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Functional characterization of histamine H4 receptor on human mast cells

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ABSTRACT

Among the four different types of histamine receptors (H1-H4), H4R is predominantly expressed in immune cells and involved in immunomodulatory response. Here, in this study we determined the expression of H4R in human mast cells (HMC-1, LAD-2 and primary cord blood derived CD34+ human mast cells) and characterized its functional properties. Interestingly, we found that human mast cells responded to both histamine (natural ligand) and 4-methylhistamine (selective H4R agonist) for sustained intracellular calcium mobilization, degranulation and cytokine production. However, only histamine induced the release of cAMP, but 4-methylhistamine down regulates cAMP indicating that H4R mediates its effect through Gαi/o protein and H1R via Gαq protein. Furthermore, both histamine and 4-methylhistamine induced the production of cysteinyl leukotrienes and LTB4. Using human inflammation antibody array membrane, we found that H4R induced the expression of various inflammatory proteins, involving pro-inflammatory cytokines and chemokines and these are TGF-β1, TNF-α, TNF-β, PDGF-BB, TIMP-2, M-CSF, IP-10, IL-16, IL-6, IL-3, IL-10, MIP-1α, IL-1α, ICAM-1, Eotaxin-2, RANTES, IL-8, MCP-1, and IL-6sR. We also quantified the level of various inflammatory cytokines produced by human mast cells through H4R. It was observed that, the production level of Th2 cytokines IL-4(401.34 pg/ml), IL-5 (64.21 pg/ml) and IL-13 (1044 pg/ml) and classical proinflammatory cytokines IL-6 (221.27 pg/ml) and IL-1β (34.24 pg/ml) and chemokines MCP-1(106 pg/ml) and IL-8 (818.32 pg/ml). Furthermore, activation of H4R caused the phosphorylation of ERK and PI3 K in a time dependent manner. Taken together these data demonstrate that, the activation of H4R in human mast cells produced not only inflammatory mediators that are associated with allergic reactions but also other inflammatory conditions.

1. Introduction

Histamine is an important mediator that orchestrates inflammatory and allergic responses (Shahid et al., 2009; Salcedo et al., 2013). The pleiotropic effects of histamine are mediated through at least pharmacologically distinct receptors namely H1R, H2R, H3R and H4R, which are all members of GPCR family (Thurmond et al., 2004). Accumulating evidence suggests that, histamine contributes to the progression of allergic inflammatory responses by enhancing the secretion of pro-inflammatory cytokines like IL-1β, IL-6 as well as chemokines such as IL-8 and RANTES in inflammatory cells and local tissues as well (Umetsu et al., 2002; Dy and Schneider, 2004).

Previously it was thought that histamine modulates inflammatory and allergic responses mainly through its classical receptors H1R and H2R. However, after the discovery of the fourth receptor H4R, which is predominantly expressed on the cells of the immune system (Thurmond et al., 2008; Hodge et al., 2013) is found to be responsible for the selective recruitment and accumulation of inflammatory cells to the sites of allergic inflammation (Hofstra et al., 2003; Lim et al., 2005).

Activation of H4R triggers a series of signal transduction events such as, calcium mobilization, actin polymerization, cell shape change, up regulation of adhesion molecules leading to immune cell migration into sites of inflammation (Hofstra et al., 2003; Zampeli and Tiligada, 2009). Also, studies have shown that, asthmatic patients have increased number of degranulated mast cells in

Abbreviations: LAD-2, Laboratory of allergic diseases 2; HMC-1, Human mast cell line-1; MCP-1/CCl2, Monocyte chemo attractant protein-1; H4R, Histamine H4 receptor; MC, Mast cells; LTB4, Leukotriene B4; CyLTs, Cysteinyl leukotrienes; cAMP, Cyclic AMP; RANTES, Regulated on Activation T cell Expressed and Secreted; TIMP-2, Tissue Inhibitor of Metalloproteinases–2; 4-MH, 4-methylhistamine; Mep, Mepyramine maleate.

