Ruolo delle piastrine nella patogenesi dell’asma bronchiale allergico

Marco Cattaneo

Università degli Studi di Milano

Inflammation and bronchial asthma

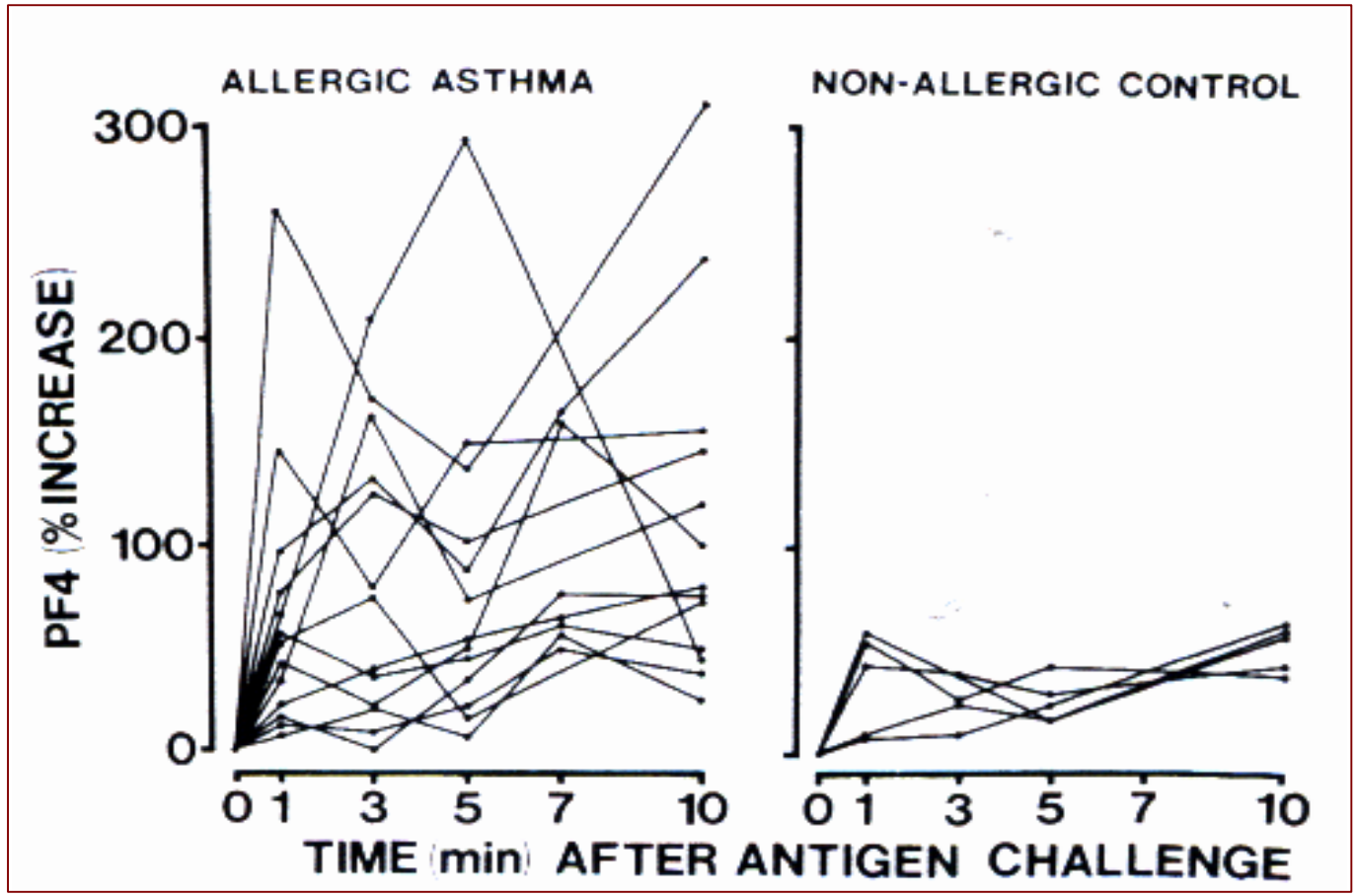
- Asthma is characterised by paroxysmal and reversible obstruction of the airways
- It is increasingly understood as a chronic inflammatory condition combined with bronchial hyper-responsiveness
- Allergic asthma is the consequence of a specific, IgE-mediated, immune response to an exogenous allergen

Platelets express IgE receptors

- Joseph M, Gounni AS, Kusnierz JP, et al. Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors. *Eur J Immunol* 1997;27:2212-8.

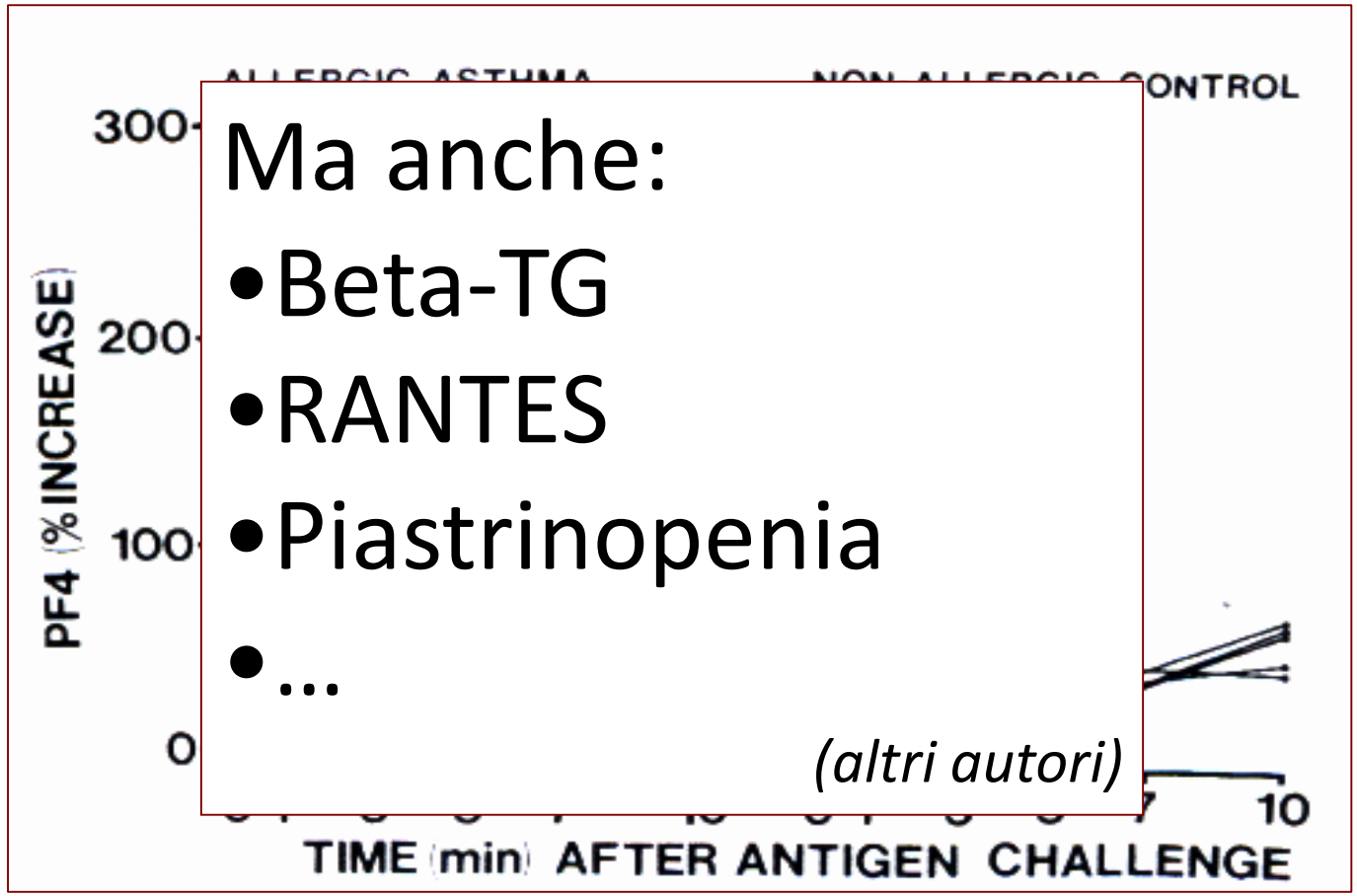
- Capron M, Joseph M. The low affinity receptor for IgE on eosinophils and platelets. *Monogr Allergy* 1991;29:63-75.

Increase of Platelet Factor 4 in allergic asthma



Knauer KA et al, N Engl J Med 1981

Increase of Platelet Factor 4 in allergic asthma

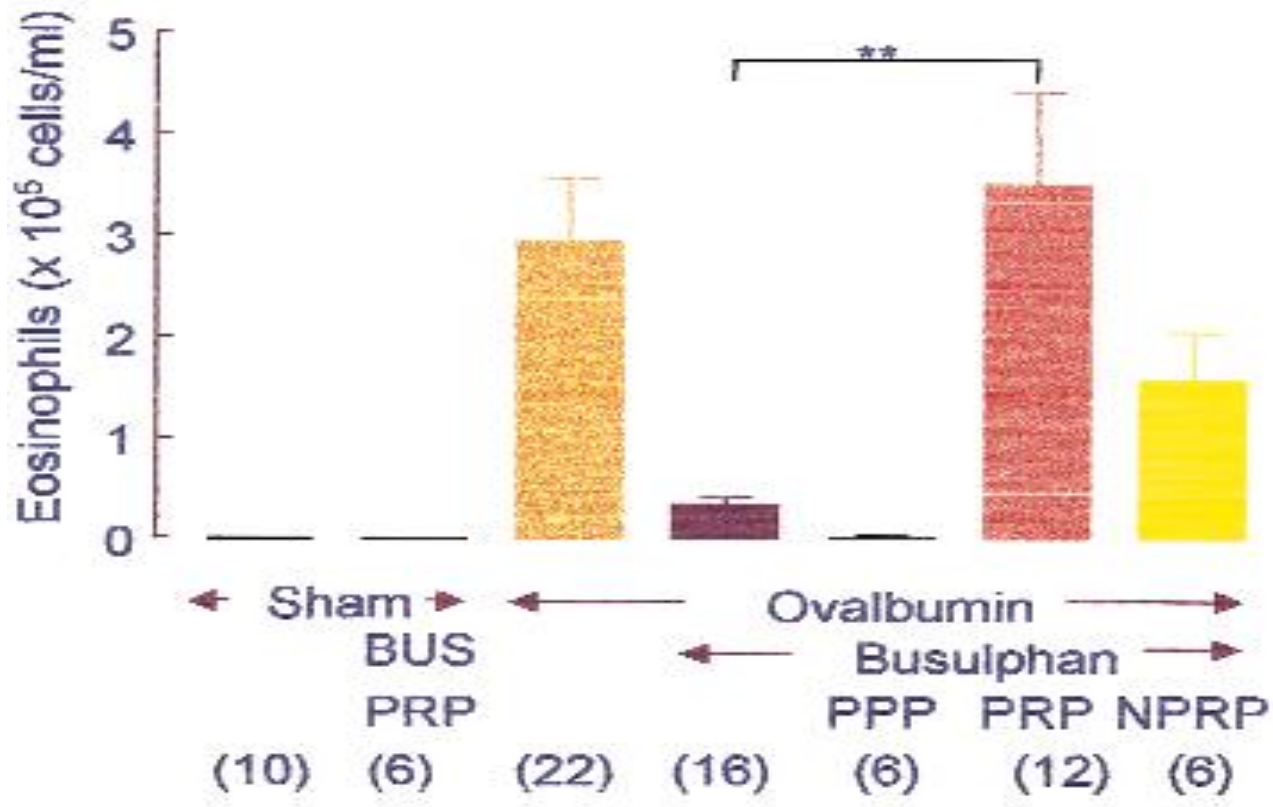


Ma anche:

- Beta-TG
- RANTES
- Piastrinopenia
- …

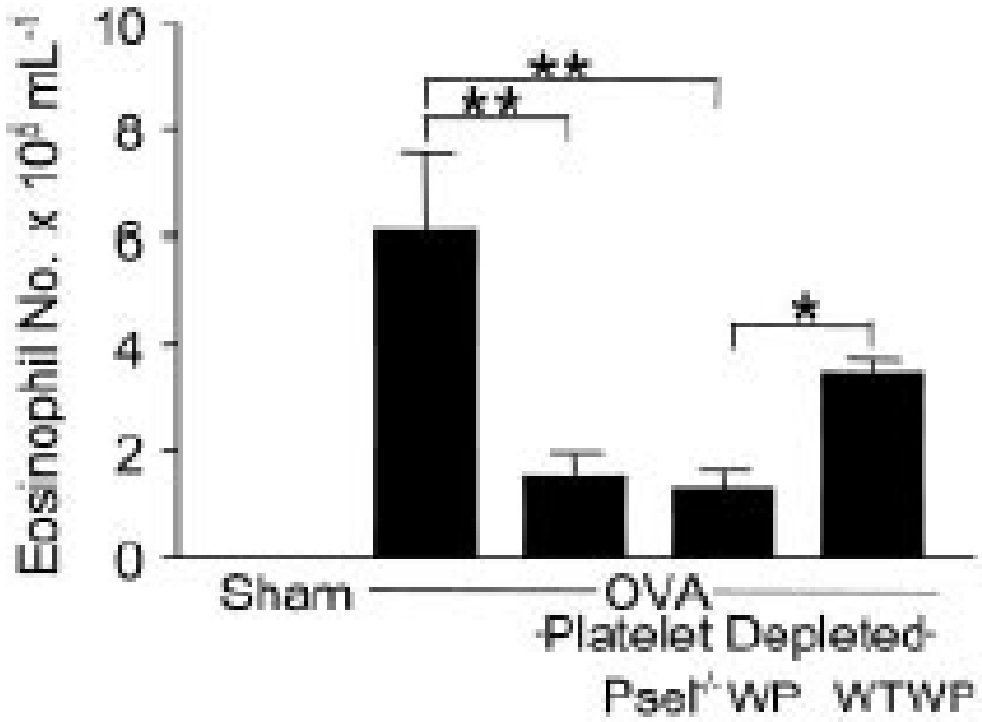
(altri autori)

