Platelets are both targets and mediators of innate immunity.

John W. Semple

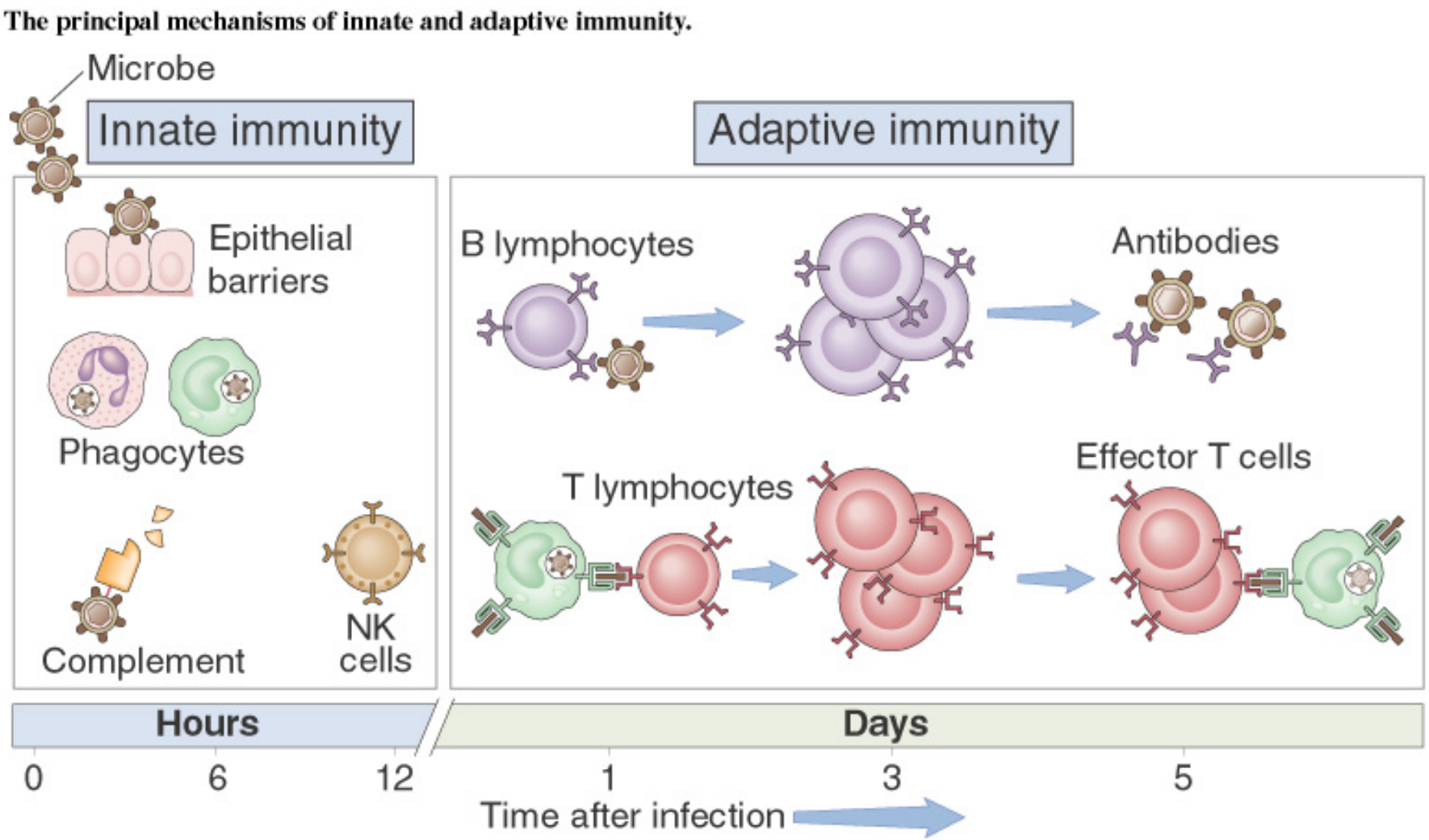
Toronto Platelet Immunology Group,
St. Michael's Hospital,
Toronto, Ontario, Canada.

Outline of talk:

1. Innate and adaptive immunity principles.
2. A crash course on platelets.
3. How platelets are seen by innate immune responses.
4. Platelets also mediate innate immunity.
5. Conclusions.

