ELSEVIER

International Immunopharmacology 6 (2006) 1577–1585

International Immunopharmacology

www.elsevier.com/locate/intimp

STAT1 phosphorylation and cleavage is regulated by the histamine (H4) receptor in human atopic and non-atopic lymphocytes

Bianca Horr [a], Hannelore Borck [a], Robin Thurmond [b], Sabine Grösch [c], Friedhelm Diel [a,*]

[a] *Institut für Umwelt und Gesundheit (IUG) and University of Applied Sciences, FB Oe, Biochemistry, Marquardstrasse 35, D-36039 Fulda, Germany*

[b] *Johnson & Johnson, Pharmaceutical Research & Development, L.L.C., 3210 Merryfield Road, San Diego, CA 92121, USA*

[c] *Uniklinik Frankfurt, Institut für Klinische Pharmakologie, Pharmazentrum Frankfurt ZAFES, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany*

Received 21 February 2006; received in revised form 31 May 2006; accepted 6 June 2006

Abstract

Histamine can modulate the balance between T helper lymphocytes 1 and 2 (Th1 and Th2), and there is evidence that allergic reactions can be associated with excessive histamine production causing shifts toward Th2 responses. As signal transduction in Th-cells is specifically correlated to signal transducer and activator of transcription (STAT) activation and expression, the question arises whether histamine acting through histamine receptors (HR) induces modulation of the Janus kinase (JAK)-STAT pathway. Peripheral blood mononuclear cells (PBMC) from atopic and non-atopic donors were stimulated with phytohemagglutinin (PHA). Initial interleukin-4 (IL-4) levels were higher in the atopic group compared to the non-atopics and interferon-γ (IFN-γ) levels were lower. This was correlated to lower levels of STAT1 expression and phosphorylation. Furthermore, Western blots showed a 118-kDa STAT1 band at the start of the PHA stimulation that was apparently cleaved to STAT1α (91 kDa) and a 28 kDa-fragment with further stimulation. Histamine or the H4R agonist, clobenpropit, led to a significant suppression of the formation and phosphorylation of STAT1α in the non-atopic group after 48 h of PHA stimulation, but had no effect in the atopic group where STAT1α levels were already reduced. The STAT1α levels in the non-atopic group were enhanced by the H4R antagonist JNJ7777120. The phosphorylation of STAT1 could also be potentiated by the H4R antagonist, mimicking the precursor responses of STAT1α. JNJ7777120 also increased the binding of STAT1 to DNA and this effect could be reversed by histamine. As for histamine, the effects of the H4R antagonist were only seen in the non-atopic group. These results suggest that, in non-atopic individuals, histamine acting via the H4R can influence STAT1 regulation, but that this pathway is not present in atopics perhaps due to constitutive suppression of STAT1 activity.

Keywords: Histamine; Histamine receptor; STAT1; H4-receptor antagonist; EMSA; Allergy

* Corresponding author. Tel.: +49 661 71003; fax: +49 661 71019 or Tel./fax: +49 661 603242.
E-mail address: friedhelm.diel@he.fh-fulda.de (F. Diel).

1567-5769/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.intimp.2006.06.005

1. Introduction

In allergic disease, T cells are believed to be involved in the regulation of the local inflammation in the lung, skin tissue and different target organs [reviewed in 1]. The balance between 1 and 2 (Th1 and Th2) is thought to be important in allergic reactions with a shift toward Th2 responses inducing the class switch to IgE expression. It has been demonstrated that histamine can modulate the Th1/Th2 balance and its production during an allergic reaction could be one of the main factors that drives Th2 skewing resulting in allergic symptoms [2,3]. Regarding distinct phases of stimulated Th1 and Th2 cell differentiation, allergy-specific polymorphisms of cytokine receptor subunit expression must also be taken in consideration. Furthermore, sensitized mast cells and basophils contribute not only histamine but also interleukins (IL) and other immune mediators to the regulatory orchestra of adaptive immune system responses in inflammatory disease [4]. In most studies histamine was found to suppress Th1 related interferon-γ (IFN-γ) production via H2 receptors (H2R), but can also modulate IFN-γ-antagonistic IL-4 in atopic and non-atopic human lymphocyte cultures [5–7]. Th-cell differentiation can also be modulated by another regulatory pathway that originates in the thymus gland. This type of CD4+CD25+Treg-cell can be antigen independent and processes specific nuclear factors such as FOXp3 [8].