Effect of platelet depletion and restoration on pulmonary eosinophil recruitment in allergic mice



Pitchford et al. J. Allergy Clin. Immnol 2003;112:109-118

Platelet P-selectin is required for pulmonary eosinophil recruitment in allergen-sensitized mice



Pitchford et al, Blood 2005

[Some of]
The Players

1. P2Y receptors
2. Cysteinyl Leukotriens (Cys-LT)

[Some of]
The Players

1. P2Y receptors
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

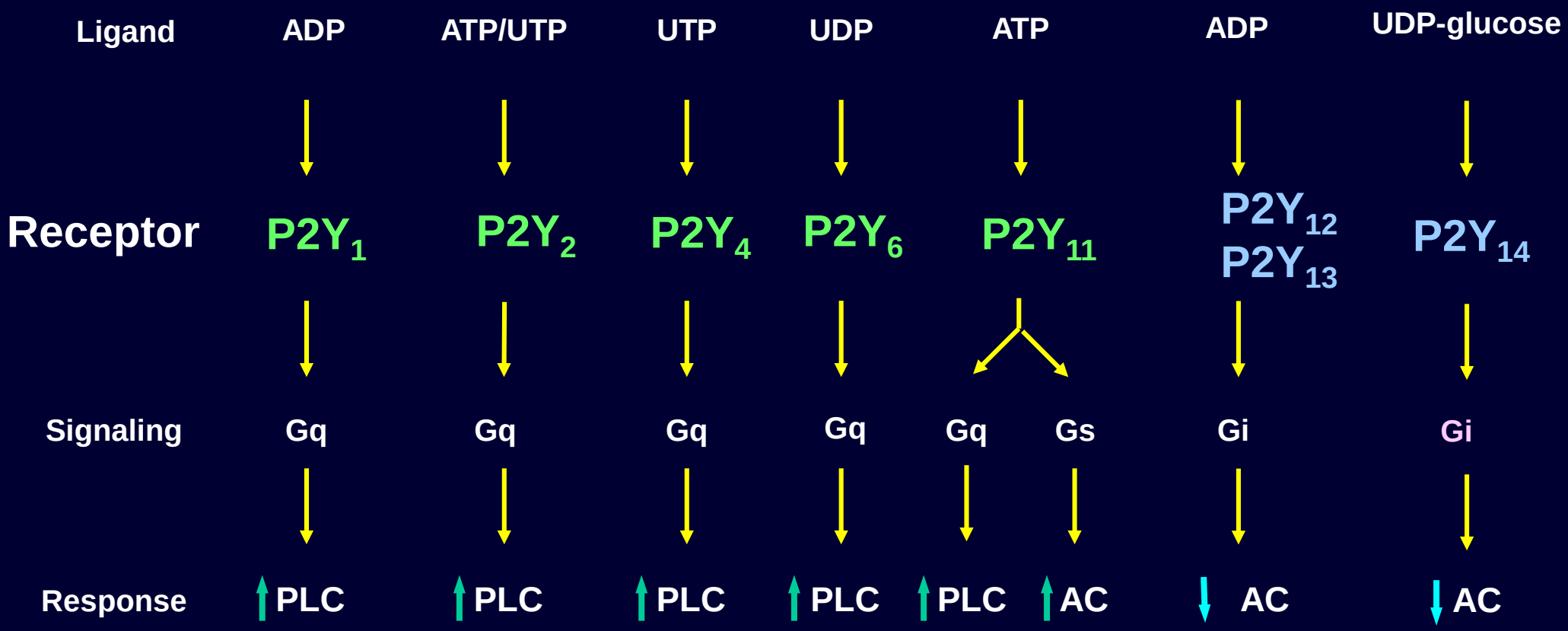
Receptors for cys-LT

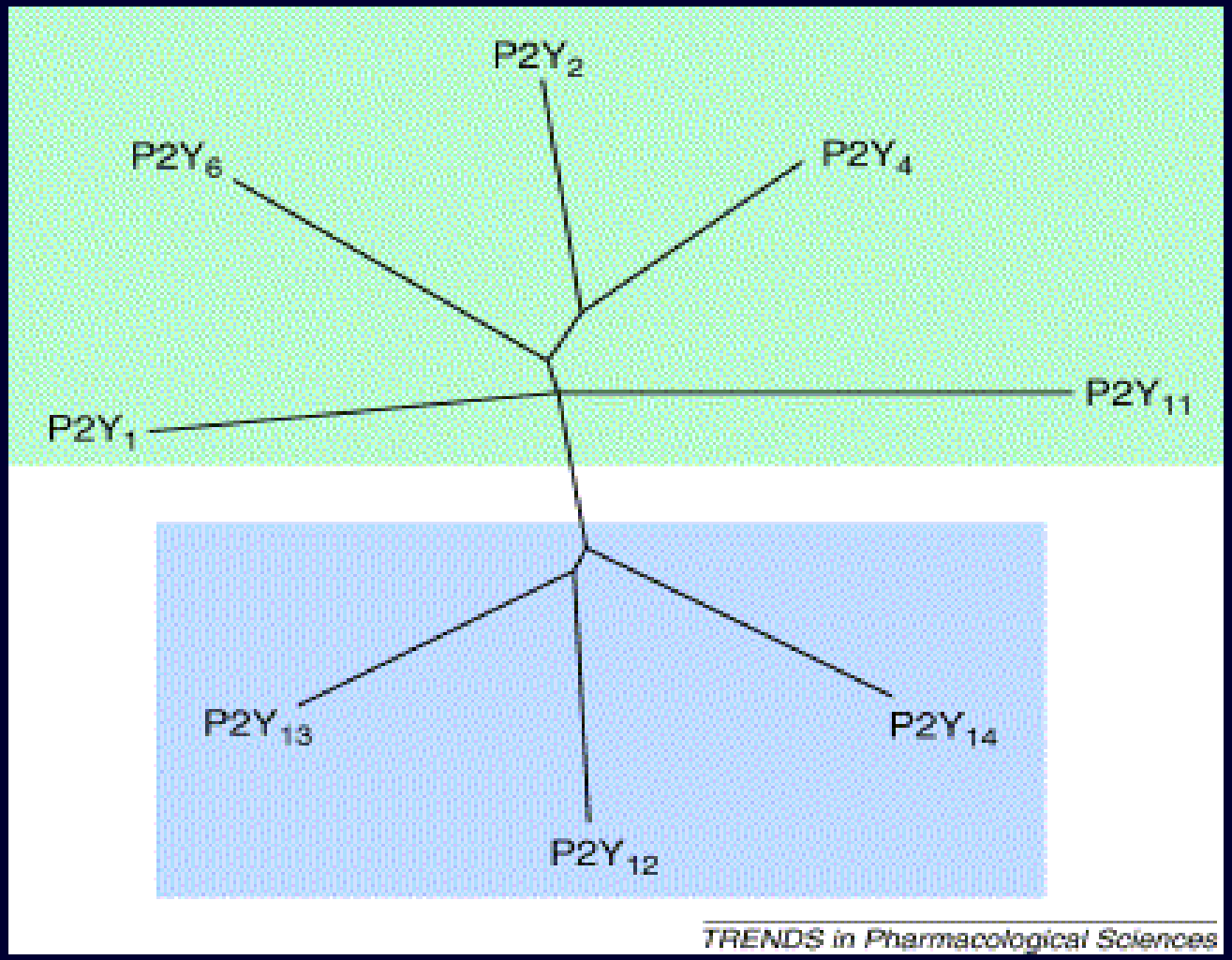
- Two G protein–coupled receptors for cys-LTs, termed type 1 and type 2 cys-LT receptors (CysLT1R and CysLT2R), have been cloned and characterized, which share 38% aminoacid identity

[Some of]
The Players

1. P2Y receptors
2. Cysteinyl Leukotriens (Cys-LT)

Mammalian G Protein-Coupled P2Y Receptors

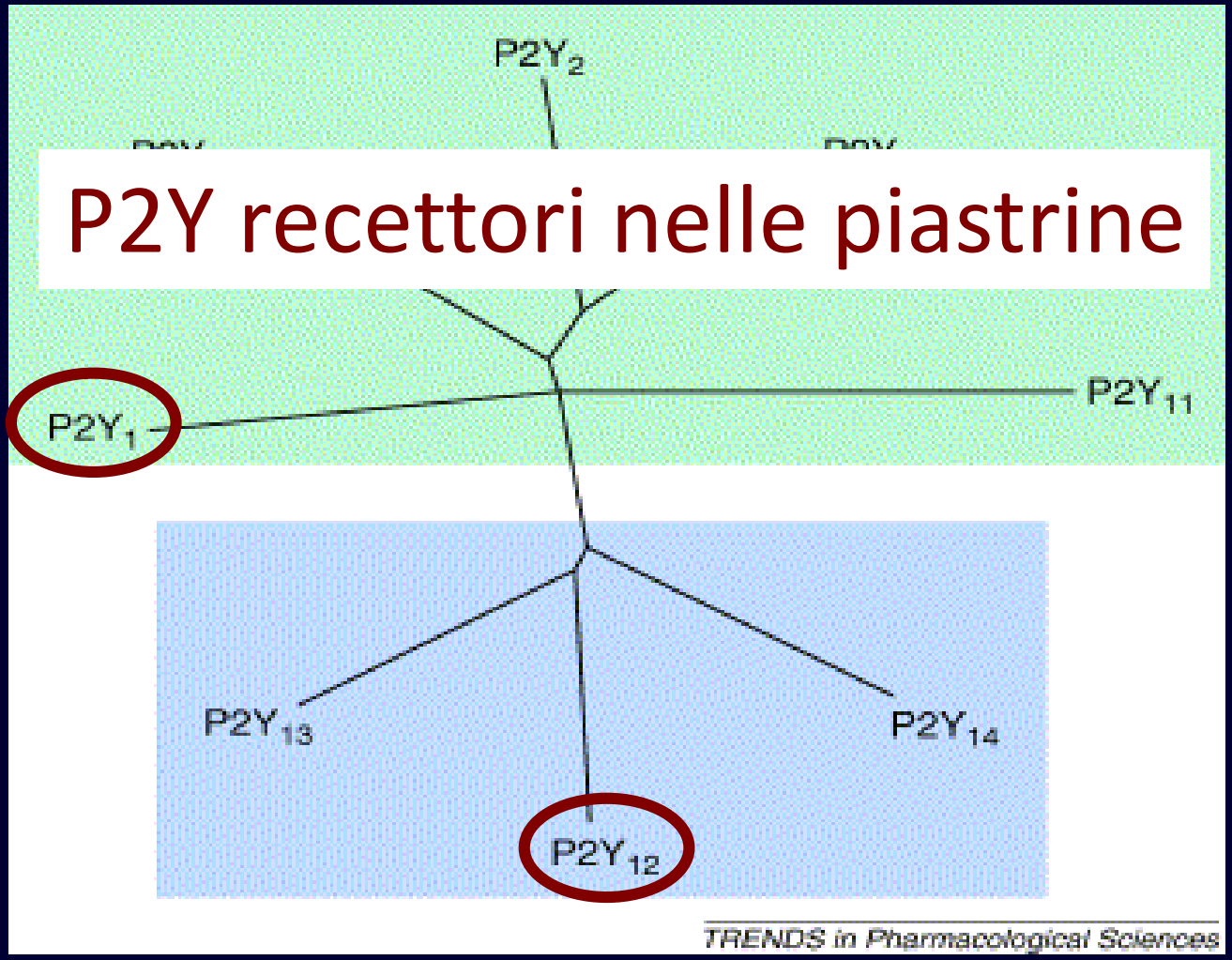




A phylogenetic tree (dendrogram) showing the relationships among the current members of the P2Y receptor family (human P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12 and P2Y13, and P2Y14 receptor). The P2Y receptors can be divided into two subgroups shown with green and blue backgrounds. Sequences were aligned using Image and the tree was built using the Image software.

