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Research article

Histamine induces NF-κB controlled cytokine secretion by orbital fibroblasts via histamine receptor type-1

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ABSTRACT

Mast cells and their products are likely to be involved in regulating orbital fibroblast activity in Graves' Ophthalmopathy (GO). Histamine is abundantly present in granules of mast cells and is released upon mast cell activation. However, the effect of histamine on orbital fibroblasts has not been examined so far. Orbital tissues from GO patients and controls were analyzed for the presence of mast cells using toluidine blue staining and immunohistochemical detection of CD117 (stem cell factor receptor). Orbital fibroblasts were cultured from GO patients and healthy controls, stimulated with histamine and cytokines (IL-6, IL-8, CCL2, CCL5, CCL7, CXCL10 and CXCL11) were measured in culture supernatants. Also hyaluronan levels were measured in culture supernatants and hyaluronan synthase (*HAS*) and hyaluronidase (*HYAL*) gene expression levels were determined. In addition, histamine receptor subtype gene expression levels were examined as well as the effect of the histamine receptor-1 (HRH1) antagonist loratadine and NF-κB inhibitor SC-514 on histamine-induced cytokine production. Mast cell numbers were increased in GO orbital tissues. Histamine stimulated the production of IL-6, IL-8 and CCL2 by orbital fibroblasts, while it had no effect on the production of CCL5, CCL7, CXCL10, CXCL11 and hyaluronan. Orbital fibroblasts expressed HRH1 and loratadine and SC-514 both blocked histamine-induced IL-6, IL-8 and CCL2 production by orbital fibroblasts. In conclusion, this study demonstrates that histamine can induce the production of NF-κB controlled-cytokines by orbital fibroblasts, which supports a role for mast cells in GO.

1. Introduction

Graves' Ophthalmopathy (GO) is an extra-thyroidal manifestation of Graves' disease (GD) and is characterized by an orbital infiltrate, consisting of T cells, macrophages, some B cells and plasma cells (Bahn, 2010; van Steensel et al., 2012b; Wang and Smith, 2014). These immune cells produce cytokines, growth factors as well as stimulatory autoantibodies against thyrotropin-receptor (TSHR) and insulin-like growth factor-I receptor (IGF1R) that stimulate orbital fibroblasts to proliferate, produce excess glycosaminoglycans (mainly hyaluronan) and inflammatory mediators and to differentiate into adipocytes and myofibroblasts (Bahn, 2010; Virakul et al., 2014). All this contributes to orbital inflammation, tissue expansion/remodeling and fibrosis typical of GO (Bahn, 2010; Virakul et al., 2014; Wang and Smith, 2014).

Mast cells have also been observed in GO orbital tissue, especially in the late phase of disease that is associated with tissue remodeling and fibrosis (Ludgate and Baker, 2002; Raikow et al., 1990; van Steensel et al., 2012b; Wegelius et al., 1957). Moreover, recruitment of mast cells into orbital tissue has been observed in a murine GO model (Banga et al., 2015). The cause of mast cell accumulation in GO orbital tissue is unknown, but stem cell factor (SCF, a growth factor for mast cells) is increased in serum from GD patients and may facilitate this (Yamada et al., 1998). Serum levels of IgE can be increased in GD patients and positive correlations between elevated serum IgE levels and the presence of GO have been described (Molnar et al., 1996; Sato et al., 1999). In addition to

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this, immunohistochemical studies demonstrated the presence of IgE in orbital muscle fibers from GO patients (Raikow et al., 1990). IgE binds and cross-links FcRε on mast cells, resulting in mast cell degranulation and the release of a plethora of mediators (Theoharides et al., 2007). IgE molecules with specificity to the TSHR have been described in GO patients, which may possibly be involved in regulating orbital mast cell recruitment and degranulation (Metcalfe et al., 2002). Although the above data suggest involvement of mast cells in the pathogenesis of GO their contributing role is so far poorly studied.

Orbital tissue mast cells are often localized in close proximity to orbital fibroblasts or adipocytes and show features of degranulation (Boschi et al., 2005). Within the secretory granules mast cells store an extensive variety of preformed mediators, including many different cytokines and growth factors (da Silva et al., 2014). Co-cultures of orbital fibroblasts with the mast cell line HMC-1 revealed that mast cell-derived prostaglandin D2 (PGD_2) stimulated hyaluronan and prostaglandin E2 (PGE_2) production by orbital fibroblasts (Guo et al., 2010; Smith and Parikh, 1999). Previous studies from our group showed that mast cells in orbital tissue from GO patients are a rich source of platelet-derived growth factor (PDGF)-BB (van Steensel et al., 2012b), a potent mitogen and stimulus of production of hyaluronan, cytokines, adipogenesis and TSHR expression by orbital fibroblasts (van Steensel et al., 2012a; van Steensel et al., 2010; van Steensel et al., 2009; van Steensel et al., 2012b; Virakul et al., 2015; Virakul et al., 2014). Although these data support a role for mast cell derived mediators in the activation of orbital fibroblasts in GO, further investigation is warranted, both from a pathophysiological as well as therapeutic point of view.