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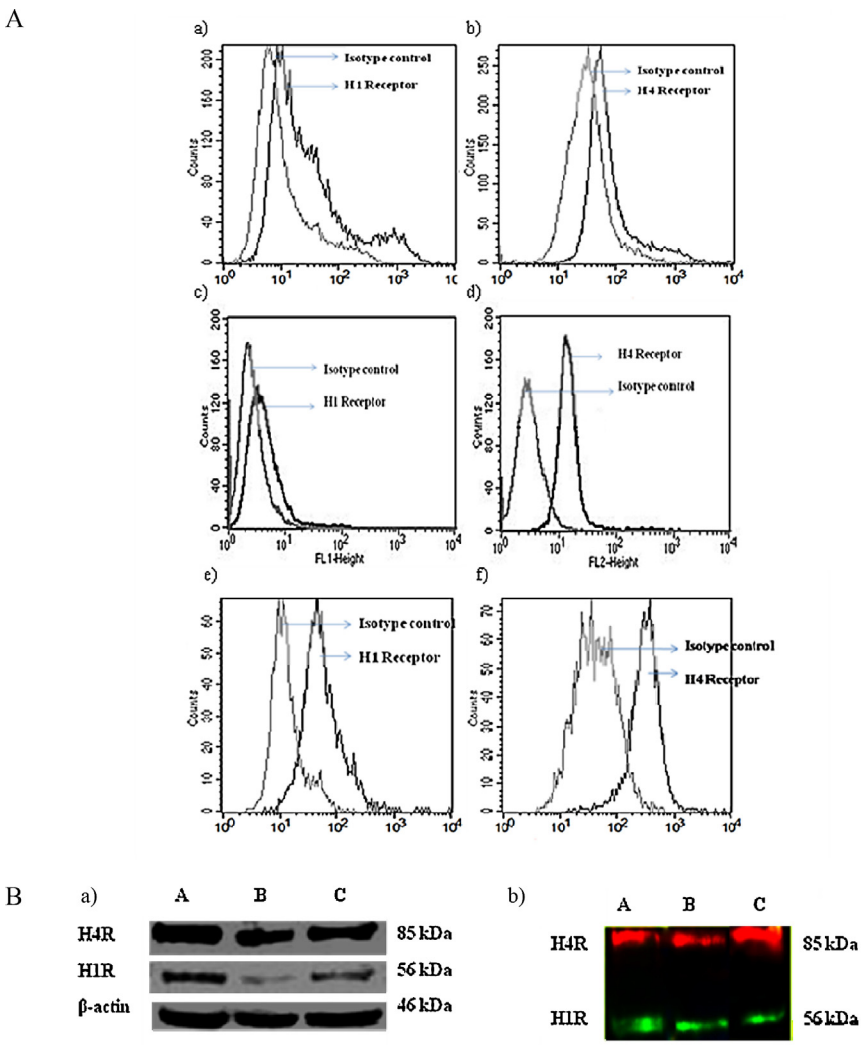

Fig. 1. A. Determination of histamine H1 and H4 receptors expression on HMC-1 cells, cord blood derived CD34+ human mast cells and THP-1 cells. Flow cytometry was performed using a fluorescent labelled H4R and H1R specific antibodies. (a and b) HMC-1 cells and (c and d) cord blood derived CD34+ human mast cells were expressed histamine H1R and H4R receptors. (e and f) THP-1 cell line that constitutively express histamine H1R and H4R was used as a positive control. Data shown are representative of three similar experiments. Fig. 1B. Expression analysis of histamine H1 and H4 receptors by western blotting. Representative results of immunoblotting for histamine H1, H4 receptors and β-actin are shown in (a) A) LAD-2, (B) HMC-1 and (C) cord blood derived CD34+ human mast cells. The proteins were visualized using ECL prime method and the image was captured by multi imaging system using Alpha view software, Cell biosciences. Multiplex western blot showing the expression of histamine H1 and H4 receptors on human immune cells. Cy3 and Cy5 linked fluorescent secondary antibodies were used to detect two different histamine H1 and H4 receptors expression on (b) (A) PBMC, (B) Granulocytes and (C) Jurkat cells. Data shown are representative of three similar experiments.

the airways as compared with normal subjects. It is well known that mast cells are the major producers of histamine (Zhang et al., 2007; Schwartz, 1994) and that mast cell activation amplifies the inflammatory response in the airways which in turn further affects lung physiology and function. Thus mast cells play a critical role in the pathogenesis of asthma (Reuter et al., 2010; Rivera, 2002). Though a great deal of research has been focused on mast cell activation via FcεRI, but very few reports highlight the involvement of H4R in cells associated with allergic asthma and the contribution of H4R mediated mast cell effects in allergic inflammation still remains little understood. Therefore, in order to better understand the functional aspects of H4R, we have utilized HMC-1, LAD-2 and cord blood derived CD34+ human mast cells to elucidate the histamine and 4-methylhistamine (selective H4R agonist) induced mediator release in human mast cells and the standard H4R antagonist JNJ7777120 was employed to confirm the H4R mediated functional activation and also the downstream signal transduction pathways. Here, we demonstrate the novel finding that H4R activation leads to the production of not only Th2 cytokines (IL-5, IL-4 and IL-13) but also various other inflammatory proteins in human mast cells.

2. Materials and methods

2.1. Chemical and reagents

StemPro-34 medium, SCF, Penicillin–Streptomycin, L-glutamine, IMDM, rhIL-6 and CysLTs kit were purchased from Gibco invitrogen, USA. Recombinant human IL-3 was purchased from R & D Systems USA. Rabbit antihuman H4R antibody, mouse antihuman H1R antibody, Mouse IgG1, rabbit IgG, goat anti rabbit IgG-HRP, goat anti mouse IgG-HRP conjugates, goat anti rabbit-PE, goat anti mouse-FITC and 4- methylhistamine (H4R agonist) were purchased from Santacruz Biotechnology, USA, Human IgE from Abcam, Anti human IgE, JNJ 7777120 (H4R antagonist), Histamine and Mepyramine maleate (H1R antagonist) from Sigma, USA, Human inflammation antibody array from Ray Biotech, Leukotriene B4 EIA Kit and Cyclic AMP Kit from Cayman Chemical Company, USA. ELISA Kits were purchased from ebioscience, USA and R & D Systems USA. ECL prime was purchased from GE Healthcare, USA. Phospho Akt antibody, Total Akt antibody, Phospho ERK1/2 (Thr202/Tyr204) antibody and total ERK antibody were