



**Review**

## **Cellular Immune Activation, Neopterin Production, Tryptophan Degradation and the Development of Immunodeficiency**

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**Abstract.** Cellular (Th1-type) immune response is centrally involved in the pathogenesis of various diseases. Within the immunological cascades of Th1-type immunity, interferon  $\gamma$  (IFN- $\gamma$ ), among other cytokines, is critically involved. It triggers a series of immune-relevant reactions mostly directed towards forward regulation of the antigen specific immune response. However, in chronic states of immune activation, systemically increased IFN- $\gamma$  is no longer antigen specific and is associated with the development of immunodeficiency. IFN- $\gamma$  also stimulates the production of neopterin, a low-mass compound, in human monocytes/macrophages. Accordingly, neopterin concentrations in humans reflect the degree of Th1-type immune activation. Since IFN- $\gamma$  also stimulates the release of reactive oxygen species (ROS) from immunocompetent cells, the amount of neopterin produced also serves as an indirect estimate of oxidative stress. In parallel, IFN- $\gamma$  activates the degradation of tryptophan, which appears to limit the growth of intracellular pathogens and the proliferation of cells, including T lymphocytes. Thus, during persisting states of immune activation, the production of IFN- $\gamma$  is not only associated with forward regulation of the immune response, but also with immunosuppressive mechanisms. The increased formation of neopterin and degradation of tryptophan may result in a decreased T cell responsiveness and development of immunodeficiency.

**Key words:** cellular immune response; neopterin; tryptophan; immunodeficiency.

The immune system has developed highly specific strategies to encounter pathogenic challenges and to detect “self-aberrant” macromolecules and cell structures. The specific immune response is triggered and controlled by a panel of immunocompetent cells, including T-helper (Th) cells, and by signalling molecules such as cytokines, chemokines and other mediators of inflammation. Th1-type, also called “cellular”, immune response involves several specific cytokines

such as interferon  $\gamma$  (IFN- $\gamma$ ), interleukin 2 (IL-2), IL-12 or IL-18. The clonal selection and proliferation of Th cells, which is preferentially initiated by IL-2 upon stimulation by antigen presenting cells, guarantees a restricted and highly specialized immune response against a specific antigenic cell surface structure. Th2 cells mediate cross-reactivity, the so-called anti-inflammatory immune response, which is exerted by the action of specific Th2-type cytokines such as IL-4, IL-5,

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IL-10, IL-13, IL-14 or IL-16. Beyond this, the immune system employs several other strategies to meet pathogenous challenge, such as activation of complement, mononuclear phagocytosing cells and natural killer (NK) cells. Activation of the so-called unspecific immune system is characterized by unrestricted phagocytosis, complement activation, opsonisation with immunoglobulins, lysozym digestion and oxidative destruction of "non-self" targets.

In most diseases, host immune reaction may involve both the Th1 and the Th2 branches of the immune system, which down-regulate each other<sup>35</sup>. This cross-regulation seems to be responsible for the change of the pattern of immune response during the course of diseases<sup>4</sup>.

Various approaches are applied to estimate the extent of immune activation in patients. Classically, skin test reactivity to intradermally exposed antigens is applied *in vivo*, whereas *in vitro* the responsiveness of T cells upon stimulation with antigens or mitogens is tested to determine the degree of cellular responsiveness. Cytokine production and the proliferation rate of T cells estimated in *in vitro* tests usually agree well with *in vivo* skin test reactivity. Direct *in vivo* measurement of circulating cytokines is very limited, although an almost unlimited number of sensitive tests is available today. Usually, concentrations of cytokines are low and their biological half-life is extremely short, partly due to high affinity interaction between cytokines and their specific receptors. In addition, locally restricted cytokine production does not necessarily reach the circulation, thereby remaining undetectable in the peripheral blood. Thus, circulating cytokine levels are frequently below the detectable range. Alternatively, cytokine mRNA expression in circulating immunocompetent cells or the concentration of soluble cytokine receptors, e.g., soluble IL-2 receptor (sIL-2R) or soluble tumor necrosis factor receptor (sTNF-R)<sup>6, 36</sup>, as well as the amount of  $\beta_2$ -microglobuline<sup>3</sup> or neopterin<sup>10</sup> are measured to evaluate the degree of immune activation. Quantifying cytokine mRNA expression is specific but primarily allows detection of the status of circulating cells and not insight into the local situation of immune response. Neopterin, a low molecular mass (253 Da) compound, is particularly useful for monitoring immune activation because its diffusion rate within the circulation and its biological stability are high. Neopterin is a sensitive surrogate parameter for IFN- $\gamma$  production and provides information about Th1-type immune response. Similarly, the rate of tryptophan degradation allows one to monitor IFN- $\gamma$  activity and its determination represents a stable method for recording the Th1-type immune response within the complex cytokine network.

