ANTIALLERGIC ACTIVITY OF A NOVEL α4β1 INTEGRIN ANTAGONIST REQUIRES BLOCKADE OF MAST CELLS AND EOSINOPHIL ADHESION AND DEGRANULATION

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Mast cells and eosinophils are key effector cells in the pathogenesis of allergic inflammation. Symptoms of allergy occur after the prompt activation and degranulation of mast cells or basophils whereas IgE only serves as one of the key messengers.

The recruitment of different cells subsets into sites of inflammation is a multifactorial and multistep process, involving endothelial interactions through adhesion molecules and local generation of chemotactic agents that direct cell migration into the inflamed area.

Mast cells are considered one of the first cells of the immune system capable to interact with environmental antigens, toxins, or invading pathogens. Fully differentiated mast cells express α4β1 (VLA-4, or CD49d/CD29) integrin. Firstly, we ascertained that this integrin is present in HMC 1.1 cells, a well established and widely used human cell line exhibiting many characteristics of mast cells. In agreement with the literature, in vitro cellular adhesion of this cell line to VCAM-1 is mediated by integrin α4β1.

Also, fibronectin (FN) enhancement of β-hexosaminidase release (a marker of mast cell degranulation) is inhibited by specific antibodies as well as by RGD- and LDV-derivated peptides that abrogate α5β1 binding to vitronectin (VNR) and α4β1 binding to FN, respectively. These results demonstrate that β1 integrins expressed on rat mast cells bind to fibronectin and play an important role in regulating mast cells activation both in vitro and in vivo.

Eosinophils are activated later and release oxidants such as superoxide anion, eosinophil peroxidase (EPO), nonoxidants such as leukotriene C4 (LTC4), eosinophil cationic protein (ECP), and proinflammatory mediators.

Integrin α4β1 is constitutively expressed in these cells: it has a role in cell degranulation and superoxide anion production in stimulated eosinophils. It is now established that cell adhesion molecules participate in the activation of inflammatory eosinophilic functions. Eosinophil adhesion to VCAM-1 can either stimulate superoxide anion generation and potentiate activation by N-formyl-methionylleucyl-phenylalanine; eosinophil degranulation is strongly dependent on cell adhesion. Selective activation of VLA-4 by cytokines has provided one mechanism of more efficient recruitment of eosinophils to the lung as IL-5 induces a change in the affinity of VLA-4 in responding leukocytes.

In this study the small-molecule VLA-4 antagonist DS70 was assessed for its effects on mast cells and eosinophils. We investigated DS70-mediated inhibition of VLA-4/VCAM-1 binding as a mean for the modulation of mast cells and eosinophil functions, in vitro and in vivo.

In HMC1.1 cells, we observed a significant decrease in β-hexosaminidase release, triggered by VCAM-1. Similar results were obtained in EOL1 cell line, where pre-treatment with compound DS70 is able to significantly lower EPO release stimulated by VCAM-1. According to previous data, DS70 effects on the early phase reaction seem to be mediated by the blockade of mast cells activation. We corroborated our hypothesis in an in vivo model of EAC (experimental model of allergic conjunctivis): DS70 was administered topically into guinea pigs conjunctival sac 30 min and 10 min before OVA challenge. DS70 is able to prevent mast cells degranulation in conjunctival tissue sections (Giemsa staining) and, therefore, the consequent release of preformed granules. In the same sections, we found an impressive decrement of infiltrating eosinophils performing Luna staining: we also detected a decrease in CCL5 and CCL11 expression in conjunctival tissue, providing a possible mechanism explaining impaired eosinophils recruitment.

In conclusion, we propose that integrin α4β1 antagonists could represent an effective therapy for the blockade of eosinophil chemotaxis, recruitment and mast cell activation.