M. P. Abbracchio, et al. Trends Pharmacol.l Sci. 24:52-55, 2003

P2Y recettori nelle piastrine



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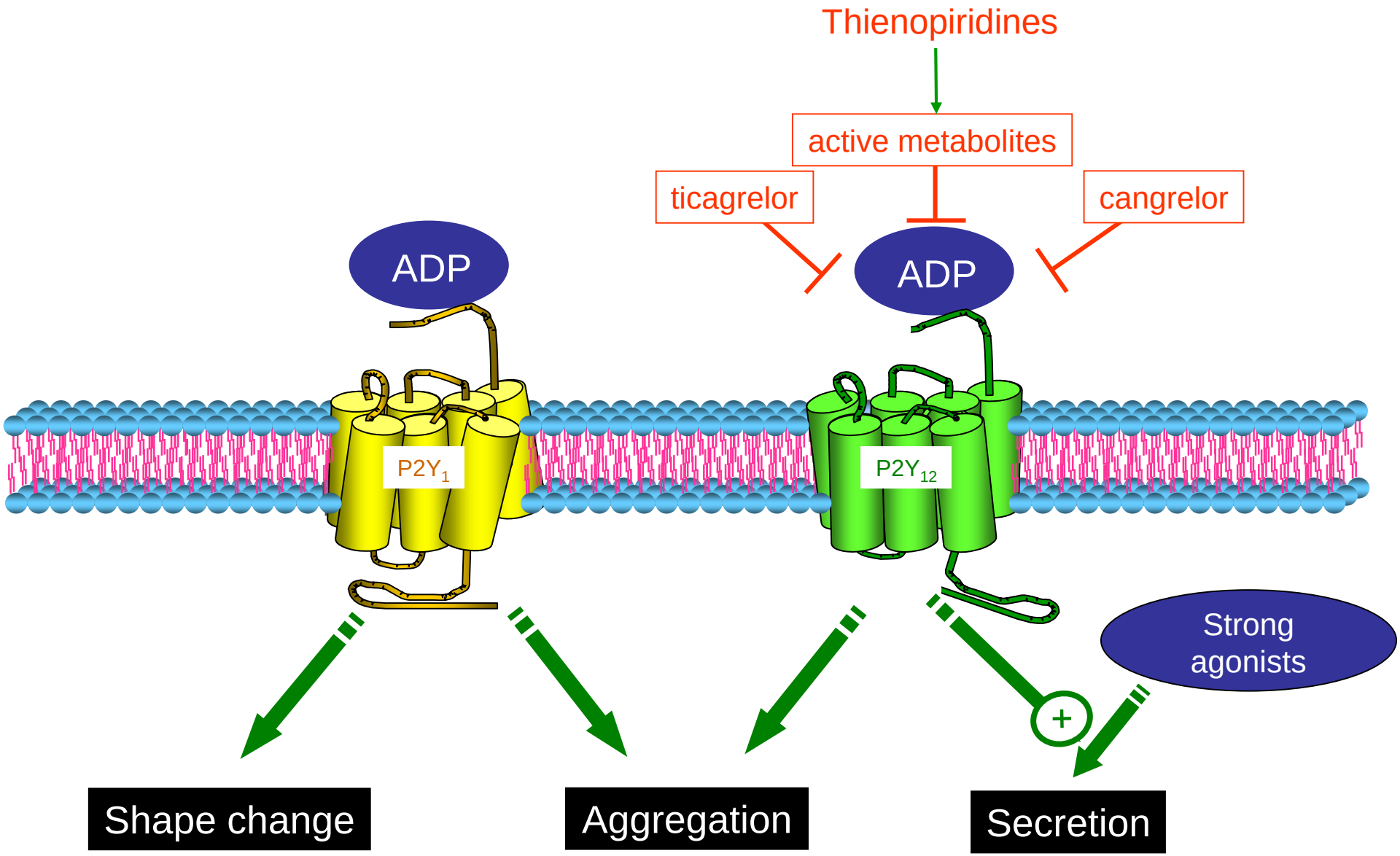
M. P. Abbracchio, et al. Trends Pharmacol.l Sci. 24:52-55, 2003

Platelet Receptors for ADP



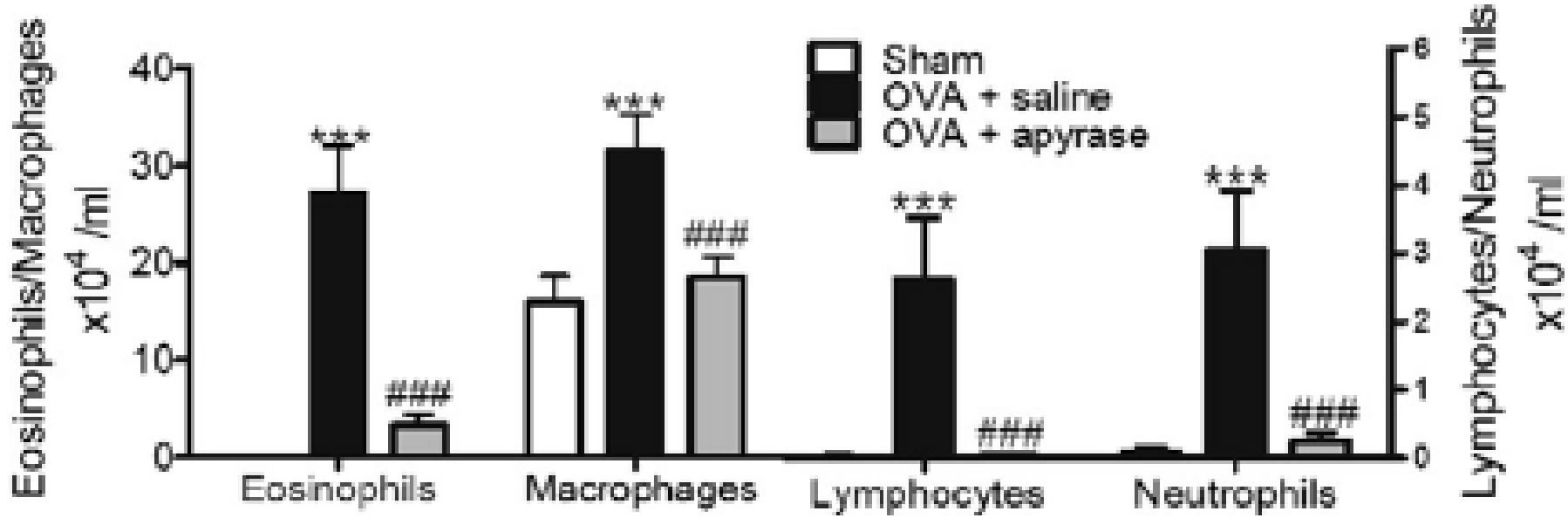
Mechanisms of allergy and clinical immunology

RhoA signaling through platelet $P2Y_1$ receptor controls leukocyte recruitment in allergic mice

Richard T. Amison, MSci,[a] Stefania Momi, 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, PhD[a] *London, United Kingdom, and Perugia, Italy*

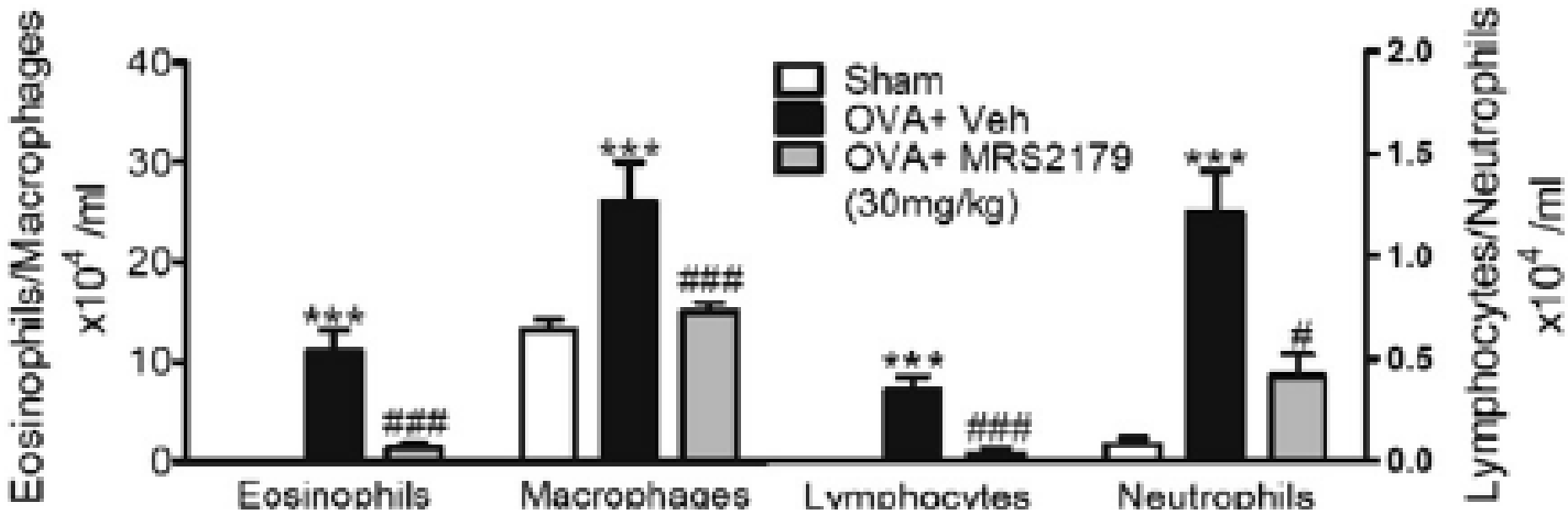
Amison RT et al, J Allergy Clin Immunol 2015

Effects of **apyrase** on pulmonary leukocyte recruitment in OVA-sensitized and challanged mice



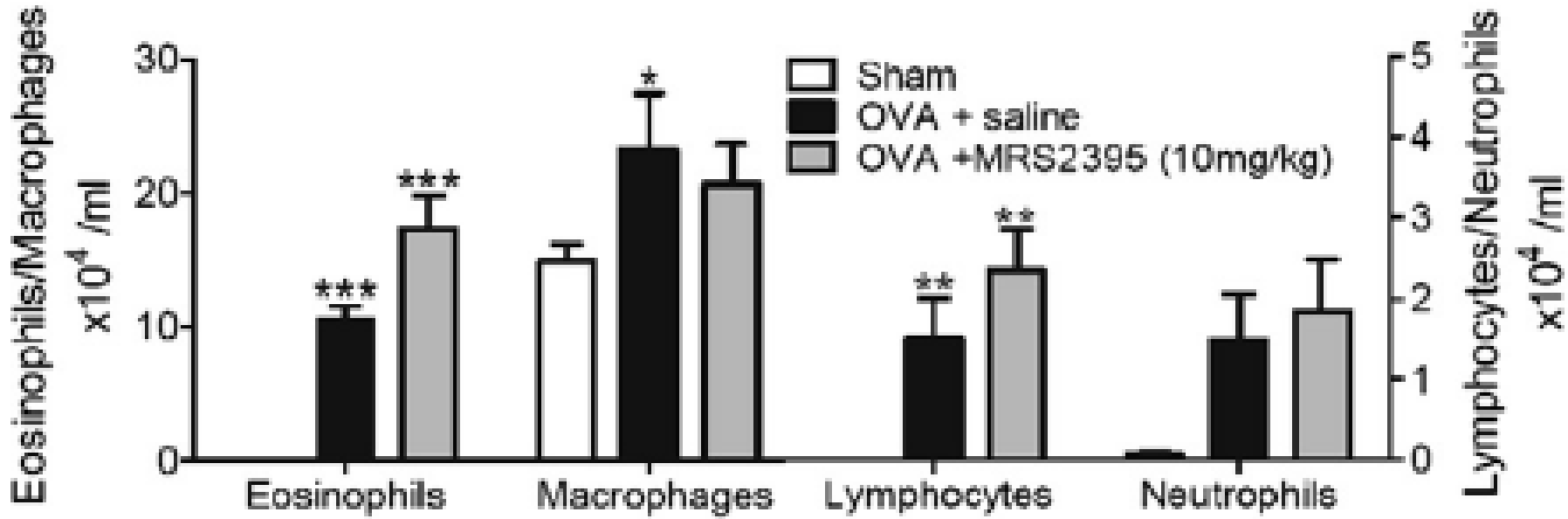
Amison RT et al, J Allergy Clin Immunol 2015

Effects of **$P2Y_1$** antagonism on pulmonary leukocyte recruitment in OVA-sensitized and challanged mice



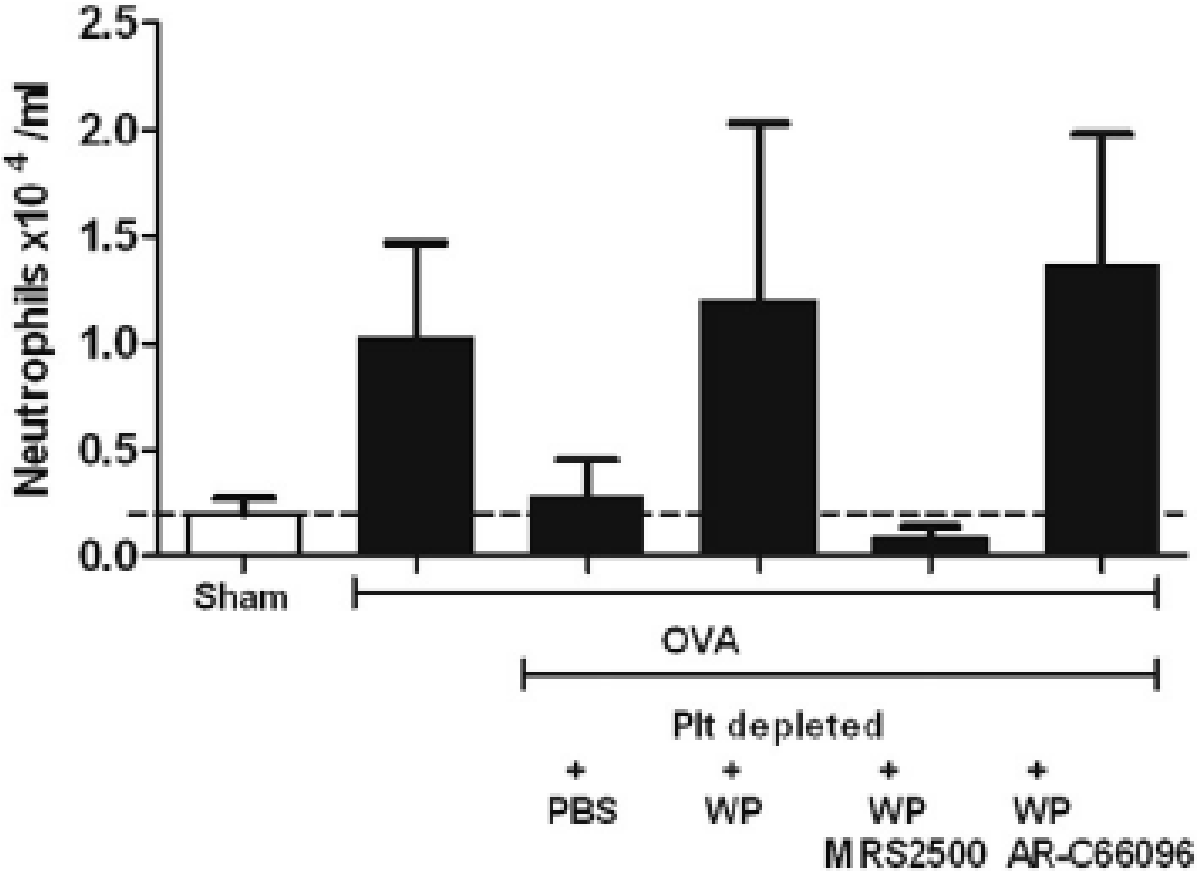
Amison RT et al, J Allergy Clin Immunol 2015

