration of neopterin was <100 nmol/L. As neopterin concentrations in the nanomolar range are commonly observed in patients suffering from infections, sepsis, or uremia, our results suggest that neopterin formation might also serve as a feedback mechanism to slow down TRP degradation *in vivo*.

Keywords: indoleamine 2,3-dioxygenase; macrophages; neopterin; T-cell activation.

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Introduction

Increased formation of the low-molecular-mass (253 Da) compound neopterin (D-erythro 1′,2′,3′-trihydroxypropylpterin) is observed in various diseases associated with activated cell-mediated immunity including infections, autoimmune diseases and certain malignancies [1]. Neopterin belongs to the class of pteridines, the most prominent members of which are vitamin folic acid and 5,6,7,8-tetrahydrobiopterin (BH4), an essential cofactor of several aromatic amino acid monooxygenases and nitric oxide (NO) synthases (NOS) [2]. The key enzyme for the biosynthesis of pteridines is GTP cyclohydrolase-I (GCH, EC 3.5.4.16), which is inducible by pro-inflammatory stimuli and preferentially by the Th1-type cytokine interferon-γ (IFN-γ). Human and primate monocyte-derived macrophages (MΦ) and dendritic cells (DC) are unique as compared with other human cells or cells from other species due to a relative deficiency of the subsequent enzyme in the biosynthesis of BH4, pyruvoyl tetrahydropterin synthase (PTPS) [3, 4]. PTPS catalyses the conversion of intermediate 7,8-dihydroneopterin triphosphate to sepiapterin and BH4. In the absence of relevant PTPS activity in human MΦ and DC, 7,8-dihydroneopterin triphosphate is converted by phosphatases to neopterin and 7,8-dihydroneopterin [2]. Therefore, once stimulated with IFN-γ, these cells produce neopterin at the expense of BH4 [3–5].

The pro-inflammatory cytokine IFN-γ is released in large amounts during Th1-type immune responses, and it represents probably the most important anti-proliferative cytokine [6]. It induces several biochemical pathways including the formation of reactive oxygen species (ROS)

[6] and the expression and/or activity of pteridine-forming enzyme GCH [2] and tryptophan (TRP)-degrading enzyme indoleamine 2,3-dioxygenase (IDO, EC 1.13.11.52) [7, 8]. Degradation of the essential amino acid TRP to kynurenine (KYN) by IDO lowers available TRP, which is required for the biosynthesis of proteins. As a consequence of decreased TRP availability, the growth of pathogens and proliferating cells is arrested [9, 10]. Accordingly, activation of IDO represents an important antimicrobial and/or antitumoral defense mechanism [8]. IDO is expressed by various cells such as MΦ, DC, fibroblasts, endothelial and epithelial cells in various species. Beside IFN-γ, which is the main inducing stimulus, lipopolysaccharide (LPS), tumor necrosis factor α (TNF-α) or other pro-inflammatory stimuli also may induce IDO expression and activity [7, 8].

Moreover, hepatic TRP 2,3-dioxygenase (EC 1.13.11.11) is able to degrade TRP; therefore the estimation of other immune activation markers such as neopterin, IFN-γ or other cytokines is necessary to finally link TRP degradation to Th1-type situations [7, 8, 11].

In human MΦ and DC, TRP breakdown occurs in parallel to the formation of neopterin [7]. Thus, *in vitro* and in patients suffering from distinct clinical conditions like viral infections, autoimmune diseases, allograft rejections and several malignant diseases, elevated neopterin concentrations are usually detected concomitantly with enhanced IDO activity as reflected by increasing KYN concentrations and a decline of TRP [10]. However, TRP degradation can also suppress the proliferation of T cells leading to immunodeficiency as an unwanted side-effect [12, 13].

Earlier, neopterin was reported from *in vitro* studies to interfere with various redox-sensitive intracellular signaling pathways, thereby stimulating, e.g., translocation and activation of nuclear factor-κB (NF-κB), the expression of inducible nitric oxide synthase (iNOS) and cytokine TNF-α, as well as of intercellular adhesion molecule-1, and inducing apoptosis [14–18]. Furthermore, neopterin levels can be used as a measure of cellular immune activation and oxidative stress *in vivo*, as its production is associated with increased ROS and low serum concentrations of antioxidants [1].

