Ruolo delle piastrine nella patogenesi dell’asma bronchiale allergico

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Inflammation and bronchial asthma

- Asthma is characterised by paroxysmal and reversible obstruction of the airways

- It is increasingly understood as a chronic inflammatory condition combined with bronchial hyperresponsiveness

- Allergic asthma is the consequence of a specific, IgE-mediated, immune response to an exogenous allergen
Platelets express IgE receptors


Increase of Platelet Factor 4 in allergic asthma

Increase of Platelet Factor 4 in allergic asthma

Ma anche:
• Beta-TG
• RANTES
• Piastrinopenia
• ...

(altri autori)
Effect of platelet depletion and restoration on pulmonary eosinophil recruitment in allergic mice

Platelet P-selectin is required for pulmonary eosinophil recruitment in allergen-sensitized mice
[Some of] The Players

1. P2Y receptors
2. Cysteinyl Leukotriens (Cys-LT)
[Some of] The Players

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2. CysteinyL Leukotriens (Cys-LT)
Cysteinyl leukotrienes

- Cysteinyl leukotrienes (cys-LT) [LTC4, LTD4, LTE4] are lipid inflammatory mediators generated in vivo by 5-lipoxygenase of mast cells, eosinophils, basophils, macrophages.
- Cys-LTs abound in mucosal inflammation, play a validated role in human asthma, and are important mediators in mouse models of pulmonary inflammation, remodeling, and fibrosis.
Receptors for cys-LT

- Two G protein–coupled receptors for cys-LTs, termed type 1 and type 2 cys-LT receptors (CysLT1R and CysLT2R), have been cloned and characterized, which share 38% aminoacid identity.
[Some of] The Players

1. P2Y receptors
2. Cysteinyl Leukotriens (Cys-LT)
# Mammalian G Protein-Coupled P2Y Receptors

<table>
<thead>
<tr>
<th>Ligand</th>
<th>ADP</th>
<th>ATP/UTP</th>
<th>UTP</th>
<th>UDP</th>
<th>ATP</th>
<th>ADP</th>
<th>UDP-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>P2Y₁</td>
<td>P2Y₂</td>
<td>P2Y₄</td>
<td>P2Y₆</td>
<td>P2Y₁₁</td>
<td>P2Y₁₂</td>
<td>P2Y₁₄</td>
</tr>
<tr>
<td>Signaling</td>
<td>Gq</td>
<td>Gq</td>
<td>Gq</td>
<td>Gq</td>
<td>Gq</td>
<td>Gq</td>
<td>Gi</td>
</tr>
<tr>
<td>Response</td>
<td>↑PLC</td>
<td>↑PLC</td>
<td>↑PLC</td>
<td>↑PLC</td>
<td>↑PLC</td>
<td>↑PLC</td>
<td>↓AC</td>
</tr>
</tbody>
</table>

- **Gq**: Gq protein
- **Gs**: Gs protein
- **Gi**: Gi protein
- **PLC**: Phospholipase C
- **AC**: Adenylyl cyclase

The diagram illustrates the signaling pathways and response for various P2Y receptors in mammalian systems, showing the ligands and their corresponding G protein-coupled signaling responses.
A phylogenetic tree (dendrogram) showing the relationships among the current members of the P2Y receptor family (human P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12 and P2Y13, and P2Y14 receptor). The P2Y receptors can be divided into two subgroups shown with green and blue backgrounds. Sequences were aligned using Image and the tree was built using the Image software.

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Platelet Receptors for ADP

- Thienopiridines
  - active metabolites
    - ticagrelor
    - cangrelor

- Strong agonists
- ADP
  - P2Y₁
  - P2Y₁₂

- Shape change
- Aggregation
- Secretion
Mechanisms of allergy and clinical immunology

RhoA signaling through platelet P2Y₁ receptor controls leukocyte recruitment in allergic mice

Richard T. Amison, MSci,a Stefania Momì, PhD,b Abigail Morris, PhD,a Giorgia Manni, BSc,b Sandra Keir, PhD,a Paolo Gresele, MD,b Clive P. Page, PhD,a and Simon C. Pitchford, PhDa  London, United Kingdom, and Perugia, Italy
Effects of **apyrase** on pulmonary leukocyte recruitment in OVA-sensitized and challenged mice

Amison RT et al, J Allergy Clin Immunol 2015
Effects of P2Y₁ antagonism on pulmonary leukocyte recruitment in OVA-sensitized and challenged mice

Amison RT et al, J Allergy Clin Immunol 2015
Effects of $\text{P2Y}_{12}$ antagonism on pulmonary leukocyte recruitment in OVA-sensitized and challenged mice

Amison RT et al, J Allergy Clin Immunol 2015
The effect of P2Y₁ on pulmonary leukocyte recruitment in OVA-sensitized and challenged mice is platelet-specific.
Leukotriene E₄–induced pulmonary inflammation is mediated by the P2Y₁₂ receptor

Sailaja Paruchuri,¹,³ Hiroyuki Tashimo,¹,³ Chunli Feng,³ Akiko Maekawa,¹,³ Wei Xing,¹,³ Yongfeng Jiang,¹,³ Yoshihide Kanaoka,¹,³ Pamela Conley,⁴ and Joshua A. Boyce¹,²,³

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LTE₄-mediated amplification of allergen-induced eosinophil recruitment and pulmonary inflammation

LTE$_4$-mediated amplification of allergen-induced globet cell metaplasia

Platelet P2Y$_{12}$ dependence of the LTE$_4$ effect on bronchial inflammation
Platelet P2Y$_{12}$ dependence of the LTE$_{4}$ effect on globet cell metaplasia

(Refer to the figure for the graphical representation of the data.)