Effects of **$P2Y_{12}$** antagonism on pulmonary leukocyte recruitment in OVA-sensitized and challanged mice



Amison RT et al, J Allergy Clin Immunol 2015

The effect of $P2Y_1$ on pulmonary leukocyte recruitment in OVA-sensitized and challanged mice is platelet-specific



Amison RT et al, J Allergy Clin Immunol 2015

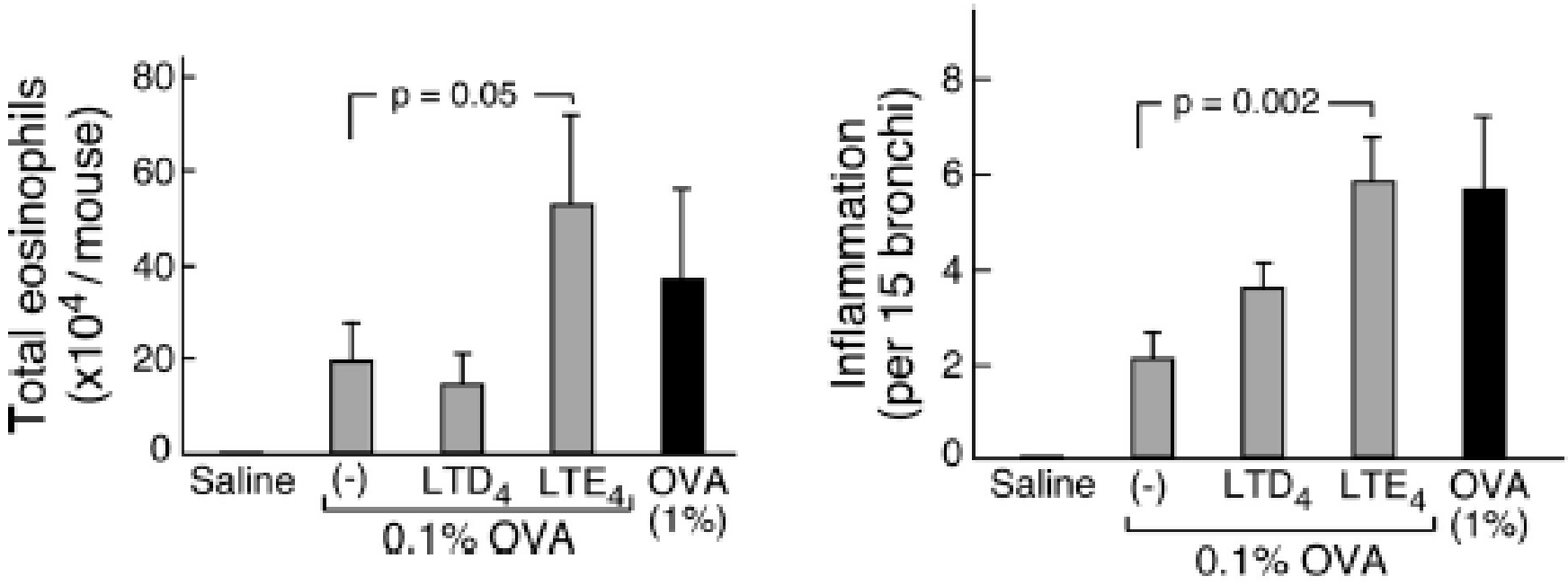
Leukotriene E_4–induced pulmonary inflammation is mediated by the $P2Y_{12}$ receptor

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. Boyce[1,2,3]

[1]Department of Medicine and [2]Department of Pediatrics, Harvard Medical School, Boston, MA 02115
[3]Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, Boston, MA 02115
[4]Portola Pharmaceuticals, San Franciso, CA 94127

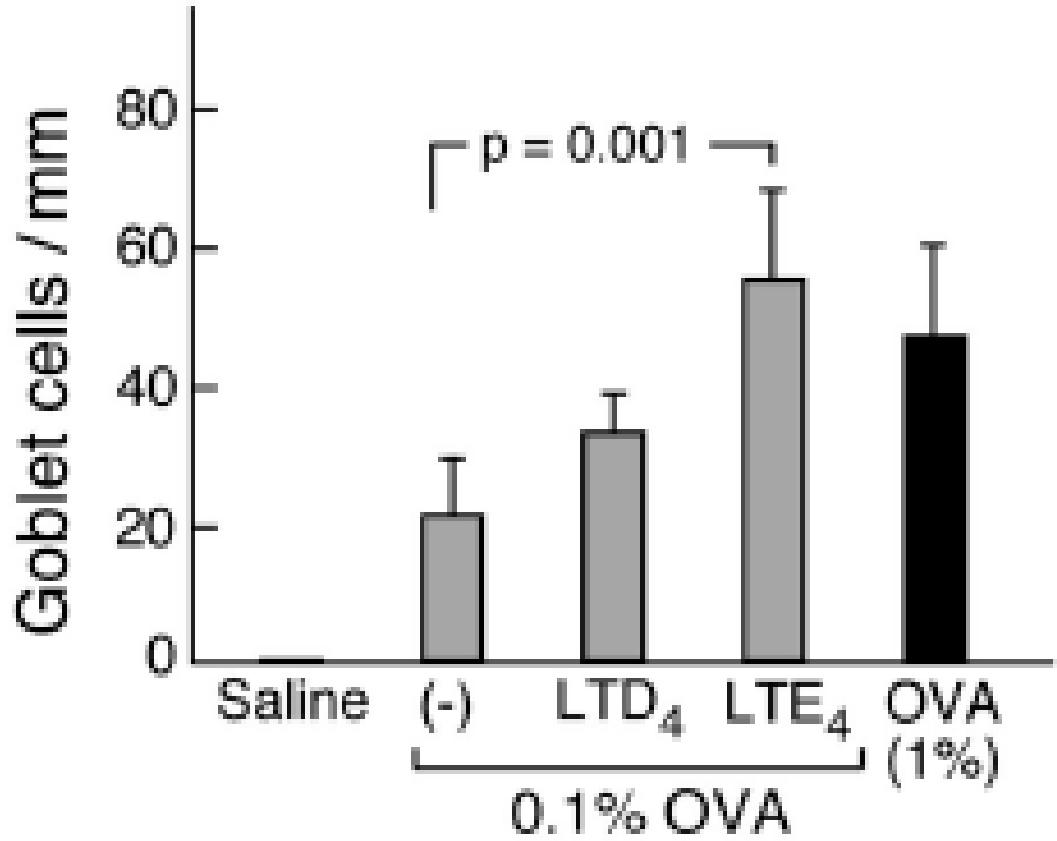
Paruchuri S.et al. J Exp Med 2009; 206(11): 2543-2555.

LTE_4-mediated amplification of allergen-induced eosinophil rectruoitment and pulmonary inflammation



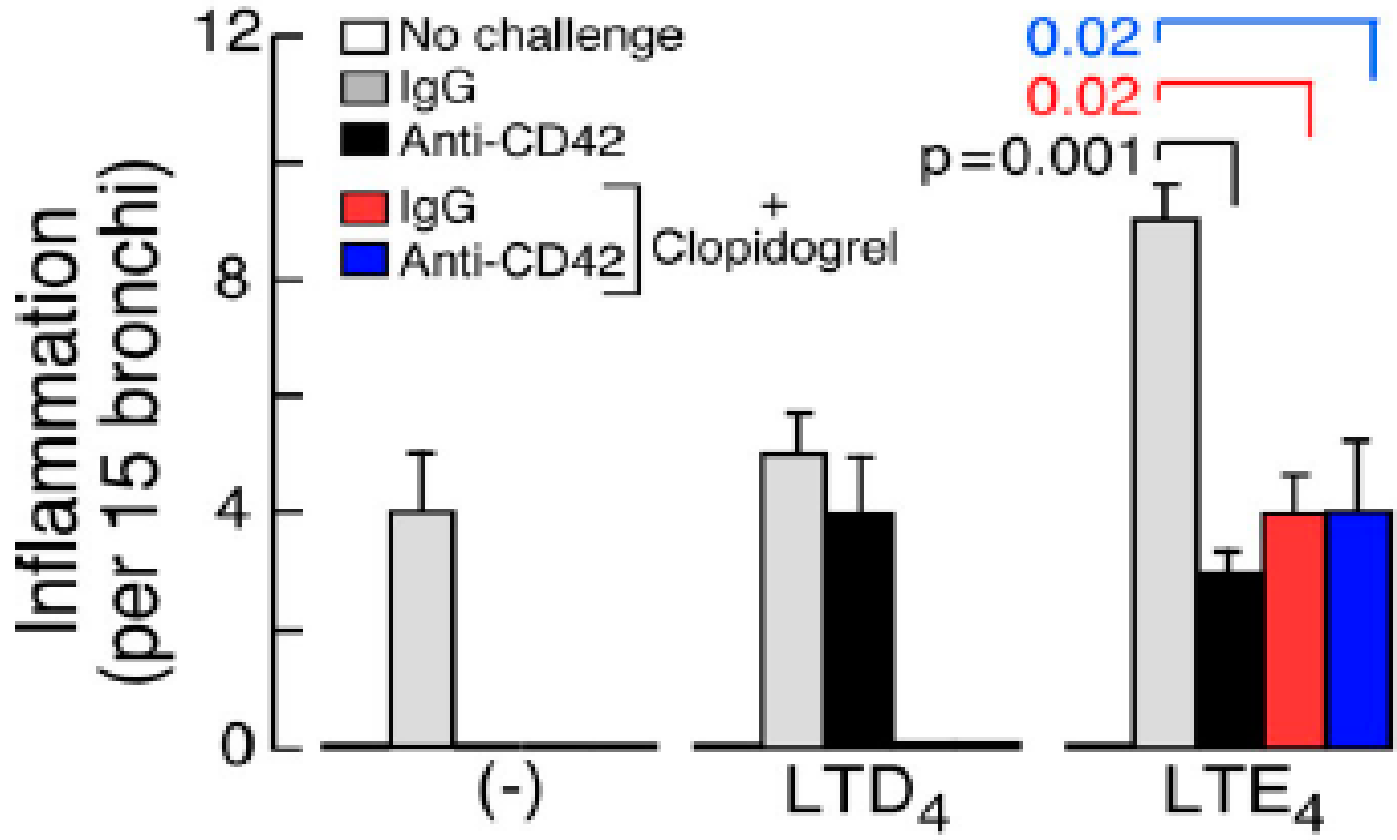
Paruchuri S.et al. J Exp Med 2009; 206(11): 2543-2555

