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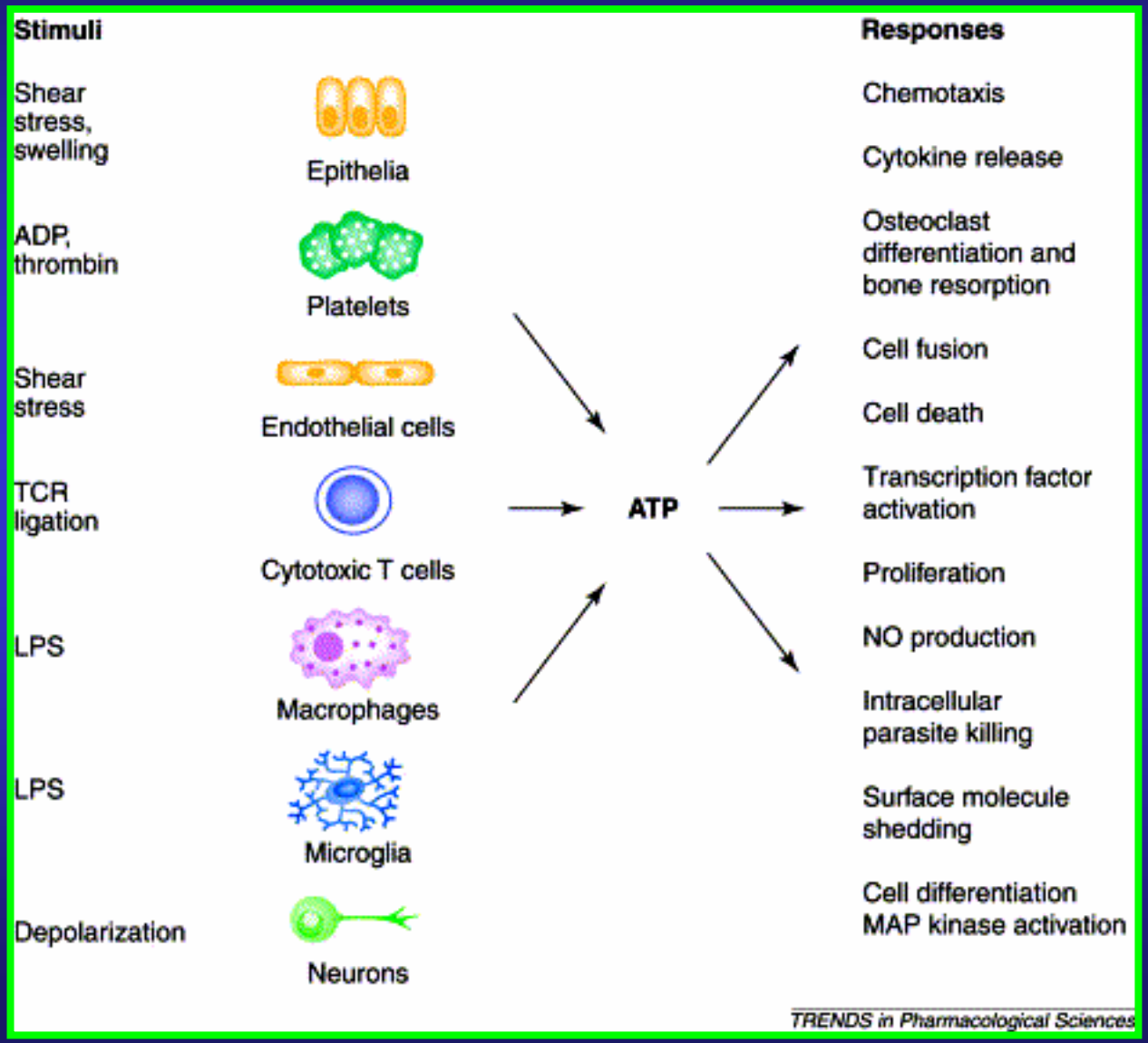
University of Siena
1240
Purinergic system and the inflammatory response

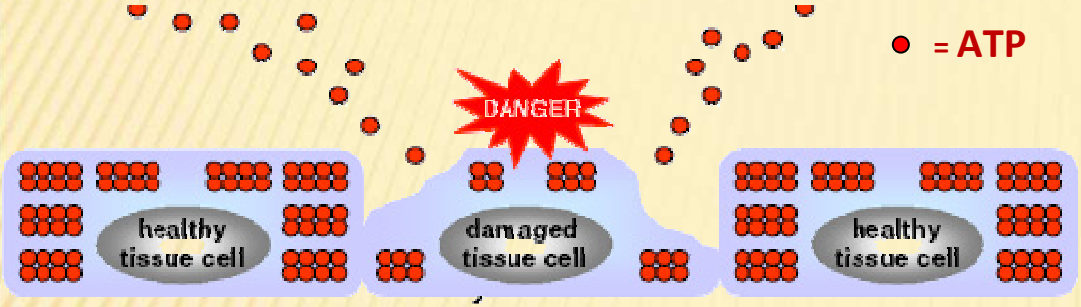
AGENDA

- General overview
- P2X7R
- A2AR
- Rheumatoid Arthritis
- “The odd couple”

ATP: production stimuli and cellular responses

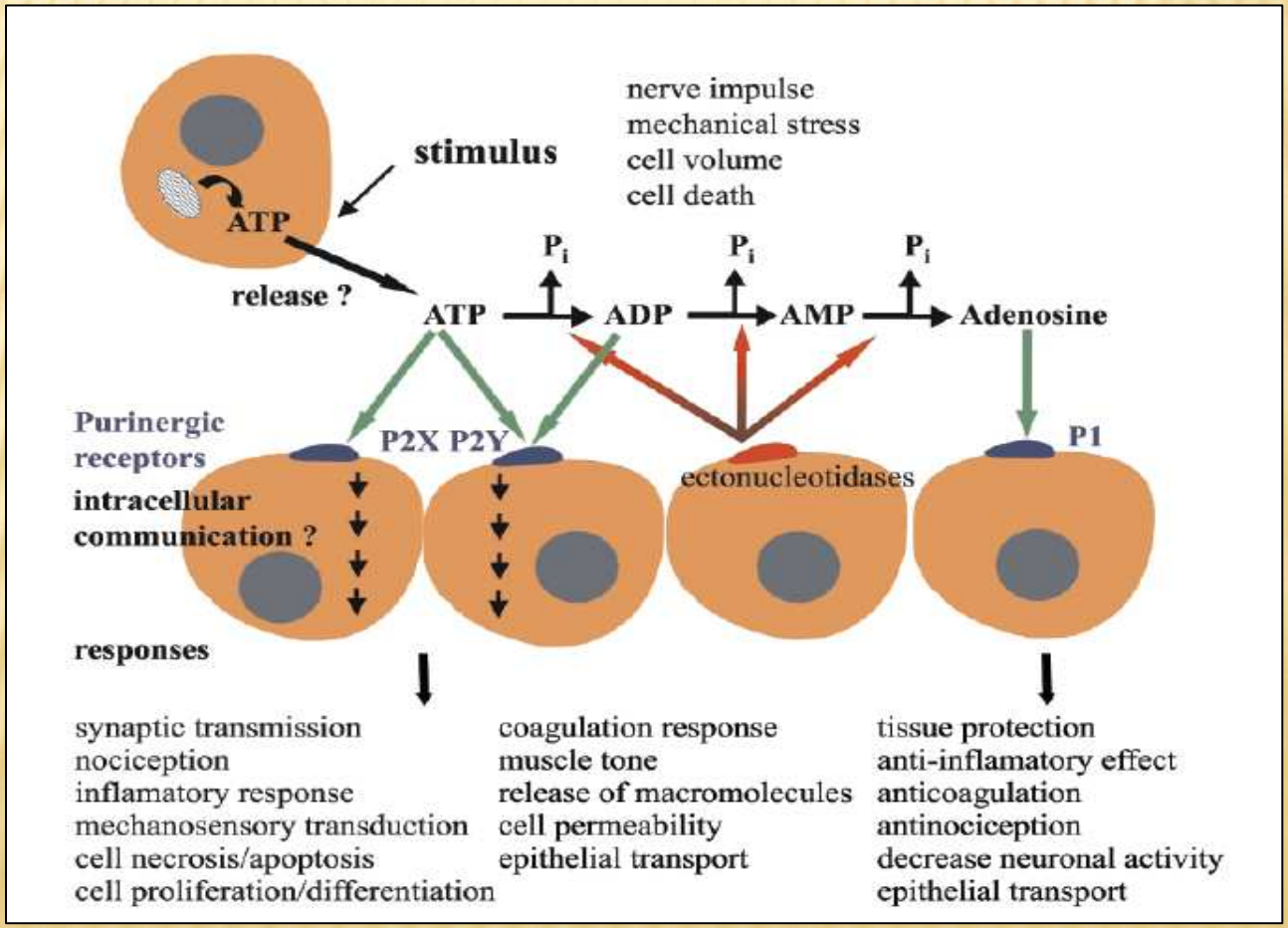


COMMUNICATING VIA ATP



PURINE AND PYRIMIDINE

NUCLEOTIDE RELEASE

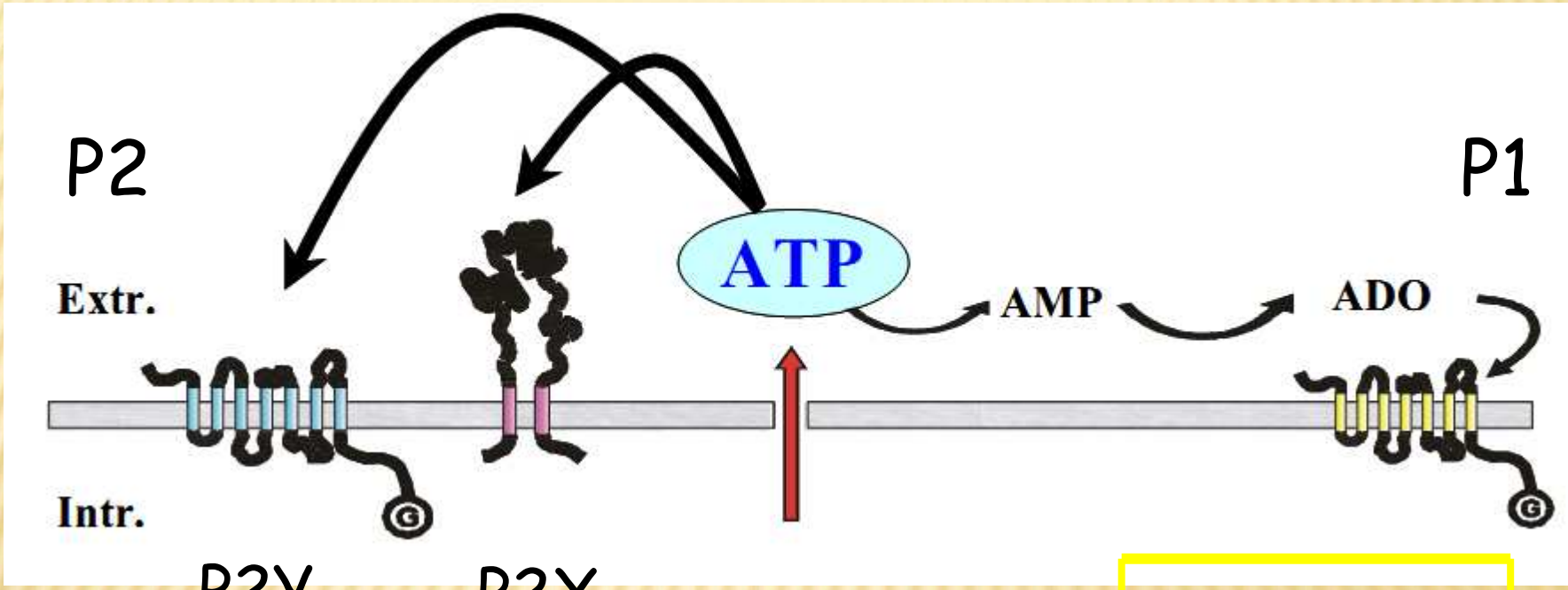


PURINERGIC CASCADE

PURINERGIC SIGNALLING

Novak, 2003

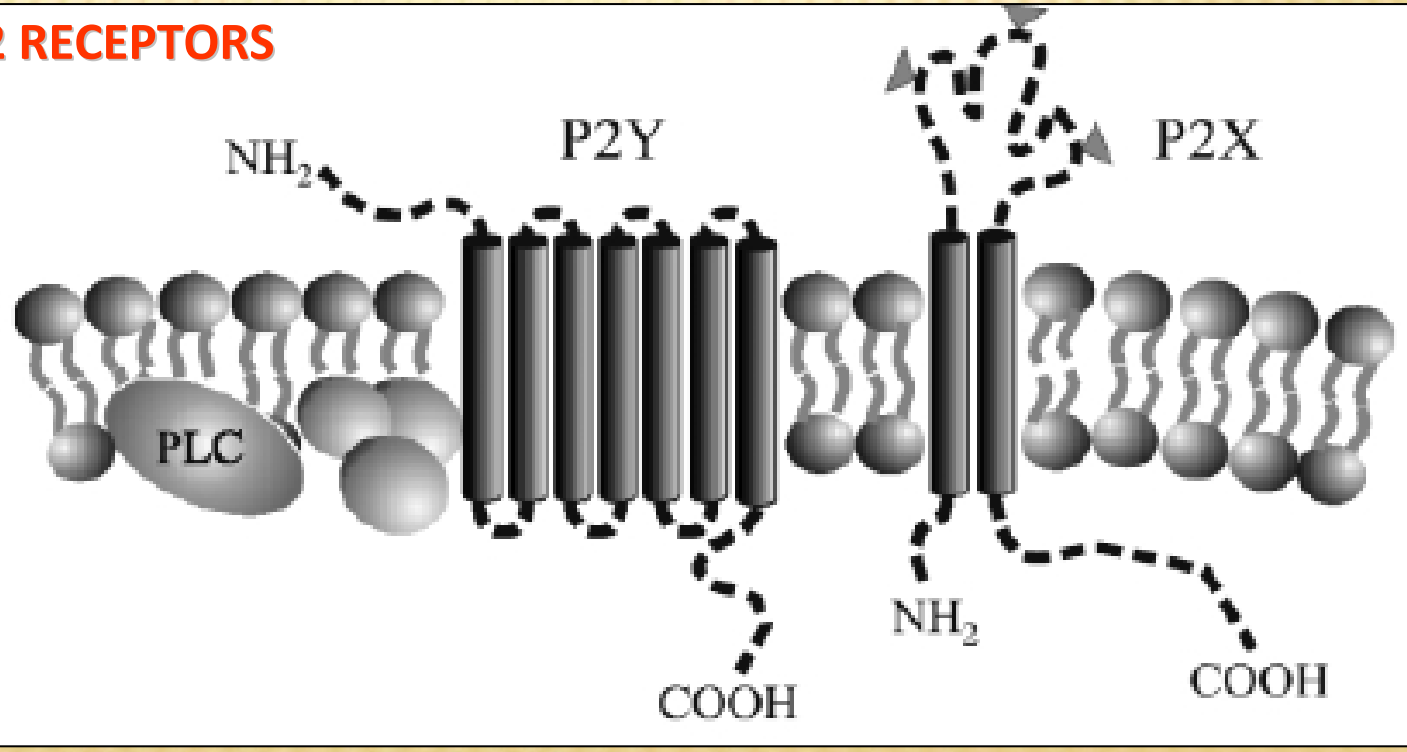
PURINERGIC RECEPTORS



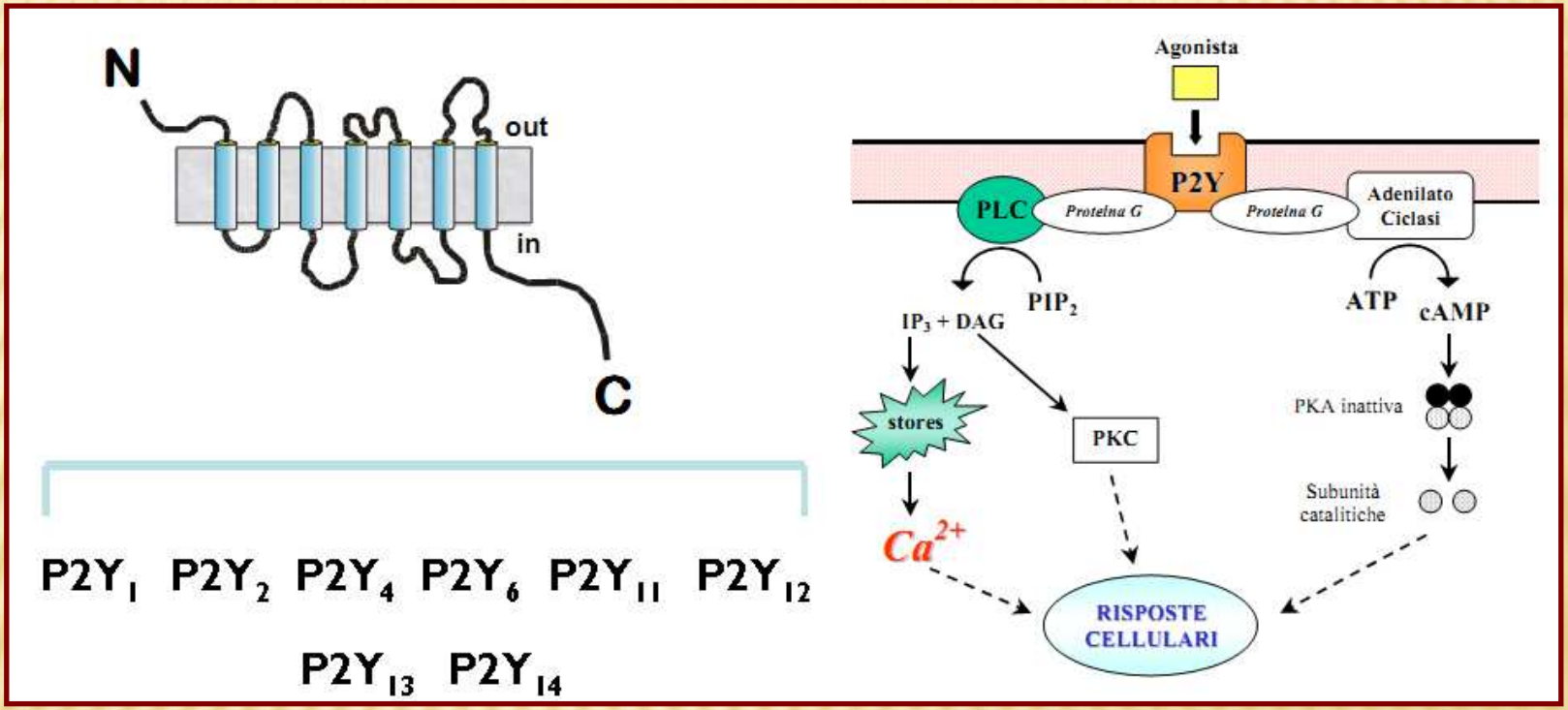
PURINERGIC RECEPTORS

P1 RECEPTORS

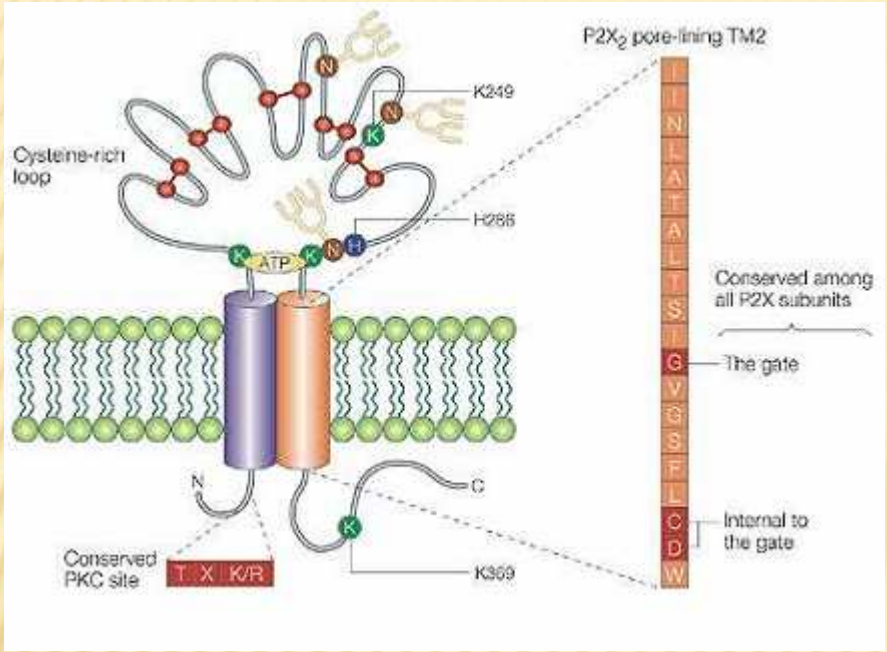
P2 RECEPTORS

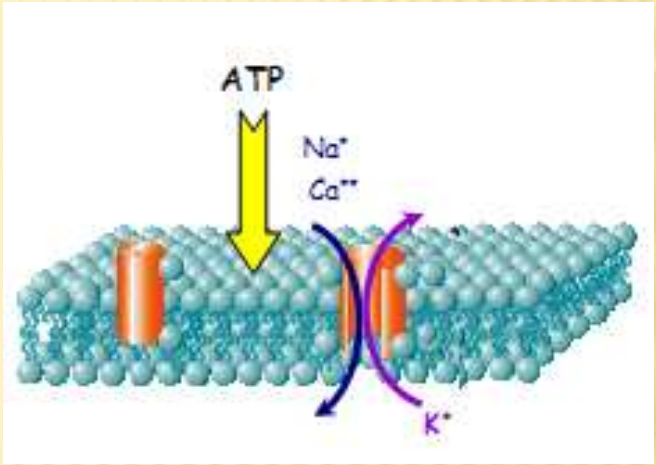


P2Y Receptors



P2X Receptors





$P2X_1$ $P2X_2$ $P2X_3$ $P2X_4$ $P2X_5$ $P2X_6$ $P2X_7$

AGENDA

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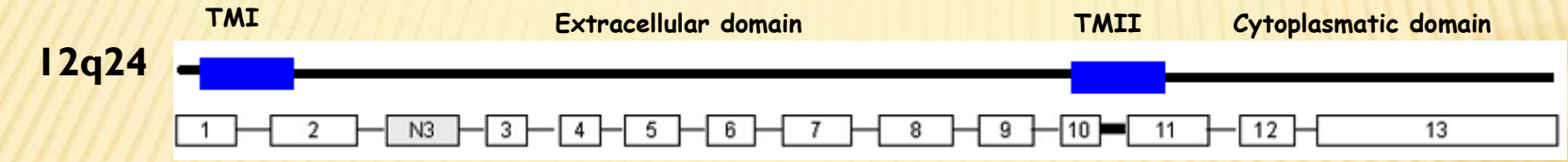
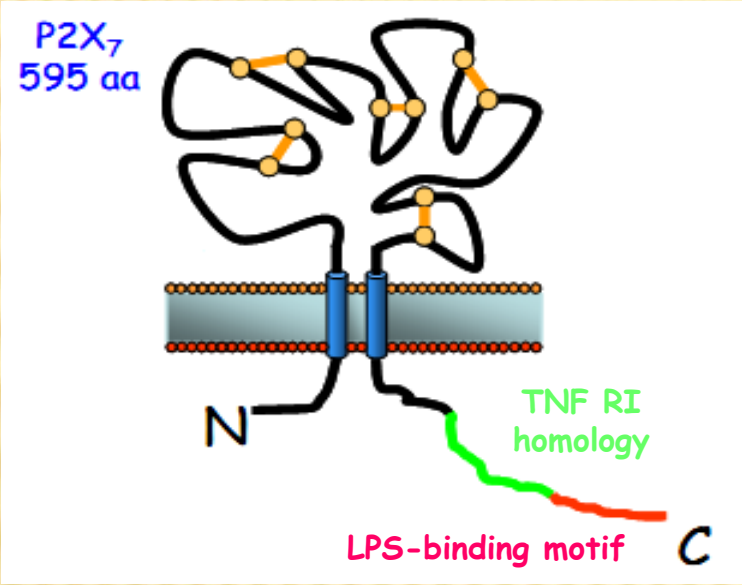
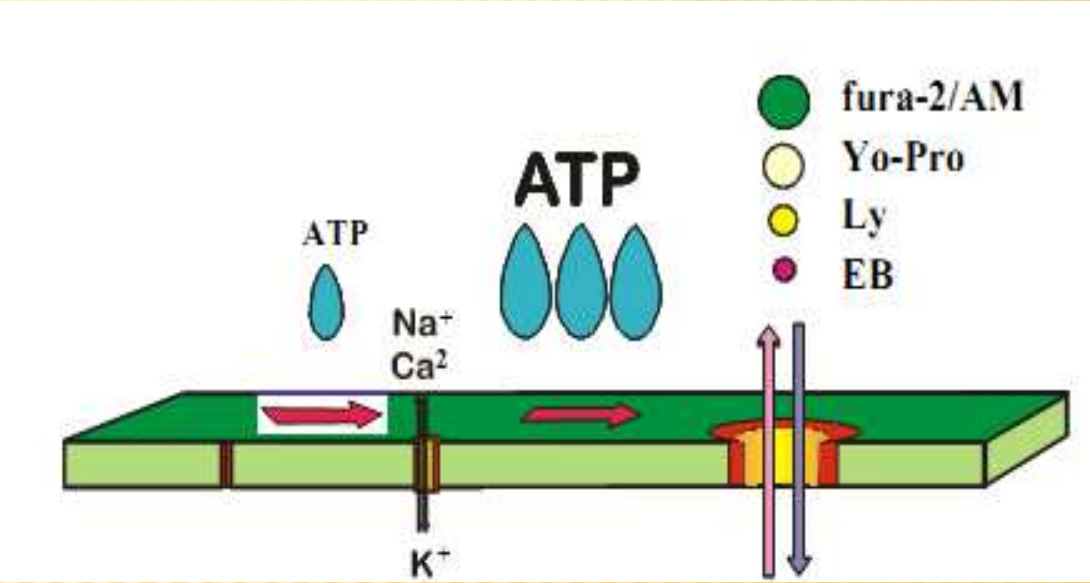
P2X7 Receptor

