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ScienceDirect
Immunobiology 212 (2007) 47–55
Immunobiology
www.elsevier.de/imbio

Stimulation of Vγ9/Vδ2 T-lymphocyte proliferation by the isoprenoid precursor, (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate

Sabine Amslinger[a,*], Stefan Hecht[a], Felix Rohdich[a], Wolfgang Eisenreich[a], Petra Adam[a], Adelbert Bacher[a], Stefan Bauer[b,**]

[a] *Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstr. 4, 85747 Garching, Germany*
[b] *Institut für Immunologie, BMFZ, Philipps-Universität Marburg, Hans-Meerwein-Str. 2, 35043 Marburg, Germany*

Received 28 April 2006; received in revised form 4 August 2006; accepted 7 August 2006

Abstract

(*E*)-1-Hydroxy-2-methyl-but-2-enyl 4-diphosphate, a recently discovered intermediate in the deoxyxylulose phosphate pathway of isoprenoid biosynthesis, has been shown to act as a potent immunomodulator. In cultures of human peripheral blood mononuclear cells from eight non-related donors, the compound stimulated the proliferation of Vγ9/Vδ2 T lymphocytes with a median EC_{50} of 70 pM when 10 U/ml of IL-2 was used as costimulant. Isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP) and some structural analogs of (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate also stimulated Vγ9/Vδ2 T-cell proliferation, albeit at much higher concentrations. The Vγ9/Vδ2 T-cell proliferation is highly dependent on the seeding density used in culture. All phosphoantigens tested elicited the proliferation of two T-lymphocyte populations with different apparent ratios between the expression level of Vδ2 and Vγ9 chains.

Keywords: Deoxyxylulose phosphate; γδ T-cell activation; 1-Hydroxy-2-methyl-but-2-enyl 4-diphosphate; Isoprenoid biosynthesis

Introduction

Innate immune recognition plays an important role in the early detection of pathogens (Medzhitov and Janeway, 1997). A variety of molecular species characteristic of microbial pathogens such as lipopolysaccharide, *N*-formylated peptides and bacterial DNA have been shown to be recognized as "pathogen-associated molecular patterns" via invariant receptors on antigen-presenting cells or lymphocytes (for review see Lindahl et al., 1995; Akira et al., 2001).

Various phosphate and diphosphate esters present in bacterial extracts (collectively designated as phosphoantigens in the literature) have been reported to stimulate the proliferation of γδ T lymphocytes expressing Vγ9/Vδ2 receptors (Constant et al., 1994; Tanaka et al., 1994; Morita et al., 1995). Among the group of phosphoantigens, the universal terpene biosynthesis precursors, isopentenyl diphosphate (IPP, **6**) and dimethylallyl diphosphate (DMAPP, **7**) were relatively potent stimulators (Tanaka et al., 1995).

Abbreviations: FITC, fluorescein isothiocyanate; IL-2, interleukin-2; PBMC, human peripheral blood mononuclear cells; PE, phycoerythrin isothiocyanate

*Corresponding author. Current address: Institut für Organische Chemie, Universität Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany. Tel.: +1 49 941 943 4650.
**Also to be corresponded to.
E-mail addresses: sabine.amslinger@chemie.uni-regensburg.de (S. Amslinger), stefan.bauer@staff.uni-marburg.de (S. Bauer).

0171-2985/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.imbio.2006.08.003

Whereas the biosynthesis of IPP and DMAPP in vertebrates via the mevalonate pathway has been studied in considerable detail during a period of several decades (for reviews see Qureshi and Porter, 1981; Bloch, 1992; Bach, 1995; Bochar et al., 1999), a second pathway for their biosynthesis via 1-deoxy-D-xylulose 5-phosphate (**3**) was recently found in many pathogenic microorganisms (Fig. 1) (for reviews see Rohmer, 1999; Arigoni and Schwarz, 1999; Eisenreich et al., 2001; Rohdich et al., 2001, 2003). A recently discovered intermediate of that pathway, (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (**5**) (Hecht et al., 2001; Adam et al., 2001; Rohdich et al., 2002), is the most potent phosphoantigen described hitherto (Hintz et al., 2001; Eberl et al., 2002). This paper reports quantitative studies on the stimulation of $\gamma\delta$ T-cell proliferation by that deoxyxylulose phosphate pathway intermediate and by structural analogs.