LTE_4-mediated amplification of allergen-induced globet cell metaplasia



Paruchuri S.et al. J Exp Med 2009; 206(11): 2543-2555

Platelet $P2Y_{12}$ dependence of the LTE_4 effect on bronchial inflammation



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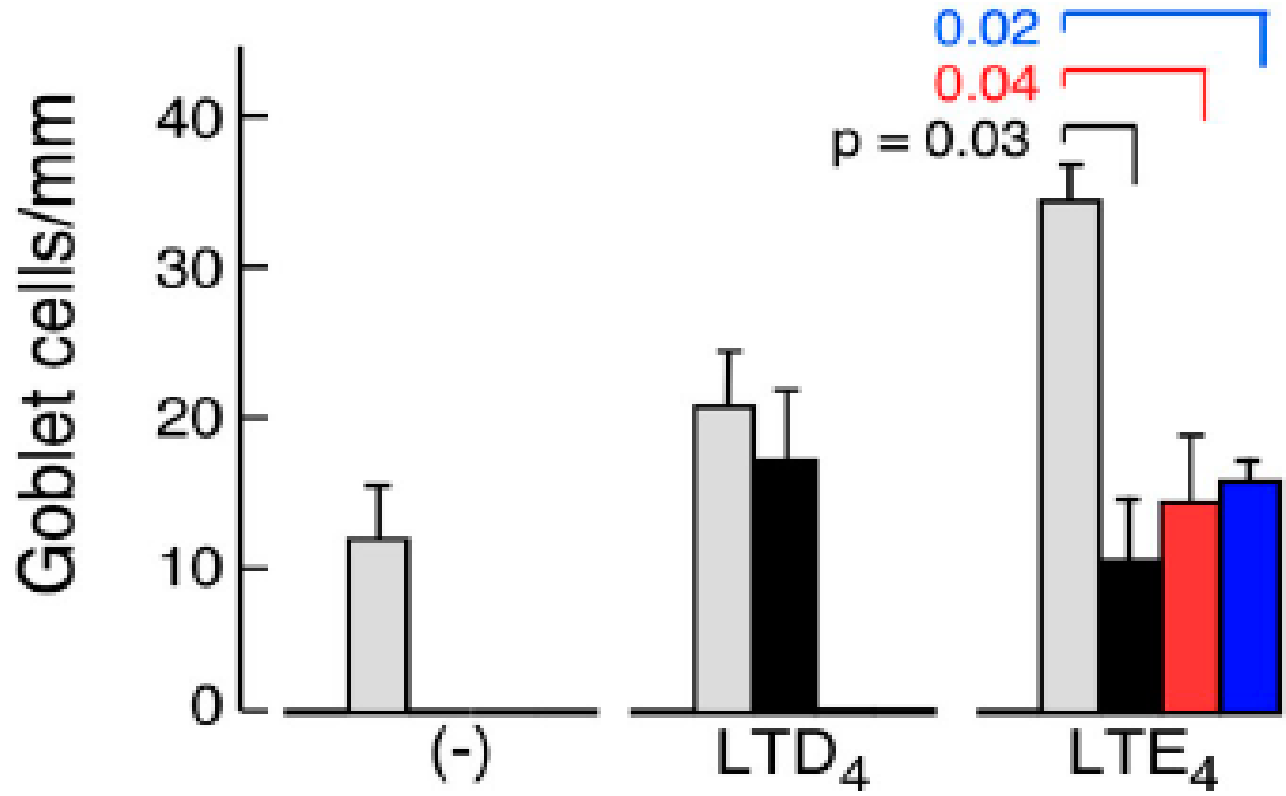
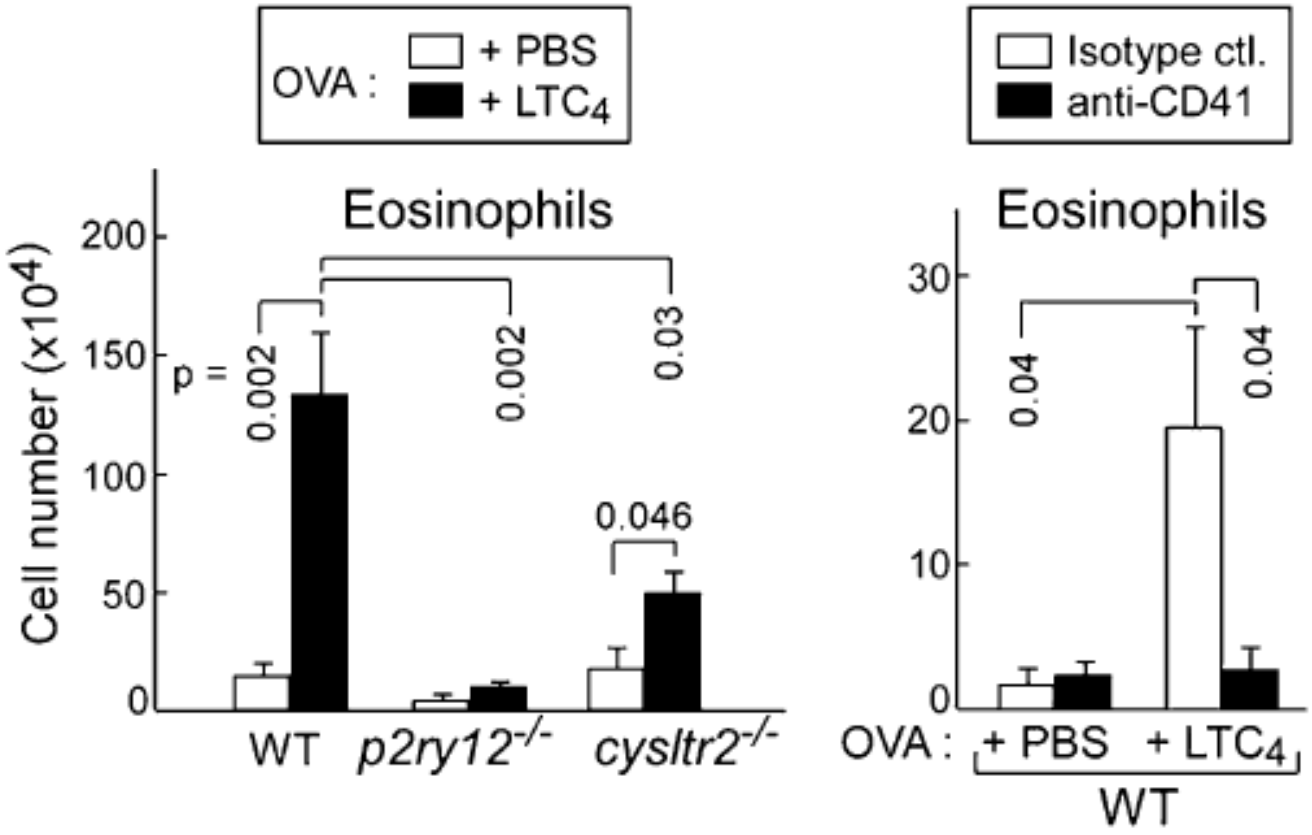


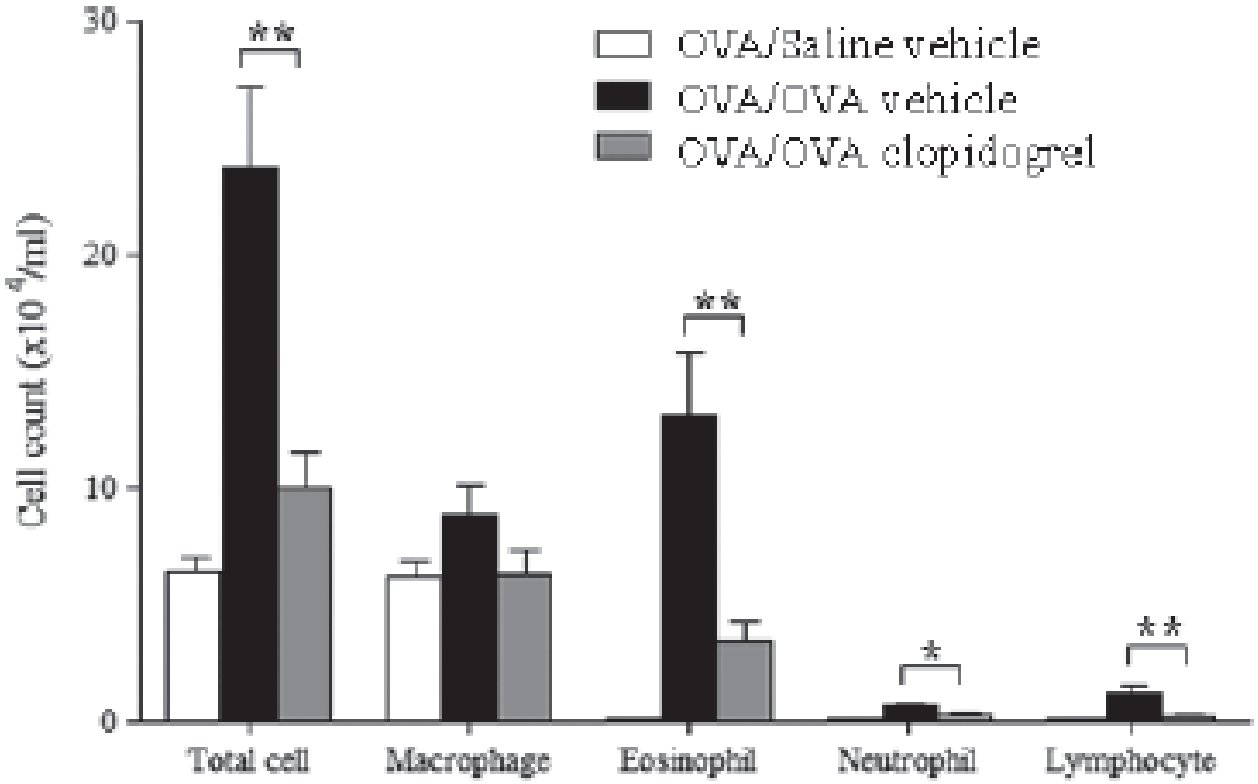
Paruchuri S.et al. J Exp Med 2009; 206(11): 2543-2555

LTC_4 amplifies allergen-induced pulmonary eosinophil recruitment in a platelet, $CysLT_2R$ and $P2Y_{12}$-dependent manner



Cummings HE et al, J Immunol 2013

Effect of clopidogrel treatment in a mouse model of asthma



Suh et al, J Cell Mol Med 2016

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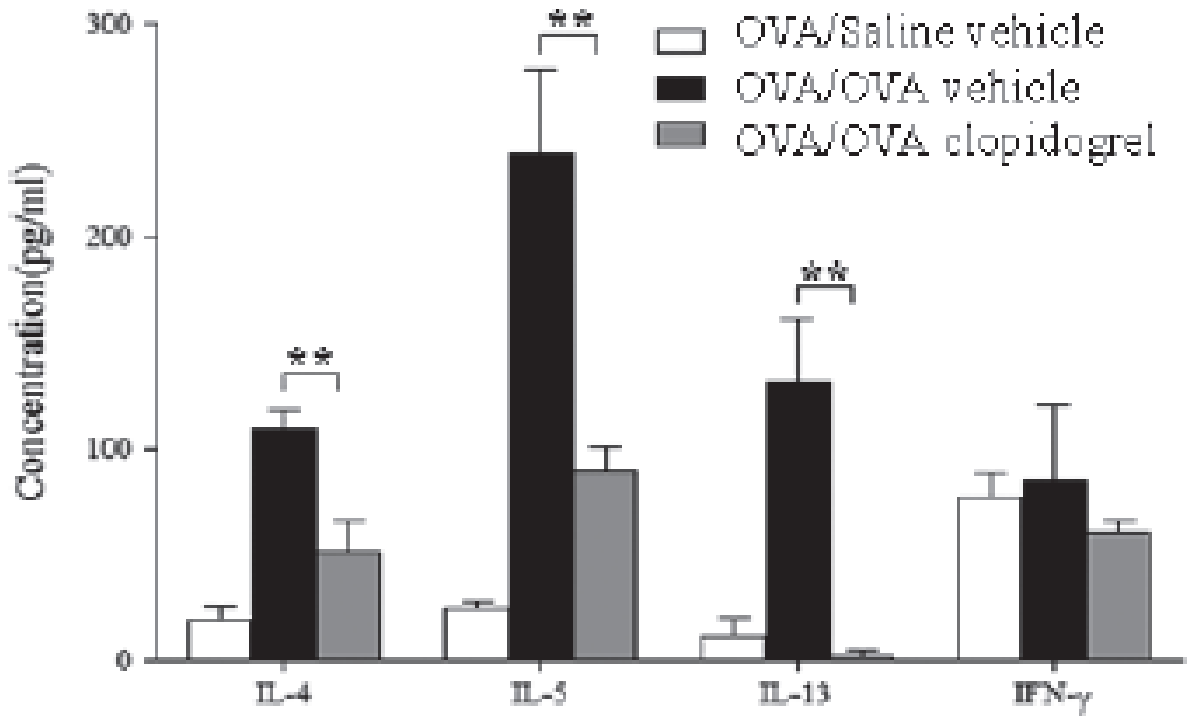
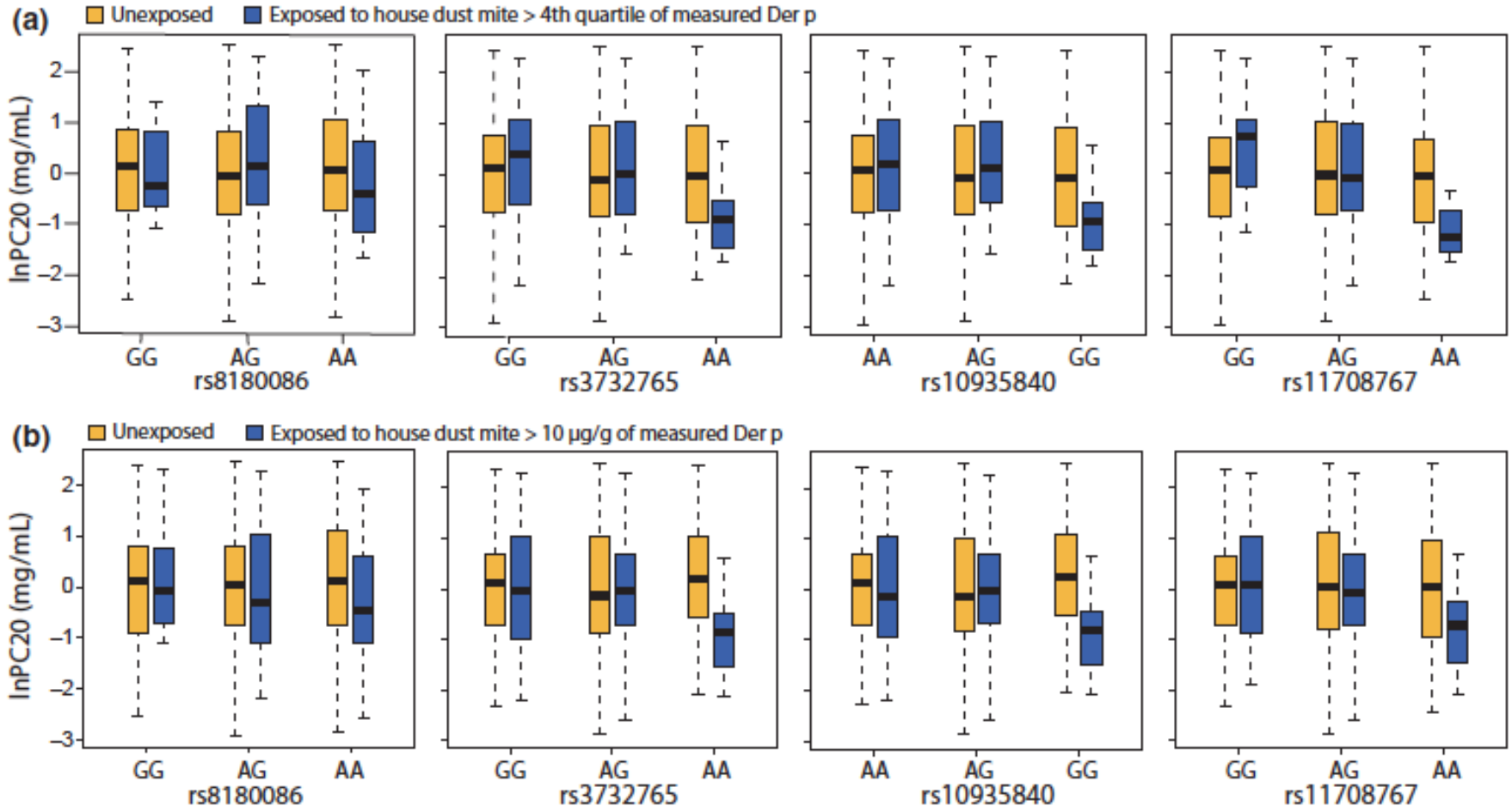