Interestingly, apparently contradictory results are obtained when comparing circulating cytokine levels with skin test reactivity and T cell responsiveness. This is true in acquired immunodeficiency during infections, as well as in malignant and autoimmune diseases.

### Neopterin Release during Cellular Immune Activation

Neopterin (D-erythro-1', 2', 3'-trihydroxypropyl-pterin) is produced and released in large quantities by human monocytic cells upon induction by IFN- $\gamma$ <sup>19</sup>. The key enzyme in the biosynthesis of neopterin is guanosine triphosphate (GTP) cyclohydrolase I, converting GTP to 7,8-dihydroneopterin triphosphate. 7,8-Dihydroneopterin triphosphate is the precursor in the synthesis of various biologic pteridine derivatives including 5,6,7,8-tetrahydrobiopterin, a cofactor for certain monooxygenases and the nitric oxide synthases. Human and primate monocytes/macrophages are unique in as much as they almost exclusively produce neopterin and 7,8-dihydroneopterin at the expense of biopterin derivatives. Recent findings point to a role of neopterin as part of the oxidative armature of stimulated monocytes/macrophages<sup>28, 43, 36, 47</sup>.

*In vitro* experiments on the myelomonocytic cell line THP-1 showed that IFN- $\gamma$  is the most potent inducer of neopterin production. TNF- $\alpha$  was found to enhance the effect of IFN- $\gamma$  in a synergistic manner<sup>45</sup>. Similarly, in peripheral blood mononuclear cells, IL-2 or IL-12 enhanced neopterin production via stimulation of Th cell subpopulations, whereas Th2-type cytokines such as IL-4, IL-5 or IL-10 suppressed the formation of neopterin<sup>44</sup>.

Since GTP cyclohydrolase I is mainly stimulated by IFN- $\gamma$ , high neopterin concentrations can be found in human body fluids during cellular immune response. Neopterin is excreted via the kidneys and urine neopterin concentrations correlate well with serum levels<sup>15</sup>. Measurement of neopterin provides useful information regarding the extent of cellular immune activation. In a variety of diseases, neopterin concentrations were determined and found to be related to concentrations of other markers of immune activation such as sTNF-R and sIL2-R as well as to IFN- $\gamma$  production (Table 1)<sup>5, 6, 11, 21, 37, 38</sup>. High correlations are found throughout and the data suggest neopterin to be an element of the IFN- $\gamma$ /TNF- $\alpha$  system.

Laboratory diagnostic applications of neopterin measurement include the surveillance of allograft recipients for early detection of immunological complica-

**Table 1.** Exemplary list of diseases in which close associations (+) were found between increased concentrations of neopterin, soluble TNF-receptors (sTNF-Rs) and interferon  $\gamma$  (IFN- $\gamma$ ) as well as tryptophan degradation via activation of indoleamine 2,3-dioxygenase (IDO)

| Disease                                                                                                     | Neopterin | IDO | sTNF-R | IFN- $\gamma$ |
|-------------------------------------------------------------------------------------------------------------|-----------|-----|--------|---------------|
| Infections<br>e.g. HIV, Lyme neuroborreliosis, brucellosis                                                  | +         | +   | +      | +             |
| Autoimmune diseases<br>e.g. systemic lupus erythematosus, rheumatoid arthritis,<br>Wegener's granulomatosis | +         | +   | +      | not done      |
| Malignant disorders<br>e.g. hematological neoplasias, gynecological tumors                                  | +         | +   | +      | +             |

tions such as rejection episodes or infections<sup>23</sup>, the easy assessment of disease activity in autoimmune disorders and infectious diseases<sup>10, 37</sup>, and the additional screening of blood donations for otherwise undetected infections<sup>39</sup>. In several malignant diseases<sup>26, 32, 33</sup> and HIV infection<sup>7, 14</sup>, neopterin was proved to be an independent prognostic parameter for disease progression and survival.

### Cellular Immune Activation and the Degradation of Tryptophan

A wide variety of cells expresses the enzyme indoleamine 2,3-dioxygenase (IDO), which is induced by IFN- $\gamma$  and converts tryptophan to N-formylkynurenine<sup>42</sup>, subsequently deformed to kynurenine. Levels of tryptophan and kynurenine in serum can be determined by high performance liquid chromatography after deproteinization<sup>49</sup>. The kynurenine to tryptophan quotient provides a good method for estimating activation of IDO and, thus, IFN- $\gamma$  production<sup>12</sup>. This association is substantiated by several studies dealing with infectious, malignant and autoimmune diseases, revealing a strong correlation between the kynurenine to tryptophan quotient and neopterin concentrations as well as the concentrations of soluble cytokine receptors (Table 1)<sup>16</sup>. Therefore, the degradation rate of tryptophan, like the determination of neopterin, can serve as an indirect monitor for endogenously released IFN- $\gamma$ .