So far, parallel TRP degradation and neopterin production are linked due to the common upstream inductor IFN-γ, and concomitant determination of neopterin levels is used to confirm IDO-mediated TRP degradation in Th1-type settings *in vitro* and *in vivo*. This study investigates whether exogenously added neopterin influences TRP levels in a model system of peripheral blood mononuclear cells (PBMC) treated or not with mitogen to stimulate TRP degradation [19].

Materials and methods

Chemicals

Neopterin was obtained from Schircks Laboratories (Jona, Switzerland), and phytohemagglutinin (PHA) from Sigma Aldrich (Vienna, Austria). Substances were dissolved in phosphate buffered saline (PBS) and stored at –20°C until use.

Cell culture experiments

For cell culture experiments, PBMC were isolated from fresh blood of healthy volunteer blood donors, from whom written informed consent was obtained that their donated blood might be used for scientific purposes in case it was not selected for transfusion. After dilution in PBS, the separation of blood cells was performed by density centrifugation using Biocoll solution (MedPro, Vienna, Austria). The isolated cells were washed three times with PBS supplemented with 0.3% EDTA (0.5 mmol/L) (Merck, Darmstadt, Germany). PBMC were cultivated in RPMI 1640 (MedPro, Vienna, Austria) containing 10% heat-inactivated fetal calf serum (FCS), 2 mmol/L of L-glutamine and 50 μg/mL of gentamicin (both from Serva, Heidelberg, Germany), and incubated in moist atmosphere at 37°C and 5% CO_2 [19]. For the experiments, cells were seeded at a density of 1.5×10^6 cells/mL. At first, PBMC were preincubated for 30 min with or without increasing concentrations of neopterin, then they were stimulated with 10 μg/mL of PHA or not. The concentration of 10 μg/mL PHA has been documented earlier to allow optimal stimulation of cells without reaching a plateau [19]. Every experiment was performed in duplicates and repeated at least three times using cells of different donors. After 48 h, the experiments were stopped and culture supernatants were harvested by centrifugation. At this time point, the accumulated TRP breakdown and neopterin formation reached a plateau [19].

IC_{50} concentration was calculated by using the CalcuSyn software from Biosoft (Cambridge, UK) [20]. The viability of cells was tested by the CellTiter Blue assay (Promega, Madison, WI, USA).

Determination of TRP and kynurenine (KYN) concentrations

Concentrations of TRP and KYN were measured in cell culture supernatants using high-performance liquid chromatography (ProStar Varian, Palo Alto, CA, USA) with 3-nitro-L-tyrosine (Sigma-Aldrich) as internal standard [21]. KYN and 3-nitro-L-tyrosine (Sigma-Aldrich) concentrations were determined by means of their UV absorption at 360 nm of wavelength. Fluorescence at 366 nm of wavelength emitted by TRP was measured under exposure to light at 286 nm of wavelength (ProStar Varian). KYN/TRP ratio was calculated to estimate IDO activity and expressed in micromoles of KYN per millimoles of TRP [21, 22].

Statistical analysis

TRP concentrations are expressed as percentage of medium content, and KYN and KYN/TRP as percentage of control cells, i.e., either

for unstimulated cells or for cells stimulated with PHA. Results are shown as mean values±standard error of the mean (SEM). Results for the tests with neopterin concentrations of between 10 and 100 nmol/L were summed up as one group to increase the statistical power of group comparisons. For comparison of groups, the Kruskal-Wallis and Mann-Whitney U-tests for nonparametric analysis were applied, because not all data sets showed normal distribution. p Values below 0.05 were considered to indicate significant differences.

Results

RPMI medium supplemented with 10% FCS contained 37.0 µmol/L of L-TRP. The average TRP concentration measured in supernatants of unstimulated cells was 26.2±1.8 µmol/L (=70% of initial medium content). Average KYN concentrations were 1.7±0.2 µmol/L, and average KYN/TRP was 70±12 µmol/mmol.

Treatment of unstimulated cells with neopterin showed no significant influence on TRP concentrations (Figure 1). However, upon exposure to neopterin (0.01–50 µmol/L), KYN concentrations decreased significantly and dose dependently to a minimum of 26±6.3% of baseline at a neopterin concentration of 50 µmol/L (all $p<0.05$) (Figure 1). Similarly, the exposure of cells to neopterin decreased KYN/TRP dose dependently to a minimum of 26.8±6.8% of baseline at 50 µmol/L of neopterin (all $p<0.05$) (Figure 1).