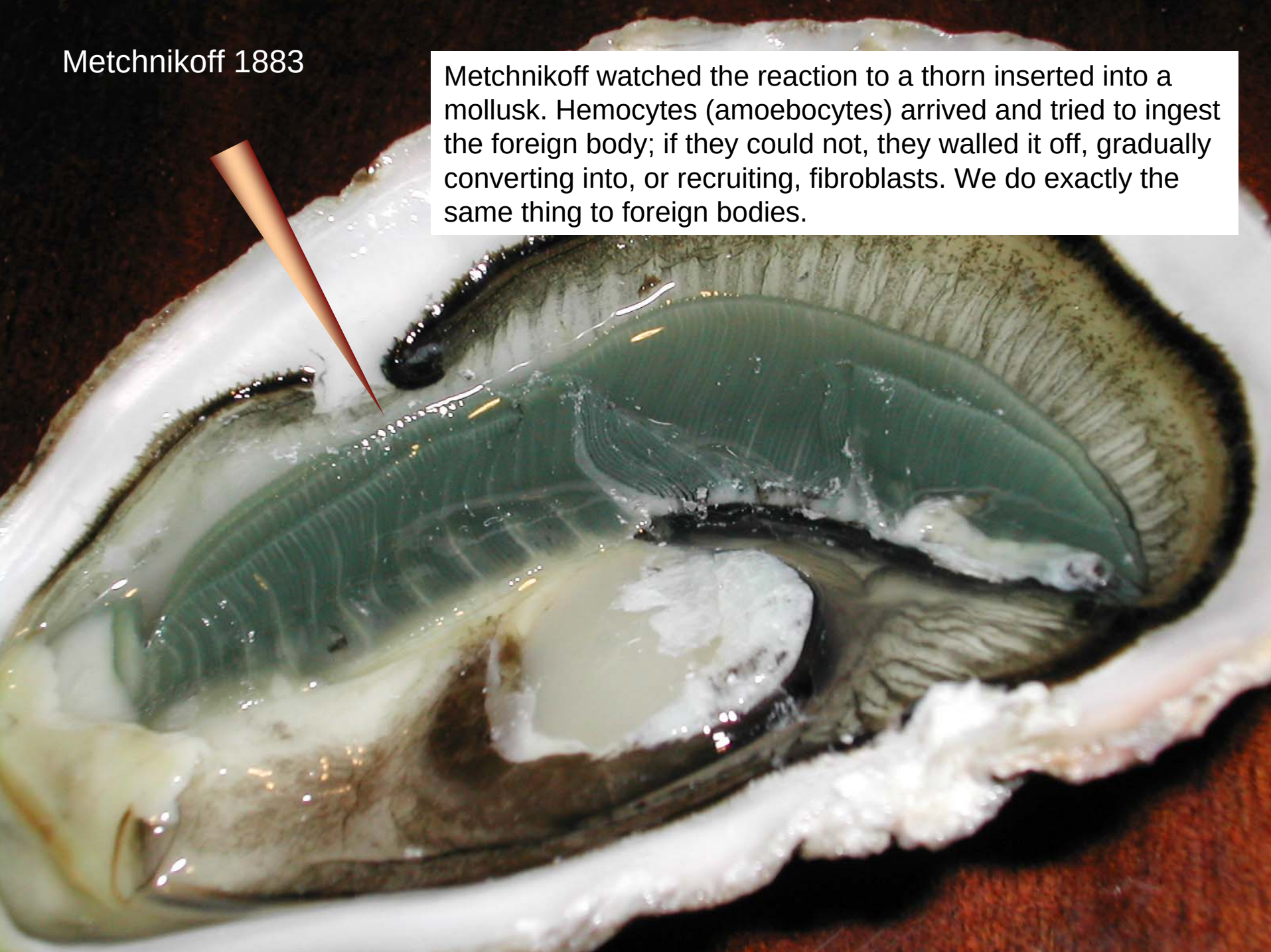
1. **Innate and adaptive immunity principles.**

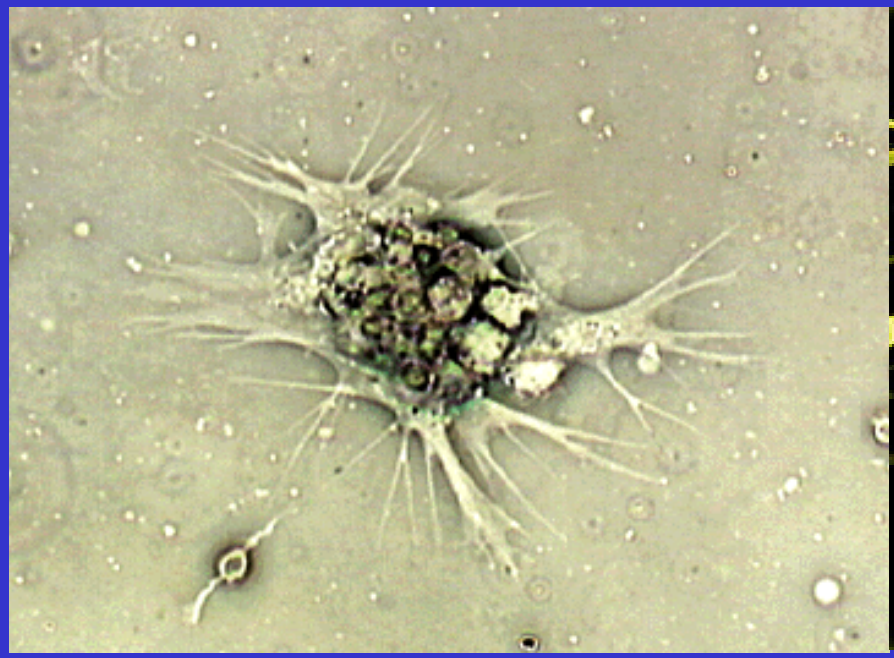
The Immune System:



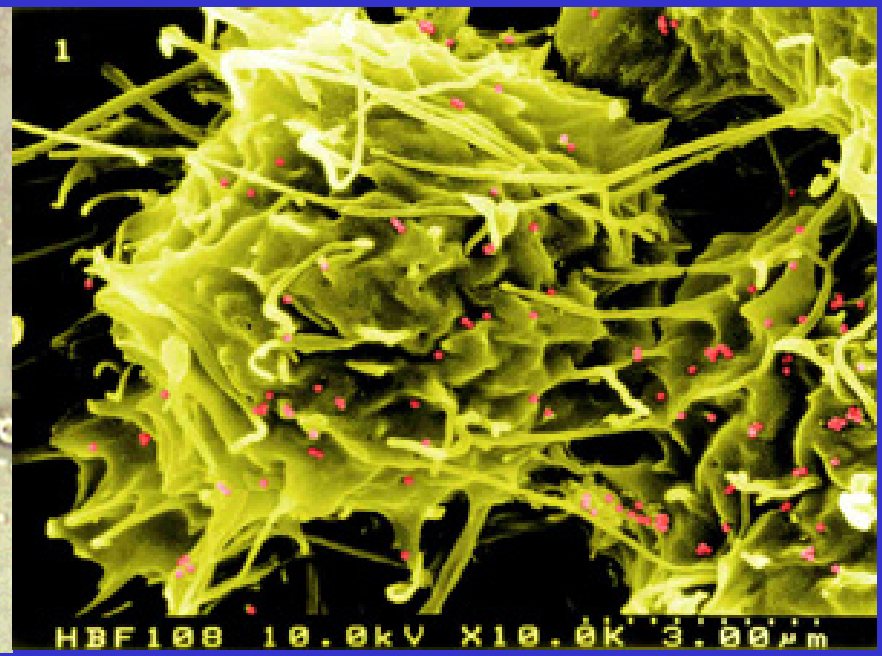
Metchnikoff 1883

Metchnikoff watched the reaction to a thorn inserted into a mollusk. Hemocytes (amoebocytes) arrived and tried to ingest the foreign body; if they could not, they walled it off, gradually converting into, or recruiting, fibroblasts. We do exactly the same thing to foreign bodies.





Oyster hemocyte



Mouse macrophage

The similarity between an invertebrate phagocyte and our own is striking; they also use many of the same mechanisms, including the production of reactive oxygen species.

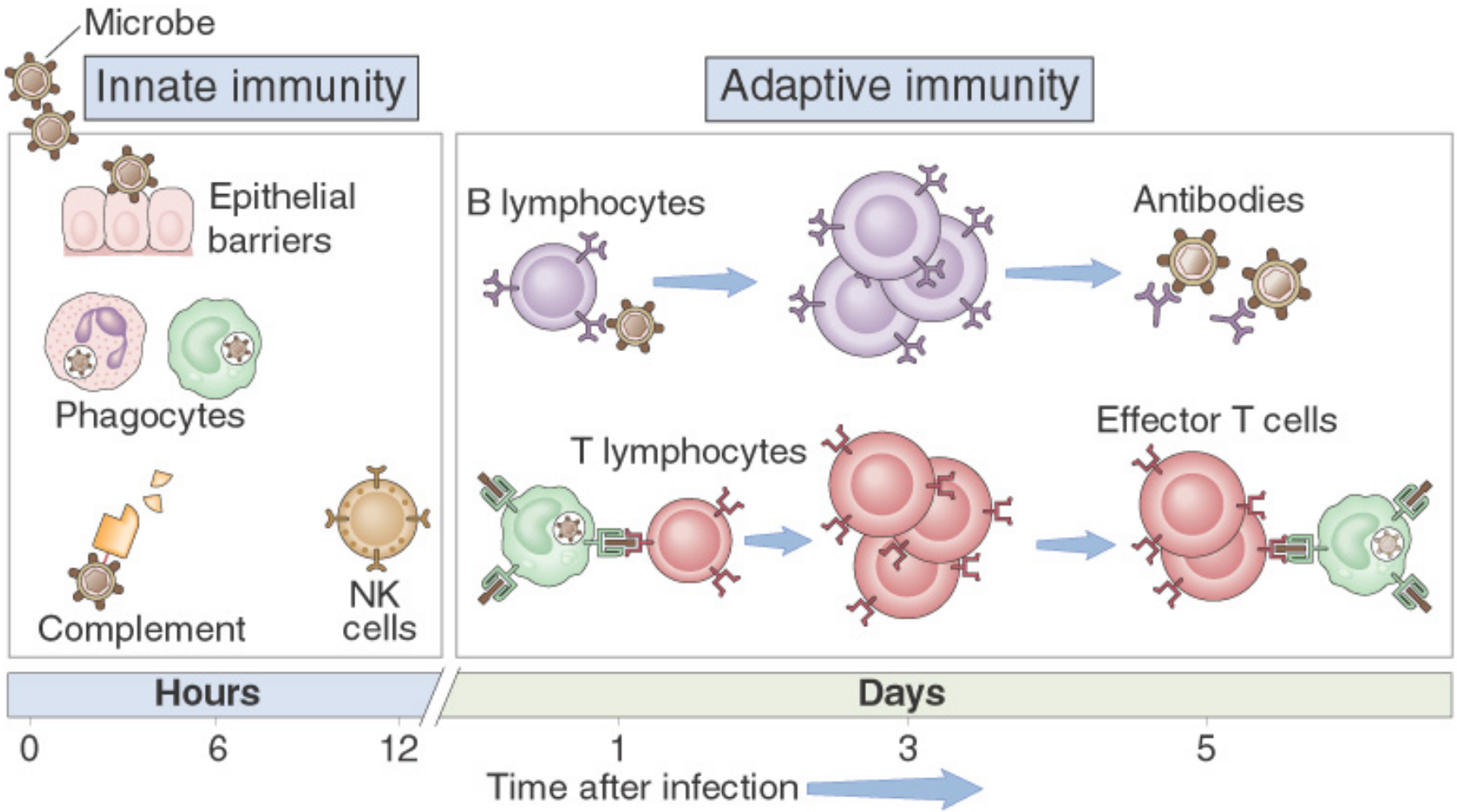
Evolution of Immunity:

In ecology: there are r-strategists and K-strategists:

Oysters: are r-strategists: have huge numbers of progeny but invest little in the survival of any individual. So they have the simple innate immune response, but cannot amplify it, nor do they adapt to specific challenges.

Humans: are K-strategists; small numbers of progeny, heavy investment in individual survival. So we (jawed vertebrates) evolved the adaptive immune response which vastly increases our chances of fighting infection (PIDs, Bubble Boy: born without adaptive immunity and unable to live in the real world).

The principal mechanisms of innate and adaptive immunity.



