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In vivo administration of the frog skin peptide frenatin 2.1S induces immunostimulatory phenotypes of mouse mononuclear cells

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ABSTRACT

Host-defense peptides secreted by epithelial cells exhibit cytotoxic and immunoregulatory effects in order to protect the organism against invading microorganisms. Antimicrobial peptides derived from frog skin display both immunostimulatory and immunosuppressive actions as demonstrated by *in vitro* cytokine production by macrophages. Frenatin 2.1S, first isolated from skin secretions of the frog, *Sphaenorhynchus lacteus* (Hylidae), enhances the *in vitro* production of pro-inflammatory IL-1β, TNF-α and IL-23 by mouse peritoneal cells. In order to test whether the immunostimulatory action of frenatin 2.1S may be reproduced *in vivo*, effects of intraperitoneal injections of this peptide on mononuclear cells in the peritoneum and spleen were determined 24 h after administration. The data indicate that frenatin 2.1S enhances the activation state and homing capacity of Th1 type lymphocytes and NKT cells in the mouse peritoneal cavity, as evaluated by increased expression of early activation marker CD69 among T and NKT cells and chemokine receptor CXCR3 among T cells. Frenatin 2.1S significantly increases the percentage of ($F4/80^+CD11c^+CD206^+$) pro-inflammatory M1 macrophages and enhances the expression of MHC class II molecules on $F4/80^+CD11c^+$ macrophages in the mouse peritoneal cavity. Additionally, injection of frenatin 2.1S, in the presence or absence of lipopolysaccharide, increases the percentage of peritoneal B cells of the ($CD19^+CD11b^+CD5^+$) B1a phenotype thus contributing to an inflammatory milieu. We suggest that the immunostimulatory effect of frenatin 2.1S may have therapeutic relevance in disease states, such as certain types of cancer, in which an enhanced inflammatory response may be beneficial.
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Introduction

Cationic host-defense peptides, such as cathelicidins and defensins, secreted by epithelial cells are considered to be components of the system of innate immunity and exhibit various antimicrobial and immunoregulatory effects in order to protect the animal against invading microorganisms [9]. These effects include both inhibition and stimulation of pro- and/or anti-inflamatory cytokines, lipopolysaccharide (LPS) neutralization, mast cell degranulation, and chemokine production with consequent chemotaxis of leukocytes as well as cytotoxic activities [3,9,14]. Peptides with antibacterial and/or antifungal actions have been isolated from the skin secretions and/or skin extracts of numerous frog species belonging to the *Alytidae*, *Bombinatoridae*, *Dicroglossidae*, *Hylidae*, *Hyperoliidae*, *Leiopelmatidae*, *Leptodactylidae*, *Myobatrachidae*, *Pipidae*, and *Ranidae* families [4]. To date, the Antimicrobial Peptide Database (http://aps.unmc.edu/AP) contains around 1000 amphibian host-defense peptides [34].

The Orinoco lime tree frog *Sphaenorhynchus lacteus* (Daudin, 1802) belongs to the subfamily Hylinae in the family Hylidae and inhabits the Amazon basin of Columbia, Venezuela, Peru, Brazil and Ecuador and also the Guianas and the island of Trinidad and Tobago [11]. Frenatin 2.1S (GLVGTLLGHIGKAILG.NH_2), 2.2S (GLVGTLLGHIGKAILS.NH_2) and 2.3S (GLVGTLLGHIGKAILG) are structurally related host-defense peptides that have been isolated from norepinephrine-stimulated skin secretions of *S. lacteus* (6). Frenatin 2.1S and 2.2S potently inhibit the growth of clinical isolates of Gram positive methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* [6]. Additionally, frenatin 2.1S ($LC_{50} = 80 \pm 6$ μM) and 2.2S ($LC_{50} = 75 \pm 5$ μM) are cytotoxic against non-small cell lung adenocarcinoma A549 cells but are less hemolytic against human erythrocytes ($LC_{50} = 167 \pm 8$ μM for frenatin 2.1S and 169 ± 7 μM for 2.2S).

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