ImmunoTools *special* Award 2023

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Leukocyte integrin ligands as promising treatment for inflammatory-based diseases.

Background. Integrins are cell adhesion receptors which play a relevant role in cell–cell and cell–extracellular matrix communication by regulating crucial aspects of cellular functions[1]. Given their fundamental contribution in human physiology, specific integrin dysregulation phenomena are linked to the pathogenesis of many diseases, including inflammation and autoimmune diseases. Within the integrin family, $\alpha_4\beta_1$ and β_2 integrins are mainly expressed on leukocyte cell surface and are involved in the modulation of leukocyte recruitment, a multistep process initiated by capture of rolling leukocytes from the bloodstream and terminated by their extravasation into the nearby inflamed tissue. In particular, $\alpha_4\beta_1$, $\alpha_L\beta_2$ and $\alpha_M\beta_2$ integrins are deeply involved in leukocyte firm adhesion preceding extravasation.

Since many inflammatory diseases are characterized by a dysregulated migration of leukocytes, there is a keen interest in finding and testing compounds able to modulate these processes[2].

Aim of the project. Targeting integrins has already proven to be a successful therapeutic strategy especially in inflammatory-based pathologies and several agents targeting integrins have already been approved for clinical use[3]. Therefore, considering integrins as a valuable drug target, the current study aimed to evaluate the *in vitro* ability of some β-lactam derivatives to modulate integrin-mediated immune cells recruitment.

Methods and Preliminary Results. The pharmacological activity of new compounds was at first evaluated by adhesion assays performed on Jurkat E6.1 (expressing $\alpha_4\beta_1$ and $\alpha_L\beta_2$) or HL-60 cells (expressing $\alpha_M\beta_2$, differentiated for 5 days with DMSO 1.25%). For those compounds capable to modulate integrin-mediated cell adhesion, the ability to regulate $\alpha_4\beta_1$ integrin-mediated signal transduction was also evaluated, through the study of ERK, Akt and JNK phosphorylation by Western Blot. Based on these preliminary results, we have found selective

and potent agonists and antagonists of $\alpha_4\beta_1$ and $\alpha_M\beta_2$ leukocyte integrins, able to modulate cell adhesion and intracellular signaling.

Future Perspectives. We're evaluating the effects of $\alpha_M\beta_2$ ligands on integrin mediated signal transduction, so we need ImmunoTools HRP-linked mouse anti-rabbit IgG (H+L chain) secondary antibody to perform Western Blots and complete our understanding on compounds' effects on signal transduction.

We will also investigate new antagonist ability to reduce leukocyte transendothelial migration (TEM), to reduce the number of immune cells recruited into an inflamed tissue. For this purpose, we developed an *in vitro* model able to mimic the *in vivo* leukocyte recruitment from the bloodstream into the inflamed tissue. The model foresees the use of Transwell®, HMVEC primary cells (Human Dermal Microvascular Endothelial cells) and human neutrophils. HMVEC, seeded on Transwell® upper chamber, are treated with TNF-α, mimicking an inflammatory condition of endothelial cells. That's why ImmunoTools TNF-α would be useful for our project. The day of the assay, neutrophil treated with leukocyte integrin antagonists/agonist will be added into the upper chamber of Transwell® and they will be induced to migrate across endothelial cell monolayer adding a chemotactic stimulus into the lower chamber.

To confirm that the effect of agonist/antagonist treatment observed in adhesion assays as well in TEM is specifically determined by the interaction between leukocyte integrin and our ligands, we would like to perform the assays blocking integrin function with ImmunoTools anti-human CD11a/CD11b/CD18/CD49d purified monoclonal antibodies (functional application).

Moreover, treating cells with integrin ligands may result in different integrin expression on leukocyte cell surface, due to possible internalization and recycling processes. So, we would also evaluate integrin expression on different cell lines treated with different concentration of the compounds by flow cytometry. For this purpose, we require ImmunoTools FITC-labelled CD11a/CD11b/CD18/CD49d monoclonal antibodies.

The aforementioned ImmunoTools reagents would allow us to better characterize the interaction between new *β*-lactam integrin ligands and leukocyte integrin as well as their effects on leukocyte recruitment, in order to develop innovative pharmacological treatment for inflammatory based diseases.

Reference.

1. Cox D, Brennan M, Moran N. Integrins as therapeutic targets: lessons and opportunities. Nat Rev Drug Discov. 2010 Oct;9(10):804-20. doi: 10.1038/nrd3266. PMID: 20885411.
2. Herter J, Zarbock A. Integrin Regulation during Leukocyte Recruitment. J Immunol. 2013 May 1;190(9):4451-7. doi: 10.4049/jimmunol.1203179. PMID: 23606722.

3. Tabary O, Corvol H, Boncoeur E, Chadelat K, Fitting C, Cavaillon JM, Clément A, Jacquot J. Adherence of airway neutrophils and inflammatory response are increased in CF airway epithelial cell-neutrophil interactions. Am J Physiol Lung Cell Mol Physiol. 2006 Mar;290(3):L588-96. doi: 10.1152/ajplung.00013.2005. Epub 2005 Nov 4. PMID: 16272177.

ImmunoTools *special* AWARD for **Andrea Maurizio** includes 10 reagents

FITC - conjugated anti-human CD11a, CD11b, CD18, CD49d

anti-human CD11a, anti-CD11b, CD18 and CD49d as function application antibody

anti-rabbit IgG (H+L-chain) HRP secondary antibody

recombinant human cytokines: rh TNFalpha

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