ELSEVIER

International Immunopharmacology 6 (2006) 1847–1858

International Immunopharmacology

www.elsevier.com/locate/intimp

Inflammatory biomarker, neopterin, enlarges splenic mast-cell-progenitor pool: Prominent impairment of responses in age-related stromal cell-impairment mouse SCI/SAM

Toshitaka Fukumoto [a], Isao Tsuboi [a,*], Tomonori Harada [a], Masaki Hiramoto [a], Akihiro Minami [a], Morimichi Koshinaga [a], Yoko Hirabayashi [b], Jun Kanno [b], Tohru Inoue [c], Shin Aizawa [a]

[a] *Department of Anatomy, Nihon University School of Medicine, 30-1 Ohyaguchi-kami-machi, Itabashiku, Tokyo 173-8610, Japan*
[b] *Division of Cellular and Molecular Toxicology, National Institute of Health Sciences, Tokyo 158-8501, Japan*
[c] *Center for Biological Safety and Research, National Institute of Health Sciences, Tokyo 158-8501, Japan*

Received 25 May 2006; received in revised form 21 July 2006; accepted 3 August 2006

Abstract

Neopterin is produced by monocytes and is a useful biomarker of inflammatory responses. We found that neopterin enhances granulopoiesis, but suppresses B-lymphopoiesis triggered by the positive and negative regulations of cytokines produced by stromal cells in mice. In this study, neopterin was found to regulate mast cell development, which was confirmed in the mouse model of senescent stromal-cell impairment (SCI). In non-SCI mice (=less senescent stage of SCI mice), neopterin decreased the number of colonies of IL-3-dependent mast-cell progenitor cells (CFU-mast) from unfractionated bone-marrow cells, but *not* that from the lineage-negative bone-marrow cell population without stromal cells in a semisolid *in vitro* system. Neopterin increased the gene expression and protein production of TGF-β, a negative regulator of CFU-mast, in cultured stromal cells, indicating that neopterin suppressed CFU-mast colony formation by inducing TGF-β in stromal cells. In contrast to this *in vitro* study, *in vivo* treatment with neopterin did not significantly up-regulate TGF-β. The intravenous injection of neopterin into mice decreased the number of femoral CFU-mast and the expression level of the gene for stem cell factor (SCF), a positive regulator of CFU-mast, whereas the number of splenic CFU-mast and SCF gene expression level increased. In SCI mice, the *in vivo* and *in vitro* responses of mast cell development and cytokine gene expression level to neopterin treatment were less marked than those in non-SCI mice. These results suggest that, firstly, neopterin augments the splenic pool of CFU-mast by the production of SCF, and secondly, such neopterin function becomes impaired during senescence because of an impaired stromal-cell function, resulting in the down-modulation of host-defense mechanisms.

Keywords: Neopterin; Mast cell; Senescence-accelerated mice (SAM); Aging; Stromal cell

1. Introduction

Neopterin is a metabolite of guanosine triphosphate that is produced in the biopterin synthetic pathway [1].

* Corresponding author. Tel.: +81 3 3972 8111x2222; fax: +81 3 3973 8832.
E-mail address: tsuboi@med.nihon-u.ac.jp (I. Tsuboi).

1567-5769/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.intimp.2006.08.002