Stimulation of PBMC with PHA increased TRP breakdown significantly; TRP concentrations decreased to about 17% of medium content ($p<0.01$). Except for the lowest concentration (0.01 µmol/L), treatment with neopterin increased TRP concentrations significantly in comparison to PHA-stimulated controls (all $p<0.05$). At a concentration of 50 µmol/L of neopterin, TRP concentration was 54±3% of initial medium content (Figure 2). Neopterin administration decreased KYN production significantly; application of all dilutions led to a dose-dependent decline in KYN concentrations, reaching 9.6±6.7% with 50 µmol/L of neopterin compared to PHA-treated controls (all $p<0.05$) (Figure 2).

Treatment of PBMC with neopterin led to lower KYN/TRP, with all concentrations reaching a minimum of

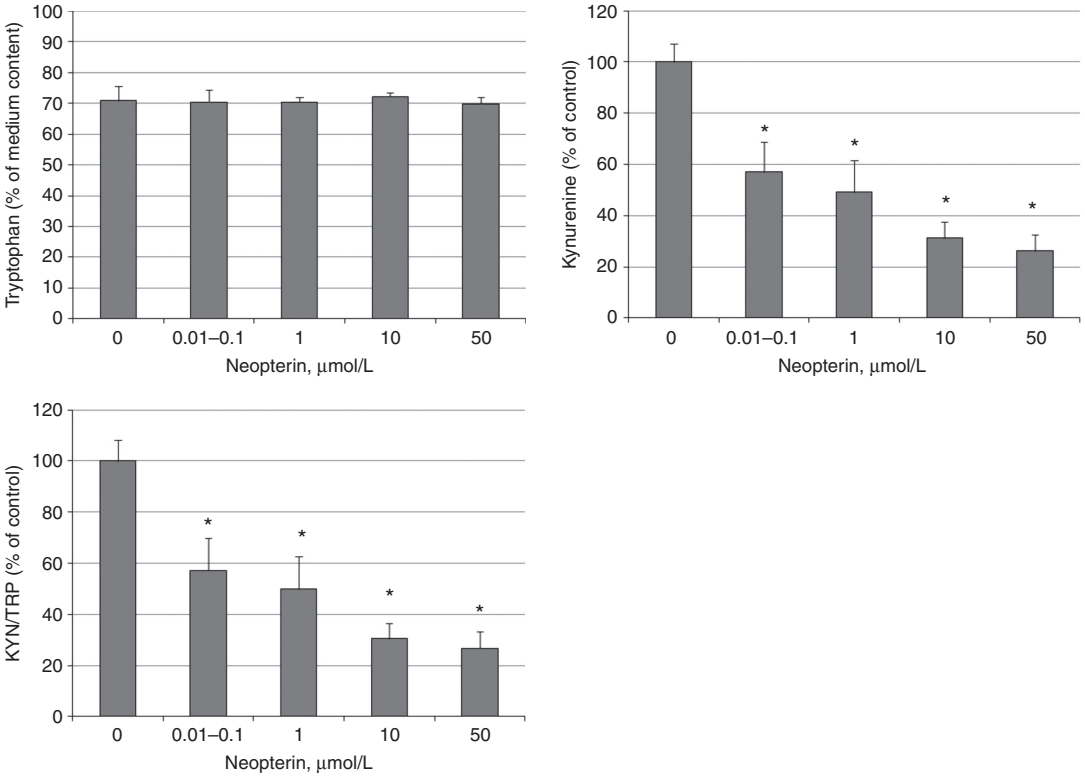

Figure 1 Concentrations of tryptophan (upper left), kynurenine (upper right), and the kynurenine-to-tryptophan ratio (KYN/TRP) in the supernatants collected from unstimulated peripheral blood mononuclear cells after exposure to increasing concentrations of neopterin for 48 h. Columns are representative for three independent experiments with cells obtained from three different donors and run in duplicates. Results are expressed as % of baseline (or as % of initial medium content for tryptophan), and columns show mean±standard error of the mean; *$p<0.05$.