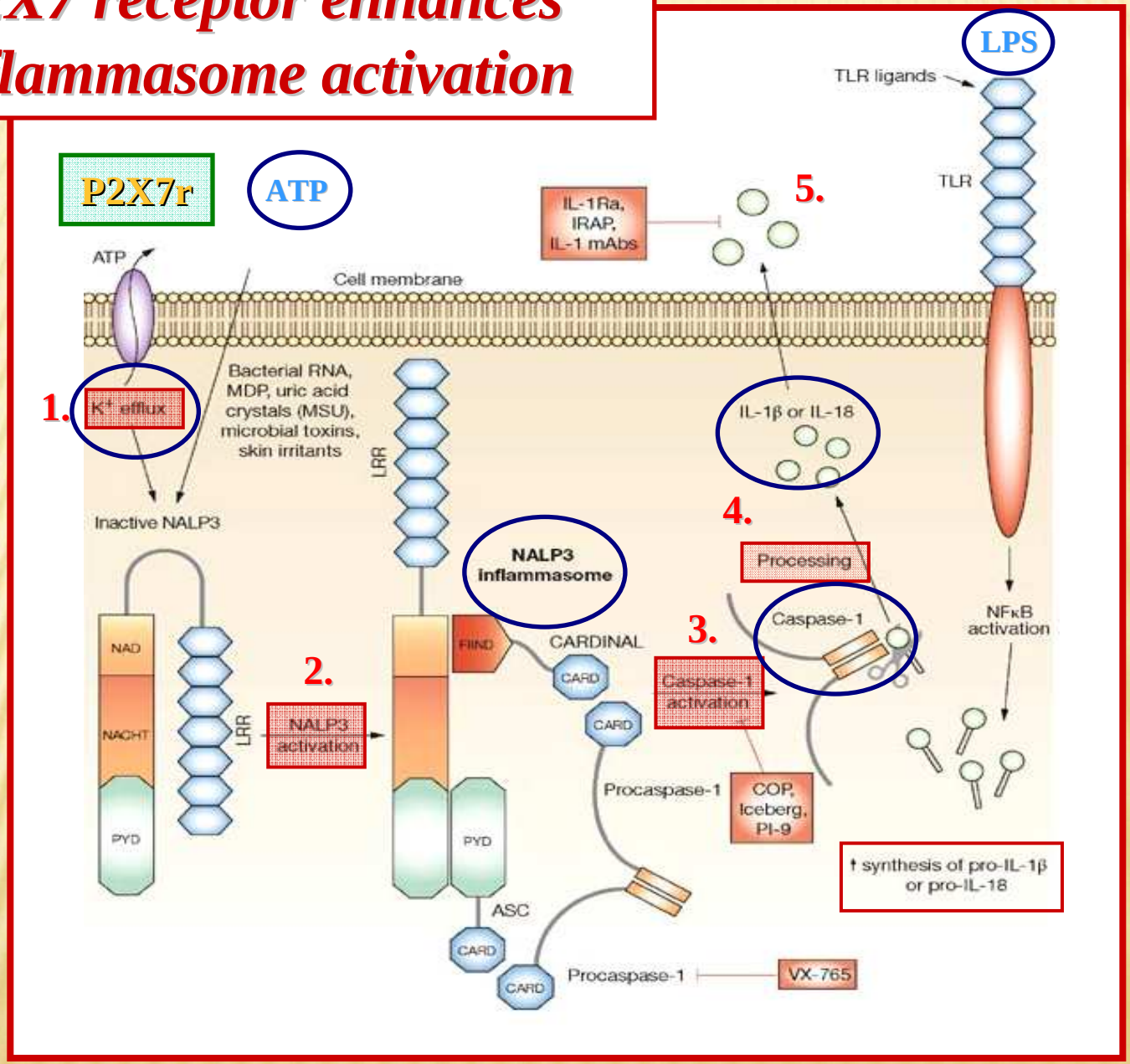


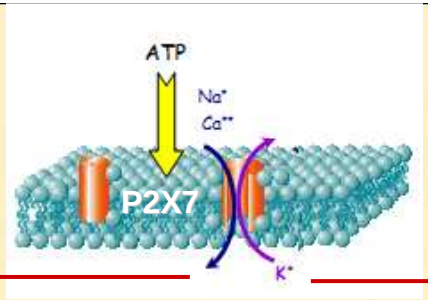




Receptor	Main Distribution	Agonists	Antagonists
$P2X_7$	Macrophages, mast cells, microglia, pancreas, skin, endocrine organs	BzATP	KN 62

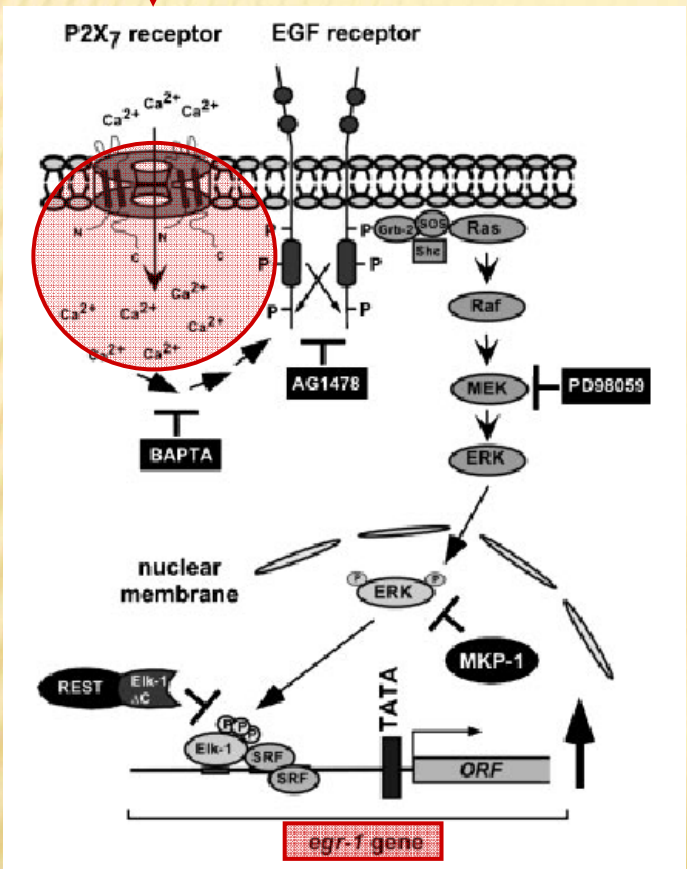
P2X7 receptor enhances inflammasome activation

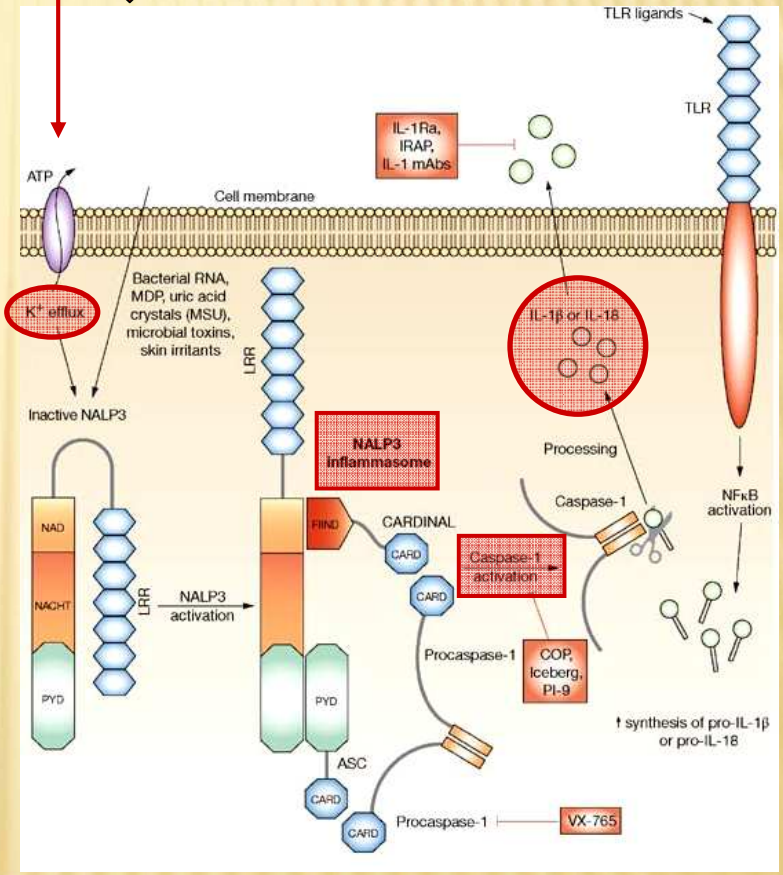




↑ [Ca++]i

↓ [K+]i



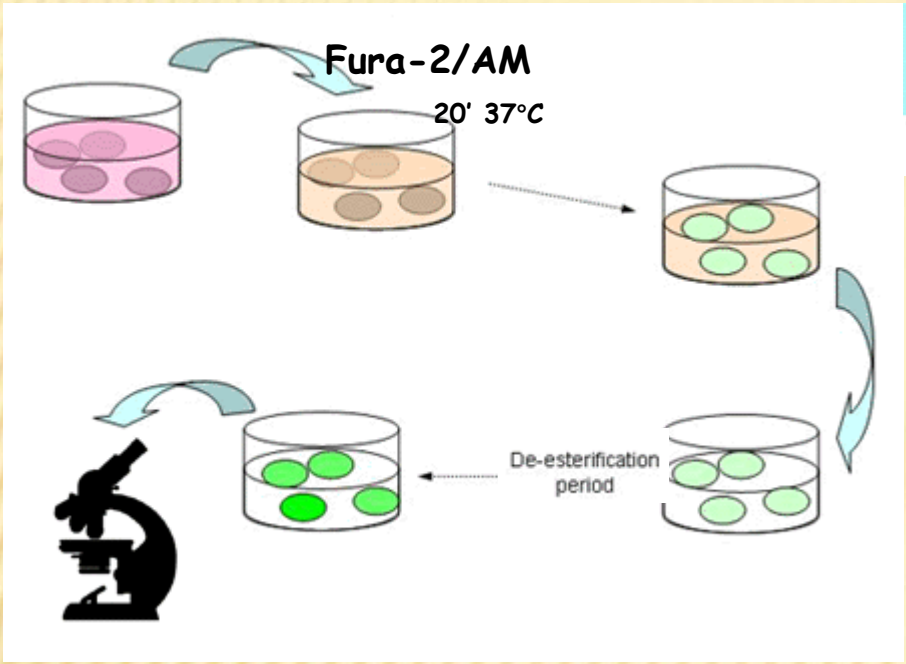


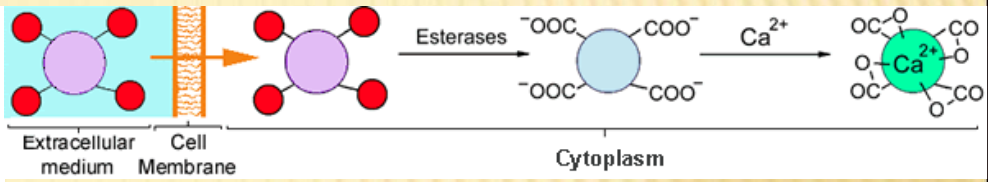
↑ IL-6 mRNA expression

↑ IL-1β and IL-18 secretion

P2X7 receptor function analysis

[Ca2+]i fluxes measurement in monocytes using a single-cell fluorescent microscopy method

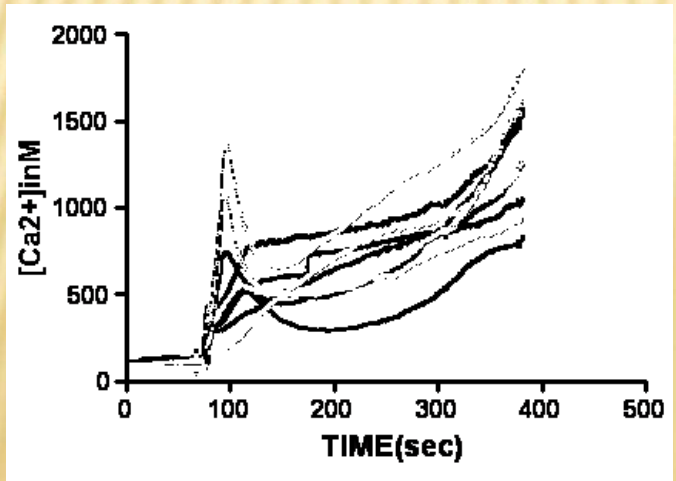


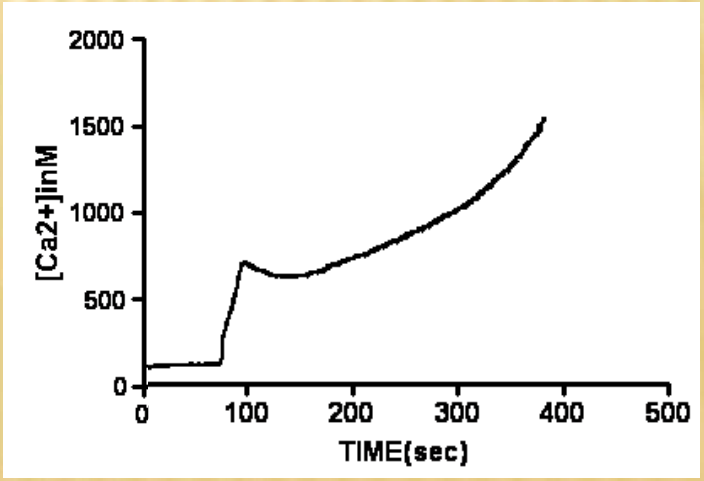


ATP 1mM

BzATP 0,5mM/0,1mM - KN 62 1µM

(30'37°C pre-incubation)

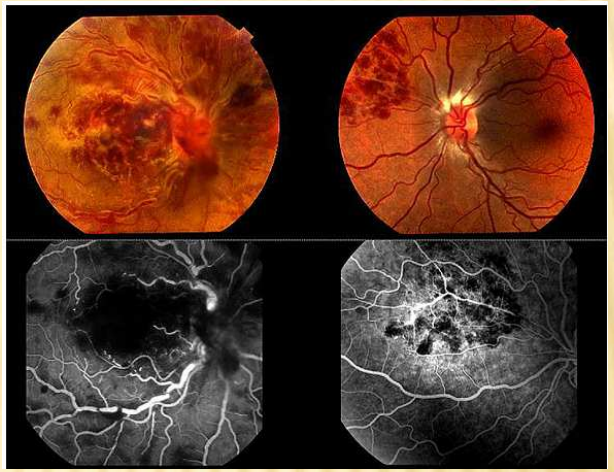




Behçet's disease

Clinical features

Oral and genitals ulcers



Ocular disease (uveitis)

