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Immunology Letters 100 (2005) 14–20
Immunology Letters
www.elsevier.com/locate/

Review

Vγ9Vδ2 T cell-mediated non-cytolytic antiviral mechanisms and their potential for cell-based therapy

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Available online 8 August 2005

Abstract

In healthy adult *Homo sapiens*, the most frequent circulating γδ T cells (Vγ9Vδ2) respond to pyrophosphomonoesters, alkylamines (together referred to as non-peptidic antigens, NpAgs) and nitrogen-containing bisphosphonates. The seemingly very low toxicity of bisphosphonate and pyrophosphomonoester drugs in vivo, may allow novel approaches to the immunotherapy of viral infections. For example, these drugs can be used to stimulate Vγ9Vδ2 T cells to release antiviral molecules that directly suppress virus replication without destroying the virus-replicating cells. In addition, the soluble molecules released by γδ T cells could boost the antiviral potency of cytotoxic T lymphocytes (CTLs) and promote antigen presentation. The relative therapeutic value of drug-induced direct antiviral and immunoregulatory activities may depend on the infecting virus as well as on the nature of protective immune responses.

Keywords: Antiviral immunity; Vγ9Vδ2 T cells; Immunotherapy; Non-peptidic antigens; Bisphosphonates

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1. Introduction

In viral infections, both adaptive and innate immune reactions cooperate to protect the host and, whenever it is possible, to eradicate or control the infection. The early synthesis of soluble factors (cytokines, chemokines) influences substantially the subsequent immune response and may, therefore, affect the course of infection. One of the important effectors of natural immunity are γδ T lymphocytes, which display a broad antiviral activity against different viruses such as retroviruses, flaviviruses, paramyxoviruses, orthomyxoviruses, picornaviruses, coronaviruses, rhabdoviruses, arenaviruses, herpesviruses, hepadnaviruses, and orthopoxviruses (reviewed in [1]). This broad antiviral activity of γδ T cells is likely to play a crucial defensive role, especially considering that their relatively large numbers (e.g., approximately one out of every 30 adult human peripheral blood lymphocytes is a Vγ9Vδ2 T lymphocyte) can respond very quickly (typically, no antigen processing

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0165-2478/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.imlet.2005.06.025

is required for the potent major histocompatibility complex (MHC)-unrestricted activities of Vγ9Vδ2 T cells) and release soluble antiviral factors.

2. Pharmacological stimulation of γδ T cells

Many natural ligands recognized by human Vγ9Vδ2 T cells are known. Probably the most important representatives of this group are intermediates of isoprenoid biosynthesis [2] (e.g., 3-formyl-1-butyl-pyrophosphate and isopentenyl-pyrophosphate) and were first isolated from mycobacteria [3–5]. Other natural and synthetic phospho-ligands [6,7], alkylamines [8] and aminobiphosphonates [9] also stimulate Vγ9Vδ2 T cells. Structure-function studies of Vγ9Vδ2-specific ligands suggest that some of these molecules could be readily docked into a putative binding site of the Vγ9Vδ2 TCR [10,11]. Functionally, mature Vγ9Vδ2 T cells express cell-surface inhibitory receptors for MHC class I molecules (INMRs) that may control TCR-mediated reactivities in the antiviral response [12,13].

Phosphostim (phosphobromohydrin) is the first drug that was designed to stimulate selectively Vγ9Vδ2 T cells. Currently, this new drug is in Phase I/Phase II clinical trials in cancer patients. Nitrogen-containing bisphosphonates (N-BPs) have been used to prevent bone demineralization in patients with osteoporosis, multiple myeloma, and certain metastatic cancers (e.g., breast and prostate). Recently, N-BPs have been shown to induce activation of Vγ9Vδ2 T cells accompanied by augmented: (a) cytotoxic activities; (b) cytokine/β-chemokine production; and (c) DNA synthetic responses in Vγ9Vδ2 T cells. These unexpected activities of N-BPs have opened new possibilities of therapeutic usage for these drugs.