Suh et al, J Cell Mol Med 2016

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?

Journal of Thrombosis and Haemostasis, 13: 136–141 DOI: 10.1111/jth.12779

BRIEF REPORT

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

F. LUSSANA,*† F. DI MARCO,‡ S. TERRANEO,‡ M. PARATI,‡ C. RAZZARI,* M. SCAVONE,* E. A. FEMIA,* A. MORO,§ S. CENTANNI‡ and M. CATTANEO*

**Divisione di Medicina Generale III, Ospedale San Paolo, Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan; †Divisione di Ematologia, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo; ‡Divisione di Pneumologia, Ospedale San Paolo, Dipartimento di Scienze della Salute, Università degli Studi di Milano; and §U.O.C. di Anatomia Patologica, Ospedale San Paolo, Dipartimento di Scienze della Salute, Università degli Studi di Milano, Milan, Italy*

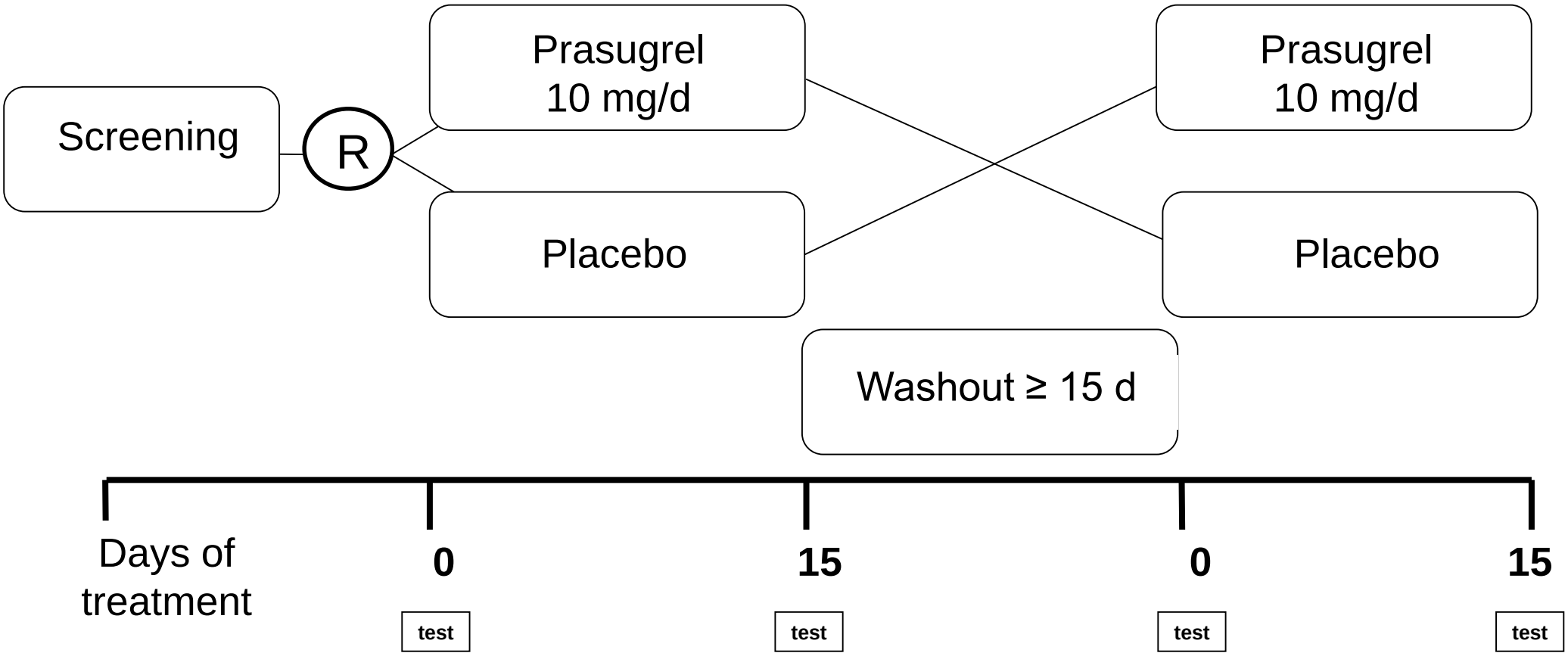
Unrestricted grant by Eli Lilly and Daiichi Sankyo

Aim of the study

- 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



Inclusion criteria

- 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

Gender, women/men	11/15
Age, mean ± SD (y)	43±13
Number of dropouts*	2

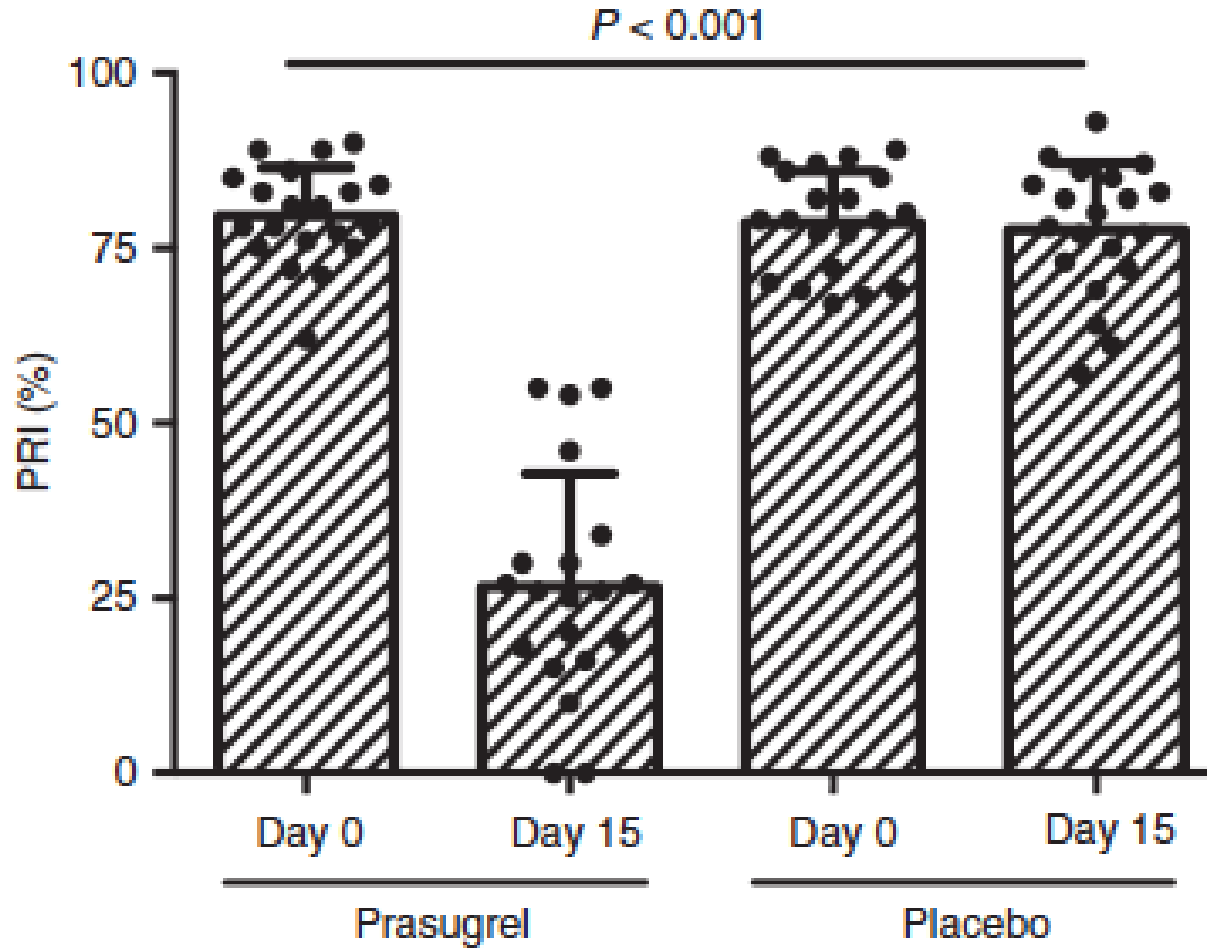
* due to asthma exacerbation: 1 during placebo treatment, 1 during prasugrel treatment

Methods

Mannitol bronchial challenge test

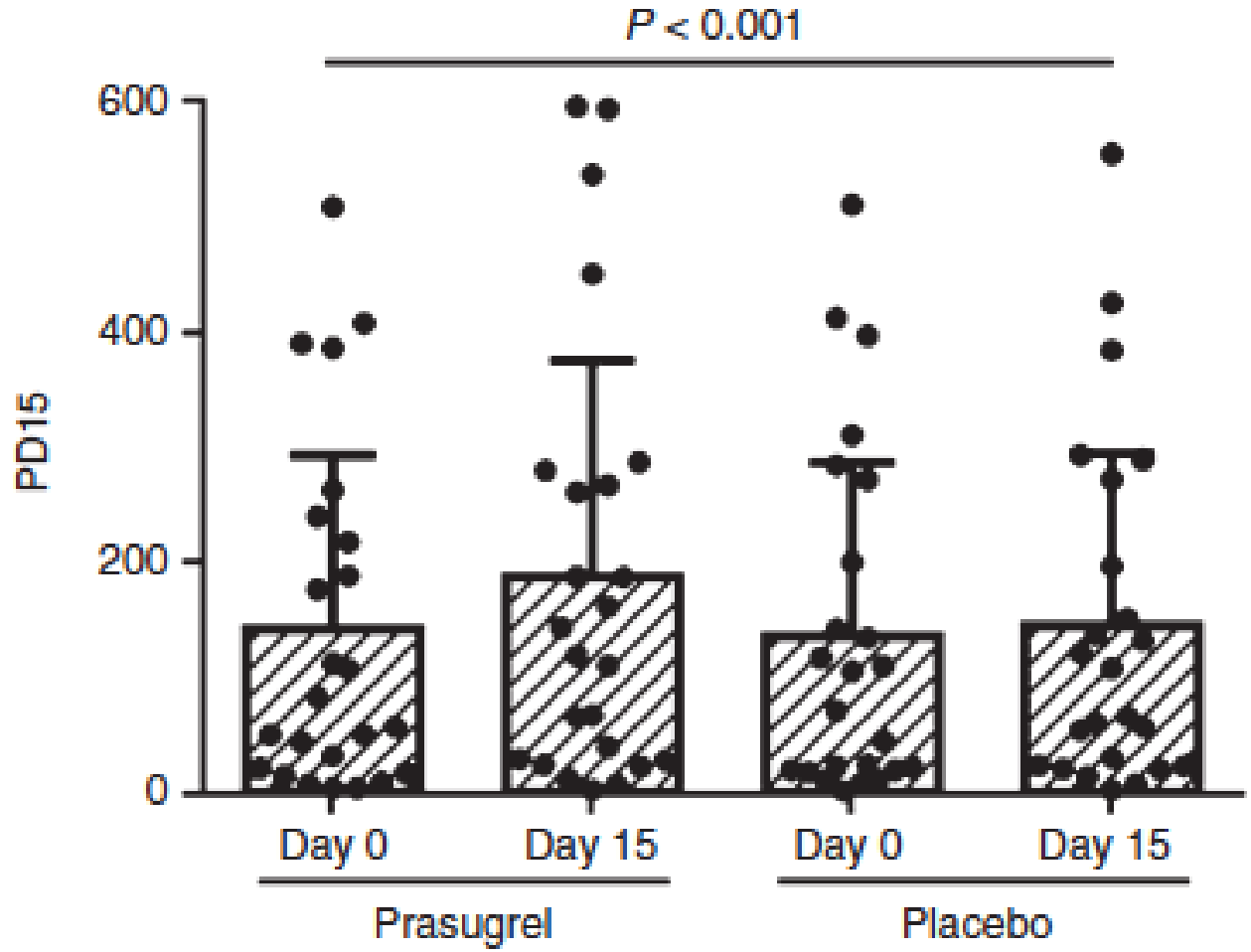
- Airway hyperresponsiveness was measured by bronchial provocation with dry powder mannitol (Osmohale™)
- The test was terminated when the fall in forced expiratory volume in 1-s (FEV1) reached at least 15% of baseline or after a cumulative mannitol dose of 635 mg.
- The provocative dose of mannitol 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