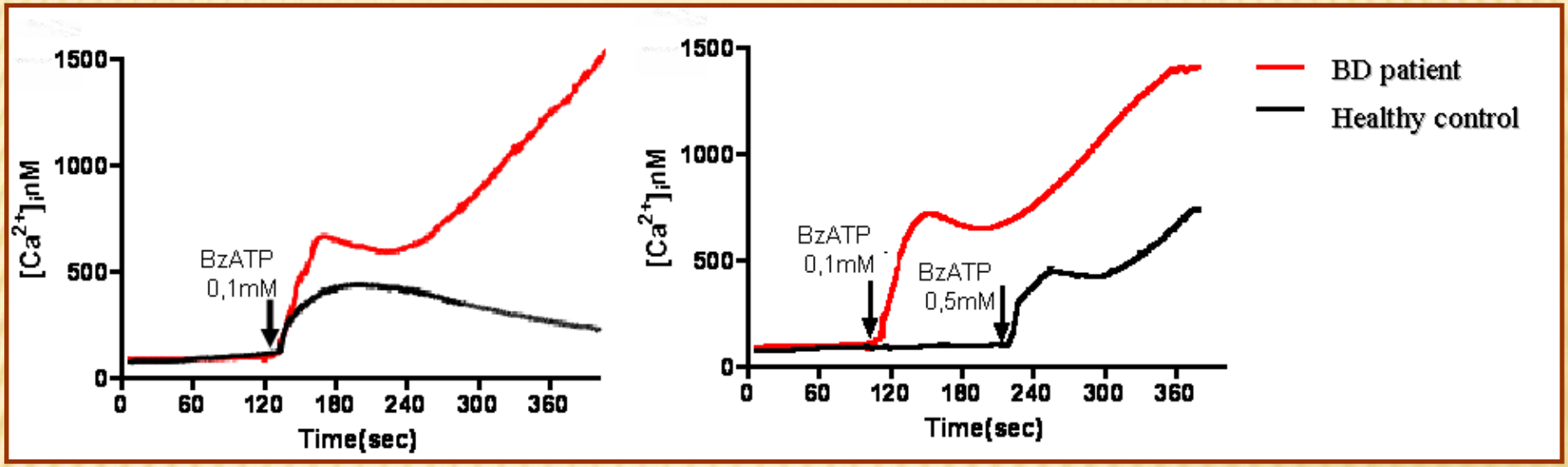
Cutaneous lesions

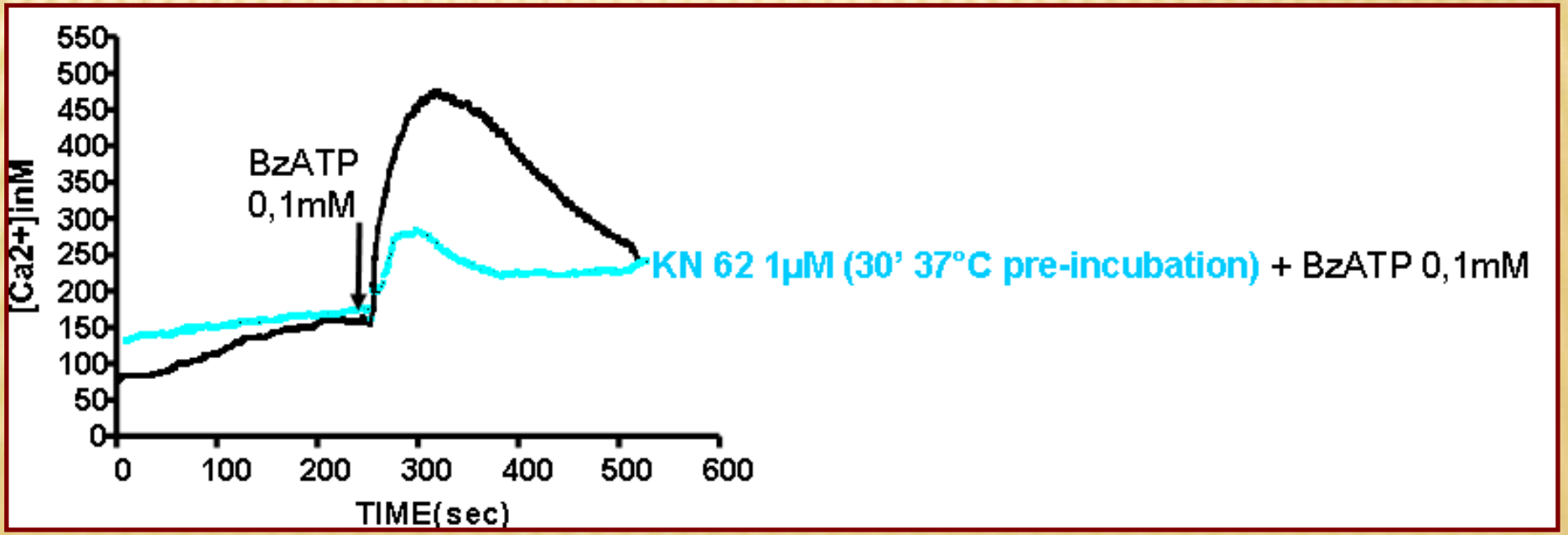
Gastrointestinal disease

sudden recurrent inflammatory attacks intercalated with periods of remission

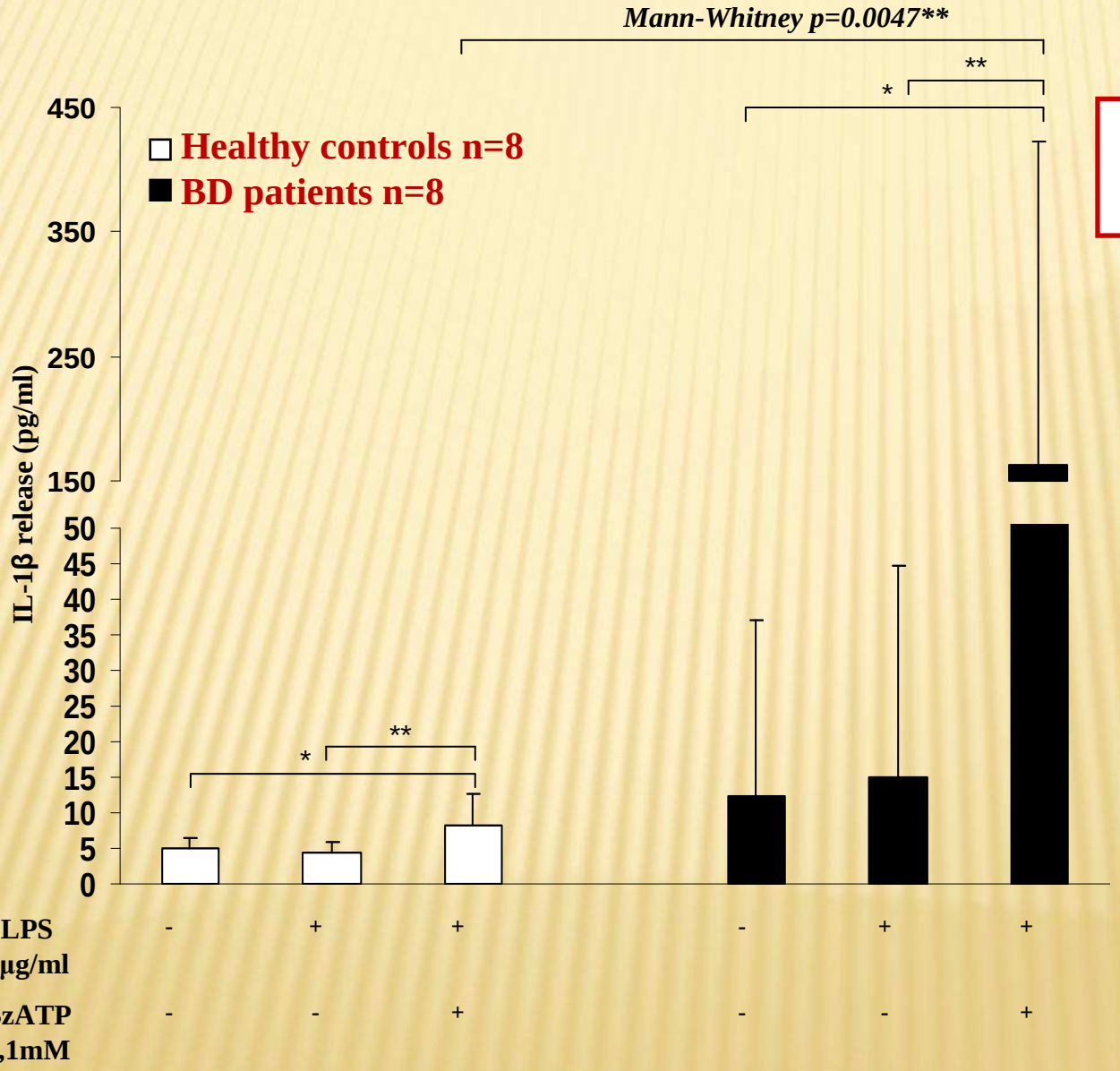
P2X$_7$ receptor function analysis

[Ca2+]i fluxes measurement in monocytes using a single-cell fluorescent microscopy method





$P2X_7$ receptor function analysis



BzATP-induced release of IL-1β

RM-ANOVA

BD patients p=0.003

** p<0,05, ** p<0,01 Dunn's*

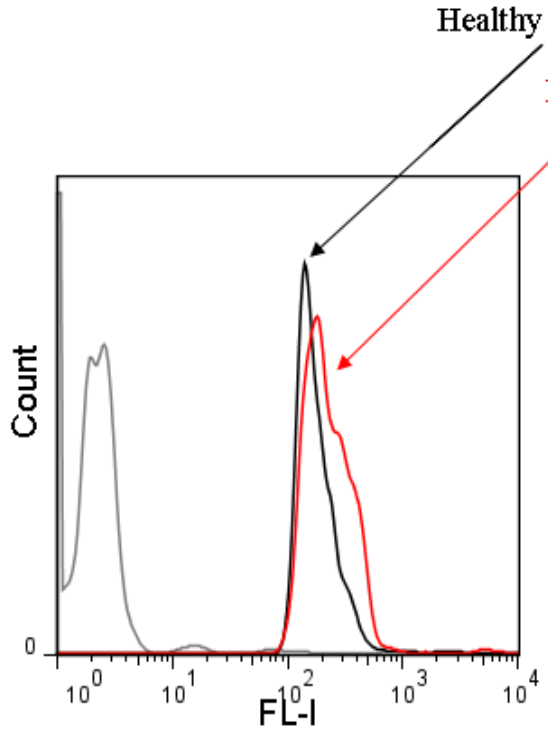
Healthy controls p=0.002

** p<0,05, ** p<0,01 Tukey-Kramer*

$P2X_7$ receptor expression analysis

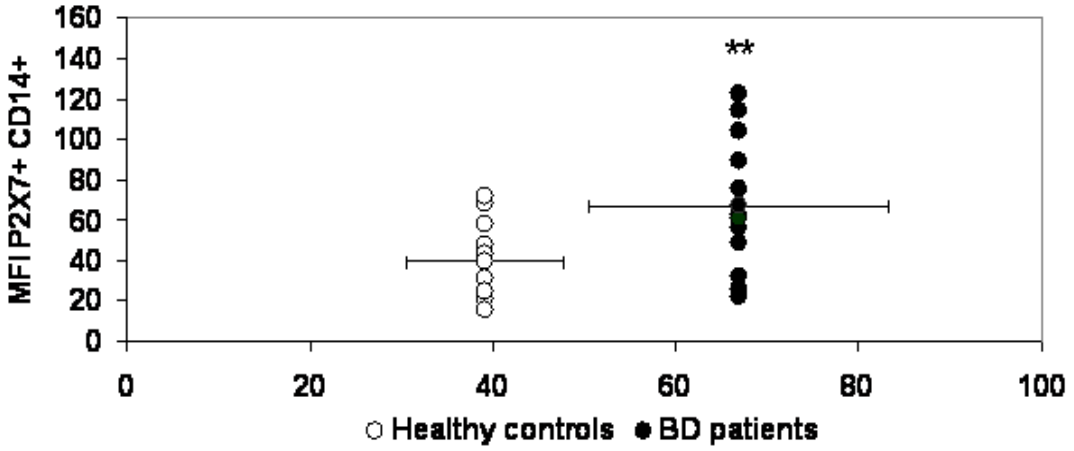
Flow cytometry assay (P2X7-FITC and CD14-PE)

Mean Fluorescence Intensity of CD14+cells (monocytes)



Healthy controls n=14

BD patients n=13

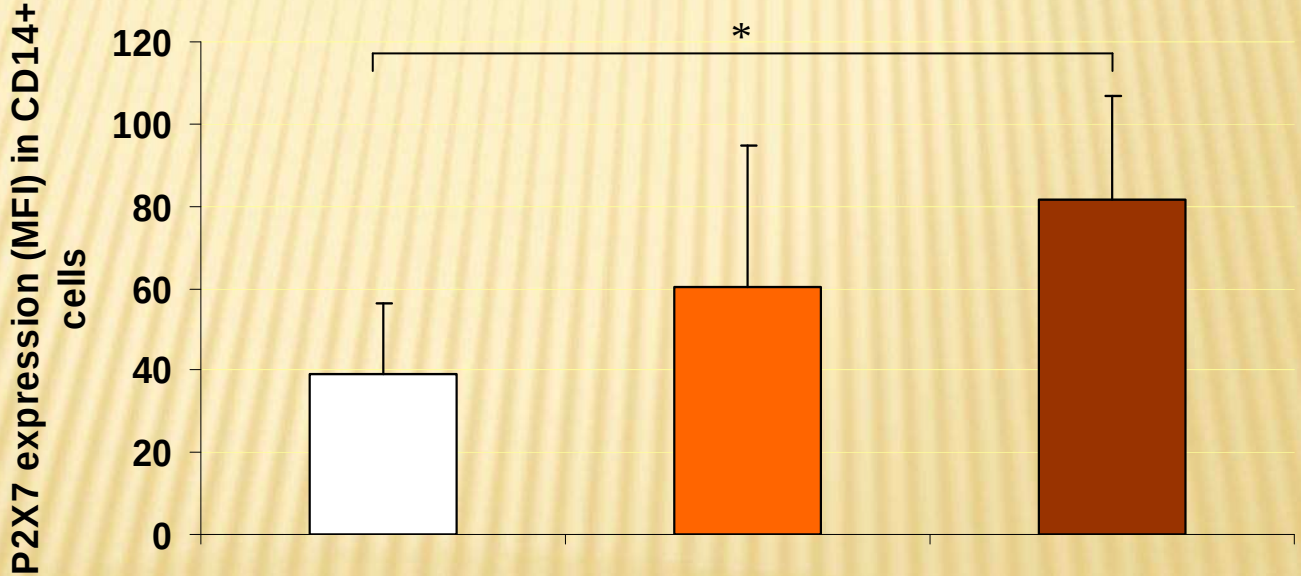


*** p<0.01 unpaired t test***

$P2X_7$ receptor in BD patients: effect of anti-TNFα treatment

- Healthy controls n=14
- BD **treated** n=11
- BD un**treated** n=4

Mean Fluorescence Intensity of CD14+ cells (monocytes)

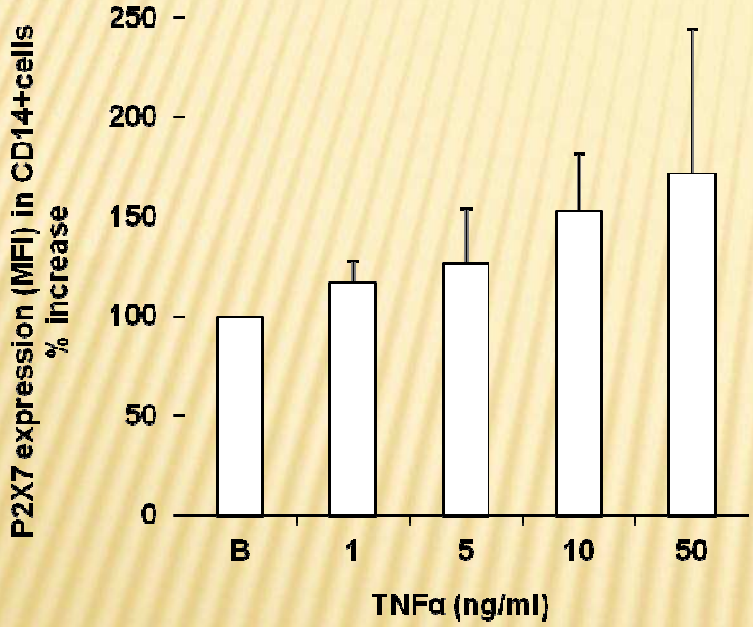


RM ANOVA: p<0.05

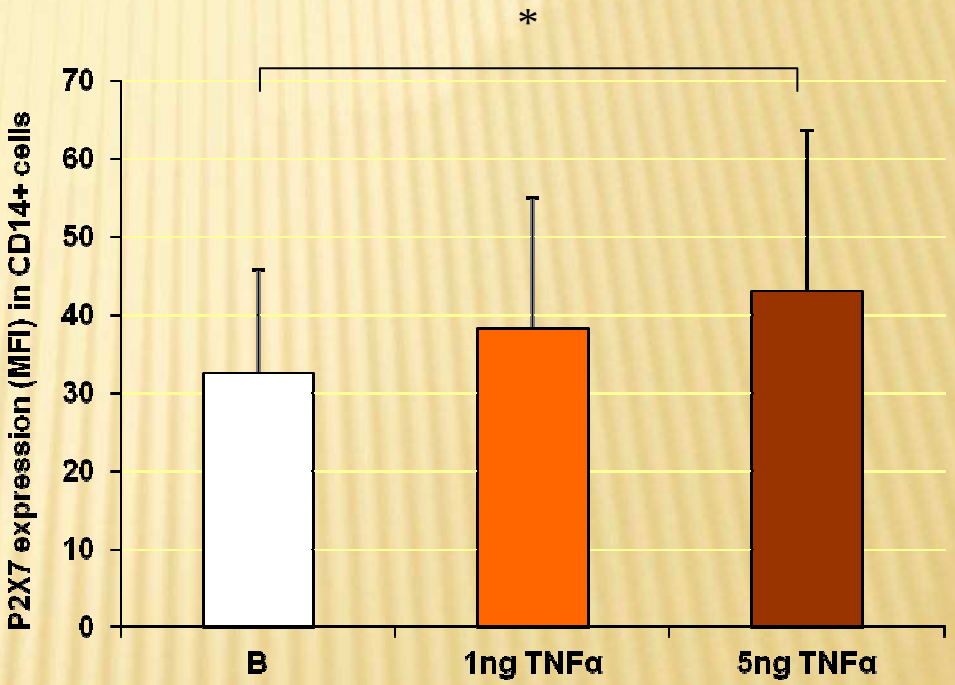
Dunn's testPost-hoc test: *p<0.05

$P2X_7$ receptor: in vitro modulating effect of TNFα

TNFα induced $P2X_7$ receptor expression



Healthy controls n=3



Healthy controls n=5

RM ANOVA: p<0.05

*Dunn's testPost-hoc test: *p<0.05*

P2X7 upregulation in Multiple Sclerosis

P2X7R is probably involved in the pathological mechanisms of MS, being upregulated in:

astrocytes (human cerebral cortex), Narcisse et al. Glia, 2005

oligodendrocytes (human and rat optic nerve), Matute et al. J Neurosci., 2007

microglia (human spinal cord), Yiangou et al. BMC Neurol., 2006

monocytes (human PBMC), Caragnano et al., J Neuroimmunol., 2012

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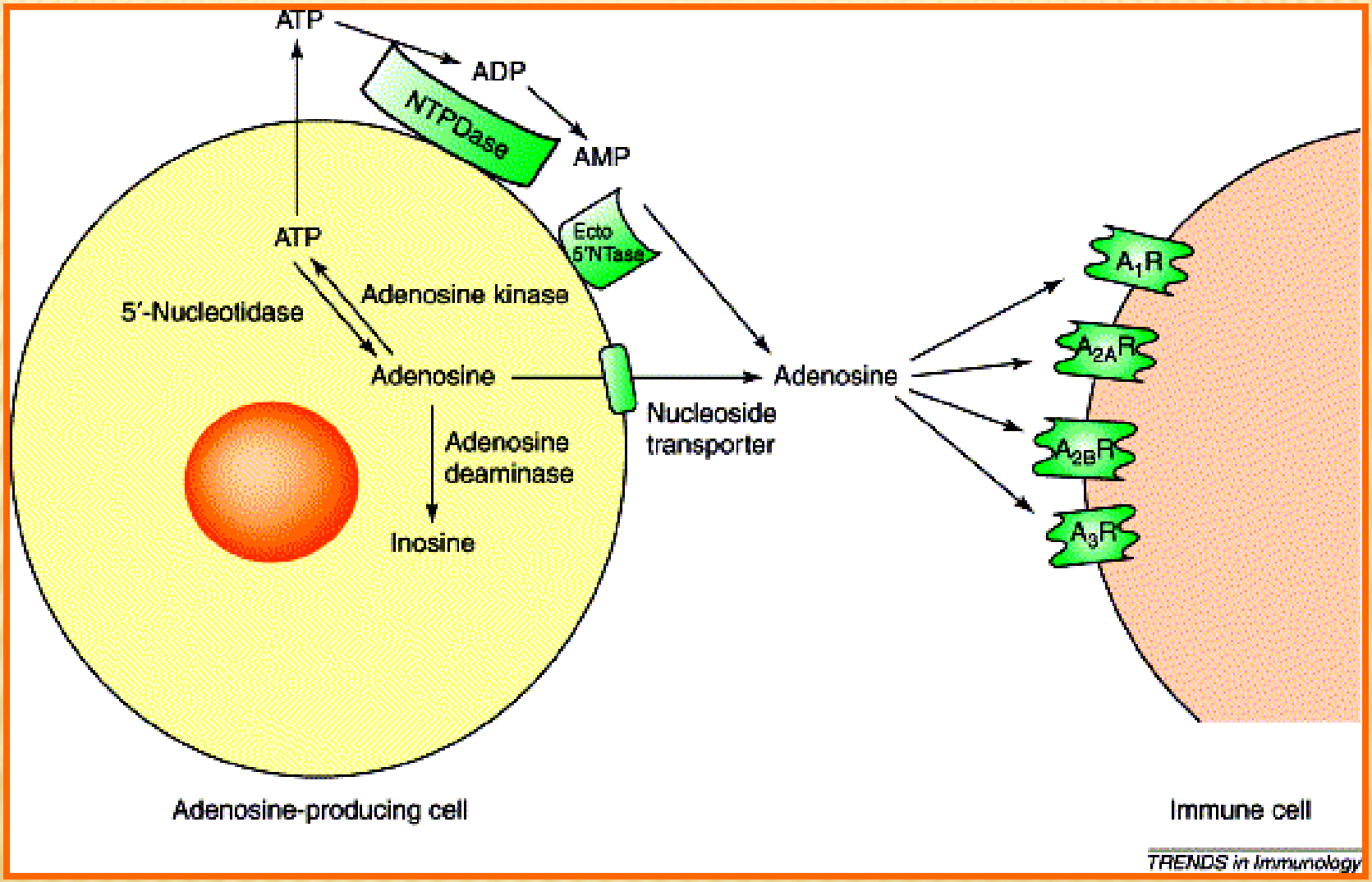
- “The odd couple”

P_1 receptors and Immune Cells



Hasko G, Cronstein BN. *Trends Immunol* 2004; 25: 33-9

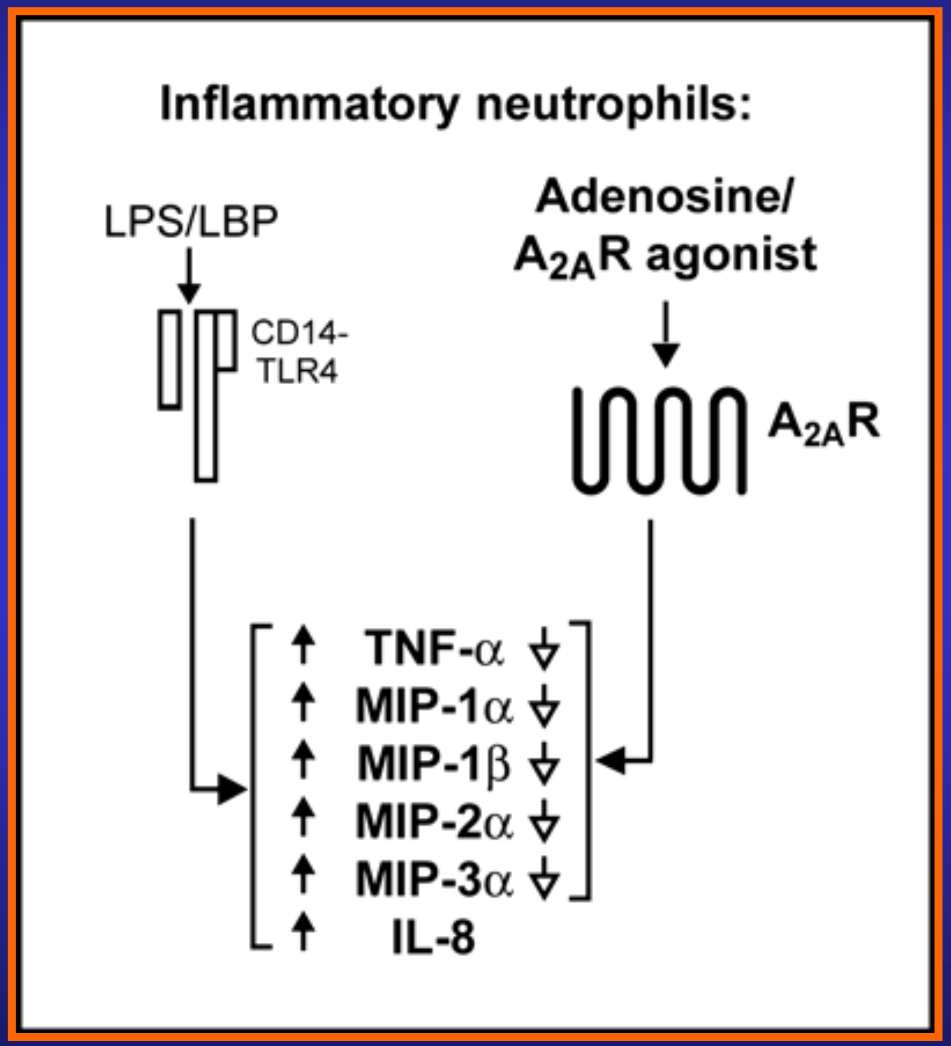
ADENOSINE RECEPTORS

Hasko G, Cronstein BN. *Trends Immunol* 2004; 25: 33-9

	A1	*A2a*	*A2b*	*A3*
Intracellular 2nd messenger pathways				
cAMP	↓	↑	↑	↓
Ca++	↑	-	-	↑
p38	↑	-	↑	-
p42/44	↑	↑	-	-
Selective agonists	CPA CCPA	GCS-21680	-	IB-MECA 2CI-IB-MECA
Selective antagonists	DPCPX PACPX	ZM241385 CSC	Alloxazine	MRS-1220
Affinity to adenosine	H	H	L	L
G-protein-coupling	Gi/o	Gs	Gs,Gq	Gi,Gq

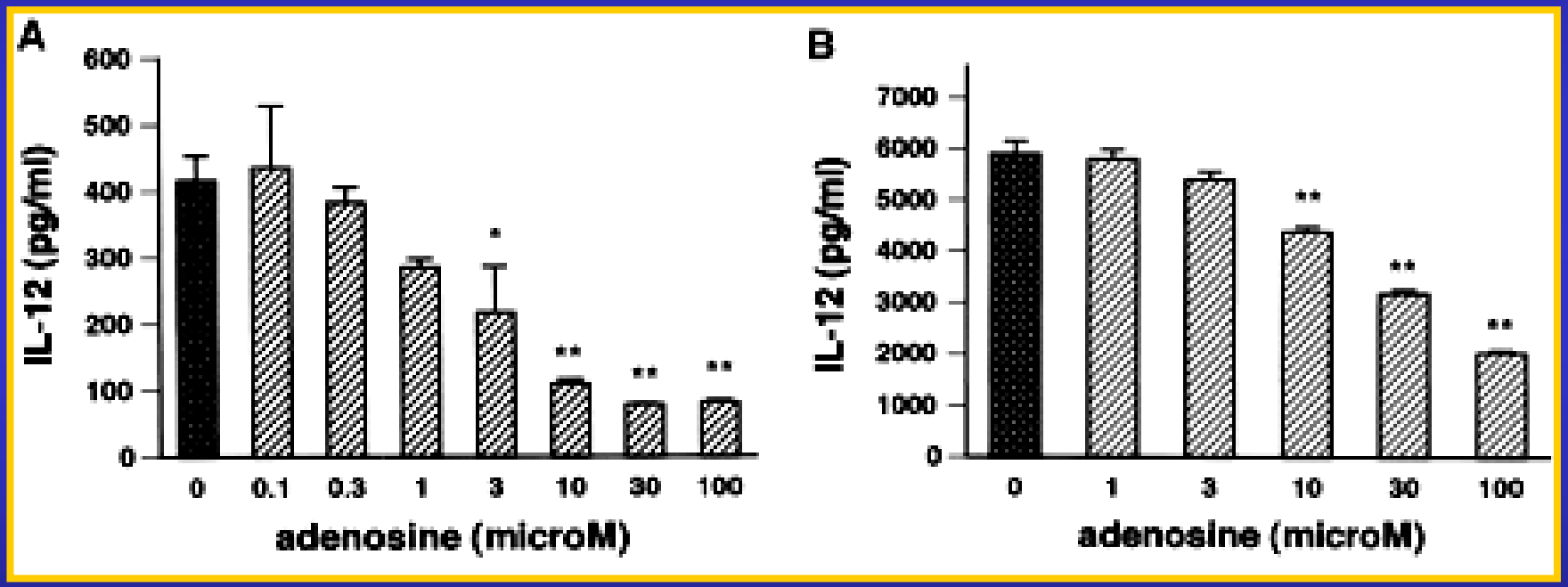
Modulation of the profile of cytokines/chemokines generated in inflammatory neutrophils by activation of the adenosine A_{2A} receptor.