The mechanism of N-BP action appears to include the inhibition of farnesyl pyrophosphate synthase activity (as one of the key enzymes in the mevalonate pathway, farnesyl pyrophosphate synthase catalyzes the sequential head-to-tail condensation of two molecules of isopentenyl pyrophosphate with dimethylallyl pyrophosphate). This leads to an accumulation of isopentenyl pyrophosphate, an essential metabolite that is directly recognized by Vγ9Vδ2 T cells [14]. Interestingly, the mevalonate pathway is critical for protein prenylation, which may be important for viral assembly (Fig. 1). In hepatoma cells exposed to lovastatin (an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate), hepatitic C virus (HCV) RNA replication is impaired due to the dissolution of the HCV replication complex [15]. The lovastatin treatment can also block respiratory syncytial virus (RSV) replication and cell-to-cell fusion in vivo and in vitro by inhibiting the isoprenylation of the cellular protein RhoA [16].

Similarly, BZA-5B (an inhibitor of protein prenylation) blocks the production of hepatitis delta virus (HDV) particles in vitro in a dose dependent manner [17]. In particular,

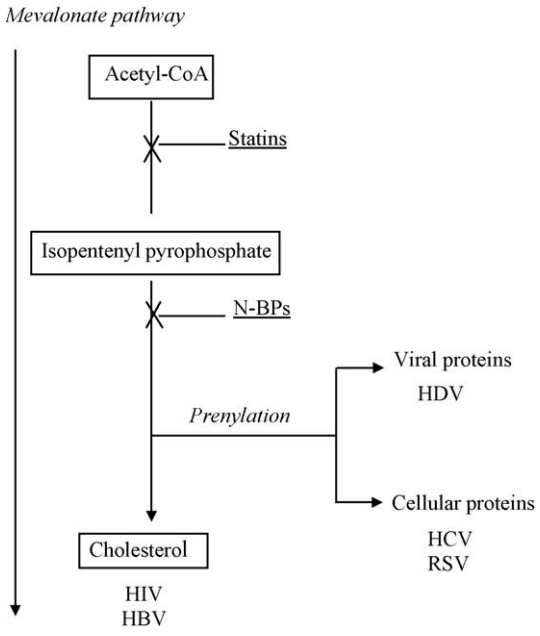

Fig. 1. The role of the mevalonate (MVA) pathway in viral assembly. The MVA pathway is critical for protein prenylation and cholesterol biosynthesis. Therefore, the MVA pathway may be important for viral assembly of particles requiring: (a) the prenylation of viral (HDV) or cellular (HCV, RSV) proteins; or (b) the synthesis of cholesterol (HIV, HBV) that is necessary for membrane budding. Blocking this pathway by statins or N-BPs interferes with both prenylation and cholesterol synthesis. However, the 'pre-IPP' statin inhibition decreases, whereas the 'post-IPP' N-BP block increases the accumulation of IPP, a potent endogenous stimulator of Vγ9Vδ2 T lymphocytes.

the inhibition of large delta antigen prenylation mediated by BZA-5B interferes with the assembly of HDV virions. In addition, the increased concentration of large delta antigen within infected cells may act as a potent *trans* dominant inhibitor of HDV replication [18].

The block of mevalonate pathway by N-BPs also results in a decreased synthesis of cholesterol. It has been reported that cholesterol is critical for HIV passage through cell membranes and that the ability of Nef protein to increase viral infectivity depends on cholesterol [19]. Specifically, Nef is involved in transporting newly synthesized cholesterol to the site of viral budding and promotes the incorporation of cholesterol into viral particles. These data suggest that an efficient cholesterol synthesis in HIV-infected cells is important for the production of infectious virions [20]. Altogether, these results open a new possibility of using the inhibitors of mevalonate pathway as double-edge swords—that is as antivirals interfering with virion production as well as stimulators of Vγ9Vδ2 T cell cytotoxic and other antiviral effects.

3. Non-cytolytic antiviral immunity mediated by Vγ9Vδ2 T cells

Immune responses to viral infections comprise both cytolytic (i.e., cytotoxicity against virus-infected cells) and