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Commentary

Biosynthesis and Significance of Neopterin in the Immune System

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NEOPTERIN [6-(D-*erythro*-1′,2′,3′-trihydroxypropyl)-pterin] is a degradation product of dihydroneopterin triphosphate (NH_2TP), the first intermediate in the biosynthesis of tetrahydrobiopterin (Fig. 1). Tetrahydrobiopterin is the natural cofactor of three aromatic amino acid hydroxylases and is essential for the biosynthesis of the neurotransmitters dopamine and serotonin [1]. In addition to the hydroxylation of aromatic amino acids, tetrahydrobiopterin might play a role in ether lipid oxidation, proline hydroxylation and mitochondrial electron transport.

Three inborn errors of metabolism are known to cause tetrahydrobiopterin deficiency: GTP cyclohydrolase I deficiency, 6-pyruvoyl tetrahydropterin synthase deficiency and dihydropteridine reductase deficiency [2]. Due to the metabolic block pterin metabolites (e.g. neopterin or biopterin) accumulate in these patients and are excreted in the urine. In patients with GTP cyclohydrolase I deficiency, no pterins at all are synthesized. These patients are characterized both by neopterin and biopterin deficiency. Although it has been claimed that neopterin or similar metabolites may play an active role in the cell-mediated immune response, cell proliferation and differentiation [3], in one patient all humoral and cellular immune functions tested were found to be normal [4]. In patients with 6-pyruvoyl tetrahydropterin synthase deficiency, neopterin accumulates and in patients with dihydropteridine reductase biopterin accumulates. However, both groups of tetrahydrobiopterin deficient patients show no immunological abnormalities.

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Experiments with cancer cells have shown that biopterin metabolism is altered during cell growth [5]. Increased neopterin excretion has been observed in patients suffering from neoplastic diseases or viral infections, including AIDS [6, 7], and it was suggested to be a new marker for T-cell activation [8]. It was recognized that the increased neopterin is related to the cell-mediated immune response because of antigen-induced neopterin release by macrophages *in vitro* [9], and it is now well known that neopterin excreted in urine originates from NH_2TP and that its levels are dependent on GTP cyclohydrolase I (EC 3.5.4.16) activity [10].

To understand the role of neopterin in the immune system, the difference in tetrahydrobiopterin biosynthesis between primates and lower mammals has to be considered [11]. Generally, the biosynthesis of pterins proceeds from GTP via the four enzymes GTP cyclohydrolase I, 6-pyruvoyl tetrahydropterin synthase, 6-pyruvoyl tetrahydropterin reductase and sepiapterin reductase. In primates, selective accumulation of NH_2TP occurs leading to the appearance of neopterin. Since no neopterin is found in non-primates, this indicates that tetrahydrobiopterin biosynthesis is regulated differently due to different rate-limiting enzymes.

Neopterin and biopterin, degradation products of NH_2TP and tetrahydrobiopterin, respectively, as well as pterin, a degradation product of former compounds, can be found in the human immune system [12]. However, the pattern of pterin metabolites is different in T-lymphocytes from that in monocytes/macrophages. Low concentrations of all pterins are found in T-cells, while the concentration increases after stimulation with mitogenic lectines