A_{2A}R, adenosine A_{2A} receptor;
LPS/LBP, lipopolysaccharide/lipopolysaccharide binding protein;
TLR4, Toll-like receptor-4.



Mc Coll SR, et al. *FASEB Journal.* 2006;20:187-189

Adenosine inhibits IL-12 production in mouse peritoneal macrophages

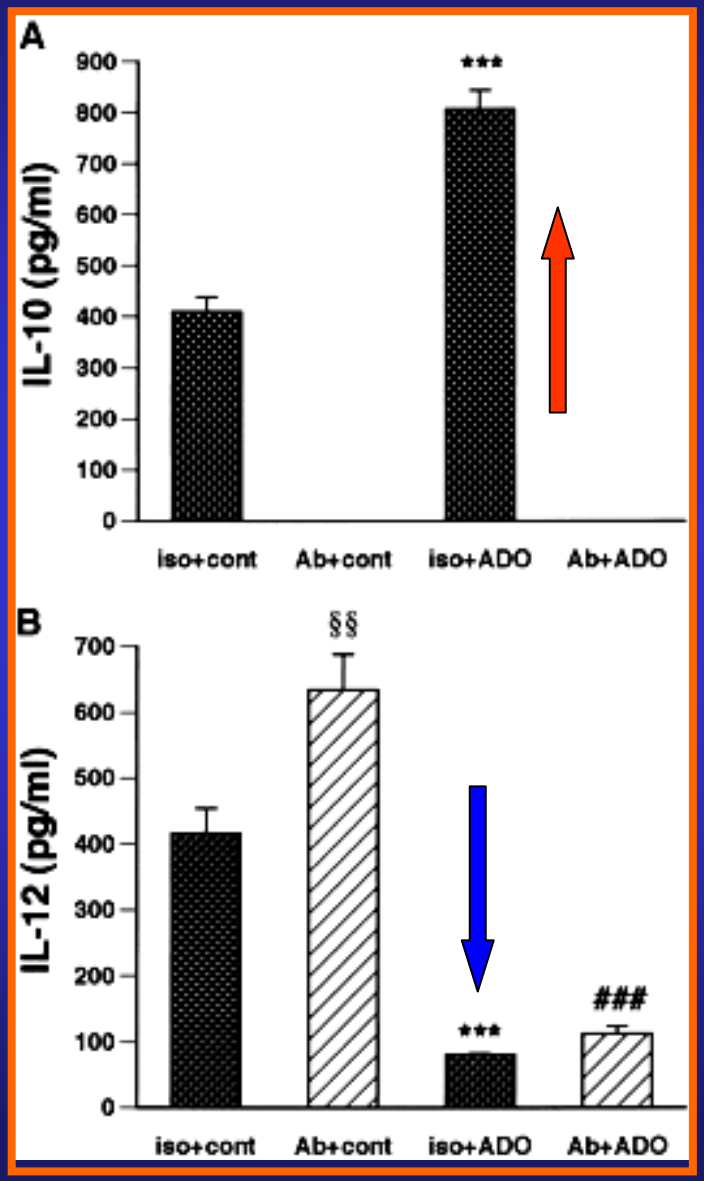


LPS **LPS + IFN-γ**

The FASEB Journal

Hasko GDG, et al. *FASEB J* 2000;14:2065-2074

Selective effect of adenosine on inflammation

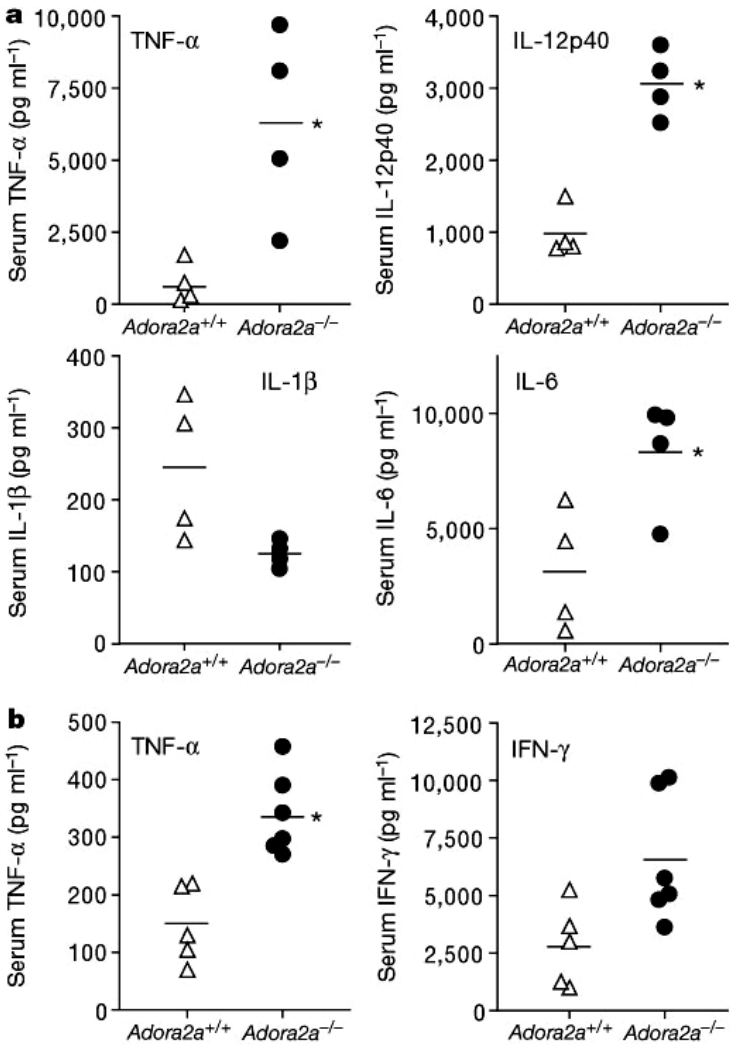


inhibition of pro-inflammatory and enhancement of anti-inflammatory cytokines

The FASEB Journal

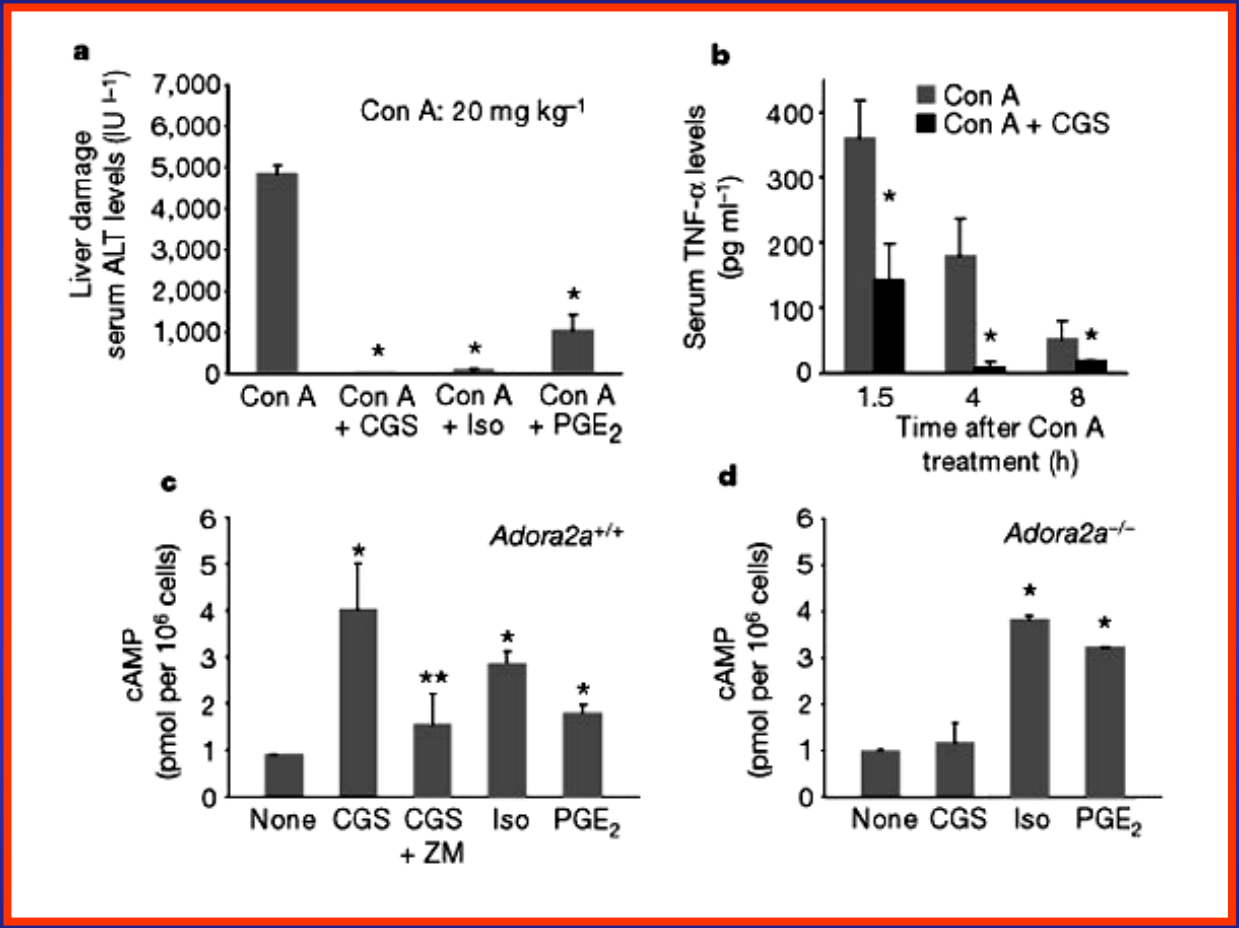
Hasko GDG, et al. *FASEB J* 2000;14:2065-2074

Enhanced accumulation of pro-inflammatory cytokines and tissue damage in A2a-receptor-deficient mice treated with endotoxin



Ohta A and Sitkovsky M. *Nature* 2001; 414, 916-920

Pharmacological activation of A2a or other G_s-protein-coupled receptors in vivo prevents Con A-induced liver damage and TNF-α accumulation

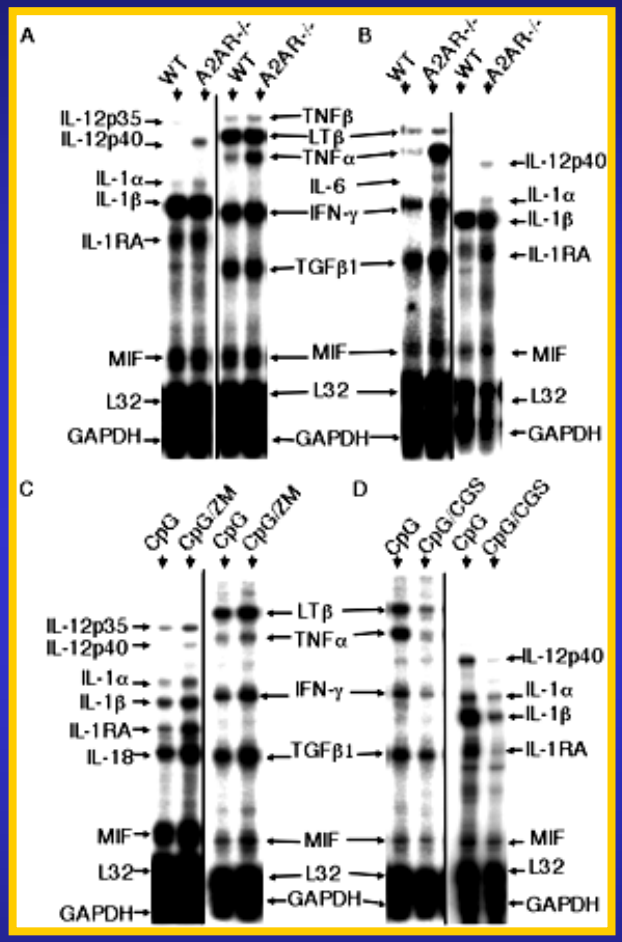


nature

Ohta A and Sitkovsky M. *Nature* 2001; 414, 916-920

increased expression of proinflammatory cytokines mRNA in A2AR-deficient mice (total spleen mRNA)

increased expression of proinflammatory cytokines' mRNA in macrophages from A2AR-deficient mice

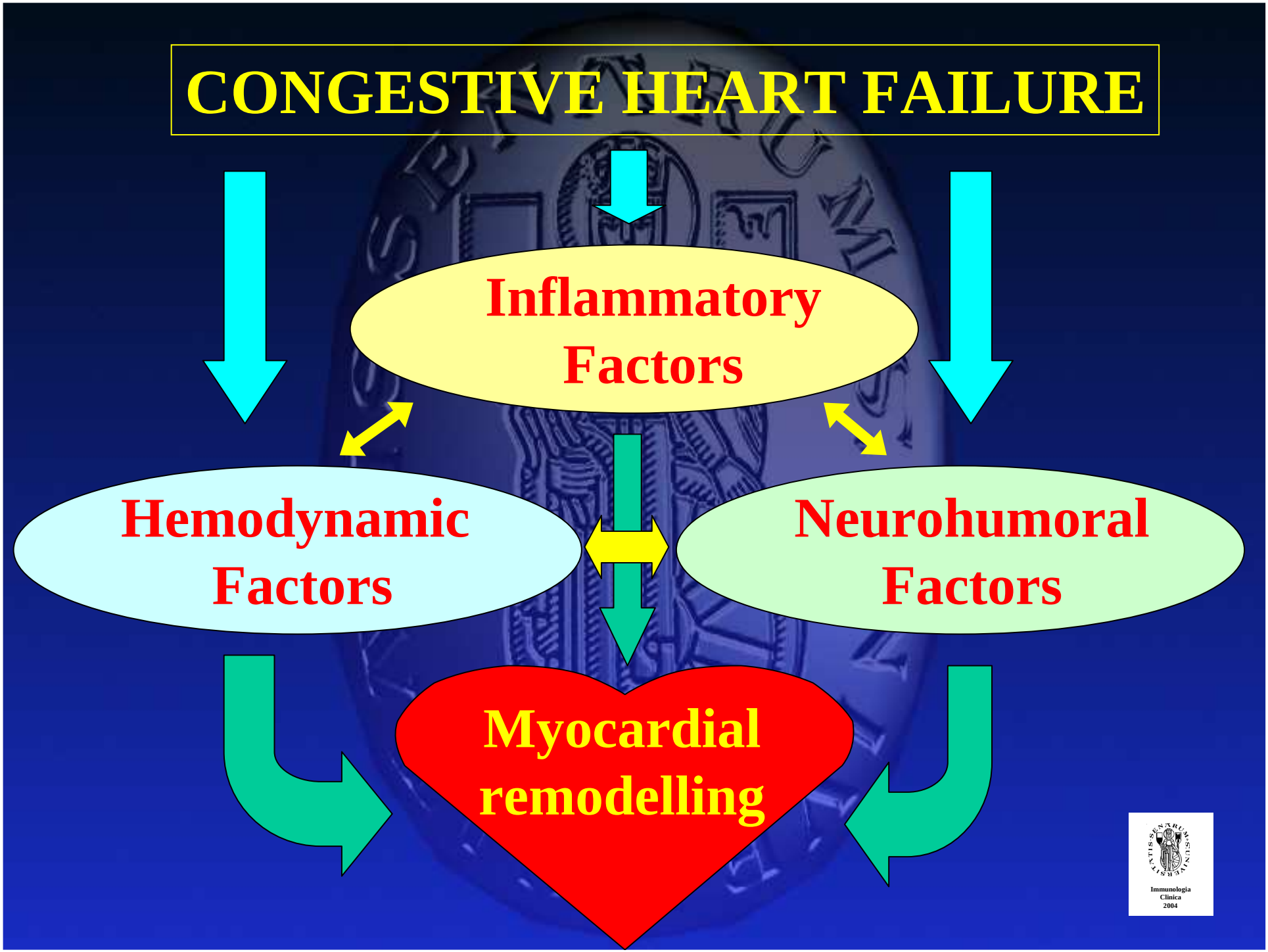


decreased expression of proinflammatory cytokine mRNA in splenocytes of mice with pharmacologically activated in vivo A2AR (CGS21680, CGS)

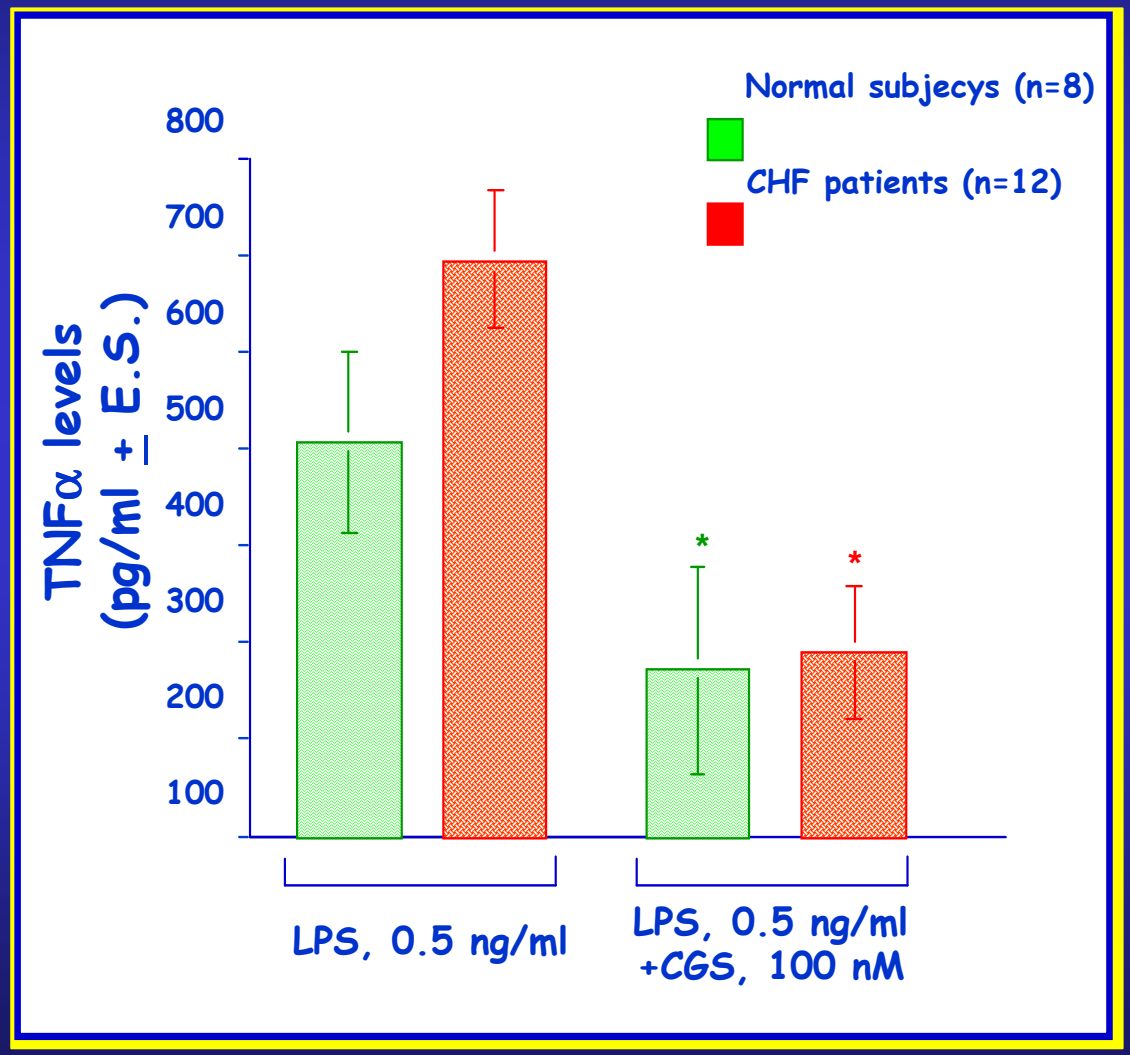
increased expression of proinflammatory cytokines mRNA in splenocytes with pharmacologically inactivated in vivo A2AR (ZM241385, ZM)

The Journal of Immunology

Lukashev D, et al J Immunol. 2004 Jul 1;173(1):21-4.