Histamine is a biogenic amine that is highly expressed in granules of mast cells and released upon their activation and which causes vasodilation, bronchoconstriction, increased capillary permeability, and smooth muscle contraction, all phenomena commonly associated with allergic and inflammatory reactions (da Silva et al., 2014). Moreover, histamine has been found to induce chemotaxis, proliferation, extracellular matrix molecule and inflammatory mediator synthesis by fibroblasts, thereby contributing to wound healing and tissue remodeling but also fibrosis (Abe et al., 2002; Dommisch et al., 2015; Franceschini et al., 2006; Garbuzenko et al., 2002, 2004; Holdsworth and Summers, 2008; Hong et al., 2015; Horie et al., 2014; Kunzmann et al., 2007; Li et al., 2007; Rankin et al., 1987; Shen, 2008; Veerappan et al., 2013; Walls et al., 1991; Wulff et al., 2012; Yang et al., 2014). Histamine effects can be mediated through four types of G-protein coupled histamine receptors; HRH1 to HRH4 (Jutel et al., 2009) but differences in specific histamine receptor involvement may exist between fibroblasts from different anatomical regions (Horie et al., 2014; Zeng et al., 2014). Although orbital fibroblast activation is at the heart of GO pathogenesis and there is data to implicate that mast cell-derived factors contribute to this (Dik et al., 2016), the contribution of histamine to GO, especially with regard to orbital fibroblast activation has not been examined so far.

The purpose of the present study was to evaluate the effect of histamine on the production of cytokines (IL-6, IL-8, CCL2, CCL5, CCL7, CXCL10 and CXCL11) previously implicated in GO as well as hyaluronan by orbital fibroblasts. Histamine receptor subtype involvement and NF-κB signaling was further investigated using pharmacological inhibitors.

2. Materials and methods

2.1. Patients and control subjects

Orbital tissues were obtained at the Rotterdam Eye Hospital (Rotterdam, The Netherlands) from five euthyroid GO patients who underwent orbital decompression surgery at an inactive stage of disease and three control subjects without known thyroid or inflammatory disease who underwent orbital surgery for other reasons. The patients had not received steroid or other immunosuppressive treatment for at least three months prior to surgery. Informed consent was obtained in accordance with the principles of the Declaration of Helsinki and the protocol was approved by the local medical ethics committee. Orbital tissue was partly snap-frozen for (immuno)histological studies, the remaining orbital tissue was used for orbital fibroblast isolation as described previously (van Steensel et al., 2009). Orbital fibroblasts were retained in culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) and antibiotics (penicillin and streptomycin; Cambrex BioWhittaker, Verviers, Belgium) and used for experiments between the 4th and 12th passage.

2.2. Mast cell detection in orbital tissues

Snap-frozen orbital tissue from GO patients and controls was sectioned (7 μm) and put onto glass slides and kept overnight at room temperature in humidified atmosphere. The slides were air-dried for 1 h prior to staining. For toluidine blue staining, the sections were fixed in ethanol for 4 min and allowed to dry for 10 min. Hereafter, tissue sections were stained with toluidine blue solution (0.5% toluidine blue in 0.5M HCl) for 3 min, subsequently rinsed with tap-water and embedded with glycerol-gelatin. For CD117 (stem cell factor receptor) staining, sections were fixed in acetone containing 0.02% H_2O_2 and air-dried for 10 min. Slides were then washed with phosphate-buffered saline (PBS) and incubated overnight in humidified atmosphere with a mouse-anti-human CD117 antibody (YB5.B8, BD Biosciences, Erembodegem, Belgium) or a mouse IgG_1 isotype control (Santa Cruz Biotechnologies, Heidelberg, Germany). Subsequently secondary biotin-labeled horse-anti-mouse antibody (Vector laboratories Ltd, Peterborough, UK) and tertiary horse-radish-peroxidase (HRP)-labeled avidin–biotin complex (ABC/HRP; Dako, Heverlee, Belgium) were added for 1 h at room temperature. HRP activity was developed by incubating slides with 3-amino-9-ethyl-carbazole (AEC; Sigma-Aldrich, St. Louis, MO, USA) for 10 min at room temperature. After adequate washing in PBS the slides were counterstained with haematoxylin, embedded in glycerol-gelatin and visualized using an Axiovert (Zeiss, Oberkochen, Germany) and photographed at 200× or 400× magnification using an AxioCam MR5 (Zeiss).

2.3. IL-6 and hyaluronan production by orbital fibroblast cultures

Orbital fibroblasts from four GO patients and three controls were used. Orbital fibroblasts were seeded at a density of 2.5×10^5 cell/well in 12-well plates in DMEM 10% FCS and allowed to reach confluence. Once a fully confluent monolayer was obtained the medium was substituted with DMEM 1% FCS overnight. Hereafter, the orbital fibroblasts were incubated in DMEM 1% FCS in the presence or absence of histamine (59964; Sigma-Aldrich) at concentrations of 1.25, 2.5 and 5.0 mM for 24 and 48 h. Culture supernatant was collected and analyzed by ELISA for IL-6 (Invitrogen, Frederick, MD, USA) and hyaluronan (R&D Systems, Abingdon, UK).

2.4. Hyaluronan synthase and hyaluronidase mRNA expression by orbital fibroblast cultures

Orbital fibroblasts from three GO patients and three controls were used. Orbital fibroblasts were seeded at 4.0×10^5 cells/well into 6-well plates in DMEM 1% FCS and allowed to adhere overnight. Hereafter, the orbital fibroblasts were incubated in DMEM 1% FCS in the presence or absence of histamine (5.0 mM) for 2, 4, 6, 8