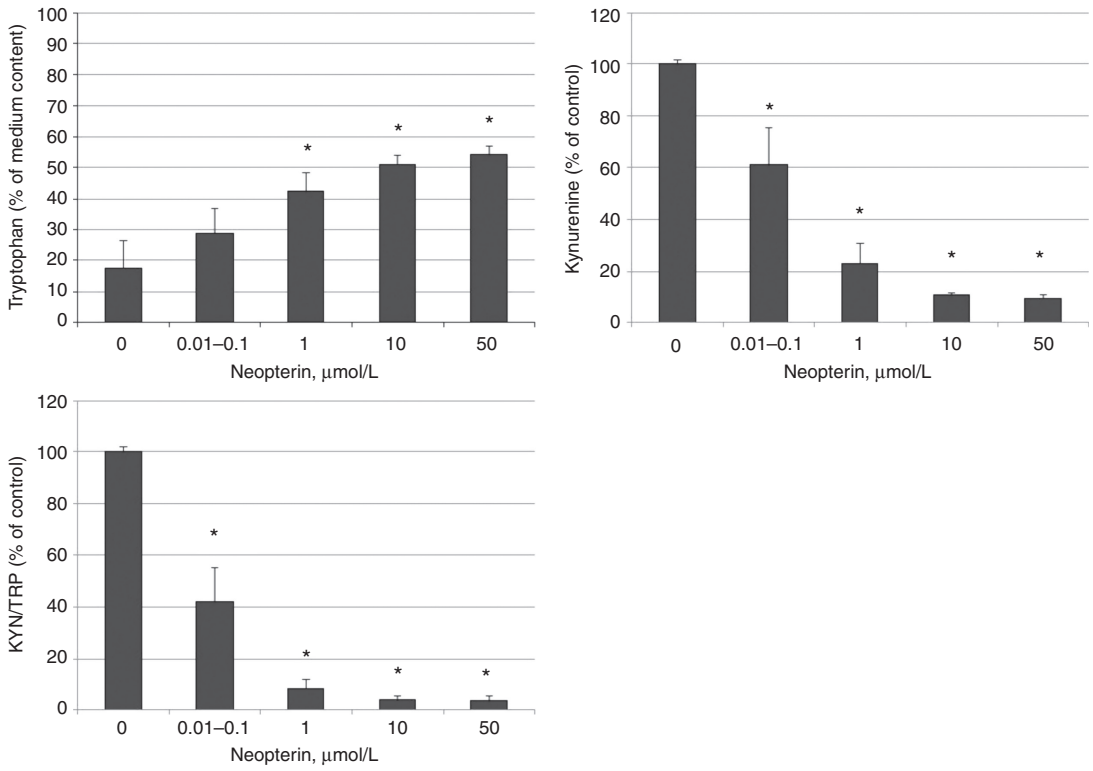

Figure 2 Concentrations of tryptophan (upper left), kynurenine (upper right), and the kynurenine-to-tryptophan ratio (KYN/TRP) in the supernatants collected from peripheral blood mononuclear cells after exposure to increasing concentrations of neopterin and stimulation with mitogen phytohemagglutinin for 48 h. Columns are representative for three independent experiments with cells obtained from three different donors and run in duplicates. Results are expressed as % of baseline (or as % of initial medium content for tryptophan), and columns show mean±standard error of the mean; *$p<0.05$.

3.7±1.9% compared to PHA-stimulated control cells when 50 µmol/L of neopterin was used (all $p<0.05$) (Figure 2).

Calculated IC_{50} concentration for KYN production was 143 nmol/L of neopterin for stimulated PBMC and 486 nmol/L for unstimulated PBMC.

Discussion

The stimulation of PBMC with mitogen PHA significantly induced IDO activity as reflected by a significant decline in TRP concentrations and a parallel increase in KYN production and KYN/TRP ratio. Pre-exposure of PBMC to neopterin suppressed this mitogen-induced TRP degradation in a dose-dependent way. The lowest active concentration of neopterin was ≤100 nmol/L; the calculated IC_{50} was 143 nmol/L in stimulated and 486 nmol/L in unstimulated PBMC. As the T-cell-derived Th1-type cytokine IFN-γ is the primary inducer of IDO and thus of TRP degradation in MΦ, our data suggest a suppressive effect of neopterin on the formation and release of IFN-γ in human mitogen-stimulated PBMC. Also, direct effects of neopterin on MΦ or on IDO enzyme itself might have contributed to the stabilization of TRP levels.