Lussana F et al, JTH 2015

PD15 before and after 14 days treatment with placebo or prasugrel in patients with allergic bronchial asthma



Lussana F et al, JTH 2015

Conclusion

1. Our proof-of-concept study suggests that inhibition of the (platelet) $P2Y_{12}$ 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 controlle clinical trials, using clinical end-points

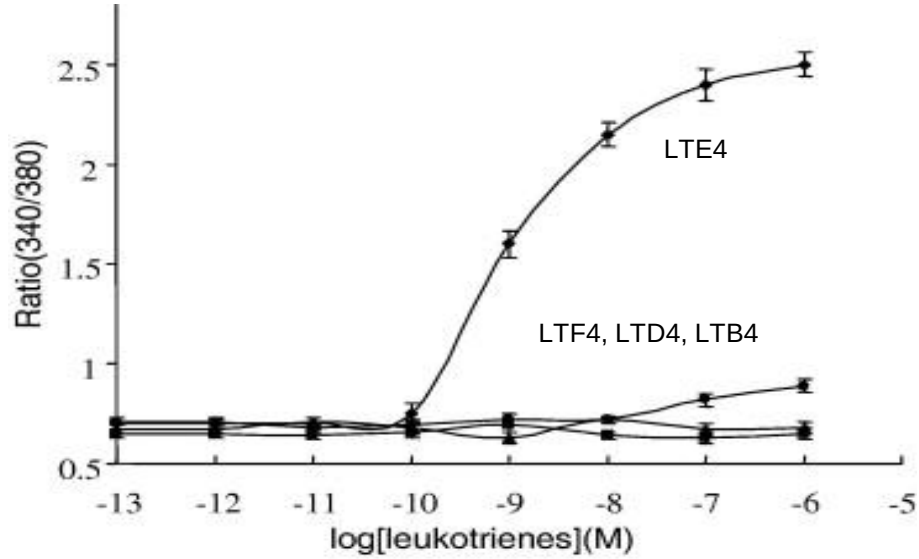
Receptors for cys-LT

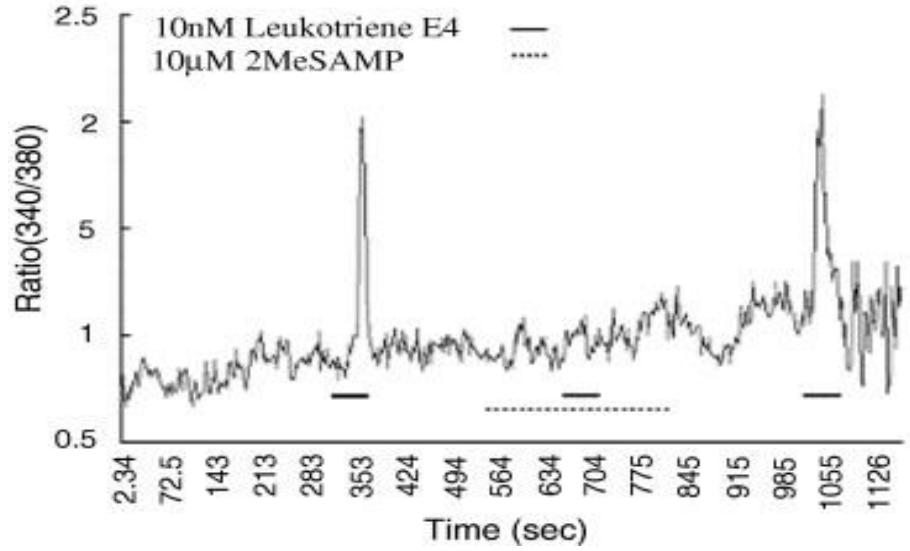
- Two G protein–coupled receptors for cys-LTs, termed type 1 and type 2 cys-LT receptors (CysLT1R and CysLT2R), have been cloned and characterized, which share 38% aminoacid identity
- Each of them is 24–32% identical to the purinergic (P2Y) class of GPCRs, suggesting a phylogenetic relationship between 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 $[Ca^{2+}]_i$ increases via the expressed $hP2Y_{12}$–$hG_{16}\alpha$ fusion proteins

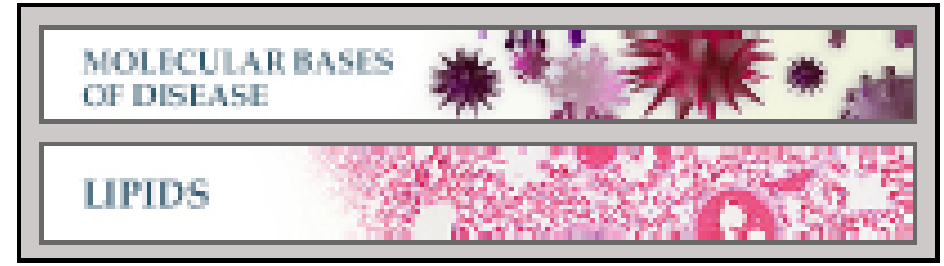




Nonaka et al, BBRC 2005

Reports:

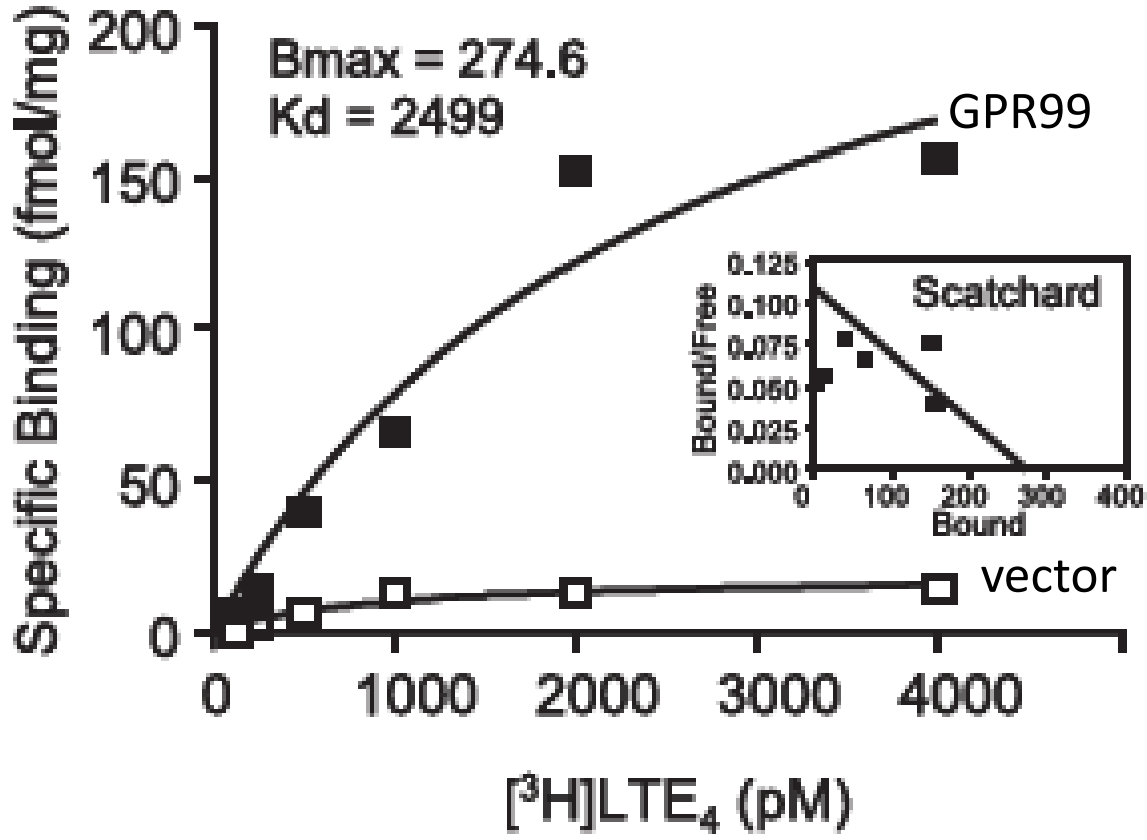
Identification of GPR99 Protein as a Potential Third Cysteinyl Leukotriene Receptor with a Preference for Leukotriene E_4 Ligand



Yoshihide Kanaoka, Akiko Maekawa and K. Frank Austen

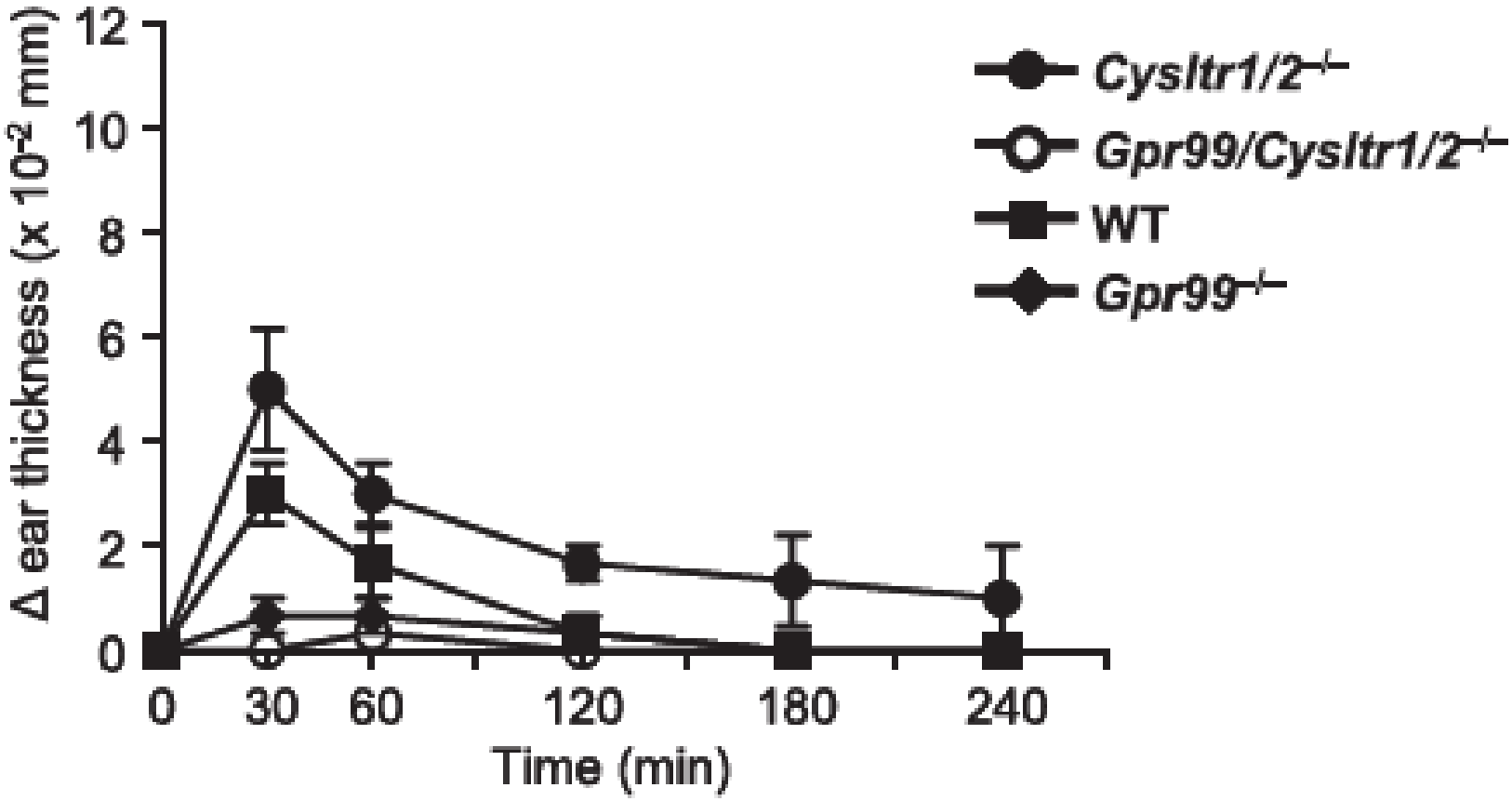
J. Biol. Chem. 2013, 288:10967-10972.
doi: 10.1074/jbc.C113.453704 originally published online March 15, 2013

Binding of [^{3}H]LTE$_4$ to microsomal membrane proteins from GPR99 and vector control transfectants