This metabolite is generated in large amounts by monocytes and macrophages in response to interferon-γ (IFN-γ) [2]. An increased neopterin production is observed following infection by viruses, bacteria and parasites [3]. Because neopterin level in body fluid reflects immune responses *in vivo*, neopterin is regarded as a sensitive marker of the activation of the cellular immune system in humans as well as a factor for prognosis and its indicator biomarker [3,4]. However, there is evidence that neopterin is not only a marker of the activity of the immune system, but it also exerts distinct biological effects; that is, neopterin induces the apoptosis of L2 rat alveolar epithelial cells [5], and inhibits NADPH-oxidase in peritoneal macrophages [6]. Neopterin has also been found to inhibit erythropoietin gene expression [7], induce the expression of genes for the proto-oncogene c-*fos* [8], and activate the transcription of nuclear factor-kappa B (NF-κB) [9]. Furthermore, because the expression of the inducible nitric oxide synthase (iNOS) gene and the subsequent release of nitric oxide (NO) from vascular smooth muscle cells (VSMCs) following incubation with neopterin are documented, neopterin is supposed to play the role of a modulator in Gram-negative-bacterium-induced endotoxemia that contributes to the overproduction of NO [10,11]. Neopterin, on the other hand, augments the colony formation of granulocyte–macrophage progenitor cells (CFU-GM) in a semisolid culture system [12] and that the intraperitoneal injection of neopterin into mice stimulates granulopoiesis [13]. This stimulation of *in vitro* and *in vivo* granulopoiesis is due not to a direct effect of neopterin on hematopoietic stem cells, but rather to an indirect effect of stromal-cell-mediated hematopoietic growth factors, such as GM-CSF and IL-6 [12]. In contrast to the enhancement of granulopoiesis by neopterin, neopterin suppresses *in vitro* and *in vivo* B-lymphopoiesis by inducing the production of predominantly negative regulators by stromal cells, such as TNF-α and IL-6 (Minami et al., in press).[1] These findings indicate that neopterin is biologically active against hematopoiesis during inflammatory processes, which is induced by stimulating the production of cytokines by stromal cells.

Mast cells are derived from hematopoietic stem cells, but they do not ordinarily circulate in the mature form; instead, they undergo differentiation and maturation in the mucosal settlement after the migration of their precursors into vascularized tissues or serosal cavities in which they will ultimately reside [14–18]. The physiologic functions of mast cells include the enhancement of inflammatory responses induced by parasites and bacterial infection and immune complexes [19–21]. Because neopterin facilitates splenic immune responses, it is of interest to determine whether neopterin affects mast-cell progenitor cells (CFU-mast) in hematopoietic tissues, specifically in the spleen, during inflammatory processes.

SAM/P-1, a subline of senescence-accelerated mice (SAM) exhibiting a unique stromal cell impairment after 30 weeks of age, was used because the numbers of splenic cells and splenic hematopoietic progenitor cells start to decrease thereafter [22–25]. Therefore, stromal cell impairment (SCI) mice, SAM/P-1, are a useful tool for elucidating the possible interaction between neopterin and stromal cells during mast cell development. In this study using a stromal impairment (SCI) mouse, SAM/P-1, we investigated whether neopterin affects the pool of CFU-mast in hematopoietic tissues.

2. Materials and methods

2.1. Mice

A subline of SAM, SAM/P-1 [22], which is a senescent stromal impairment substrain (SCI mouse), was derived from an AKR/J mouse (Jackson Laboratory, Bar Harbor, ME), and established by Dr. Toshio Takeda, Professor Emeritus of the Chest Disease Research Institute, Kyoto University, Japan. The mice were bred and maintained in an experimental facility at the National Institute of Health Sciences under pathogen-free conditions. SAM/P-1 exhibits stromal cell impairment after 30–36 weeks of age. In this study, male SAM/P-1 mice designated as non-SCI (8 to 12 weeks old) and those designated as SCI mice (30–36 weeks old) were compared. These ages were selected because the numbers of splenic cells and splenic hematopoietic progenitor cells start to decrease at approximately 30 weeks of age [23,24]. The study was approved by the Committee for Institutional Animal Care and Use at National Institute of Health Sciences under the Health Guidelines for Animal Care.

2.2. Agents and antibodies

D-(+)-Neopterin was obtained from Sigma (St. Louis, MO). Neopterin was dissolved in 1 N HCl at 1 mg/mL and diluted tenfold with Dulbecco's phosphate-buffered saline (PBS). Hamerlinck reported that the concentration of neopterin is 5.89 ± 1.78 nM in normal human serum [26]. Murr et al. showed that the mean serum neopterin level in patients with serious infection by *Streptococcus pyogenes* is 152 nM (range = 71–242 nM) [27]. Thus, we used neopterin at concentrations from 40 nM to 4 μM for *in vitro* experiment and 4.0 mg/kg body weight for *in vivo* experiments, based on previous experiments [12,13,28].

[1] The manuscript has been published during the editorial process, see [47].