LTC₄ amplifies allergen-induced pulmonary eosinophil recruitment in a platelet, CysLT₂R and P2Y₁₂-dependent manner.
Effect of clopidogrel treatment in a mouse model of asthma

Effect of clopidogrel treatment in a mouse model of asthma

And in humans?
Relationship between P2Y$_{12}$ single nucleotide polymorphisms and airways responsiveness stratified by house dust mite exposure in children with bronchial asthma

Bunyavanich S et al, Clin Exp Allergy 2011
QUESTION

Does the inhibition of P2Y$_{12}$ decrease bronchial hyper-reactivity in patients with allergic bronchial asthma?
BRIEF REPORT

Effect of prasugrel in patients with asthma: results of PRINA, a randomized, double-blind, placebo-controlled, cross-over study

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Unrestricted grant by Eli Lilly and Daiichi Sankyo
The primary objective of this “proof of concept” study was to test whether or not the inhibition of the platelet P2Y$_{12}$ receptor by prasugrel reduces the bronchial hyper-reactivity in patients with chronic asthma.
Study design

double-blind, placebo-controlled, cross-over study

- **Screening**
  - Prasugrel 10 mg/d
  - Placebo

- **Washout ≥ 15 d**
  - Prasugrel 10 mg/d
  - Placebo
Patients with chronic allergic asthma, diagnosed based on the occurrence of episodic wheezing, chest tightness and/or dyspnoea and objectively confirmed according to standard criteria, such as methacholine airway hyper-responsiveness (PC20 FEV1 < 16mg/ml) and positivity of skin test to common allergens (prick test)

- Positivity of bronchial challenge testing with mannitol
- Age range of 18-74 years
- Duration of asthma >1 year
- Mild and stable asthma without chronic medication, except for the use of inhaled low dose of steroids or the use of inhaled beta2-agonist on demand
- Written informed consent
# Patients

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<table>
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<tbody>
<tr>
<td>Gender, women/men</td>
<td>11/15</td>
</tr>
<tr>
<td>Age, mean ± SD (y)</td>
<td>43±13</td>
</tr>
<tr>
<td>Number of dropouts*</td>
<td>2</td>
</tr>
</tbody>
</table>

* due to asthma exacerbation: 1 during placebo treatment, 1 during prasugrel treatment
Mannitol bronchial challenge test
• Airway hyperresponsiveness was measured by bronchial provocation with dry powder mannitol (Osmohale™)
• The test was terminated when the fall in forced expiratory volume in 1-s (FEV1) reached at least 15% of baseline or after a cumulative mannitol dose of 635 mg.
• The provocative dose of mannitol causing a 15% or greater fall in FEV1 (PD15) was calculated by linear interpolation.
VASP-P on day 14 of treatment with placebo or prasugrel in patients with allergic bronchial asthma
PD15 before and after 14 days treatment with placebo or prasugrel in patients with allergic bronchial asthma

Lussana F et al, JTH 2015
1. Our proof-of-concept study suggests that inhibition of the (platelet) $\text{P}2\text{Y}_{12}$ receptor by prasugrel may be clinically useful in the treatment of patients with chronic allergica asthma

2. This hypothesis should now be tested in randomized controlled clinical trials, using clinical end-points
Two G protein–coupled receptors for cys-LTs, termed type 1 and type 2 cys-LT receptors (CysLT1R and CysLT2R), have been cloned and characterized, which share 38% aminoacid identity. Each of them is 24–32% identical to the purinergic (P2Y) class of GPCRs, suggesting a phylogenetic relationship between these two GPCR classes.
How many receptors for LTE4?

• Among the 3 Cys-LTs, only LTE4 is stable and abundant in vivo

• Although LTE4 shows negligible activity at CysLT1R and CysLT2R, it is a powerful inducer of mucosal eosinophilia and airway hyper-responsiveness in humans with asthma

• The existence of an additional cys-LT receptor with a preference for LTE4 has long been suspected
Dose–response curves for leukotriene-evoked $[\text{Ca}^{2+}]_i$ increases via the expressed hP2Y$_{12}$–hG$_{16\alpha}$ fusion proteins

Nonaka et al, BBRC 2005
Reports:
Identification of GPR99 Protein as a Potential Third Cysteinyl Leukotriene Receptor with a Preference for Leukotriene E4 Ligand

Yoshihide Kanaoka, Akiko Maekawa and K. Frank Austen
doi: 10.1074/jbc.C113.453704 originally published online March 15, 2013
Binding of $[^3\text{H}]\text{LTE}_4$ to microsomal membrane proteins from GPR99 and vector control transfectants

Kanaoka Y et al, J Biol Chem 2013
Effect of subcutaneous injection of LTE$_4$ on ear edema in mice

Kanaoka Y et al, J Biol Chem 2013
What receptors for cysLTs are expressed on platelets?
Human and mouse platelets express CysLT$_1$R and CysLT$_2$R but not GPR99

Cummings HE et al, J Immunol 2013
CysLT$_2$R is involved in LTC$_4$–induced mouse platelet activation

Cummings HE et al, J Immunol 2013
CysLT$_2$R is involved in LTC$_4$–induced mouse platelet activation
General Conclusion

- Platelets play a role in the pathogenesis of allergic asthma
- Platelet released ADP and P2Y receptors are involved (P2Y1 and P2Y12?)
- Prasugrel slightly decreased bronchial hyperresponsiveness in a prove-of-concept RCT
- CysLT do not activate human platelets directly, but likely through ADP secretion by cells other than platelets (?)
Ringraziamenti

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  (Unità di Pneumologia, ASST SS Paolo e Carlo, Università degli Studi di Milano)

• A. Moro (Unità di Anatomia Patologica, ASST SS Paolo e Carlo, Università degli Studi di Milano)