The concentrations of neopterin, which were effective in our *in vitro* system, appear to be rather high when compared to normal neopterin levels averaging 5.2 nmol/L, which are detectable in the blood of healthy controls [1]. However, in the circulation of patients with, e.g., sepsis, HIV infection or cancer, sometimes levels higher than 50 or 100 nmol/L can be observed [1, 23–25]. In dialysis patients, neopterin concentrations as high as 500 nmol/L have been reported [26]. Even higher neopterin concentrations have been extrapolated at the site of the inflammatory response [27]. With these considerations, the effects of neopterin seen in our *in vitro* study could be of relevance also under *in vivo* conditions. The cells used in this study were human PBMC, which under stimulatory conditions themselves produce significant amounts of neopterin, which can reach up to 50 nmol/L [19]. Thus, any effects of supplemented neopterin at concentrations lower than 50 nmol/L cannot be detected easily.

In fact, increased neopterin production may represent a negative feedback loop during Th1-type immune response. It might even contribute to the development of

immunodeficiency by slowing down the responsiveness of T cells when exposed to an antigenic stimulus. The regulatory circuit is very similar to that characterized for IDO earlier: IDO induction is triggered in human monocytes/macrophages by pro-inflammatory stimuli like leukocyte-derived IFN-γ [7, 8]. Then, TRP degradation very efficiently down-regulates T-cell activation cascades either by TRP deprivation or via production of toxic catabolites and induction of regulatory T cells [12, 28].

The effect of neopterin on the TRP breakdown by PBMC may relate to the influence of the compound on redox systems. Several experimental studies demonstrated that neopterin influences the biochemical pathways of oxidative stress, e.g., neopterin was found to enhance the effects of cytotoxic ROS originating from chloramine T and hydrogen peroxide [14, 18], and neopterin triggered ROS production in neutrophils [29]. In contrast, neopterin treatment in a micromolar range has been shown to suppress the generation of superoxides by inhibiting NADPH oxidases in phorbol myristate acetate-stimulated rat macrophages [30]. In vascular smooth muscle cells, neopterin has been shown to stimulate the gene expression of iNOS with a subsequent increase in NO production [15], and neopterin activated NF-κB by modulating the production and/or the effects of ROS intermediates in these cells, which are sufficient to trigger programmed cell death [16, 17]. A close association was observed in stimulated myelomonocytic THP-1 cells between the expression of NF-κB, the production of neopterin and the degradation of TRP [31]. Furthermore, neopterin was demonstrated to augment the formation and release of TNF-α in PBMC and in MΦ stimulated with LPS [16, 32].

Summarizing these, at first glance, somewhat contradictory data, it appears that neopterin is able to down-regulate T-cell functional responses, whereas it promotes the inflammation response in which the monocyte/macrophage population is central. This bidirectional influence is quite similar to that observed regarding effects mediated by ROS [33].

Of note, neopterin is released from stimulated human monocyte-derived MΦ and DC at the expense of BH4, the cofactor of specific monooxygenases including iNOS. The iNOS product NO is a well-characterized inhibitor of the heme protein IDO [34]. The results of this study show that neopterin at moderately high concentrations inhibits IDO function. This effect is similar to that of NO. Because iNOS activity is almost completely absent in human monocytes/macrophages [34–36], neopterin might replace its effect on IDO. By contrast, monocytes-macrophages of species other than human and primates do not produce neopterin [37] but possess a functional iNOS system, which is able to down-regulate IDO as a kind of feedback to the underlying process of T-cell activation.

Increased neopterin concentrations are found together with accelerated TRP breakdown in various diseases including infections, cardiovascular disease, autoimmune diseases, malignant diseases and also certain malignancies [1, 11]. In these clinical conditions, increased neopterin production is often accompanied by a significant decline in TRP concentrations in serum or plasma and an increase in KYN production and KYN/TRP ratio [11]. The enhanced IDO activity may induce immunodeficiency, when T cells begin to suffer from TRP shortage and toxic products of TRP breakdown accumulate [12]. In addition to the negative effects of IDO activity on T-cell activation cascades, neopterin production can also contribute to the development of immunodeficiency because neopterin is released by MΦ under the same conditions when the cells exert their antiproliferative activities. Notably, patients with cancer, HIV-1 infection or on hemodialysis [22–26], who exhibit increased neopterin levels, are well known to be characterized by secondary immunodeficiency.