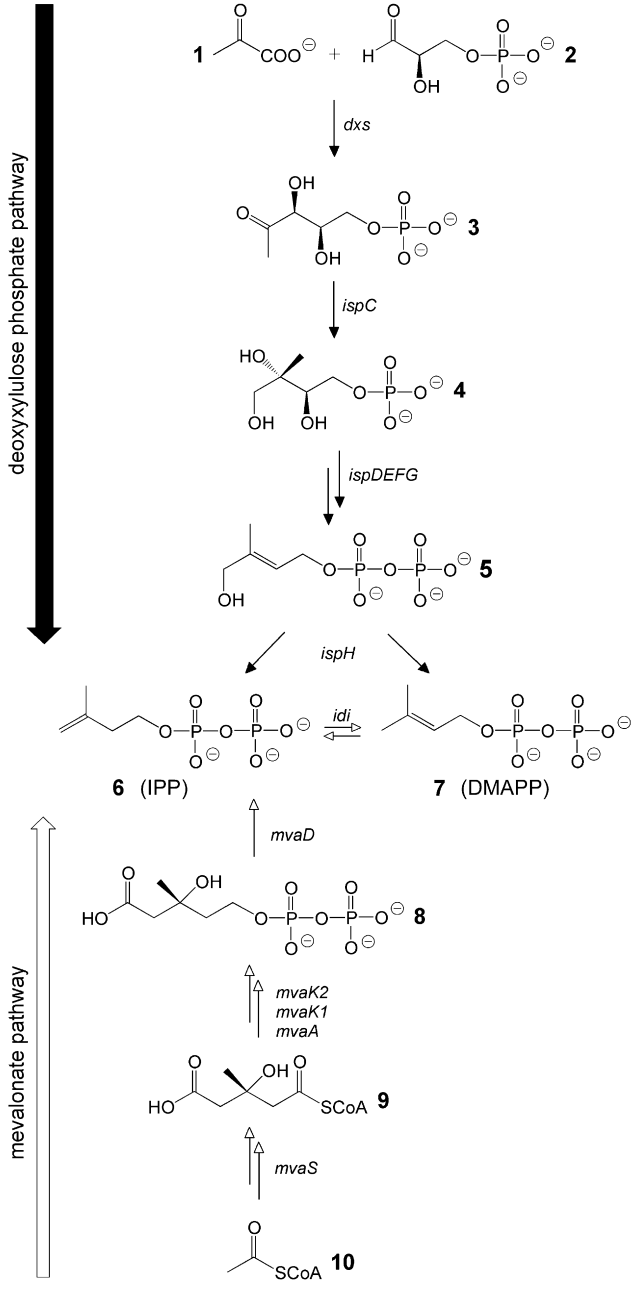

Fig. 1. Biosynthesis of isoprenoids.

Materials and methods

Materials

IPP (**6**), DMAPP (**7**), (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate (**5**) and (*E*)-3-formyl-but-2-enyl 1-diphosphate (**14**) were prepared by published procedures (Davisson et al., 1986; Amslinger et al., 2002; Hecht et al., 2002). Pooled human AB serum was supplied by Klinikum Rechts der Isar, Munich, Germany. Fluorescein isothiocyanate ($FITC^3$)-conjugated mouse anti-human Vδ2 TCR monoclonal antibody, mouse anti-human $\gamma\delta$ TCR monoclonal antibody, mouse anti-human CD3 (UCHT1, ε chain) monoclonal antibody (Beckman Coulter-Immunotech, Unterschleissheim, Germany), phycoerythrin isothiocyanate (PE)-conjugated mouse anti-human Vγ9 TCR monoclonal antibody (BD PharMingen, Becton Dickinson BD Biosciences, Heidelberg, Germany) and PE-conjugated mouse anti-human CD3 (UCHT1, ε chain) monoclonal antibody (Beckman Coulter-Immunotech, Unterschleissheim, Germany) were obtained from the suppliers indicated.

Preparation of (*E*)-1-hydroxy-2-methyl-but-2-enyl 4-phosphate (11)

A mixture containing 4.0 mg (0.020 mmol) of (*E*)-4-chloro-2-methyl-1-tetrahydropyranyloxy-but-2-ene (Amslinger et al., 2002) and 6.2 mg (0.029 mmol) of di(tetrabutylammonium) hydrogen phosphate in 500 µl of acetonitrile was kept at ambient temperature for 2 h. The mixture was brought to pH 1 by the addition of 10 µl of 37% aqueous hydrochloric acid. After 7 min, 10 µl of 40% aqueous NaOH was added. The solution was placed on top of a column of DOWEX 50 WX8 (4.5 ml, NH_4^+ form), which had been equilibrated with 10 ml of 25 mM NH_4HCO_3. The column was developed with 15 ml of 25 mM NH_4HCO_3. The effluent was lyophilized affording 2.9 mg (0.013 mmol, 68%) of **11** as a white powder. ^{1}H NMR (D_2O, 500 MHz) δ 5.45 (t, $J = 7.0$ Hz, 1H), 4.03 (d, $J = 7.1$ Hz, 2H), 3.87 (s, 2H), 1.54 (s, 3H); ^{13}C NMR (D_2O, 126 MHz) δ 140.7, 124.7, 68.7, 59.6, 14.8; ^{31}P NMR (D_2O, 101 MHz) δ 5.25.

Preparation of (*E*)-2-methyl-but-2-ene-1,4-diol (12)

An aqueous solution (500 µl) containing 16.5 mg (0.0886 mmol) of (*E*)-2-methyl 1-tetrahydropyranyloxy-