Since tryptophan is the least available amino acid in the organism, IFN- $\gamma$ -induced tryptophan deprivation has an antiproliferative effect<sup>18</sup>. For example, intracellular growth of *Toxoplasma gondii* and *Chlamydia psittaci* was inhibited by IFN- $\gamma$ , which was reversed by tryptophan supplementation<sup>2, 31</sup>. Similarly, IFN- $\gamma$ -mediated tryptophan depletion might be involved in the growth inhibition of highly proliferating tissue, such as malignant cells<sup>34, 51</sup>, but also activated immunocompetent cells, such as T cells<sup>24</sup>. This effect may be limited

to the site of inflammation, but the systemic decrease of tryptophan could also be of general importance in suppressing T cell response. Accordingly, in a murine model, tryptophan catabolism was found to be essential for the survival of the conceptus to maintain immune suppression<sup>25</sup>. Earlier findings of decreased tryptophan concentrations together with increased neopterin concentrations in pregnant women<sup>40</sup> would support the concept of tryptophan degradation to suppress T cell responsiveness as a pre-requisite for a successful gestation.

Besides the microbicidal and cytotoxic effect of tryptophan deprivation, low circulating tryptophan levels may also affect biosynthesis of the neurotransmitter 5-hydroxytryptamine (serotonin)<sup>50</sup>. Serotonin is biosynthetically derived from tryptophan in a reaction catalyzed in the first step by tryptophan hydroxylase. Thus, in patients with chronic immune activation such as infectious, autoimmune or malignant diseases, low tryptophan concentrations increase the susceptibility for neuropsychiatric disorders<sup>16</sup>. Accordingly, tryptophan depletion was found in the blood of patients with neurodegenerative diseases, e.g., Alzheimer's disease<sup>48</sup>.

### IFN- $\gamma$ and Oxidative Burst

Apart from its immunomodulatory role, IFN- $\gamma$  is a potent primer for the release of ROS from monocytes/macrophages and from neutrophils<sup>29</sup>. Hence, the oxidative potential of the cells is closely linked to the activation status of the cell-mediated immune system. A close correlation exists between IFN- $\gamma$  activity in macrophages, the release of ROS and the excretion of neopterin<sup>28</sup>. Neopterin can thus be regarded as an indirect estimate for the amount of ROS produced during the oxidative burst. When the natural antioxidative capacity of the tissue is too low to overcome the increased production of ROS, oxidative stress occurs, leading to

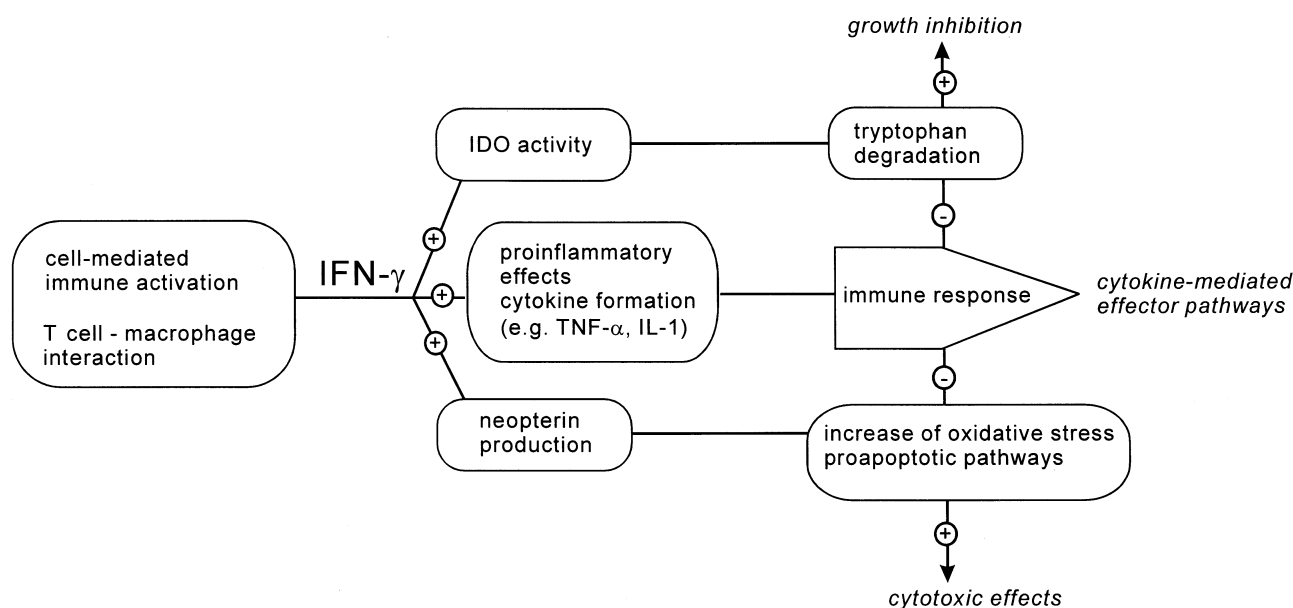
the destruction of important cell structures such as membranes, proteins or purines<sup>17</sup>. Beyond this, ROS are able to trigger specific elements of the intracellular signal transduction pathway. Oxidative stress is closely linked to the induction of apoptosis, e.g., by mediation of p53, the activity of which may be enhanced by oxidants<sup>41</sup>. Thus, the increased production of IFN- $\gamma$  via enhancement of oxidative stress may also result in T cell apoptosis and immunodeficiency. Such a scenario will be especially relevant for clinical situations of chronic immune system activation such as in HIV infection<sup>1, 13</sup>. A reduced functional response of T cell populations will result in immunodeficiency.