Since signal transduction via the Janus kinase (JAK)-STAT pathway is important in Th cells, the question arises whether histamine influences the Th-specific STAT expression and phosphoregulation [9,10]. Histamine acts through 4 known histamine receptors. H2R has been shown to be expressed in murine lymphocyte cultures [4] and Elliott et al. [11] postulated that histamine enhances IL-13 expression via H1R and H2R. It is likely that H4R also plays a role in lymphocytes [12] and recently it has been shown to be important in Th2 cell differentiation in mice [13]. The human H4R shows the highest affinity for histamine suggesting that it may play a regulatory function [14]. As histamine receptors belong to the superfamily of G protein coupled receptors (GPCR), signal transduction depends on G protein activity influencing adenylyl cyclase, phosphoinositol-triphosphate (PIP3) and phosphokinase A (PKA) activity [15–17]. The different histamine receptors activate specific downstream pathways. In recent work we have demonstrated that histamine can also interfere at the cytokine IL-4-receptor and influence the constitutive tyrosine kinase (TYK) activity [18].

Phosphorylation by JAK and TYK of the intracellular receptor domains and subsequential dimerization leads to activated STAT molecules that are capable of inducing expression of genes containing the GAS elements in concert with Th1 and Th2-typical enhancer proteins and distinct nuclear factors [19]. More recently it has been found that Th2 specific STAT6 can be cleaved by DNA coupled proteolytic enzymes in murine mast cells [20]. Hydrolyses of STATs at the C-terminal conserved transcription activator domain (TAD) attenuates promoter gene activation. Consequently, this can be a general mechanism of inactivation of STATs after translocation into the nucleus and can also be an inhibiting step in IFN-γ-induced STAT1 signal transduction. Since both histamine and STATs are implicated in the Th1/Th2 balance, the question arises whether histamine influences the Th-specific STAT expression and phosphoregulation. In previous work, we investigated the influence of histamine on STAT6 [3]. Here, we examine its effects on STAT1 expression in PHA stimulated human lymphocytes ex vivo and demonstrate the cleavage of a STAT1 fragment with a molecular weight of 28 kDa.

This study aims to show the influences of histamine on T cell cultures and the production of Th-typical STAT1, STAT1 phosphorylation and STAT1 DNA-binding in atopic and non-atopic subjects ex vivo.

2. Materials and methods

2.1. Volunteers

Blood was taken from 3 atopic (adult, IgE > 1000 IU) suffering from allergic symptoms like hay fever, atopic eczema and atopic asthma as well as from 3 sex and age matched non-atopic (IgE < 100 IU) persons by venipuncture and treated with EDTA. The peripheral blood mononuclear cells (PBMC) were isolated by gradient density centrifugation (Histopaque®1077: Sigma, Deisenhofen, Germany).

All patients gave their written informed consent for the experiments: the study had been reviewed and approved by the University of Applied Sciences Fulda joint ethic committee.

2.2. Cell cultures

Reagents: RPMI 1640, L-glutamine, penicillin/streptomycin, fetal calf serum (FCS), PHA (Phytohemagglutinin), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide), (all Sigma Deisenhofen, Germany); PBS: NaCl (149.5 mM), KCl (2.96 mM), $Na_2HPO_4 \cdot 2H_2O$ (7.98 mM), KH_2PO4 (1.62 mM), pH 7.2; (all Merck Darmstadt, Germany); JNJ7777120 was synthesized as previously described [21].

For cell proliferation, the PBMCs (2×10^6) were cultured in supplemented RPMI 1640 (2 mM L-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% FCS) and incubated at 37 °C in a humidified atmosphere of 5% CO_2 in