Dogma not so long ago:

Innate and adaptive immunity are generally separate entities.

It is adaptive immunity that is more important in the hierarchy of immunity (self/non-self era).

The adaptive immune system is normally off. The Innate immune system instructs the adaptive immune system to respond to microbial infection.

The major decision to adaptively respond or not respond to a particular ligand is decided by the genome-encoded receptors of the innate immune system (e.g. TLR, MannoseR, CD36 etc).

Non-infectious self vs. infectious non-self.

Janeway, 1989

Adaptive immunity only responds to injury, insult or "Danger" *(*'**bad cell death**').

Matzinger, 1994

Immune Diversity:

Charles Janeway

Feb 5, 1943-April 12, 2003
(91y after Titanic sunk)

Fourth generation of Prominent Physicians:

- **-him:** was at Yale.
- **-dad:** physician-in-chief, Boston Children's Hospital
- **-grandfather:** 1st full-time professor at Johns Hopkins
- **-great grand dad:** 1st New York health commissioner.
- **-Janeway** now considered: ***"Father of Innate Immunity"***

Polly Matzinger

Amazing past:

- **-playboy bunny** (Denver,1969)
- **-bar waitress** (how she found her PhD supervisor, 1974, UCDavis, Robert Schwab)
- **-accomplished Jazz musician**
- **-carpenter**
- **-dog trainer** (represented US at 2005 World Sheepdog Finals, Tullamore Ire.)
- **-section head** (NIH Allergy, Infect.Dis., Ghost lab)

IN A FULLY H-2 INCOMPATIBLE CHIMERA, T CELLS OF DONOR ORIGIN CAN RESPOND TO MINOR HISTOCOMPATIBILITY ANTIGENS IN ASSOCIATION WITH EITHER DONOR OR HOST H-2 TYPE*

BY POLLY MATZINGER AND GALADRIEL MIRKWOOD

(From the Department of Biology, University of California San Diego, La Jolla, California 92093)

Despite much recent interest and effort, the role played by major histocompatibility complex products in the regulation of T-cell responses remains perplexing. In 1972 it was observed that mouse T and B cells would only cooperate in an antibody reponse if they shared certain regions of H-2 (1). Subsequently, H-2 gene products were also found to be involved in cytotoxic T-cell reactions, and it was postulated that the killer T cell must bear H-2 molecules in common with those of its target in order to effect lysis (2–6). Later studies with radiation chimeras showed that this is not the case, but that the H-2 region must be shared between the cells used to stimulate the response and the targets; a killer T cell that was itself H-2 type A, after having grown up in an (A × B)F_1 could be stimulated to lyse H-2 type B virus-infected or trinitrophenyl-modified targets (7–9). Such chimeras were also found to contain A type helper T cells which can cooperate with B type B cells (10). It was then postulated that T-cell precursors "learn" to recognize the H-2 type of the host as self (11). Recent evidence shows that the host H-2 type of a chimera does distinctly influence the specificity of the responding T-cell population (12, 13) and that it is the H-2 type of the thymus that is important (13). Most of this work has been done with semiallogeneic chimeras (e.g., "A" bone marrow into an irradiated [A × B]F_1, or [A × B]F_1 bone marrow into an "A" or [A × C]F_1) where the responses were very strongly restricted by the H-2 type of the host. A small number of completely allogeneic chimeras was tested (e.g., "A" bone marrow into "B") and appeared to be immunoincompetent. The virtually absolute restriction of the semiallogeneic chimeras as well as the immunoincompetence of the fully allogeneic chimeras has led to much speculation and has been quoted as suggestive evidence for the dual recognition model of T-cell receptors (13).

We report here that in contrast to the results with virus-infected mice, fully allogeneic chimeras made by repopulating irradiated BALB/c(H-2^d) mice with BALB.B(H-2^b) bone marrow are well able to respond to minor histocompatibility

* Supported by U. S. Public Health Service grants CA 09174 and AI 08795.

[1] *Abbreviations used in this paper*: B10, C57BL/10Sn; C, BALB/c; C.B, BALB.B; C.K., BALB.K; Con A, concanavalin A; CTL, cytotoxic T lymphocyte; H antigen, histocompatibility antigen.