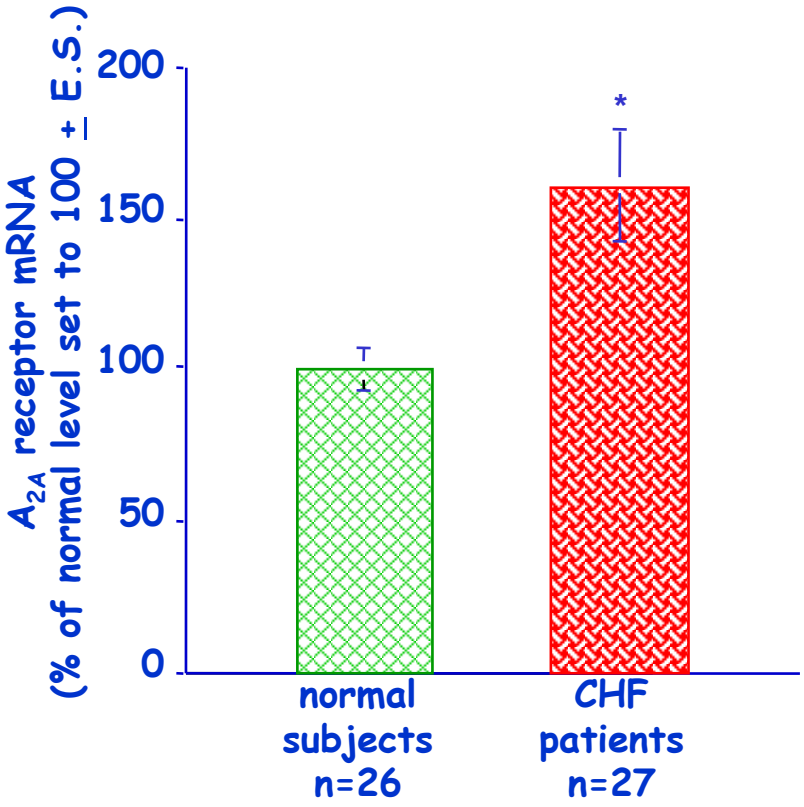

CONGESTIVE HEART FAILURE
Inflammatory Factors
Hemodynamic Factors
Neurohumoral Factors
Myocardial remodelling
Immunologia Clinica 2004

Ability of the A_{2A} receptor agonist CGS21680 to decrease the production of TNFα from cultured PBMC

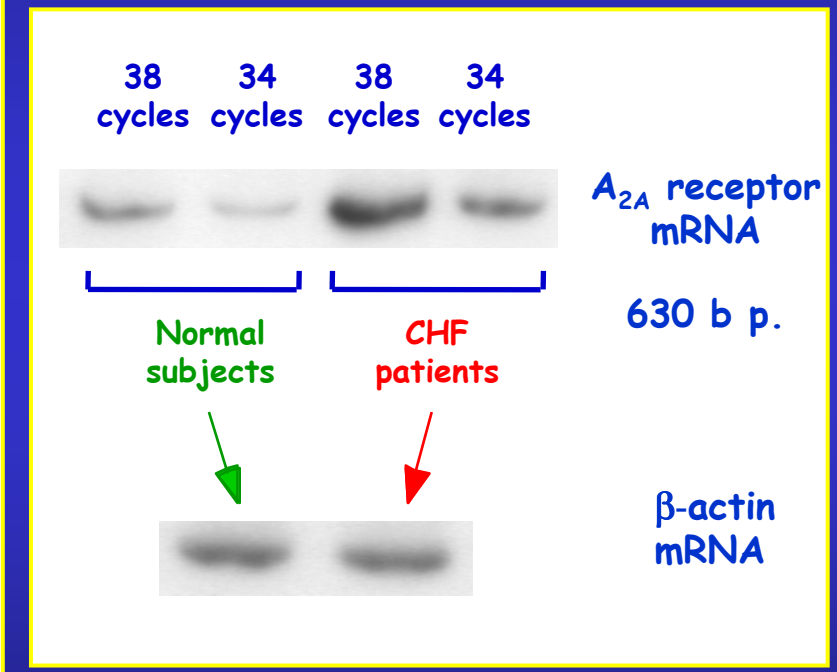


*p<0.05 with respect LPS alone, Scheffé test

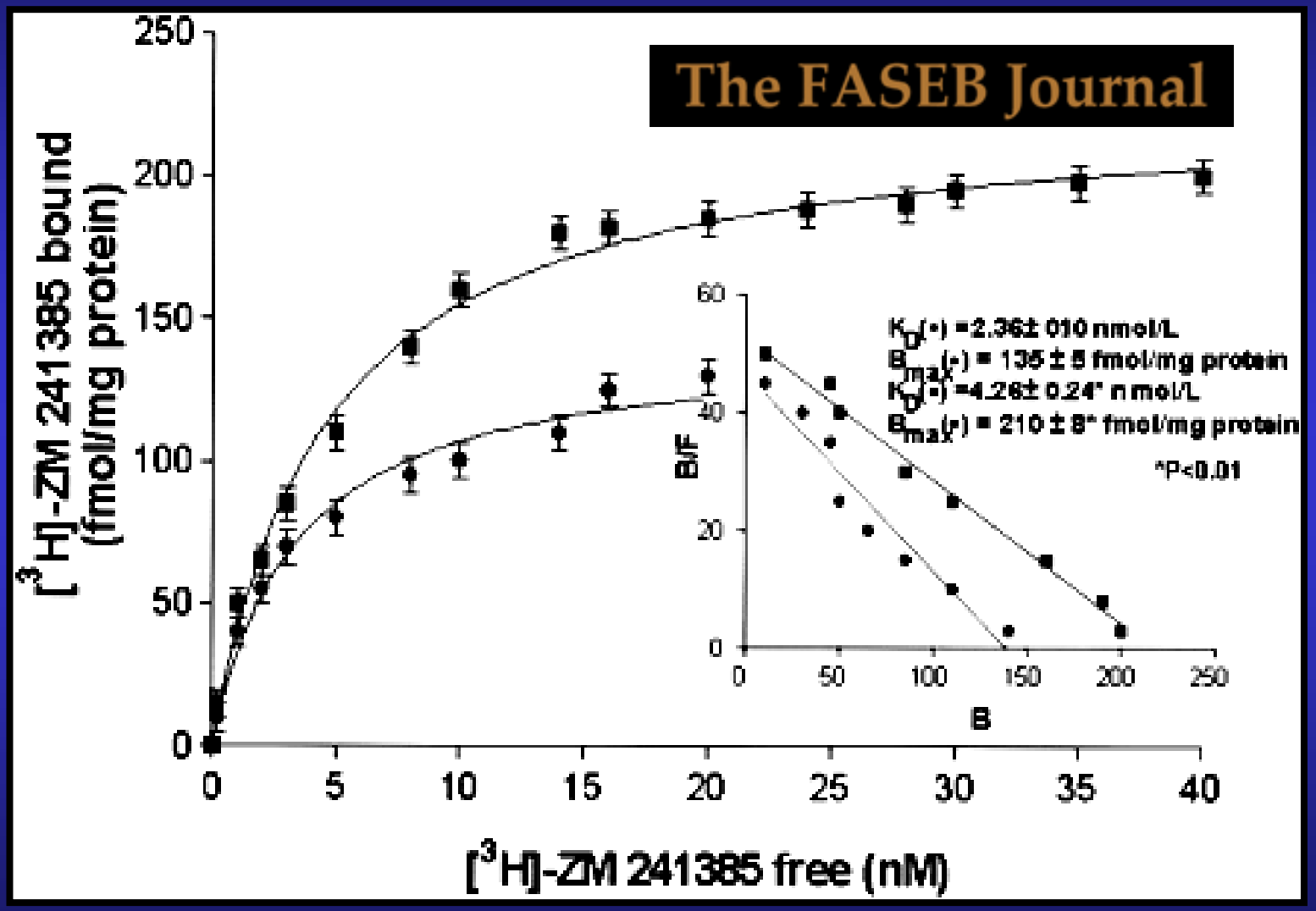
Altered expression of A_{2A} receptors in CHF PBMC



*p<0.05 with respect to normal subjects, Fischer test)



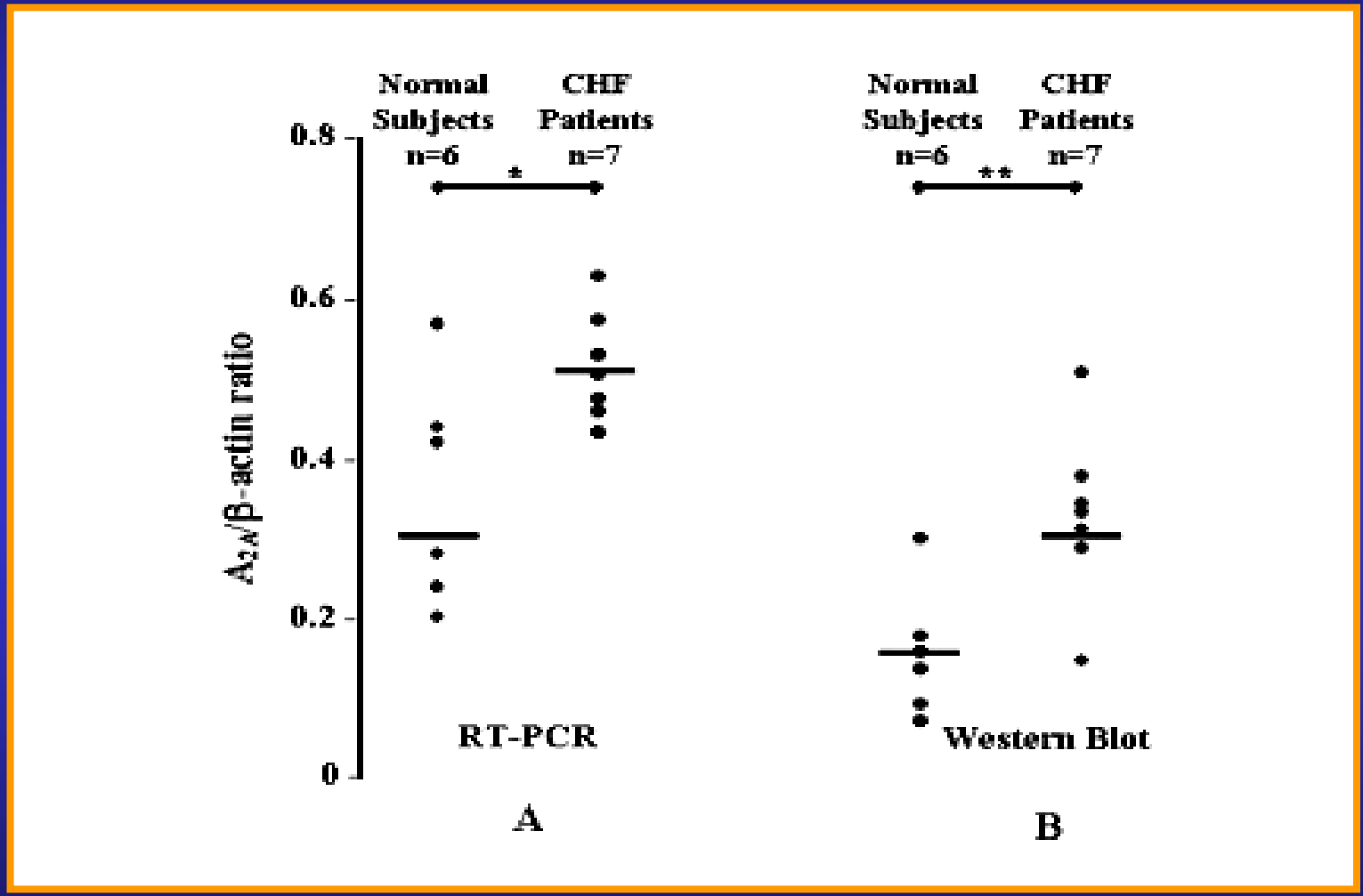
La up-regulation dei recettori A_{2A} nei PBMC rispecchia le modificazioni recettoriali del cuore scompensato



Varani K, Laghi Pasini F. et al. *FASEB J* 2003; 17: 280-282

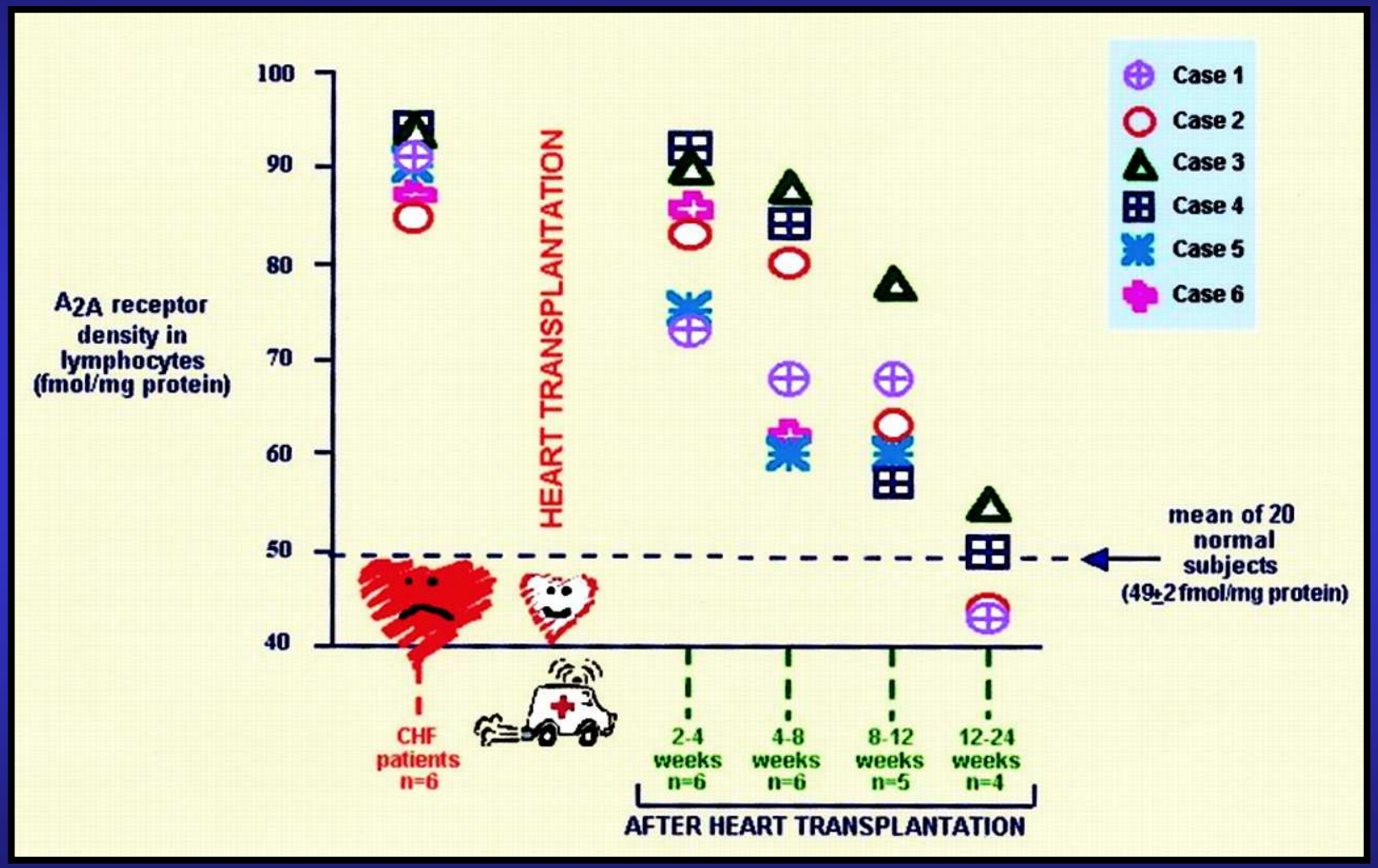
JOURNAL OF CARDIAC FAILURE

Espressione del recettore A2A in PBMC (RT-PCR ed Western blot)



PL Capecchi, et al. *J Card Fail* 2005; 11: 67-73

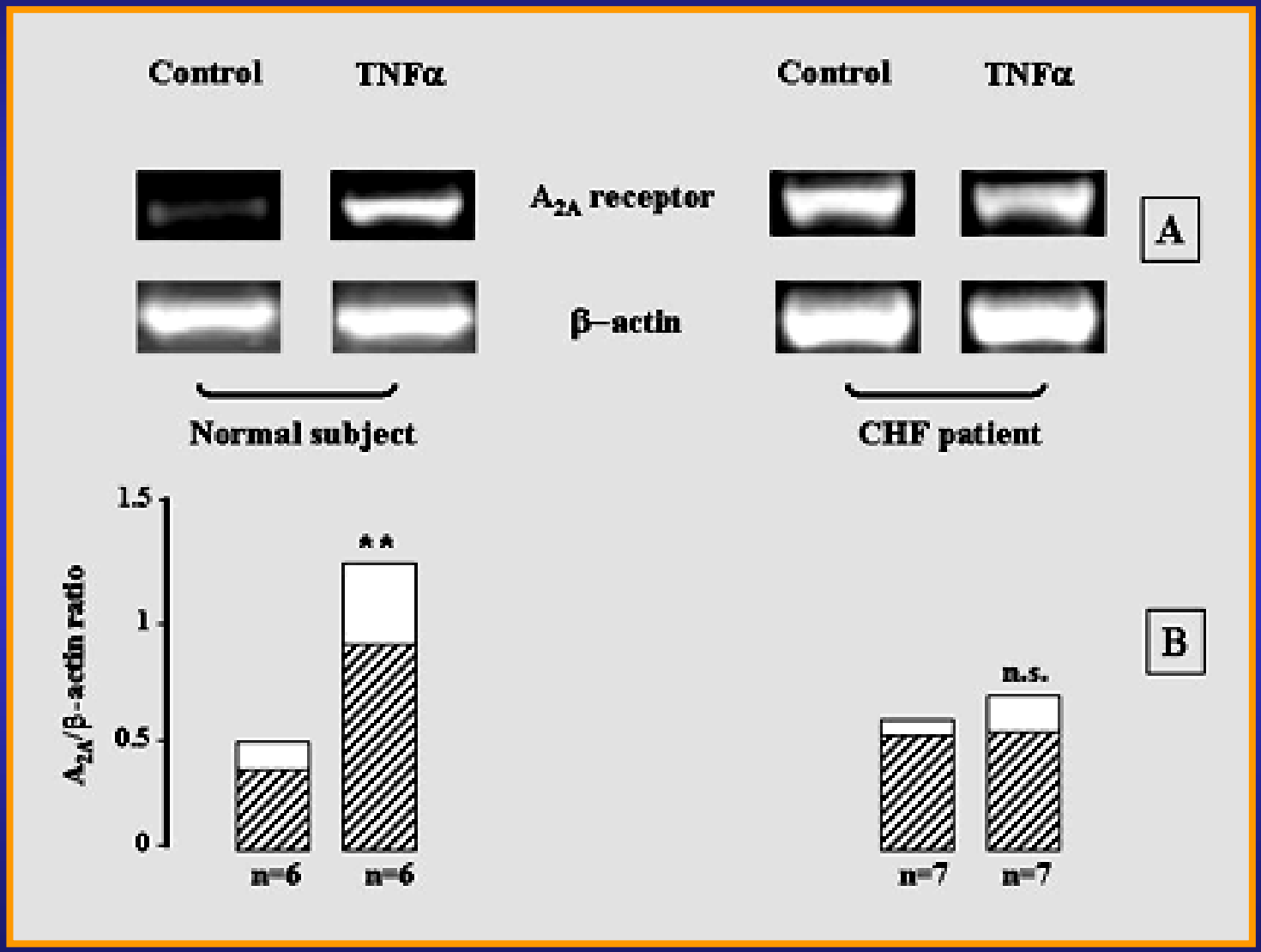
Normalizzazione dei recettori A_{2A} (PBMC) dopo il trapianto cardiaco



The FASEB Journal

Varani K, Laghi Pasini F. et al. *FASEB J* 2003; 17: 280-282

Effetto del TNF-α sulla espressione del recettore A2A in PBMC (RT-PCR)



JOURNAL OF CARDIAC FAILURE

PL Capecchi, et al. *J Card Fail* 2005; 11: 67-73

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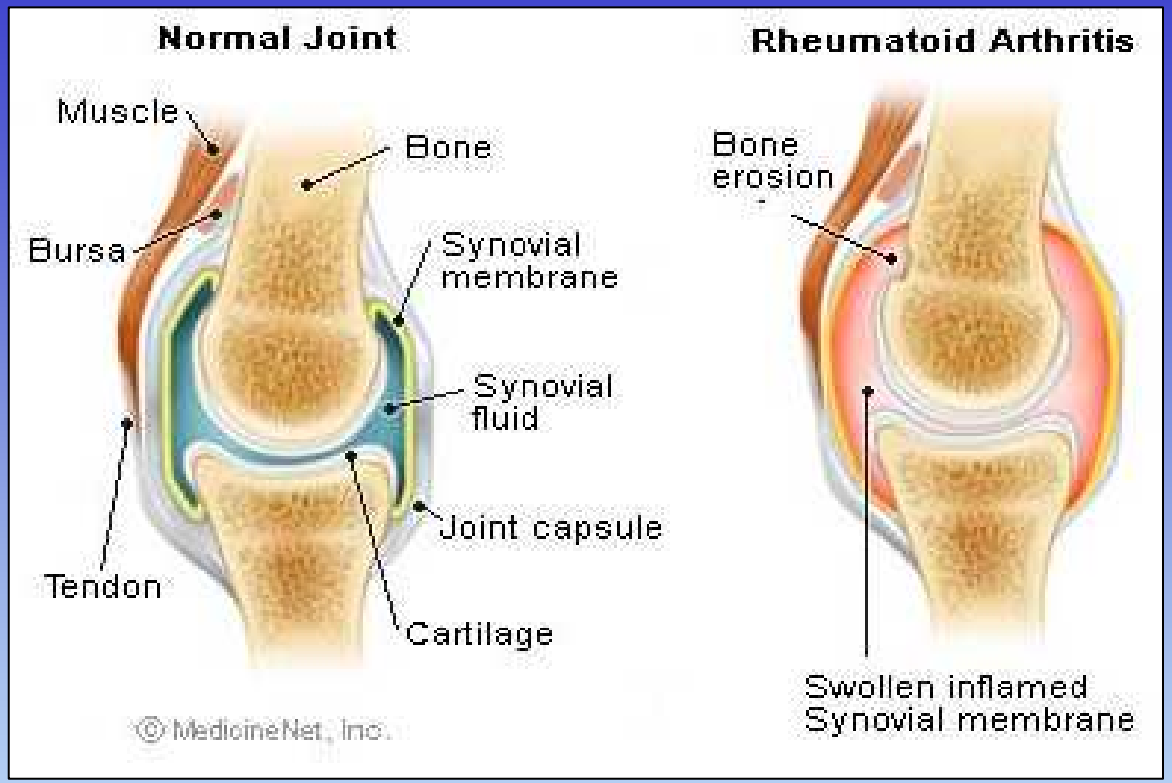
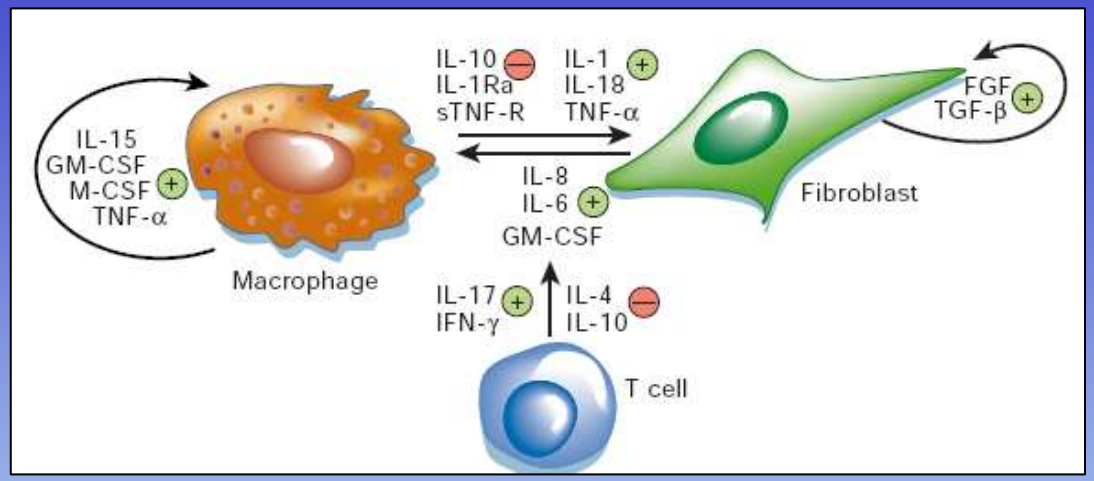
RHEUMATOID ARTHRITIS



Autoimmune disease characterized by chronic inflammation of the joints. The synovial membrane is hyperplastic and infiltrated with mononuclear cells. It is transformed into a proliferating invasive cell mass or 'PANNUS' that erodes the surrounding tissue and bone leading to painful joint deformities.