Kanaoka Y et al, J Biol Chem 2013

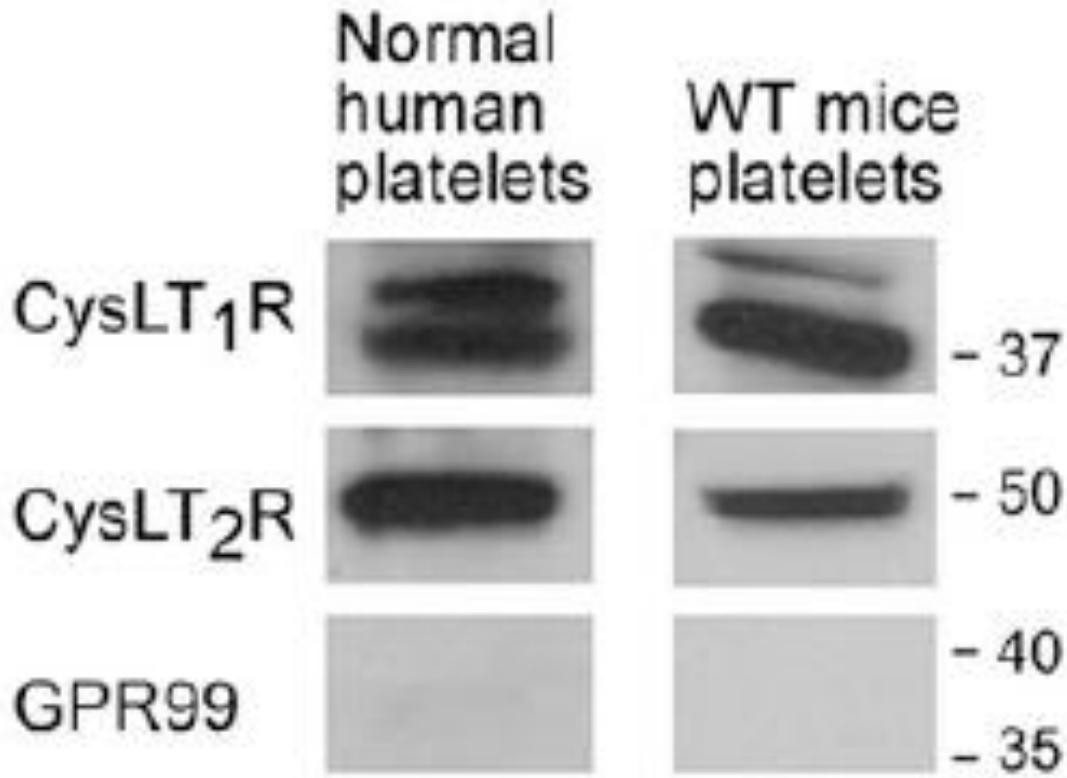
Effect of subcutaneous injection of LTE_4 on ear edema in mice



Kanaoka Y et al, J Biol Chem 2013

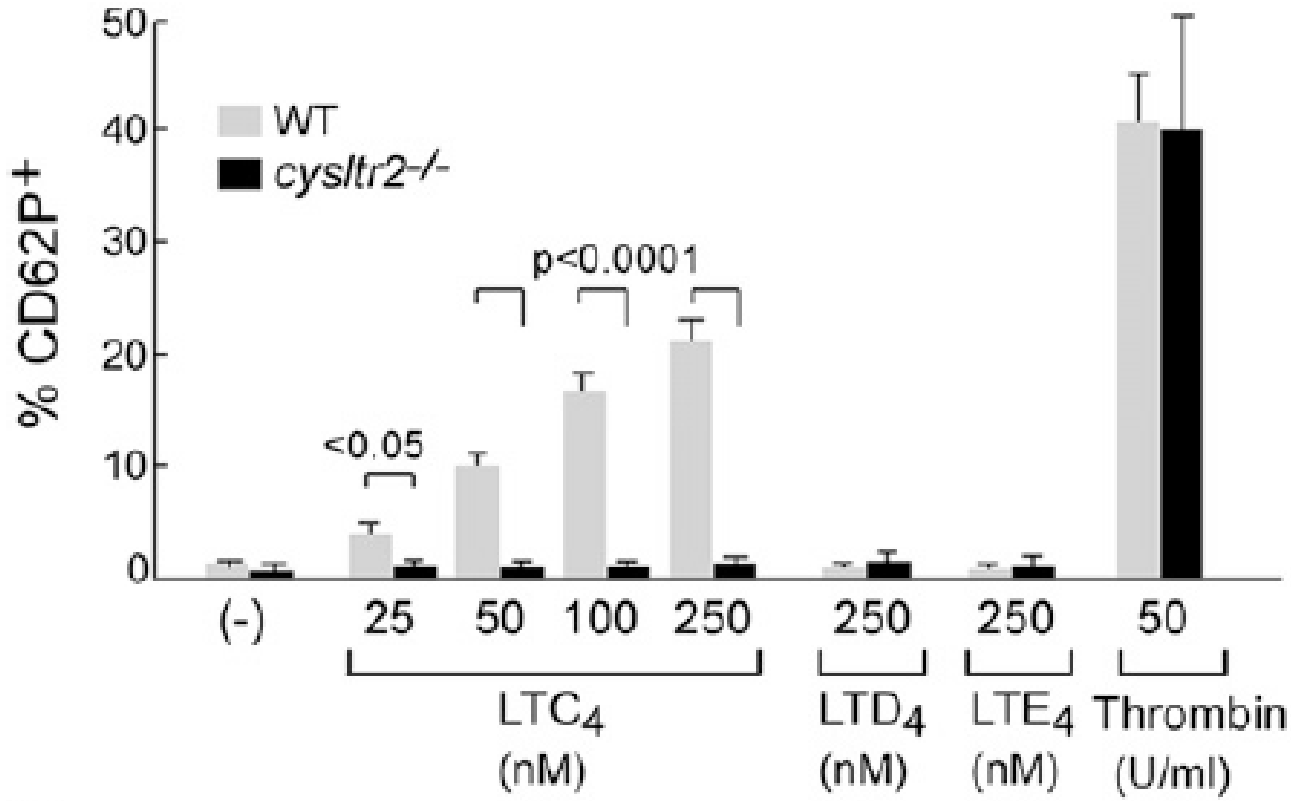
What receptors for cysLTs are expressed on platelets?

Human and mouse platelets express $CysLT_1R$ and $CysLT_2R$ but not GPR99

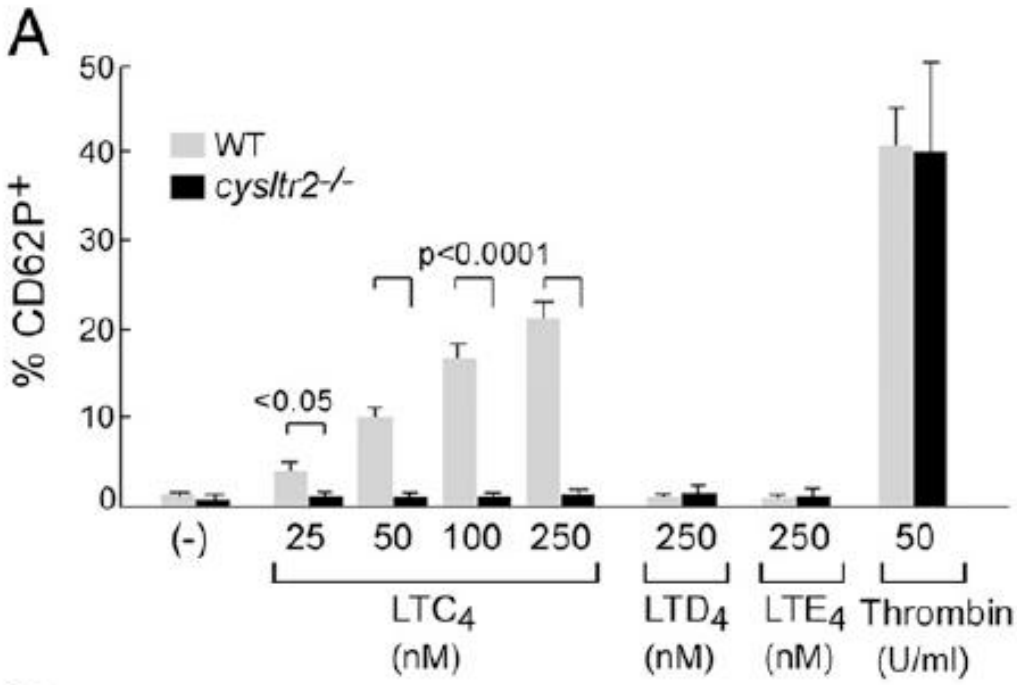


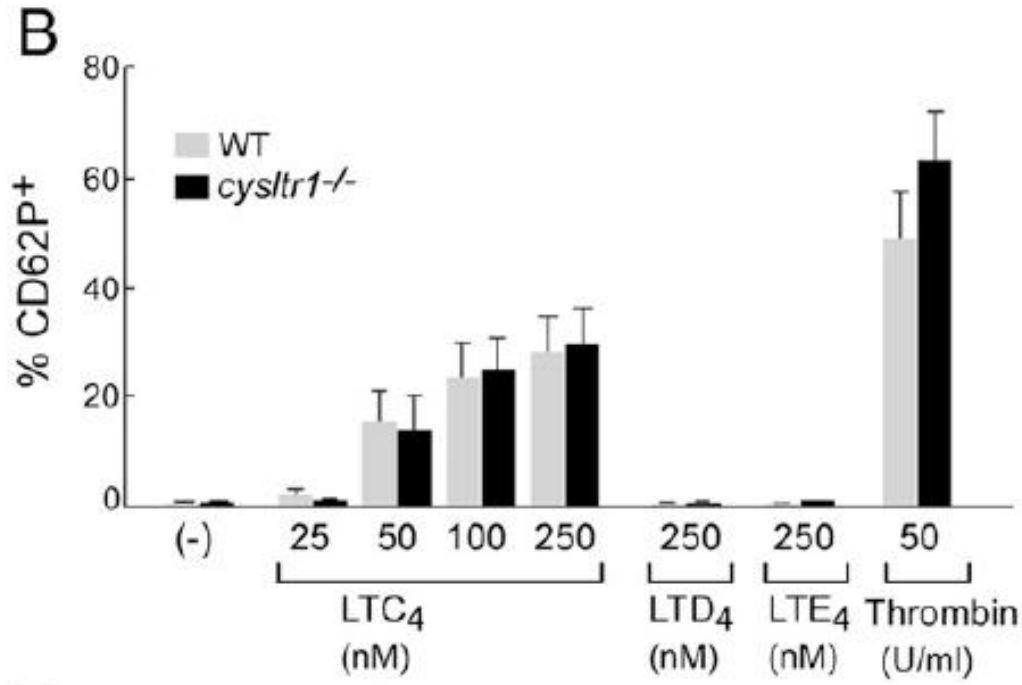
Cummings HE et al, J Immunol 2013

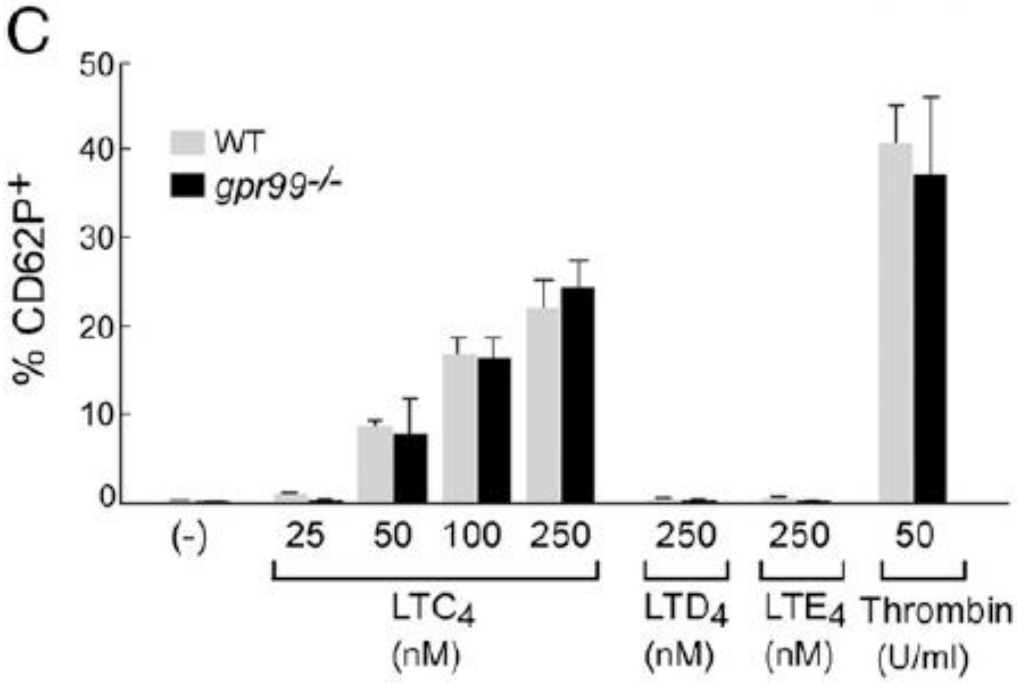
$CysLT_2R$ is involved in LTC_4–induced **mouse** platelet activation



Cummings HE et al, J Immunol 2013







$CysLT_2R$ is involved in LTC_4 –induced **mouse** platelet activation

Cummings HE et al, J Immunol 2013

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 hyper-responsiveness in a prove-of-concept RCT
- CysLT do not activate human platelets directly, but likely through ADP secretion by cells other than platelets (?)

Ringraziamenti

- V. Caroppo, C. Cheng, E.A. Femia, F. Lussana, C. Razzari, M. Scavone
- S. Centanni, F. Di Marco, M. Parati, S. Terraneo *(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)*