### IFN- $\gamma$ and Immunodeficiency

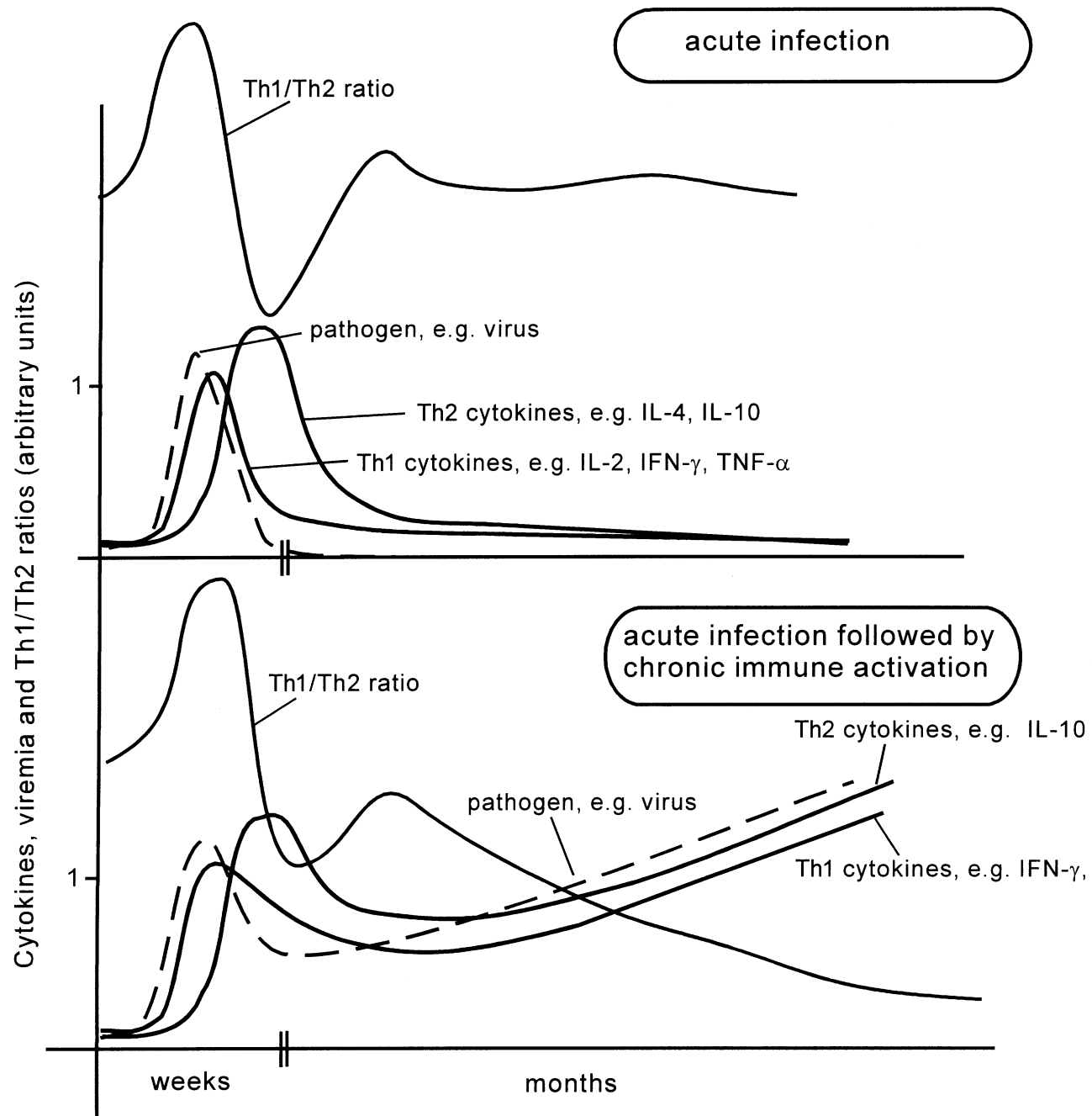
During acute cellular immune reaction, IFN- $\gamma$  acts as a positive regulator via modulation of antigen presentation and lymphocyte differentiation and proliferation (Fig. 1). Thereby, along the first wave of cellular immune activation, IFN- $\gamma$  increases T cell activity and hence supports the forward regulation of the Th1-type immune response. However, acute and even more chronic cell-mediated immune activation is also associated with the development of immunodeficiency (Fig. 2). *In vivo*, this type of immunodeficiency is determined by reduced skin-test reactivity in patients and by a re-