Our results show that neopterin down-regulates PHA-mediated TRP degradation in PBMC in a dose-dependent manner. The concentrations of neopterin effective in our *in vitro* system are commonly observed in patients suffering from infections like HIV-1 but also during sepsis and in hemodialysis patients. According to our data, neopterin production may contribute to the development of T-cell unresponsiveness in patients with sustained overwhelming neopterin production, while the function of monocytes/macrophages is supported by neopterin. Further studies are needed to clarify this point.

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References

1. Murr C, Widner B, Wirleitner B, Fuchs D. Neopterin as a marker for immune system activation. Curr Drug Metab 2002;3:175–87.
2. Werner ER, Werner-Felmayer G, Fuchs D, Hausen A, Reibnegger G, Yim JJ, et al. Tetrahydrobiopterin biosynthetic activities in human macrophages, fibroblasts, THP-1, and T 24 cells. GTP-cyclohydrolase I is stimulated by interferon gamma, and 6-pyruvoyl tetrahydropterin synthase and sepiapterin reductase are constitutively present. J Biol Chem 1990;265:3189–92.

3. Huber C, Batchelor J, Fuchs D, Hausen A, Lang A, Niederwieser D, et al. Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon-gamma. J Exp Med 1984;160: 310–6.
4. Wirleitner B, Reider D, Ebner S, Bock G, Widner B, Jaeger M, et al. Monocyte-derived dendritic cells release neopterin. J Leukoc Biol 2002;72:1148–53.
5. Fuchs D, Hausen A, Huber C, Margreiter R, Reibnegger G, Spielberger M, et al. Pteridinausscheidung als Marker für alloantigen-induzierte Lymphozytenproliferation. Hoppe Seyler's Z Physiol Chem 1982;363:661–4.
6. Nathan CF, Murray HW, Wiebe ME, Rubin BY. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med 1983;158:670–89.
7. Werner ER, Werner-Felmayer G, Fuchs D, Hausen A, Reibnegger G, Wachter J. Parallel induction of tetrahydrobiopterin biosynthesis and indoleamine 2,3-dioxygenase activity in human cells and cell lines by interferon-gamma. Biochem J 1989;262:861–6.
8. Taylor MW, Feng GS. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J 1991;5:2516–22.
9. Pfefferkorn ER. Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. Proc Natl Acad Sci USA 1984;81:908–12.
10. Uyttenhove C, Pilotte L, Théate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 2003;9:1269–74.
11. Schroecksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clin Chim Acta 2006;364:82–90.
12. Mellor AL, Keskin DB, Johnson T, Chandler P, Munn DH. Cells expressing indoleamine 2,3-dioxygenase inhibit T cell responses. J Immunol 2002;168:3771–6.
13. Weiss G, Schroecksnadel K, Mattle V, Winkler C, Konwalinka G, Fuchs D. Possible role of cytokine-induced tryptophan degradation in anaemia of inflammation. Eur J Haematol 2004;72:130–4.
14. Weiss G, Fuchs D, Hausen A, Reibnegger G, Werner E, Werner-Felmayer G, et al. Neopterin modulates toxicity mediated by reactive oxygen and chloride species. FEBS Lett 1993;321:89–92.
15. Schobersberger W, Hoffmann G, Grote J, Wachter H, Fuchs D. Induction of inducible nitric oxide synthase expression by neopterin in vascular smooth muscle cells. FEBS Lett 1996;377:461–4.
16. Hoffmann G, Frede S, Kenn S, Smolny M, Wachter H, Fuchs D, et al. Neopterin-induced tumor necrosis factor-alpha synthesis in vascular smooth muscle cells in vitro. Int Arch Allergy Immunol 1998;116:240–5.
17. Hoffmann G, Wirleitner B, Fuchs D. Potential role of immune system activation associated production of neopterin derivatives in humans. Inflamm Res 2003;52: 313–21.