CYTOKINES NETWORKS IN RHEUMATOID ARTHRITIS



TNF-α

- synovial fluids
- induces collagenase and MMPs release
- stimulates IL-8 synthesis

IL-1

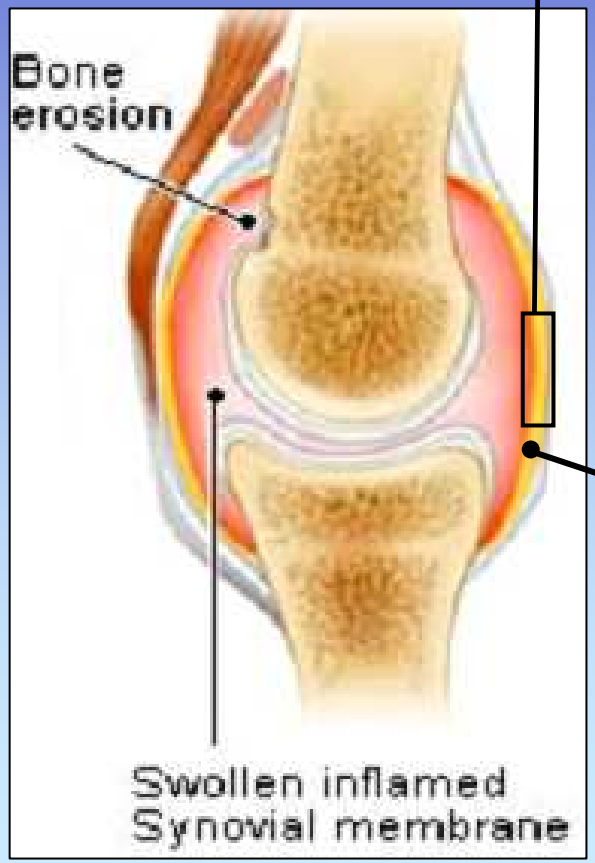
- key mediator of synovial inflammation and pannus formation
- activates T- and B-lymphocytes
- pain, swelling and tenderness

IL-6

- inflammation sites in the articular joints
- activates T- and B-lymphocytes
- synthesis of C-reactive protein

SYNOVIAL CELL TYPES

SYNOVIOCYTES



TYPE A

(macrophagic cells)

- ✓ Resident macrophages
- ✓ Possess an antigen presenting ability
- ✓ Secrete inflammatory cytokines

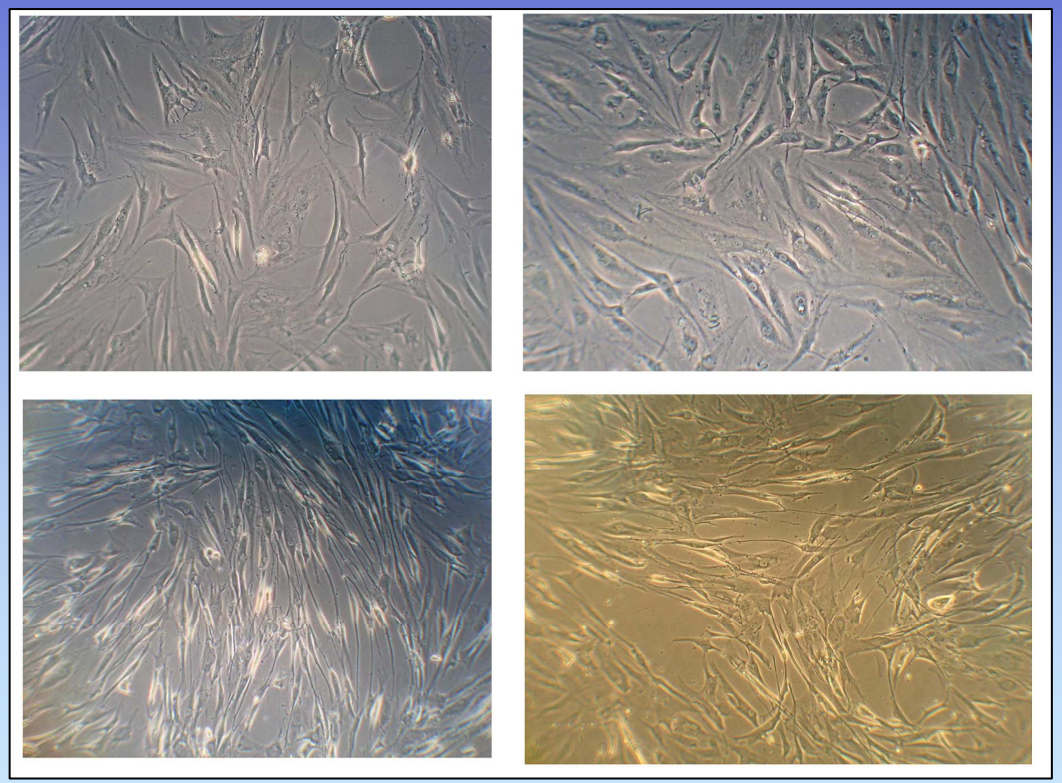
TYPE B

(fibroblast-like cells)

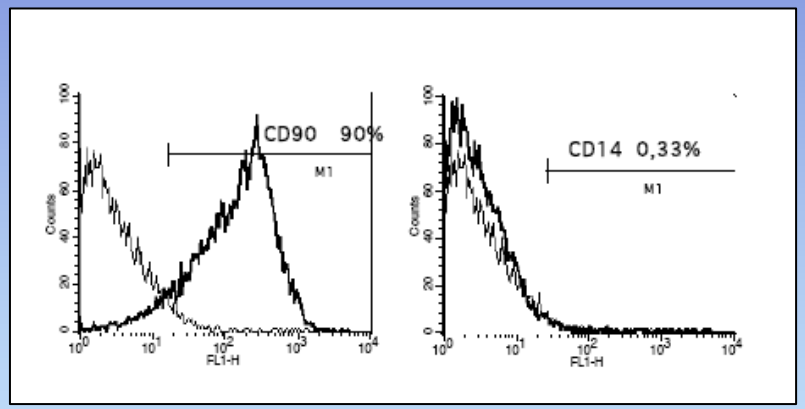
- ✓Aggressive and invasive behaviour
- ✓Partecipate in inflammation,hyperplasy of the synovial membrane
- ✓Secrete MMPs and PGs leading to the destruction of the articular cartilage and bone
- ✓Perpetuation of chronic inflammation by producing cytokines (IL-6)

MORPHOLOGY AND CHARACTERIZATION OF SYNOVIAL CELLS BY FLOW CYTOMETRY

Morphology



Flow Cytometry



CD90/Thy-1 = fibroblasts

CD14 = macrophages

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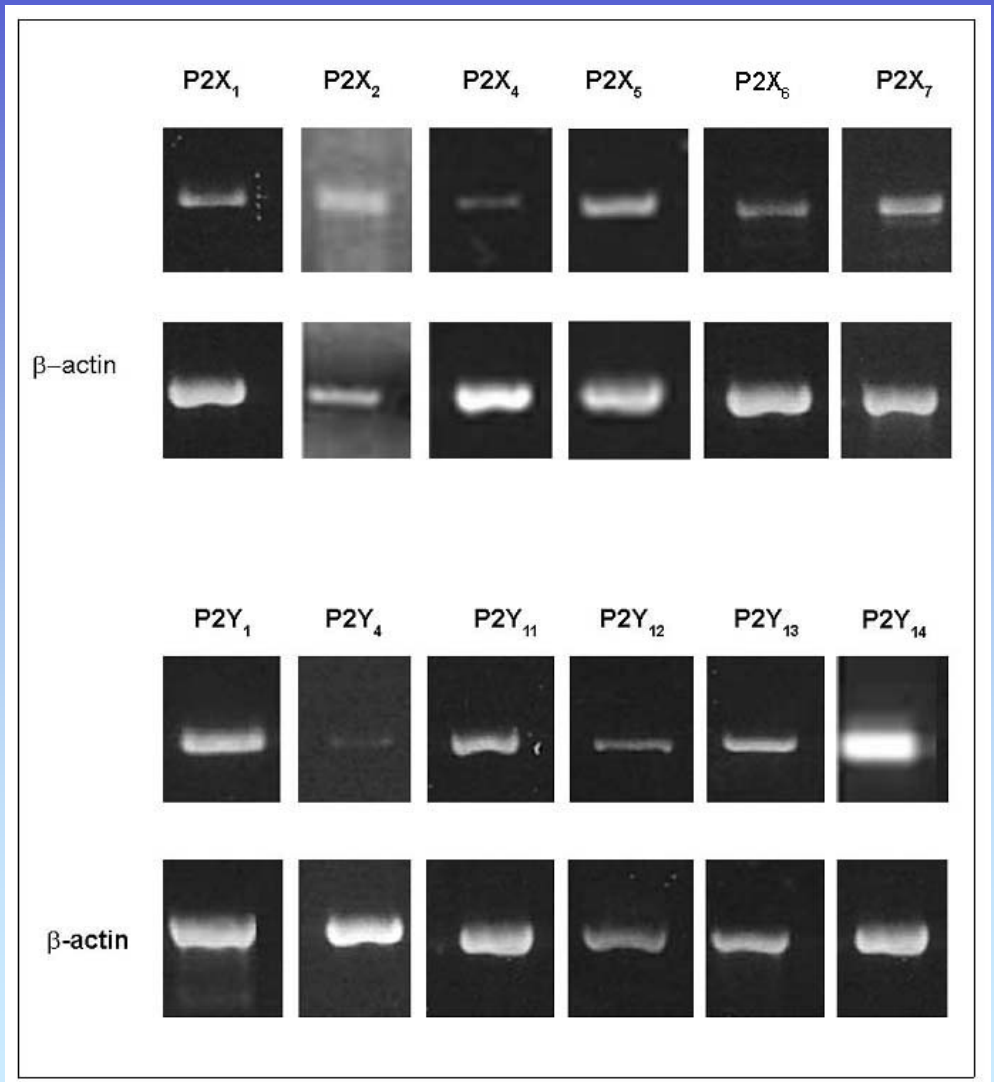
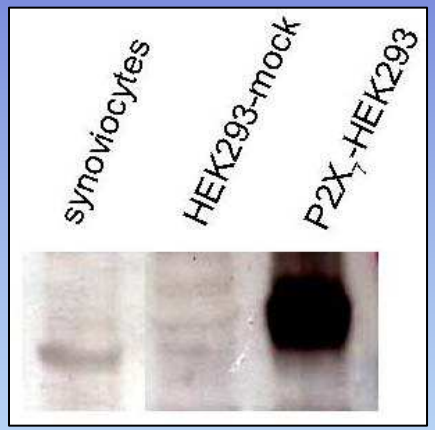
TYPE-B SYNOVIOCYTES EXPRESS P2X AND P2Y RECEPTORS SUBTYPES

RT-PCR



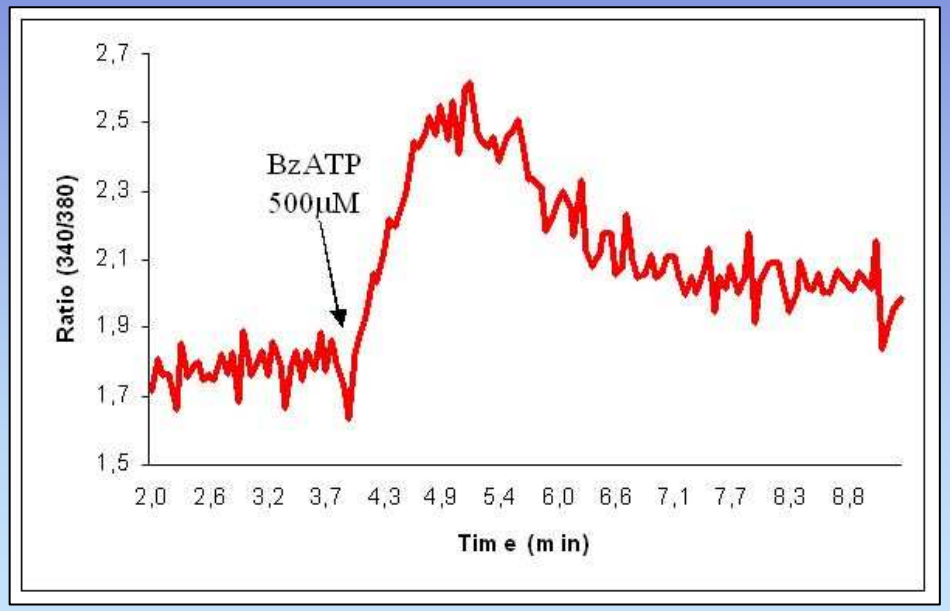
Western Blot



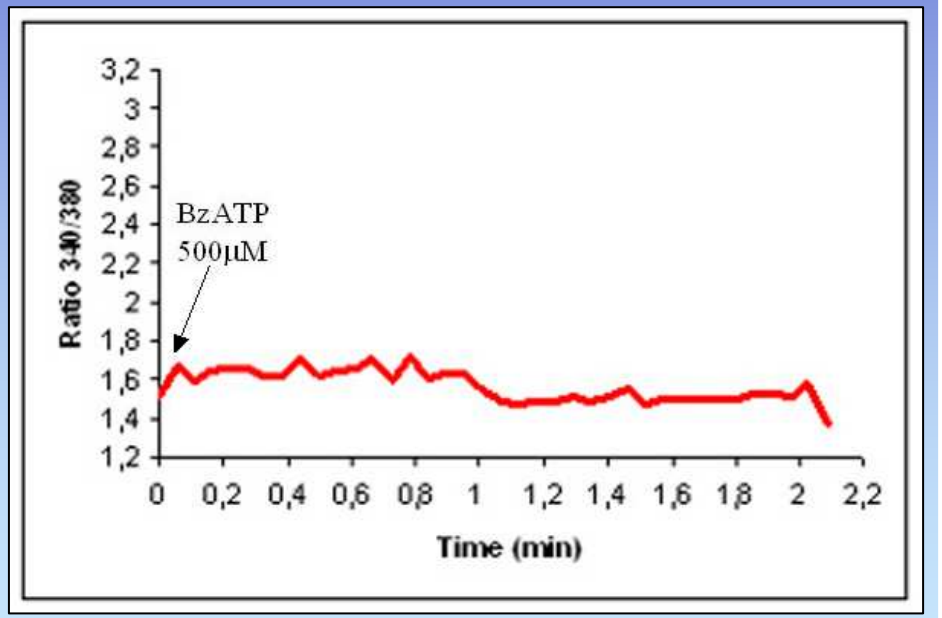
P2X	$P2X_1$	$P2X_2$	$P2X_4$	$P2X_5$	$P2X_6$	$P2X_7$
P2Y	$P2Y_1$	$P2Y_4$	$P2Y_{11}$	$P2Y_{12}$	$P2Y_{13}$	$P2Y_{14}$

ROLE OF BzATP IN MODULATION OF $[Ca^{2+}]_i$ IN TYPE-B SYNOVIOCYTES

+ Ca^{2+}



- Ca^{2+}



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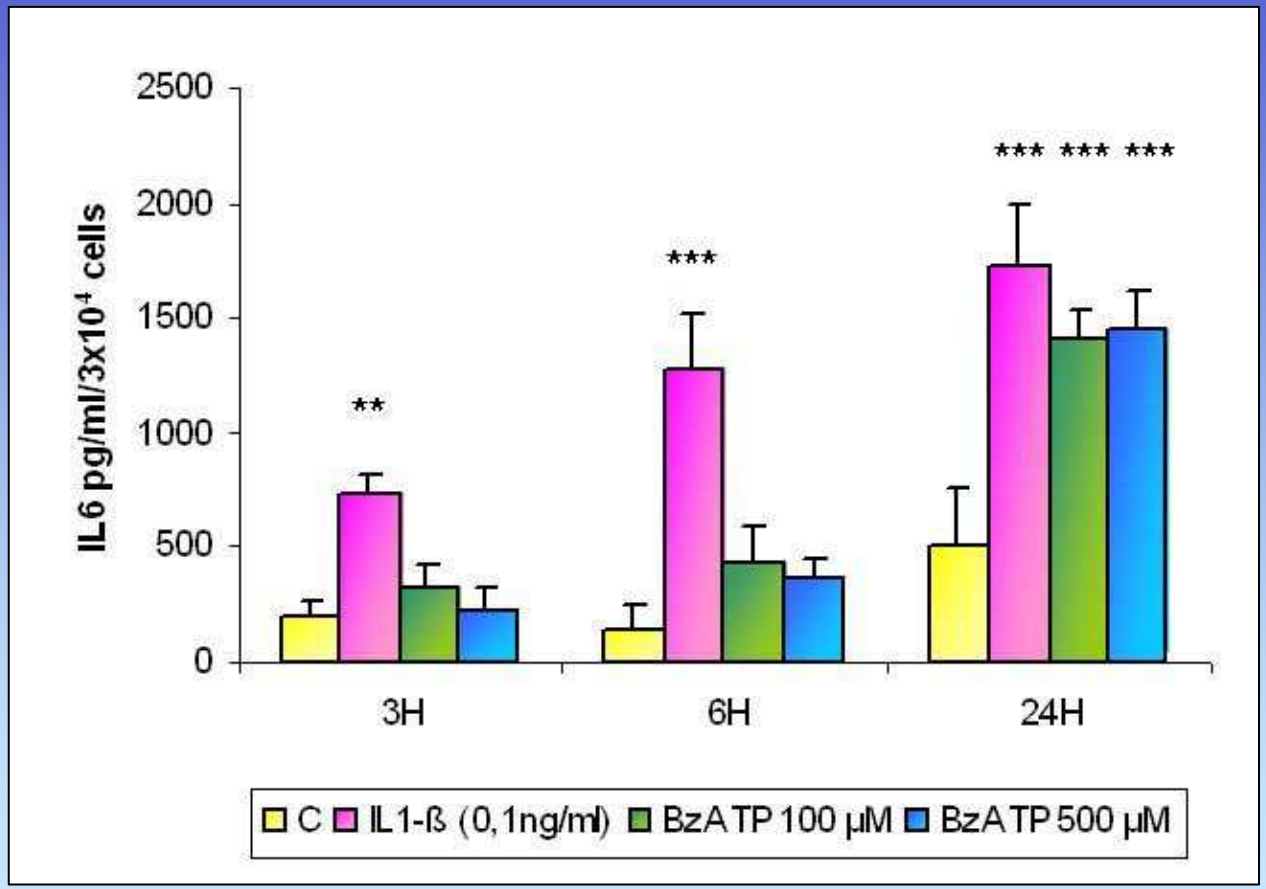
P2X$_7$ INVOLVEMENT IN IL-6 RELEASE



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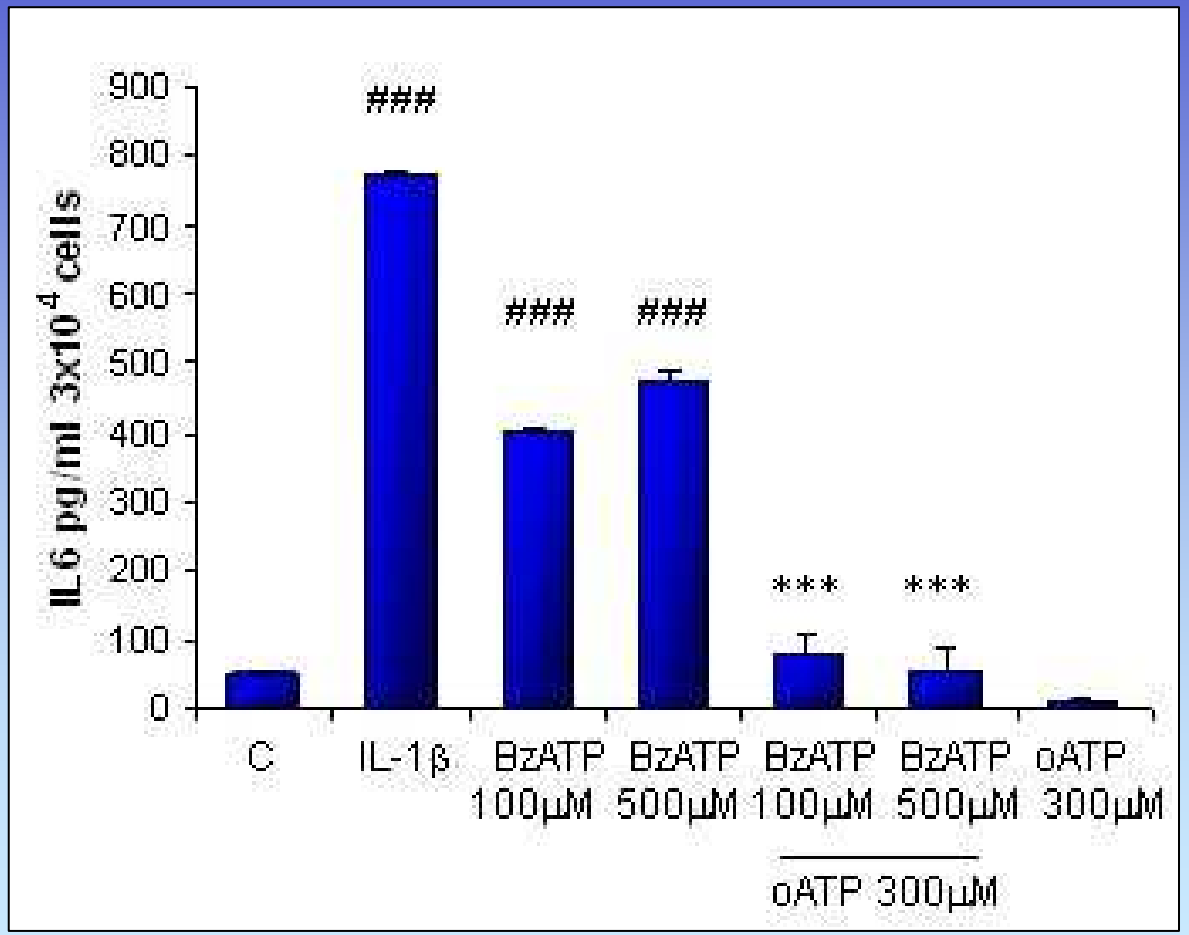
oATP INHIBITS IL-6 RELEASE IN TYPE-B SYNOVIOCYTES