duced proliferative and functional response of T cells *in vitro*. These observations suggested a functional deficiency of T cells to produce cytokines in patients with immunodeficiency. This hypothesis agreed well with early *in vivo* studies where circulating IFN- $\gamma$  was undetectable in immunodeficient patients<sup>27</sup>. However, this was due to the insufficient sensitivity of the IFN- $\gamma$  measurement. Later it became obvious that increased circulating IFN- $\gamma$  is associated with a refractory state of T cells upon secondary stimulation *in vitro* and *in vivo*<sup>13</sup>. Indeed, immunodeficiency appears to result from a chronic stimulation of cells which leads to exhaustion.

As detailed above, neopterin production preferentially depends on T cell response which is associated with IFN- $\gamma$  release. But, high neopterin concentrations are predominantly found to be associated with T cell anergy. For example, in patients with malignant disorders, higher neopterin concentrations are predictive for poor survival<sup>26, 32, 33</sup>. The same is true in patients with HIV infections in which the highest neopterin concentrations are found in later stages of infection, inversely correlating with CD4<sup>+</sup> cell counts, being a predictor of more rapid disease progression<sup>7, 14</sup>. All together, the data imply that, due to exhaustion by chronic exposure to immunological stimuli, T cells attain stages of anergy in which they are unable to respond to antigens<sup>11</sup>. This conclusion is further supported by findings from



**Fig. 1.** Interferon  $\gamma$  (IFN- $\gamma$ ) is a pivotal cytokine for a series of proinflammatory effects contributing to successful immunosurveillance. IFN- $\gamma$  also stimulates neopterin production and activates indoleamine 2,3-dioxygenase (IDO), which may support host defense by increasing cytotoxic effects and inhibiting growth. In parallel, neopterin production and tryptophan degradation may contribute to down-force immune response to enable normal immune system equilibrium



**Fig. 2.** Schematic course of the Th1-type and Th2-type immune responses and production of cytokines during acute and chronic infections. In acute infections the Th1 immune response is down-regulated by Th2-type cytokines entering a state of equilibrium. The chronic phase of immune stimulation follows the acute response when the stimulating agent (infectious pathogen) cannot be deleted. Both the Th1- and the Th2-type immune responses remain continuously active during the chronic type of immune activation, because the Th2-type immune response is actively down-regulating the Th1-type reaction, but the resistant pathogen continues to stimulate Th-1 type immunity in parallel

patients with, e.g., systemic lupus erythematosus<sup>20</sup> or HIV infection<sup>22</sup>, whose reduced functional T cell response returned to normal values after cells were cultured *in vitro* for a few days. Thus, chronic stimulation of immunocompetent cells rather than inhibition could be the underlying cause of immunodeficiency. It seems at first glance controversial that low T cell responsive-

ness goes along with high IFN- $\gamma$  levels, and, e.g., CD8<sup>+</sup> T cells might be a relevant source for IFN- $\gamma$  in chronic states of immune activation. In any case, IFN- $\gamma$  from either source would contribute to a shift towards the Th1-type immune response.

Increased production of IFN- $\gamma$  and increased neopterin concentrations themselves may act down-regula-

tory on effector functions via enhancement of oxidative stress<sup>9</sup> (Fig. 1). ROS are known to interfere with apoptotic pathways, and activation of the TNF-Rs can be involved in the induction of programmed cell death<sup>30</sup>. Stimulation of the TNF-Rs can result in a rapid rise of ROS, and both TNF expression and the release of ROS are mediated by IFNs. Along this line, in various cell lines TNF- $\alpha$ -mediated apoptosis can be inhibited by reductants or free radical scavengers. Actually, the correlation between concentrations of serum sTNF-R and neopterin was demonstrated to be considerably strong in a wide variety of diseases, such as HIV infection, malignant and autoimmune diseases<sup>6</sup>. Apoptosis due to enhanced oxidative stress could contribute to destruction of lymphoid cells, thereupon leading to immunodeficiency and anergy. Up-regulation of surface expression and shedding of TNF receptors and increased neopterin might represent markers not only for immune activation, but also for the extent of developing immunodeficiency, since neopterin appears as a consequence of IFN- $\gamma$  activity irrespective of the functional state of T cell subpopulations. There are several indications that acquired immunodeficiency in conditions of chronic immune activation is associated with increased endogenous formation of IFN- $\gamma$  whereas IL-2 is diminished<sup>8, 11</sup>. The increased production of neopterin and the enhanced degradation of tryptophan also proves that IFN- $\gamma$  is biologically active in inducing biochemical changes in target cells.