18. Cirillo P, Pacileo M, De Rosa S, Calabro P, Gargiulo A, Angri V, et al. Neopterin induces pro-atherothrombotic phenotype in human coronary endothelial cells. J Thromb Haemost 2006;4:2248–55.
19. Jenny M, Klieber M, Zaknun D, Schroecksnadel S, Kurz K, Ledochowski M, et al. In vitro testing for anti-inflammatory properties of compounds employing peripheral blood mononuclear cells freshly isolated from healthy donors. Inflamm Res 2011;60:127–35.
20. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 1984;22:27–55.
21. Laich A, Neurauter G, Widner B, Fuchs D. More rapid method for simultaneous measurement of tryptophan and kynurenine by HPLC. Clin Chem 2002;48:579–81.
22. Fuchs D, Möller AA, Reibnegger G, Stöckle E, Werner ER, Wachter H. Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. J Acquir Immune Defic Syndr 1990;3/9:873–6.
23. Krämer A, Biggar RJ, Hampl H, Friedman RM, Fuchs D, Wachter H, Goedert JJ. Immunologic markers of progression to acquired immunodeficiency syndrome are time-dependent and illness-specific. Am J Epidemiol 1992;136:71–80.
24. Ploder M, Spittler A, Schroecksnadel K, Neurauter G, Pelinka LM, Roth E, et al. Accelerated tryptophan degradation in trauma and sepsis patients is related to pro-inflammatory response and to the diminished in vitro response of monocytes. Pteridines 2009;19:54–61.
25. Sucher R, Schroecksnadel K, Weiss G, Margreiter R, Fuchs D, Brandacher G. Neopterin, a prognostic marker in human malignancies. Cancer Lett 2010;287:13–22.
26. Fuchs D, Hausen A, Reibnegger G, Werner ER, Dittrich P, Wachter H. Neopterin levels in long term hemodialysis. Clin Nephrol 1988;30:220–4.
27. Firth CA, Laing AD, Baird SK, Pearson J, Gieseg SP. Inflammatory sites as a source of plasma neopterin: measurement of high levels of neopterin and markers of oxidative stress in pus drained from human abscesses. Clin Biochem 2008;41:1078–83.
28. Fallarino F, Grohmann U, Vacca C, Bianchi R, Orabona C, Spreca A, et al. T cell apoptosis by tryptophan catabolism. Cell Death Differ 2002;9:1069–77.
29. Razumovitch JA, Fuchs D, Semenkova GN, Cherenkevich SN. Influence of neopterin on generation of reactive species by myeloperoxidase in human neutrophils. Biochim Biophys Acta 2004;1672:46–50.
30. Kojima S, Nomura T, Icho T, Kajiwara Y, Kitabatake K, Kubota K. Inhibitory effect of neopterin on NADPH-dependent superoxide-generating oxidase of rat peritoneal macrophages. FEBS Lett 1993;329:125–8.
31. Schroecksnadel S, Jenny M, Kurz K, Klein A, Ledochowski M, Uberall F, et al. LPS-induced NF-kappaB expression in THP-1Blue cells correlates with neopterin production and activity of indoleamine 2,3-dioxygenase. Biochem Biophys Res Commun 2010;399:642–46.
32. Barak M, Gruener N. Neopterin augmentation of tumor necrosis factor production. Immunol Lett 1991;30:101–6.
33. Halliwell B. The wanderings of a free radical. Free Radic Biol Med 2009;46:531–42.
34. Thomas SR, Mohr D, Stocker R. Nitric oxide inhibits indoleamine 2,3-dioxygenase activity in interferon-

gamma primed mononuclear phagocytes. J Biol Chem 1994;269:14457–64.
35. Schneemann M, Schoedon G, Hofer S, Blau N, Guerrero L, Schaffner A. Nitric oxide synthase is not a constituent of the antimicrobial armature of human mononuclear phagocytes. J Infect Dis 1993;167:1358–63.
36. Fuchs D, Murr C, Reibnegger G, Weiss G, Werner ER, Werner-Felmayer G, et al. Nitric oxide synthase and antimicrobial armature of human macrophages. J Infect Dis 1994;169:224.
37. Kwon NS, Nathan CF, Stuehr DJ. Reduced biopterin as a cofactor in the generation of nitrogen oxides by murine macrophages. J Biol Chem 1989;264:20496–501.