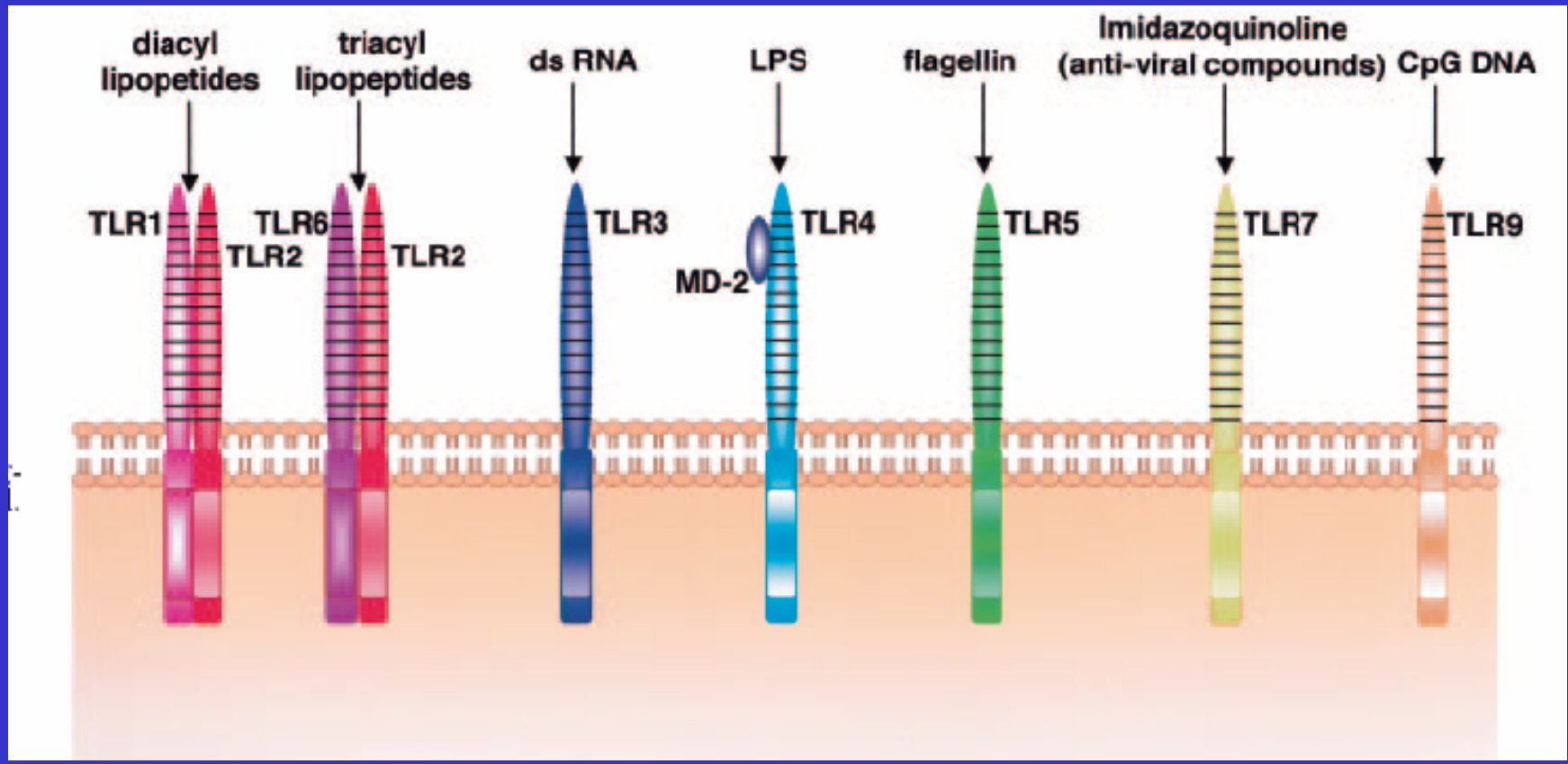
 J. EXP. MED. © The Rockefeller University Press · 0022-1007/78/0701-0084$1.00

Mirkwood: Probably the first and only published canine immunologist.

J Exp Med, 1978

J Exp Med banned her from publishing until the editor (G Kunkel) was dead.

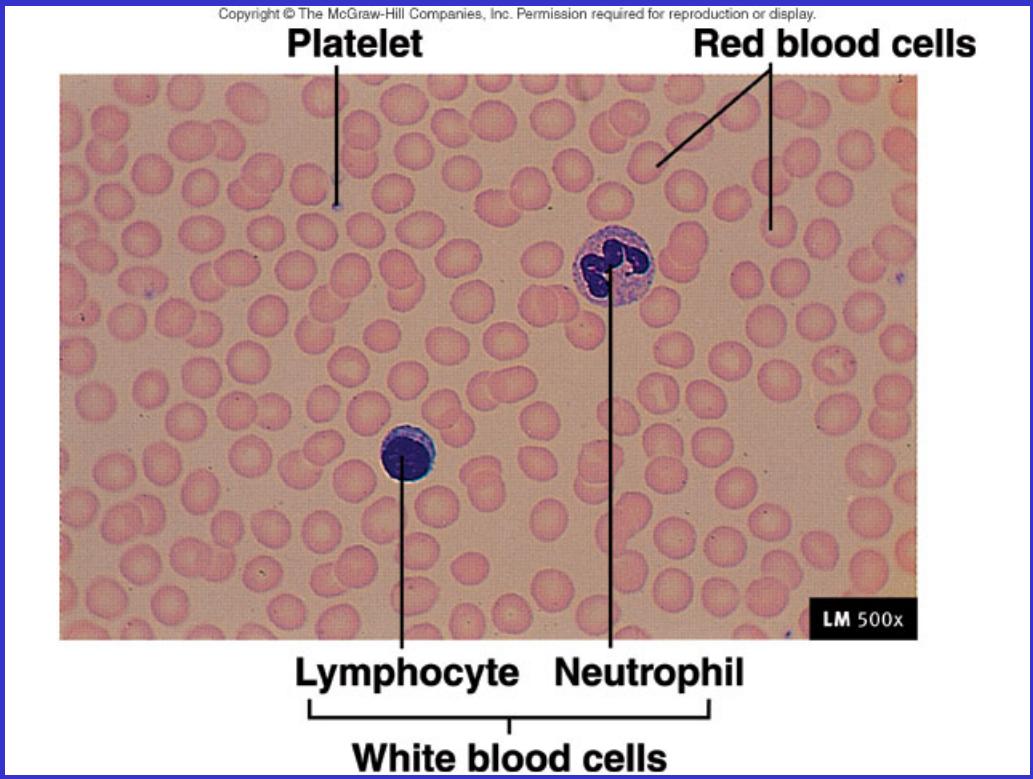
A family of genome-encoded receptors of innate immunity:



Akira *J.Biol.Chem.* 2003 278:38105–38108

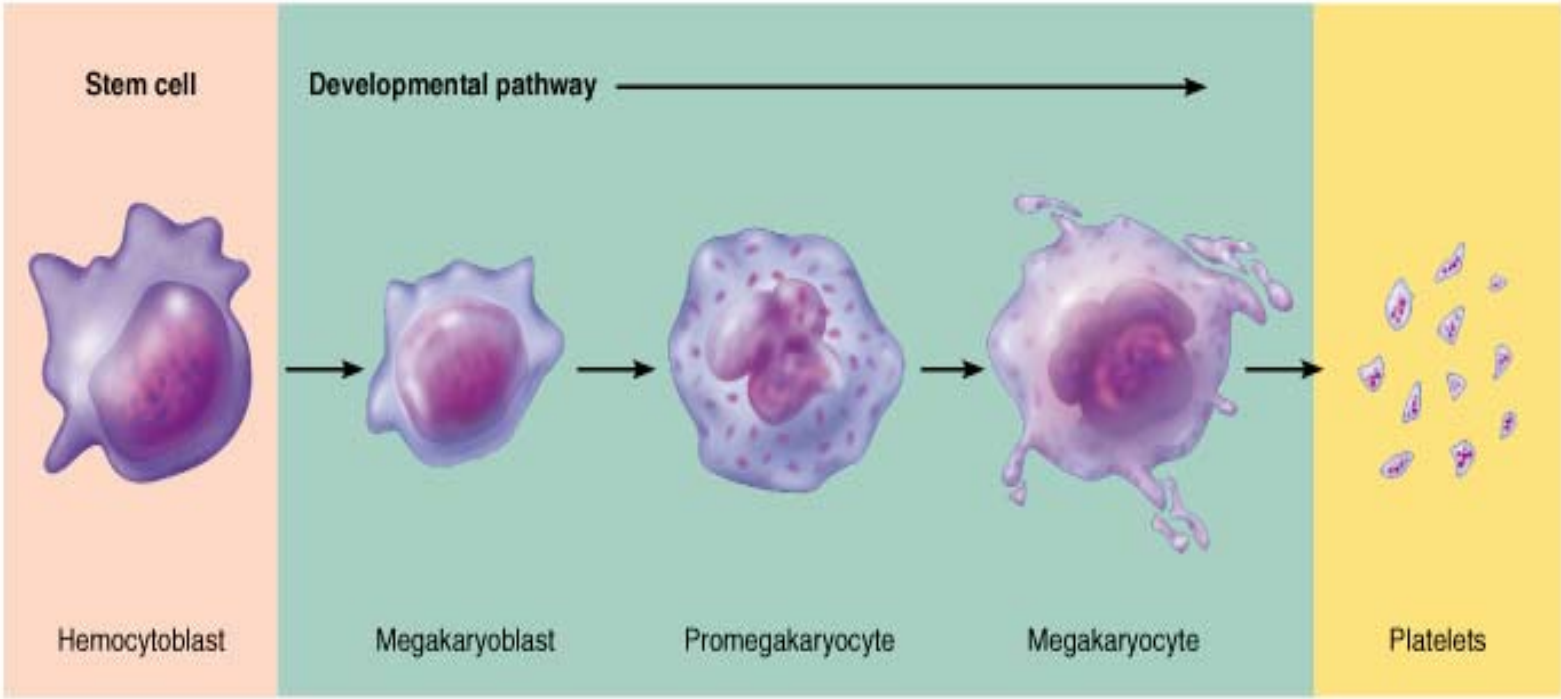
2. A crash course on platelets.

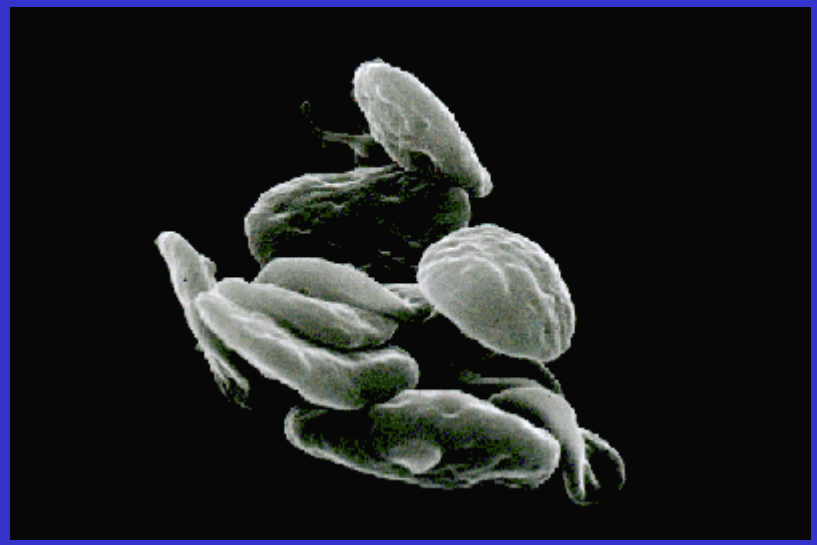
Platelets:



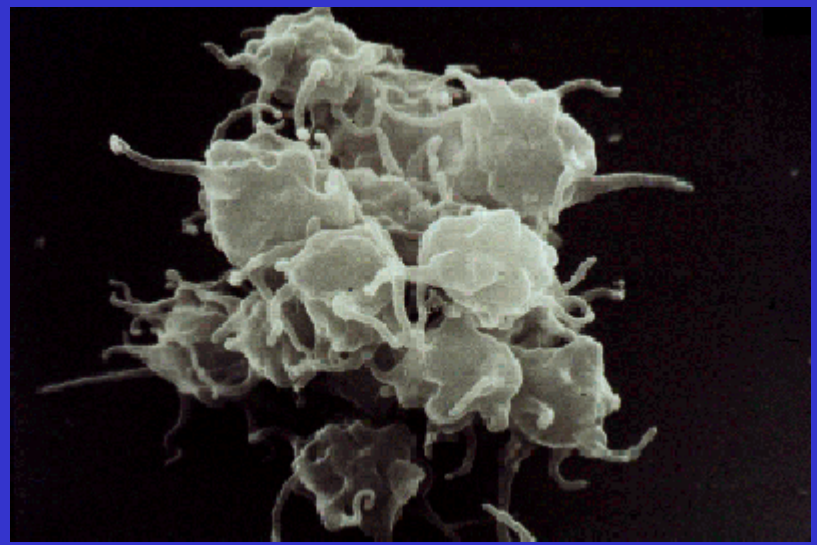
- Anucleated cell fragments pinched off from megakaryocytes in bone marrow
- Important in preventing blood loss
  - Platelet plug
  - Promote formation and contraction of clots