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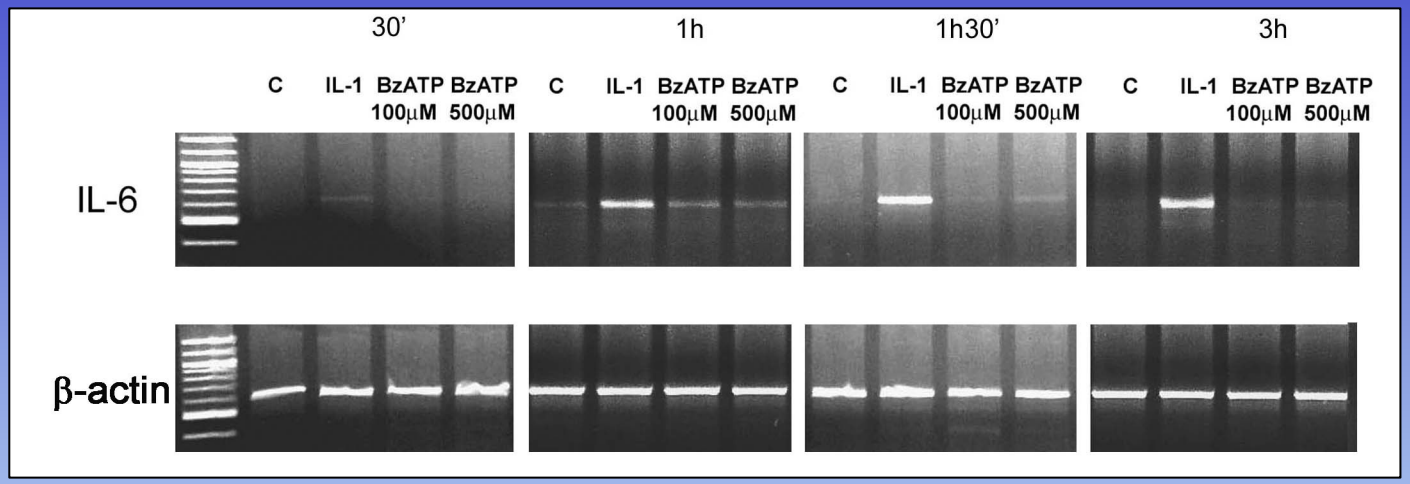
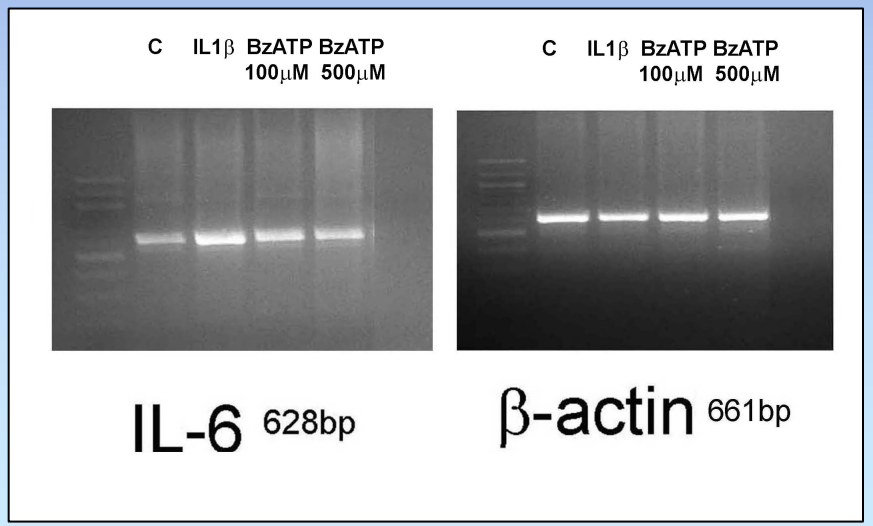
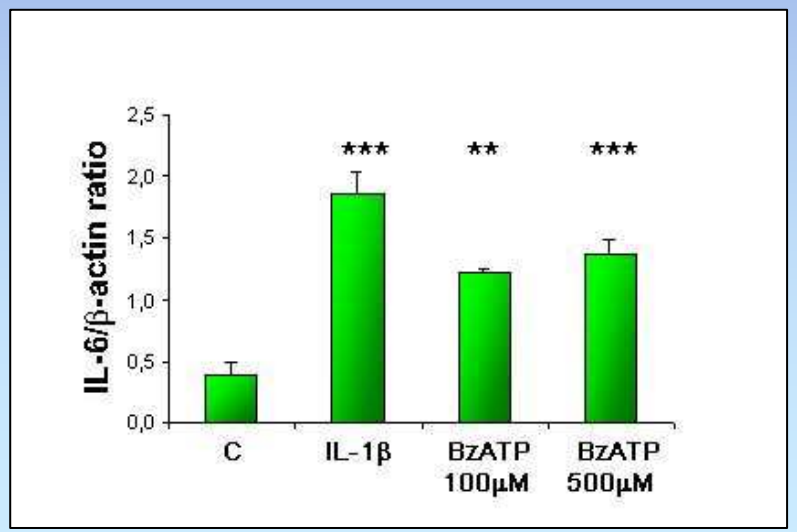
BzATP UPREGULATES EXPRESSION OF IL-6 mRNA IN HUMAN SYNOVIOCYTES







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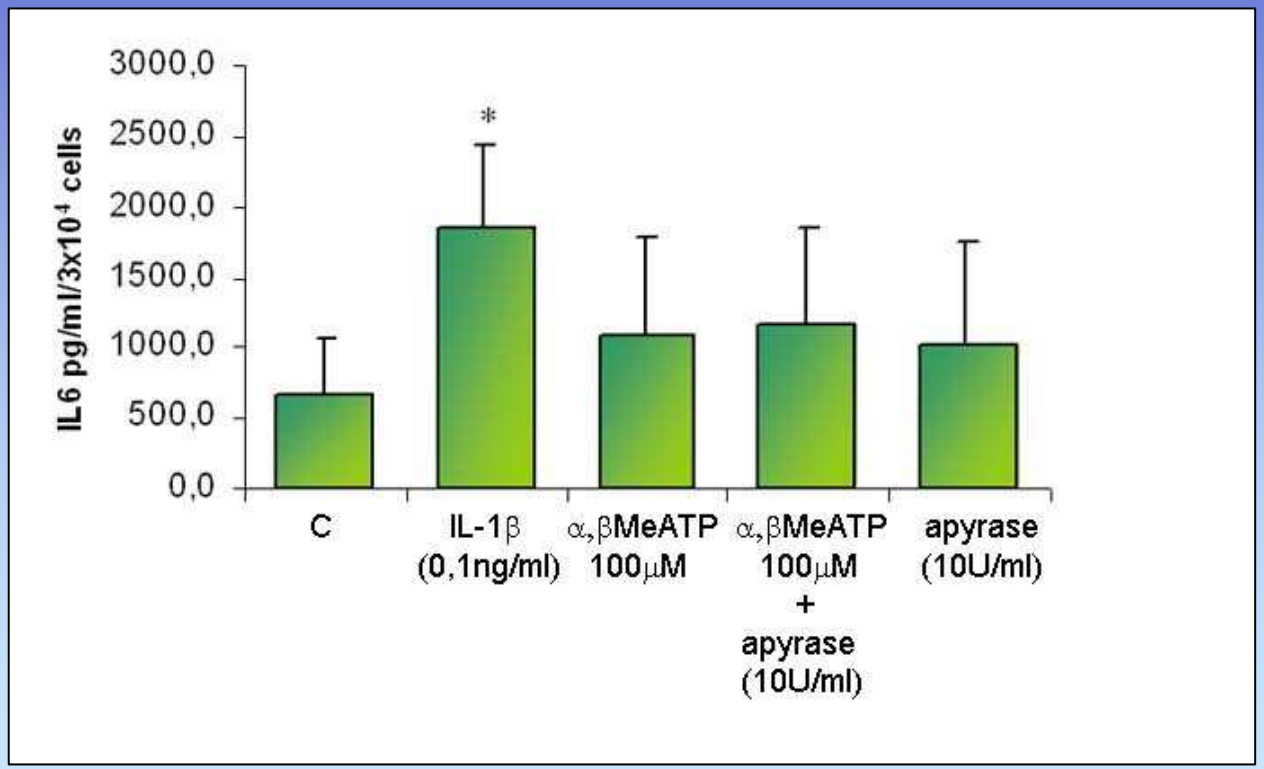
EFFECT OF α,βMeATP ON IL-6 RELEASE IN TYPE-B SYNOVIOCYTES



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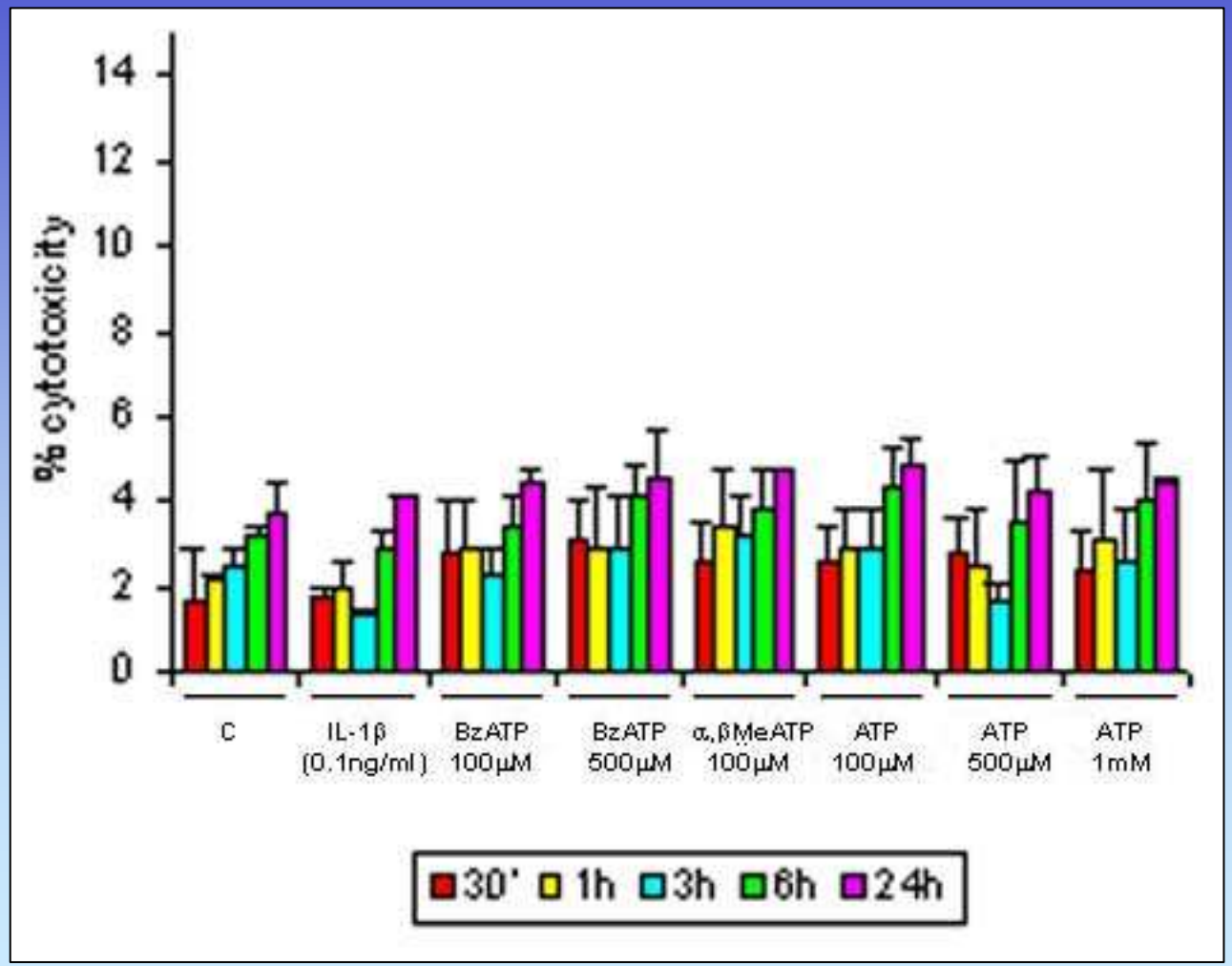
AGONIST TREATMENT DOES NOT CAUSE CELL DEATH



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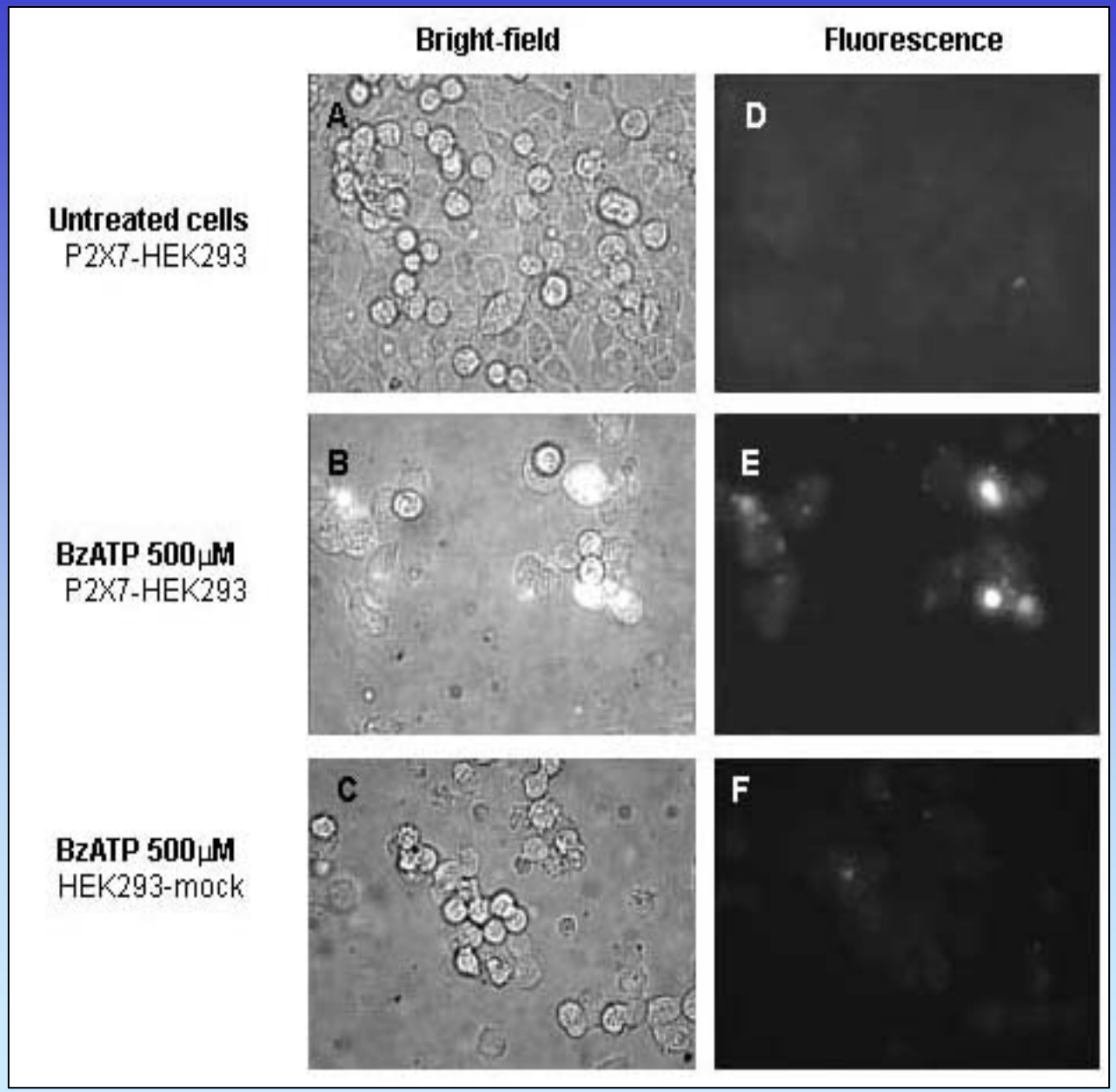
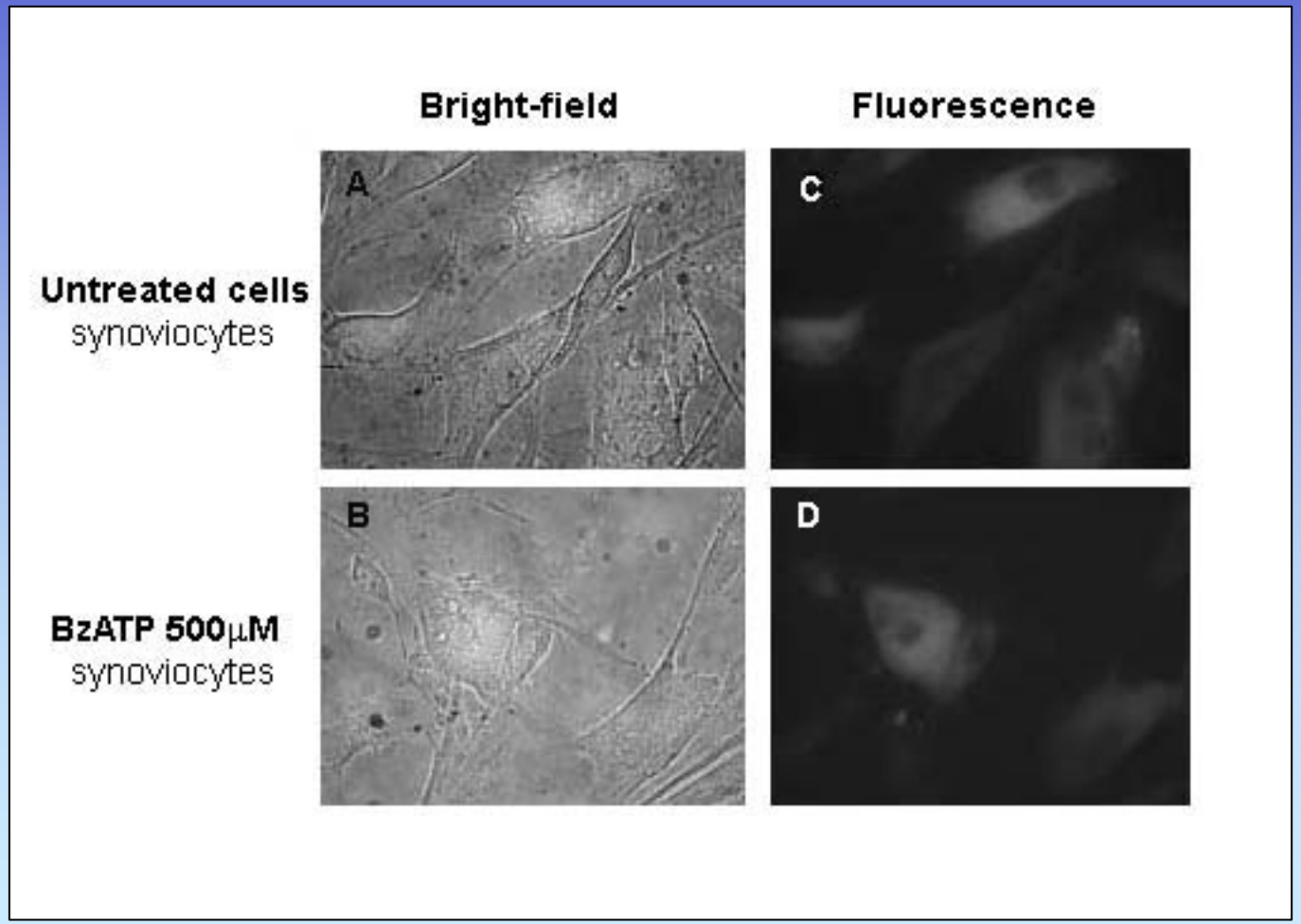
YO PRO UPTAKE IN HEK293