## Conclusion

Activation of the cellular immune system is connected with the development of immunodeficiency. IFN- $\gamma$  appears as a pivotal mediator, first activating the local antigen-specific immune response and, secondly, suppressing non-specific systemic immune effector functions, contributing to the fine regulation of the immune response, which is directed to reach an equilibrium. Tryptophan degradation and neopterin production are consequences of IFN- $\gamma$  activity which may directly contribute to the development of immunodeficiency. It may be speculated that this mechanism serves as a feedback regulation to constrain immune response and to prevent overwhelming inflammation.

## References

1. BAIER-BITTERLICH G., FUCHS D. and WACHTER H. (1997): Chronic immune stimulation, oxidative stress and apoptosis in HIV infection. *Biochem. Pharmacol.*, **53**, 755–763.
2. BYRNE G. I., LEHMANN L. K. and LANDRY G. J. (1986): Induction of tryptophan catabolism is the mechanism for gamma-interferon-mediated inhibition of intracellular *Chlamydia psittaci* replication in T24 cells. *Infect. Immun.*, **53**, 344–351.
3. CLEMENT F. (1983): Beta-2-microglobulin. *Schweiz. Med. Wochenschr.*, **113**, 838–843.
4. CLERICI M. and SHEARER G. M. (1993): A TH1-TH2 switch is a critical step in the etiology of HIV infection. *Immunol. Today*, **14**, 107–111.
5. DENZ H., FUCHS D., HUBER H., NACHBAUER D., REIBNEGGER G., THALER J. et al. (1990): Correlation between neopterin, interferon-gamma and haemoglobin in patients with haematological disorders. *Eur. J. Haematol.*, **44**, 186–189.
6. DIEZ-RUIZ A., TILZ G. P., ZANGERLE R., BAIER-BITTERLICH G., WACHTER H. and FUCHS D. (1995): Soluble receptors for tumour necrosis factor in clinical laboratory diagnosis. *Eur. J. Haematol.*, **54**, 1/8.
7. FAHEY J. L., TAYLOR J. M., DETELS R., HOFMANN B., MELMED R., NISHANIAN P. et al. (1989): The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *N. Engl. J. Med.*, **322**, 166–172.
8. FAN J., BASS H. Z. and FAHEY J. L. (1993): Elevated IFN-gamma and decreased IL-2 gene expression are associated with HIV infection. *J. Immunol.*, **151**, 5031–5040.
9. FUCHS D., BAIER-BITTERLICH G., WEDE I. and WACHTER H. (1997): Reactive oxygen and apoptosis. In SCANDALIOS J. (ed.): *Oxidative stress and the molecular biology of antioxidant defenses*. Cold Spring Harbor Laboratory Press, New York 139–167.
10. FUCHS D., HAUSEN A., REIBNEGGER G., WERNER E. R., DIERICH M. P. and WACHTER H. (1988): Neopterin as a marker for activated cell-mediated immunity. Application in HIV infection. *Immunol. Today*, **9**, 150–155.
11. FUCHS D., MALKOVSKY M., REIBNEGGER G., WERNER E. R., FORNI G. and WACHTER H. (1989): Endogenous release of interferon-gamma and diminished response of peripheral blood mononuclear cells to antigenic stimulation. *Immunol. Lett.*, **23**, 103–108.
12. FUCHS D., MÖLLER A. A., REIBNEGGER G., STÖCKLE E., WERNER E. R. and WACHTER H. (1990): Decreased serum tryptophan in patients with HIV-1 infection correlates with increased serum neopterin and with neurologic/psychiatric symptoms. *J. Acquir. Immune Defic. Syndr.*, **3**, 873–876.
13. FUCHS D., SHEARER G. M., BOSWELL R. N., CLERICI M., REIBNEGGER G., WERNER E. R., et al. (1990): Increased serum neopterin in patients with HIV-1 infection is correlated with reduced *in vitro* interleukin-2 production. *Clin. Exp. Immunol.*, **80**, 44–48.
14. FUCHS D., SPIRA T. J., HAUSEN A., REIBNEGGER G., WERNER E. R., WERNER-FELMAYER G. et al. (1989): Neopterin as a predictive marker for disease progression in human immunodeficiency virus type 1 infection. *Clin. Chem.*, **35**, 1746–1749.
15. FUCHS D., STAHL-HENNIG C., GRUBER A., MURR C., HUNSMANN G. and WACHTER H. (1994): Neopterin – is clinical use in urine analysis. *Kidney Internat.*, **46**, (S47), 8–11.
16. GASSE T., WIDNER B., BAIER-BITTERLICH G., SPERNER-UNTERWEGER B., LEBLHUBER F., WACHTER H. and FUCHS D. (1998): Abnormal tryptophan metabolism, neurologic/psychiatric disturbances and its relationship to immune activation. In QUERESHI G. A., PARVEZ H., CAUDY P. and PARVEZ S. (eds.): *Neurochemical markers of degenerative diseases and drug addiction. Progress in HPLC-HPCE*: VSP Press, Zeist, **7**, 351–382.