Normal range: 150-400x10^9/L



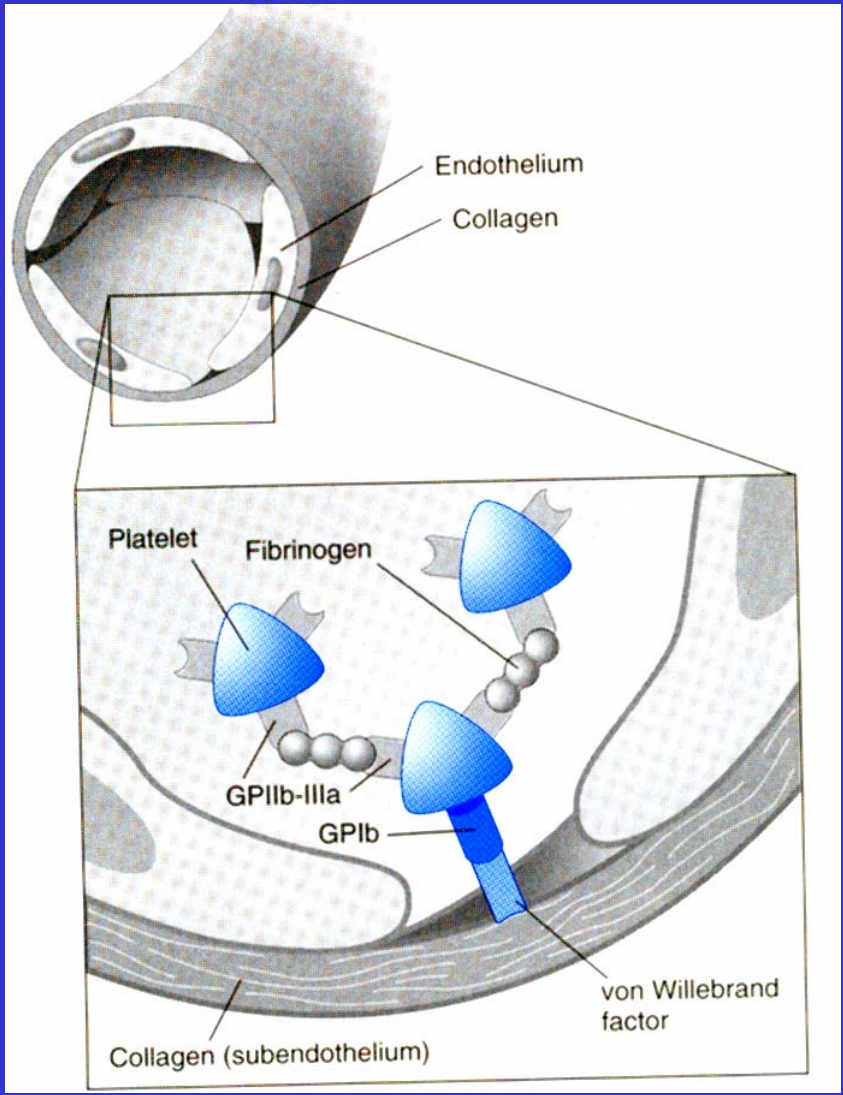


Resting

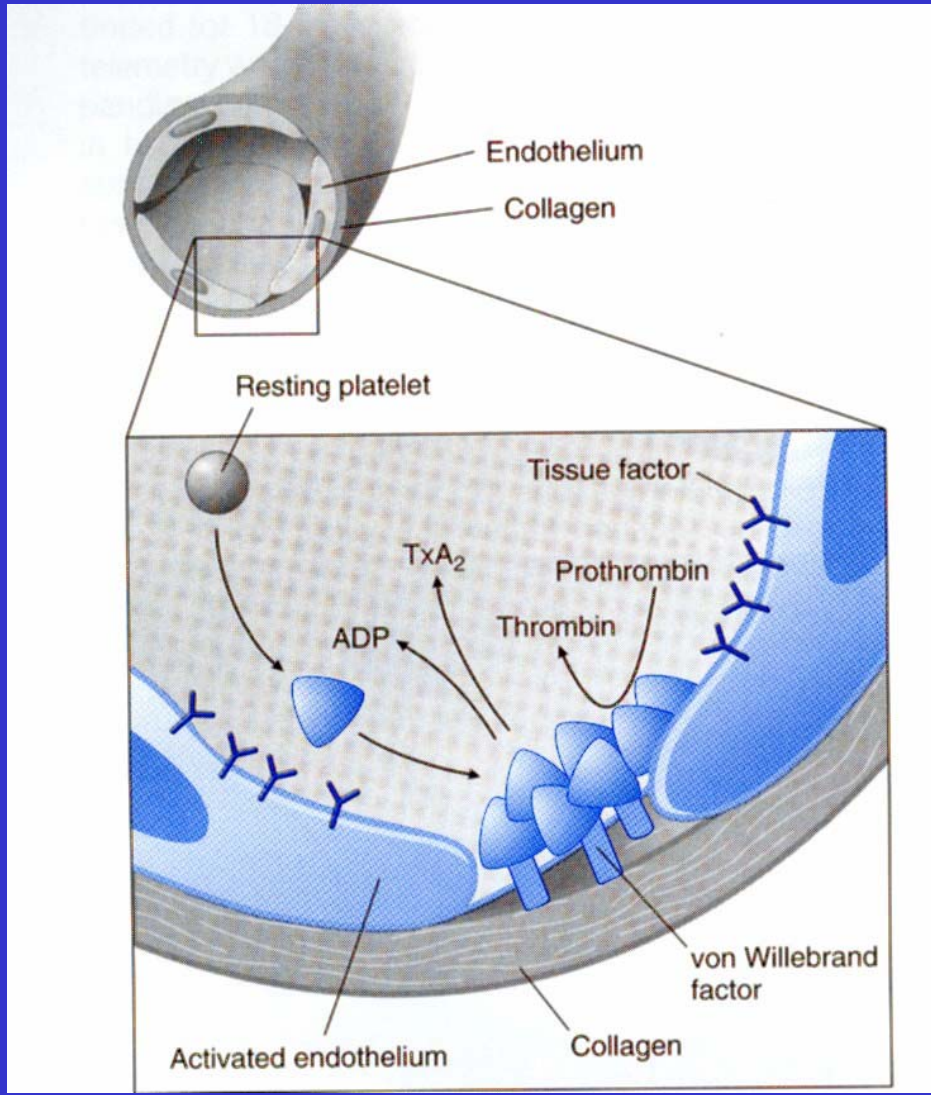


Active