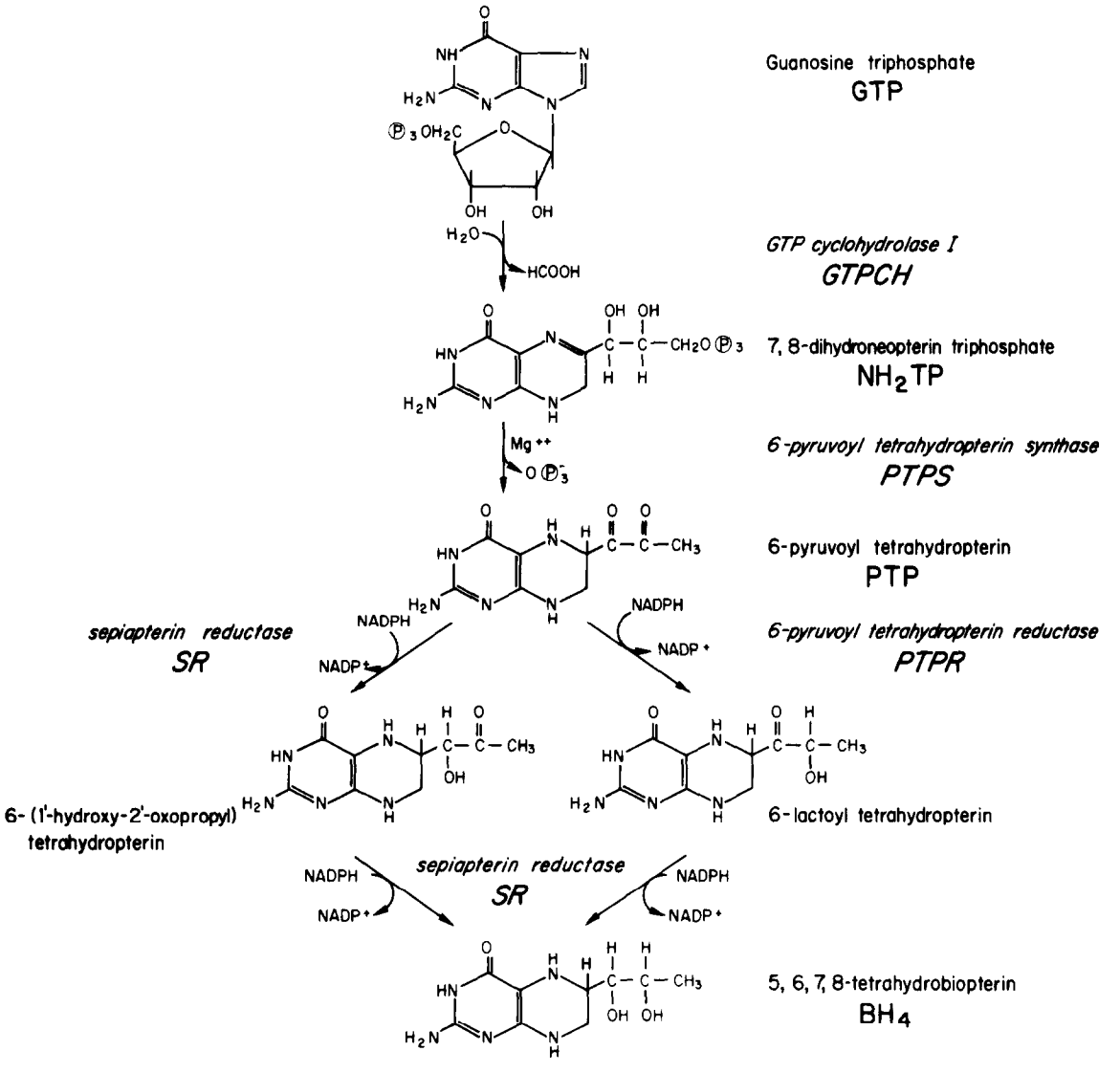

Fig. 1. Biosynthetic pathway of tetrahydrobiopterin from GTP.

or mixed lymphocyte culture. After stimulation there is also a slight but significant increase of GTP cyclohydrolase I activity, while GTP (substrate) increases up to 10 times. In human macrophages only neopterin is detected; its concentration rises drastically after stimulation with interferon-gamma [13]. GTP cyclohydrolase I activity in stimulated macrophages is 10 times higher than in T-cells and correlates well with the high GTP levels. Measurement of all tetrahydrobiopterin biosynthetic enzymes revealed that in macrophages there is no activity of 6-pyruvoyl tetrahydropterin synthase. Therefore, human macrophages are deficient in tetrahydrobiopterin, the end-product effecting feedback inhibition of GTP cyclohydrolase I. The addition of high concentrations of tetrahydrobiopterin together with interferon-gamma to the macrophage culture medium suppressed the GTP cyclohydrolase I activity and neopterin production; however, it did not affect the intracellular GTP concentration [13]. Normal cell maturation and normal immunological functions were observed in macrophages despite suppression of neopterin production.

In the mouse immune system, tetrahydrobiopterin biosynthesis shows two fundamental differences: first, no neopterin or other intermediates of the biopterin biosynthesis can be detected in either T-cells or macrophages. After stimulation, high levels of biopterin, which was found to be in tetrahydro form, were measured. Secondly, mouse macrophages do not lack 6-pyruvoyl tetrahydropterin synthase activity. Since no neopterin is found in any of the stimulated mouse immune cells, GTP cyclohydrolase I is most likely the rate limiting enzyme. Therefore NH_2TP is immediately metabolized further without any accumulation. In both man and mouse immune cells, GTP cyclohydrolase I is the only pterin-synthesizing enzyme sensitive to stimulation and thus responsible for the high levels of pterins [11, 12]. It has further been proven that in both species the increase of GTP cyclohydrolase

I activity in the immune cells is a result of the high substrate concentrations caused by stimulation. The GTP cyclohydrolase I is not induced by immune stimulation, but activated by the rising GTP pool [12].

In cultures of activated immune cells, increased excretion not only of neopterin but also of 3-hydroxyanthranilic acid was found [14]. The strict correlation of both neopterin and 3-hydroxyanthranilic acid production after interferon-gamma stimulation leads to the hypothesis that tetrahydroneopterin may be the cofactor of anthranilic acid-3-hydroxylase. However, by experiments with inhibitors of GTP cyclohydrolase I it has been shown that inhibition of neopterin production to about 20–30% did not result in a significant decrease of 3-hydroxyanthranilic acid production [15]. Furthermore it was not possible to detect any tetrahydroneopterin in macrophages stimulated with interferon-gamma. Therefore the proposed function of the tetrahydroneopterin intermediate in tryptophan degradation is rather hypothetical.

A physiological role of neopterin in the immune system was ruled out, while the role of tetrahydrobiopterin is not yet clear. There is some evidence for interaction of tetrahydrobiopterin with interleukin-2 in T-cell proliferation [16]. In conclusion, elevated neopterin excretion in man reveals an unspecific activation of macrophages by stimulation of the cellular immune system.

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