17. HALLIWELL B. (1990): Role of free radicals and catalytic metal ions in human diseases. An overview. *Methods Enzymol.*, **186**, 1–85.
18. HAYAISHI O. (1996): Utilization of superoxide anion by indoleamine oxygenase-catalyzed tryptophan and indoleamine oxidation. *Adv. Exp. Med. Biol.*, **398**, 285–289.
19. HUBER C., BATCHELOR J. R., FUCHS D., HAUSEN A., LANG A., NIEDERWIESER D. et al. (1984): Immune response-associated production of neopterin. Release from macrophages primarily under control of interferon gamma. *J. Exp. Med.*, **160**, 310–316.
20. HUANG Y. P., MIESCHER P. A. and ZUBLER R. H. (1986): The interleukin-2 secretion defect *in vitro* in systemic lupus erythematosus is reversible in rested cultured T cells. *J. Immunol.*, **137**, 3515–3520.
21. LEBLHUBER F., WALLI J., TILZ G. P., WIDNER B. and FUCHS D. (1999): Increased serum neopterin concentrations in patients with Alzheimer's disease. *Clin. Chem. Lab. Med.*, **37**, 429–431.
22. LEES O., RAMZAOUI S., GILBERT D., BORSA F., HUMBERT G., LEBLANC D. et al. (1993): The impaired *in vitro* production of interleukin-2 in HIV infection is negatively correlated to the number of circulating CD4<sup>+</sup>DR<sup>+</sup> T cells and is reversed by allowing T cells to rest in culture: arguments for *in vivo* CD4<sup>+</sup> T cell activation. *Clin. Immunol. Immunopathol.*, **67**, 185–191.
23. MARGREITER R., FUCHS D., HAUSEN A., HUBER C., REIBNEGGER G., SPIELBERGER M. et al. (1983): Neopterin as a new biochemical marker for diagnosis of allograft rejection. *Transplantation*, **36**, 650–653.
24. MUNN D. H., SHAFIZADEH E., ATTWOOD J. T., BONDAREV I., PASHINE A. and MELLOR A. L. (1990): Inhibition of T-cell proliferation by macrophage tryptophan catabolism. *J. Exp. Med.*, **189**, 1663–1372.
25. MUNN D. H., ZHOU M., ATTWOOD J. T., BONDAREV I., CONWAY S. J., MARSHALL B., BROWN C. and MELLOR A. L. (1998): Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science*, **281**, 1191–1193.
26. MURR C., FUITH L. C., WIDNER B., WIRLEITNER B., BAIER-BITTERLICH G. and FUCHS D. (1990): Increased neopterin concentrations in patients with cancer: indicator of oxidative stress. *Anticancer Res.*, **19**, 1721–1728.
27. MURRAY H. W. (1988): Interferon-gamma, the activated macrophage, and host defense against microbial challenge. *Ann. Intern. Med.*, **108**, 595–608.
28. NATHAN C. F. (1986): Peroxide and pteridine: a hypothesis on the regulation of macrophage antimicrobial activity by interferon- $\gamma$ . In GRESSER I. and VILCEK J. (eds.): *Interferon 7*. Academic Press, London, 125–143.
29. NATHAN C. F., MURRAY H. W., WIEBE M. E. and RUBIN B. Y. (1983): Identification of interferon- $\gamma$  as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. Exp. Med.*, **158**, 670–689.
30. PENDER M. P. (1999): Activation-induced apoptosis of autoreactive and alloreactive T lymphocytes in the target organ as a major mechanism of tolerance. *Immunol. Cell. Biol.*, **77**, 216–223.
31. PFEFFERKORN E. R. (1984): Interferon- $\gamma$  blocks the growth of *Toxoplasma gondii* in human fibroblast by inducing the host cells to degrade tryptophan. *Proc. Natl. Acad. Sci. USA*, **81**, 908–912.
32. REIBNEGGER G., BICHLER A., DAPUNT O., FUCHS D., FUITH L. C., HAUSEN A. et al. (1986): Neopterin as a prognostic indicator in patients with carcinoma of the uterine cervix. *Cancer Res.*, **46**, 950–955.
33. REIBNEGGER G., FUCHS D., FUITH L. C., HAUSEN A., WERNER E. R., WERNER-FELMAYER G. et al. (1991): Neopterin as a marker for activated cell-mediated immunity. Application in malignant disease. *Cancer Detect. Prevent.*, **15**, 483–490.