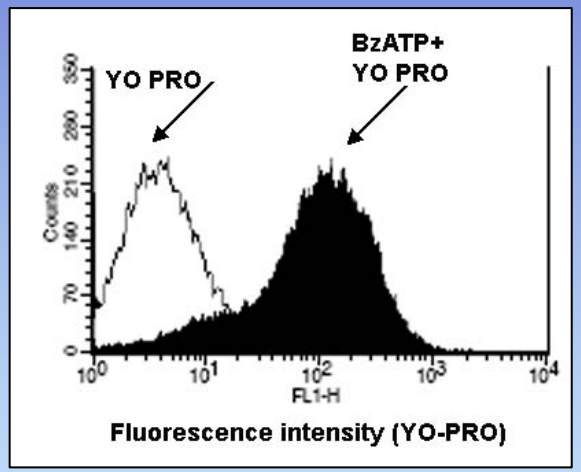


YO PRO UPTAKE IN SYNOVIOCYTES

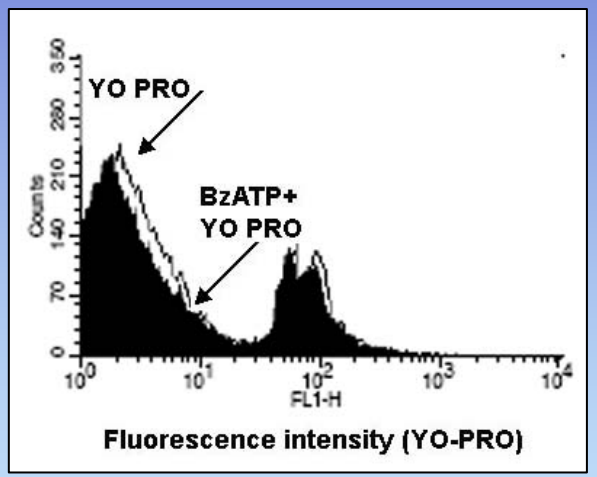


YO PRO UPTAKE IN HEK293 AND SYNOVIOCYTES

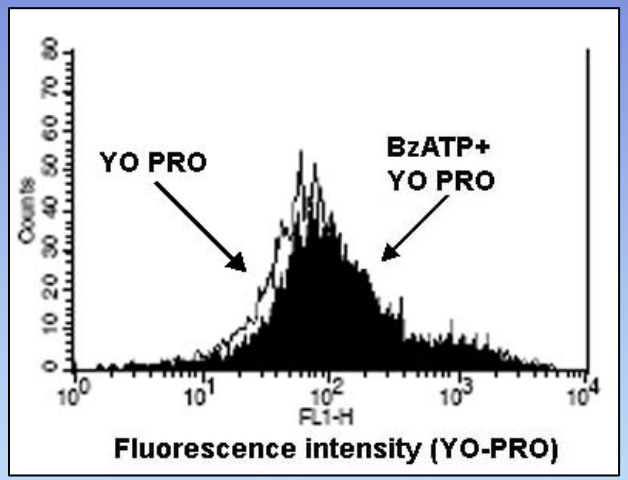
P2X$_7$-HEK 293



HEK 293 mock



synoviocytes



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CONCLUSIONS

- ✓ Human type-B synoviocytes from Rheumatoid Arthritis patients express multiple purinergic P2 receptors (P2X and P2Y receptors)
- ✓ P2 receptor subtypes are coupled to $[Ca^{2+}]_i$ mobilization
- ✓Stimulation with agonist BzATP induces IL-6 mRNA transcription and the release of the protein
- ✓IL-6 release is not the consequence of cell death
- ✓ $P2X_7$ stimulation does not cause pore formation
- ✓Purinergic receptor(s) may represent a possible target for the pharmacological manipulation of the proinflammatory activity of synoviocytes

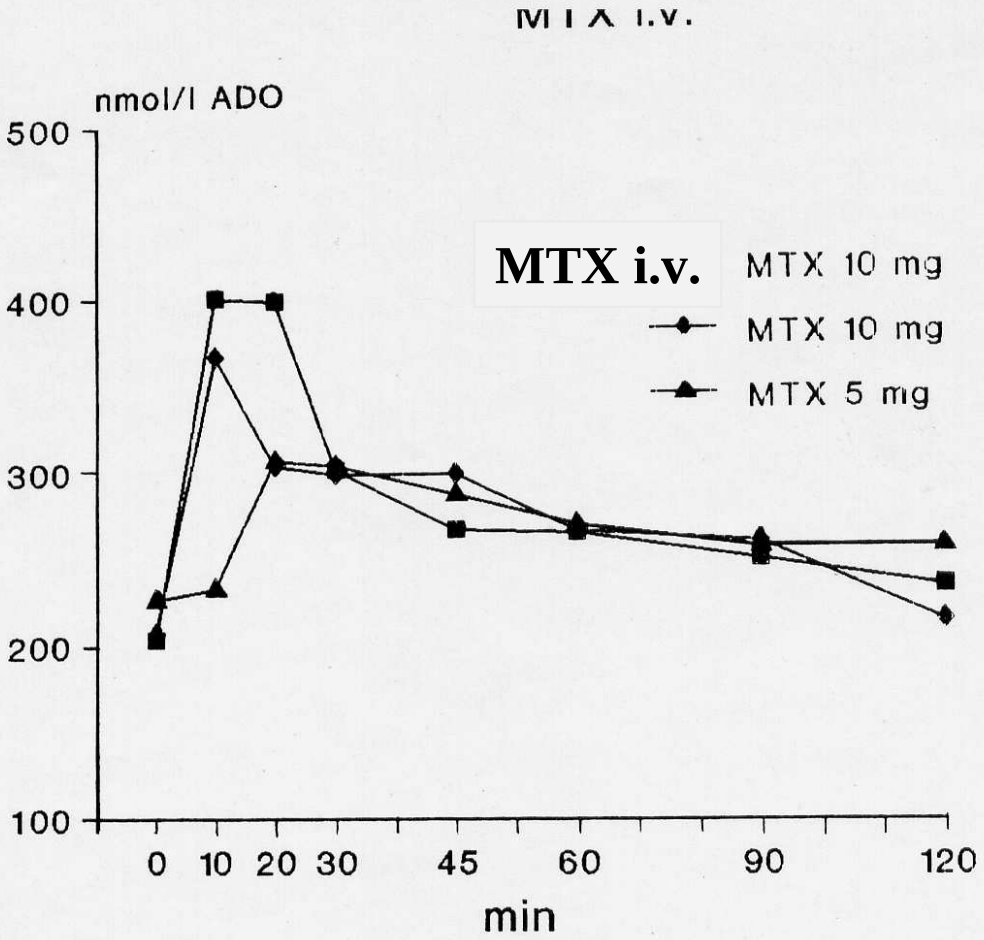
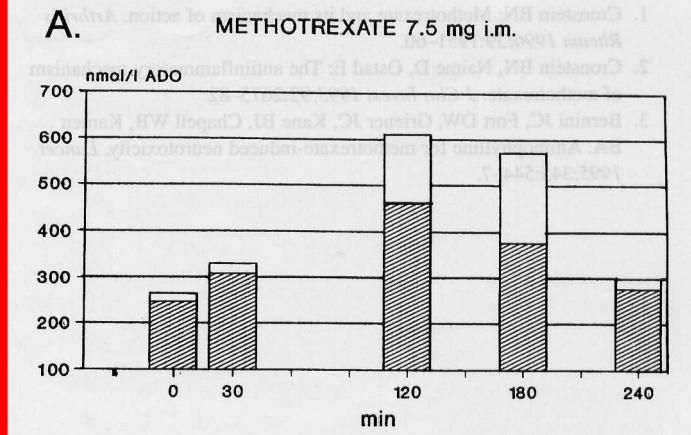
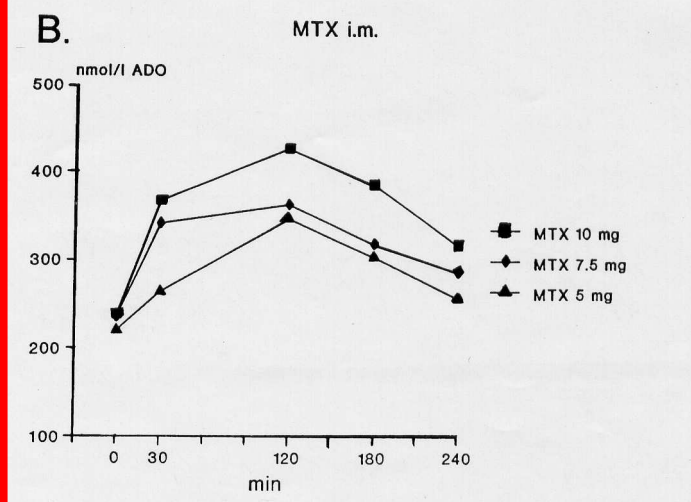


Figure 1. Plasma levels of adenosine (ADO) after iv administration of MTX.





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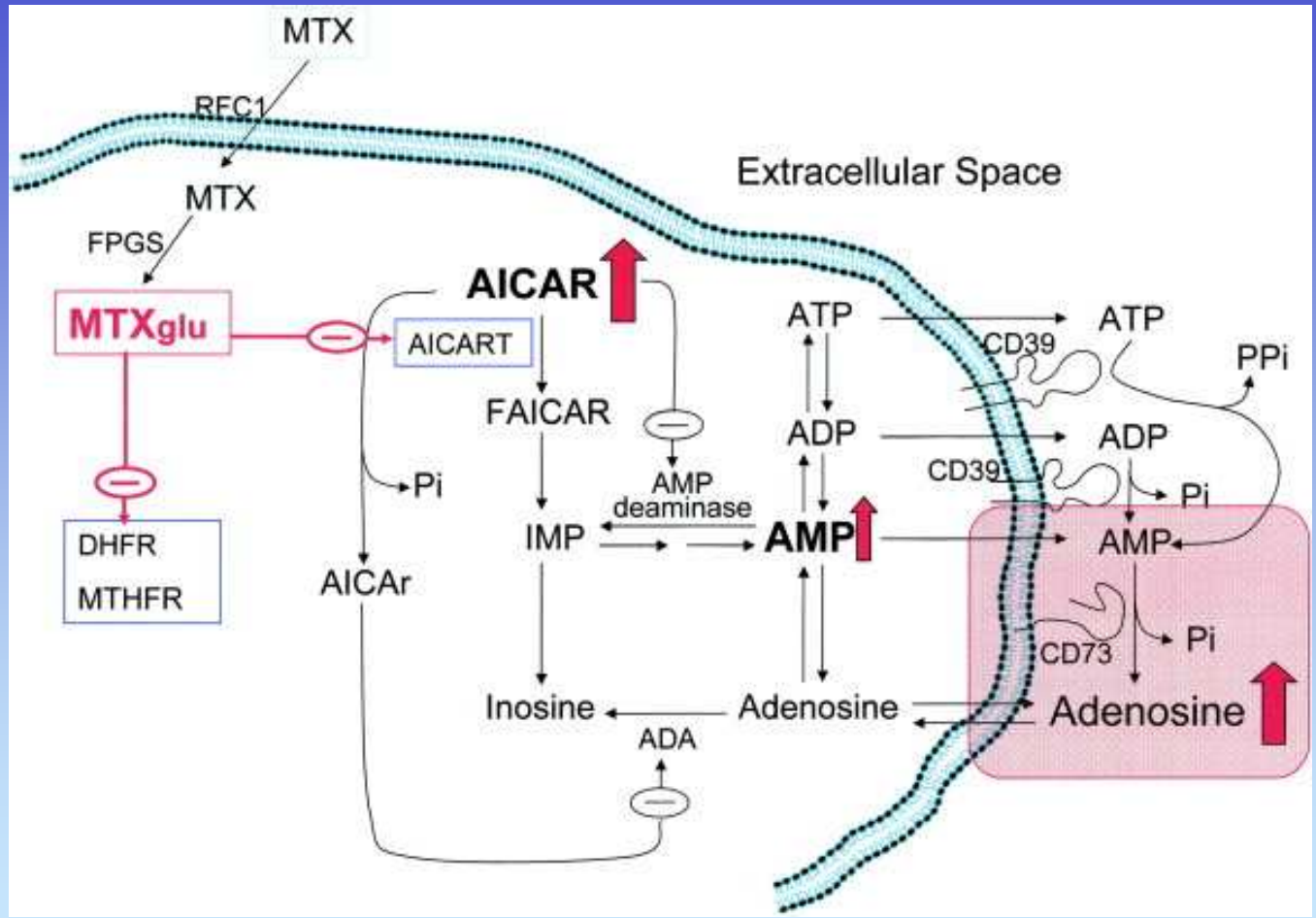
Effetto anti-infiammatorio del metotrexato ed adenosina



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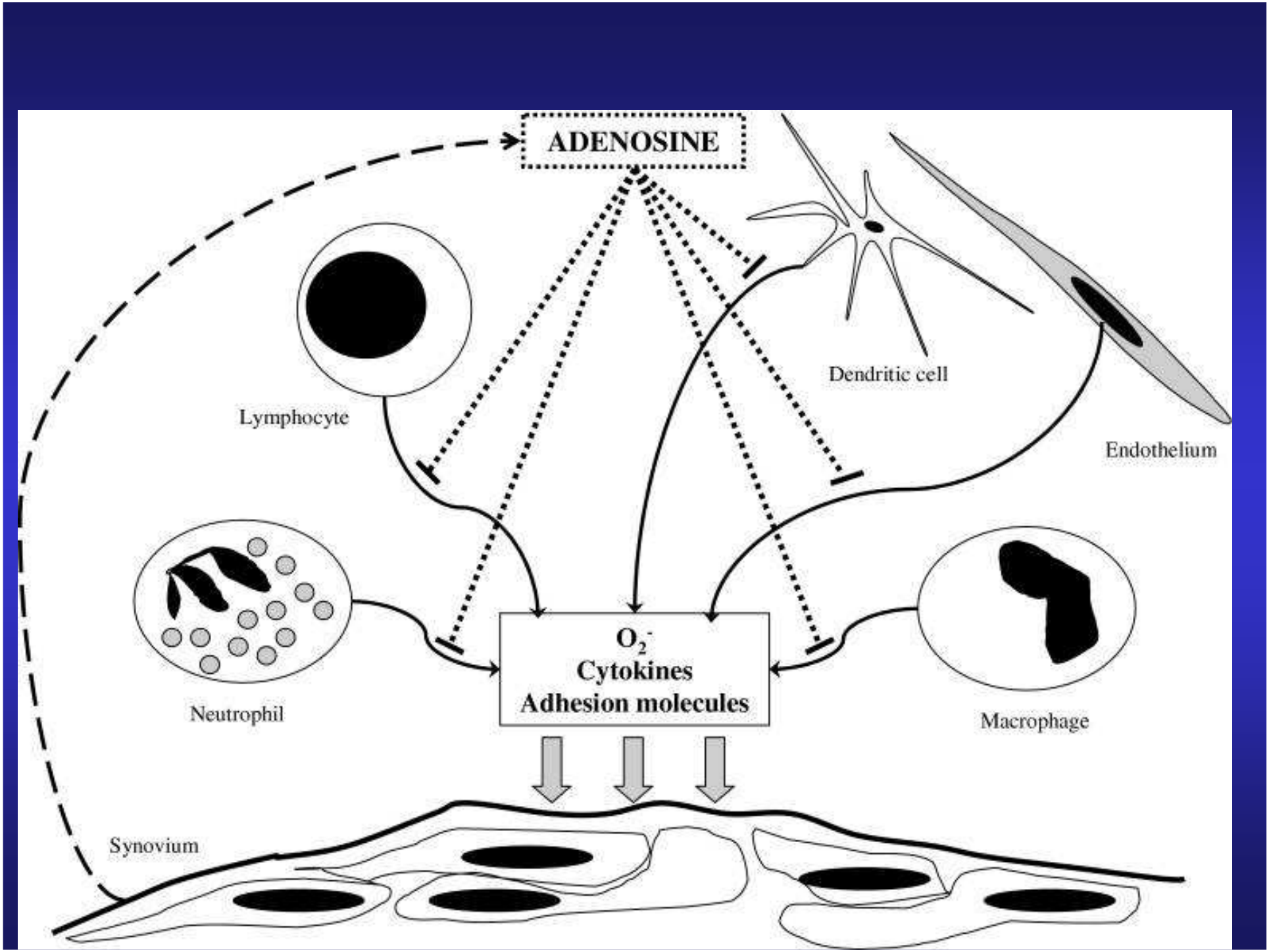
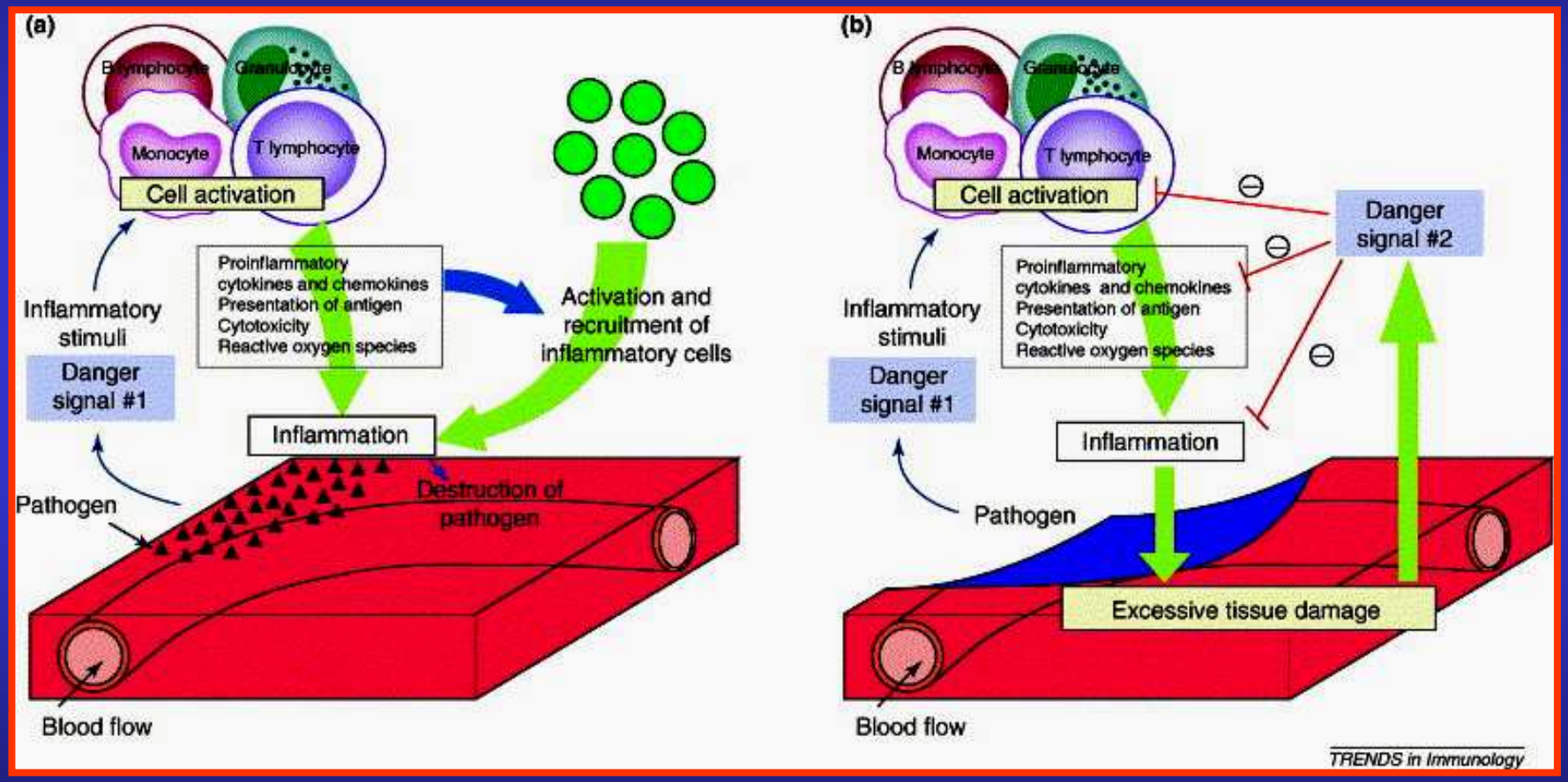

ADENOSINE
Lymphocyte
Dendritic cell
Endothelium
Neutrophil
O_2^-
Cytokines
Adhesion molecules
Macrophage
Synovium

AGENDA

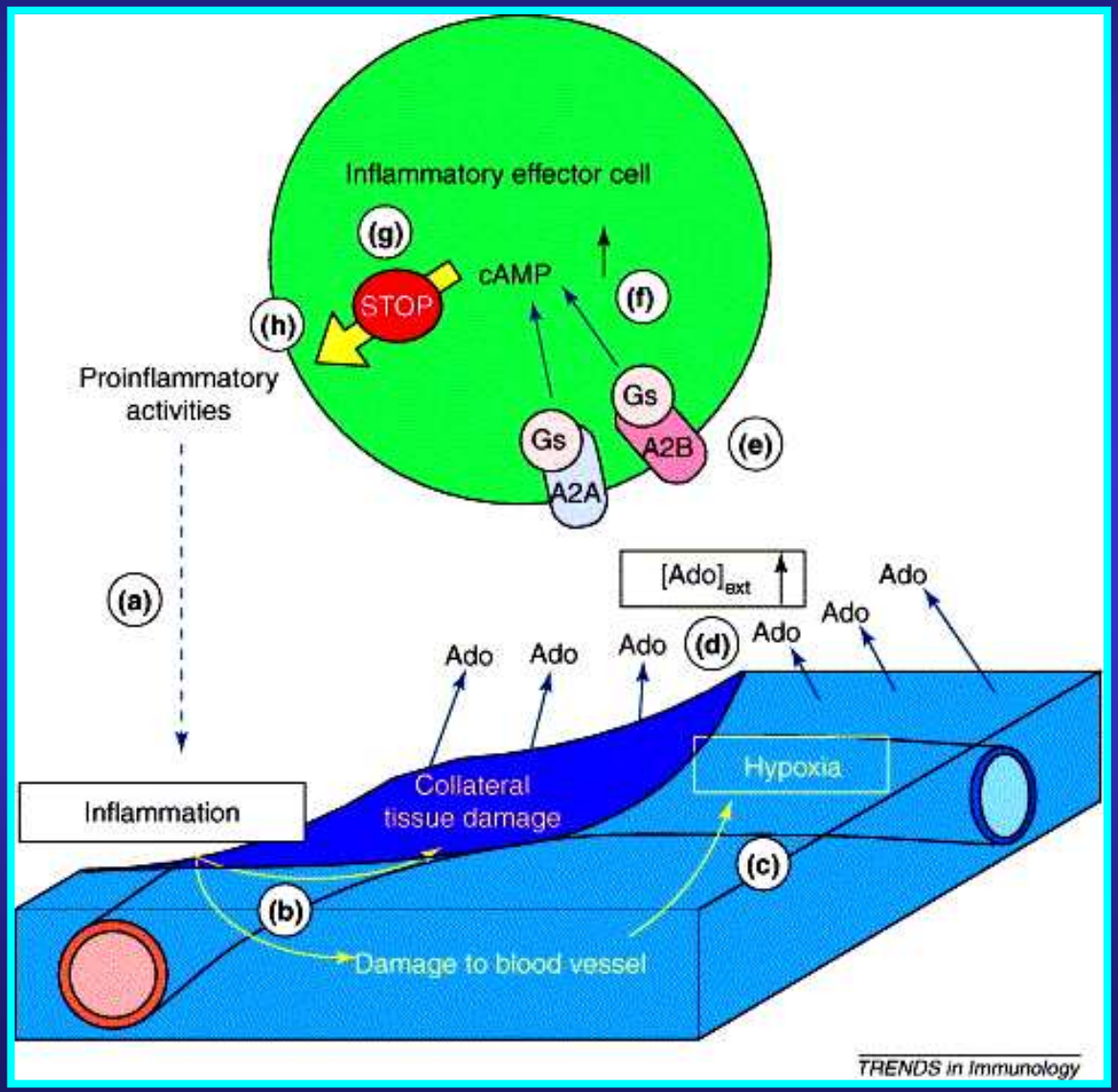
- General overview
- P2X7R
- A2AR
- Rheumatoid Arthritis
- “The odd couple”

Two danger-signal model of an immune response



Sitkovsky MV, Ohta A. *Trends Immunol* 2005; 26: 299-304

Danger signal triggers the delayed negative feedback inhibition of activated immune cells in inflamed tissue microenvironment



Sitkovsky MV, Ohta A.
Trends Immunol 2005; 26: 299-304