34. ROBERTS J., SCHMID F. A. and ROSENFELD H. J. (1979): Biologic and antineoplastic effects of enzyme-mediated *in vivo* depletion of L-glutamine, L-tryptophan and L-histidine. *Cancer Treat. Rev.*, **63**, 1045–1054.
35. ROMAGNANI S. (1997): The Th1-Th2 paradigm. *Immunol. Today*, **18**, 263–266.
36. RUBIN L. A. and NELSON D. L. (1990): The soluble interleukin-2 receptor: biology, function, and clinical application. *Ann. Intern. Med.*, **113**, 619–627.
37. SAMSONOV M. Y., TILZ G. P., EGOROVA O., REIBNEGGER G., BALABANOVA R. M., NASSONOV E. L. et al. (1995): Serum soluble markers of immune activation and disease activity in systemic lupus erythematosus. *Lupus*, **4**, 29–32.
38. SAMASONOV M. Y., TILZ G. P., PISKLAKOV V. P., REIBNEGGER G., NASSONOV E. L., NASSONOV V. A. et al. (1995): Serum-soluble receptors for tumor necrosis factor- $\alpha$  and interleukin-2, and neopterin in acute rheumatic fever. *Clin. Immunol. Immunopathol.*, **74**, 31–34.
39. SCHENNACH H., MAYERSBACH P., SCHÖNITZER D., FUCHS D., WACHTER H. and REIBNEGGER G. (1994): Increased prevalence of IgM antibodies to Epstein-Barr virus and parvovirus B19 in blood donations with above-normal neopterin concentration. *Clin. Chem.*, **40**, 2104–2105.
40. SCHRÖCKSNADEL H., BAIER-BITTERLICH G., DAPUNT O., WACHTER H. and FUCHS D. (1996): Decreased plasma tryptophan in pregnancy. *Obstet. Gynecol.*, **88**, 47–50.
41. SUZUKI Y. J., FORMAN H. J. and SEVANIAN A. (1996): Oxidants as stimulators of signal transduction. *Free Radic. Biol. Med.*, **22**, 269/285.
42. TAYLOR M. W. and FENG G. (1991): Relationship between interferon- $\gamma$ , indoleamine 2,3-dioxygenase, and tryptophan catabolism. *FASEB J.*, **5**, 2516–2522.
43. WEISS G., FUCHS D., HAUSEN A., REIBNEGGER G., WERNER E. R., BAIER-BITTERLICH G. et al. (1993): Neopterin modulates toxicity mediated by reactive oxygen and chlorine species. *FEBS Lett.*, **321**, 89–92.
44. WEISS G., MURR C., ZOLLER H., HAUN M., WIDNER B., LUDSCHER C. et al. (1999): Modulation of neopterin formation and tryptophan degradation by Th1- and Th2-derived cytokines in human monocytic cells. *Clin. Exp. Immunol.*, **116**, 435–440.
45. WERNER-FELMAYER G., WERNER E. R., FUCHS D., HAUSEN A., REIBNEGGER G. and WACHTER H. (1989): Tumor necrosis factor-alpha and lipopolysaccharide enhance interferon-induced tryptophan degradation and pteridine synthesis in human cells. *Biol. Chem. Hoppe Seyler*, **370**, 1063–1069.
46. WIDNER B., BAIER-BITTERLICH G., WEDE I., WIRLEITNER B. and FUCHS D. (1998): Neopterin derivatives modulate the nitration of tyrosine by peroxynitrite. *Biochem. Biophys. Res. Commun.*, **248**, 341–346.
47. WIDNER B., BAIER-BITTERLICH G., WEDE I., WIRLEITNER B., WACHTER H. and FUCHS D. (1998): Neopterin: indicator of oxidative stress and part of the cytotoxic armature of activated macrophages in humans. *Pteridines*, **9**, 91–102.
48. WIDNER B., LEBLHUBER F., WALLI J., TILZ G. P., DEMEL U. and FUCHS D. (2000): Tryptophan degradation and immune activation in Alzheimer's disease. *J. Neural. Transm.*, **107**, 343–353.

49. WIDNER B., WERNER E. R., SCHENNACH H., WACHTER H. and FUCHS D. (1997): Simultaneous measurement of serum tryptophan and kynurenine by HPLC. *Clin. Chem.*, **43**, 2424–2426.
50. WILLIAMS W. A., SHOAF S. E., HOMMER D., RAWLINGS R. and LINNOILA M. (1999): Effects of acute tryptophan depletion on plasma and cerebrospinal fluid and 5-hydroxyindoleacetic acid in normal volunteers. *J. Neurochem.*, **72**, 1641–1647.
51. WOLLEY P. V., DION R. L. and BONO V. H. (1974): Effects of tryptophan deprivation on L1210 cells in culture. *Cancer Res.*, **34**, 1010–1014.

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