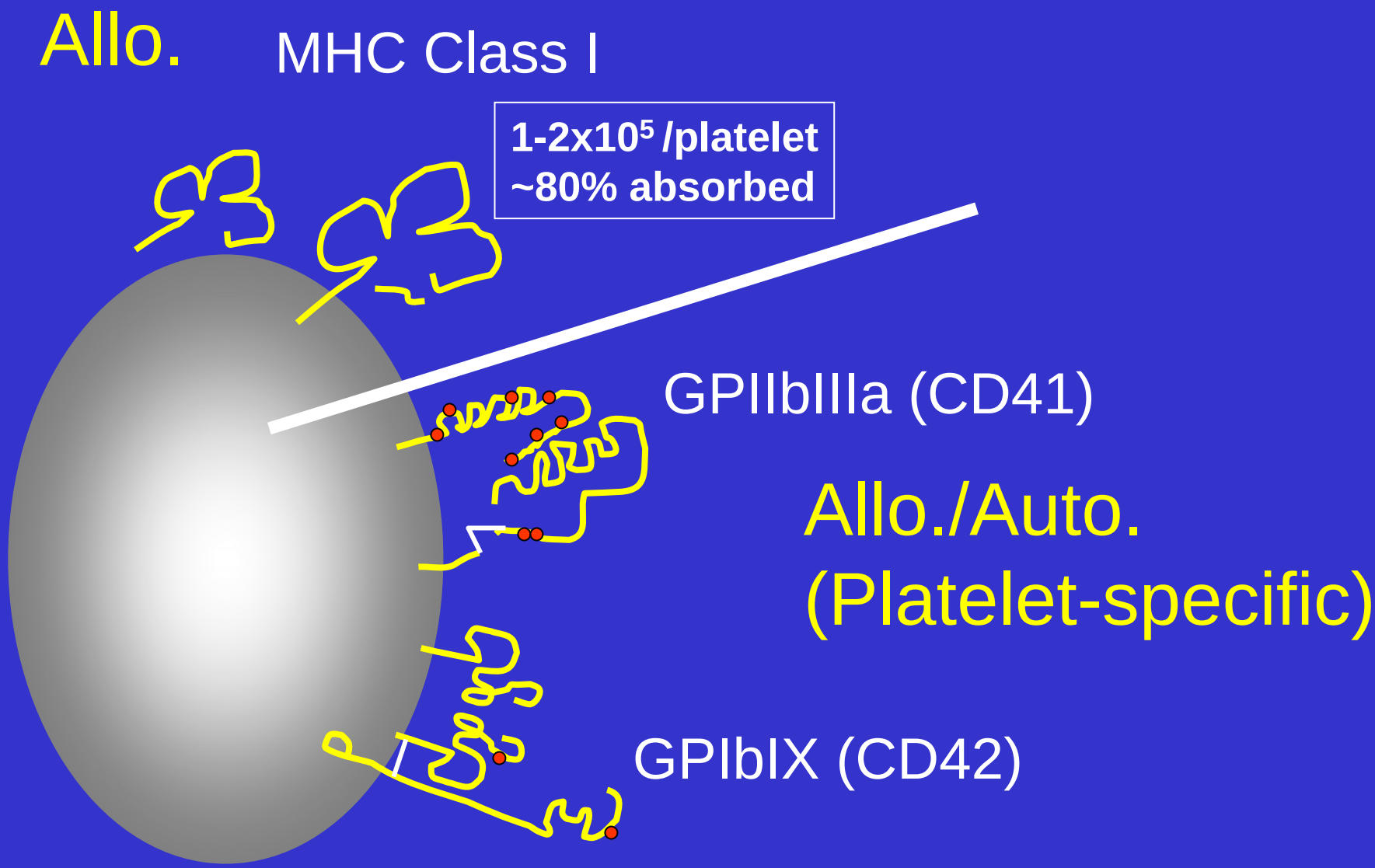
Platelet adhesion and aggregation



Platelet activation



Platelet Antigenic Profile:

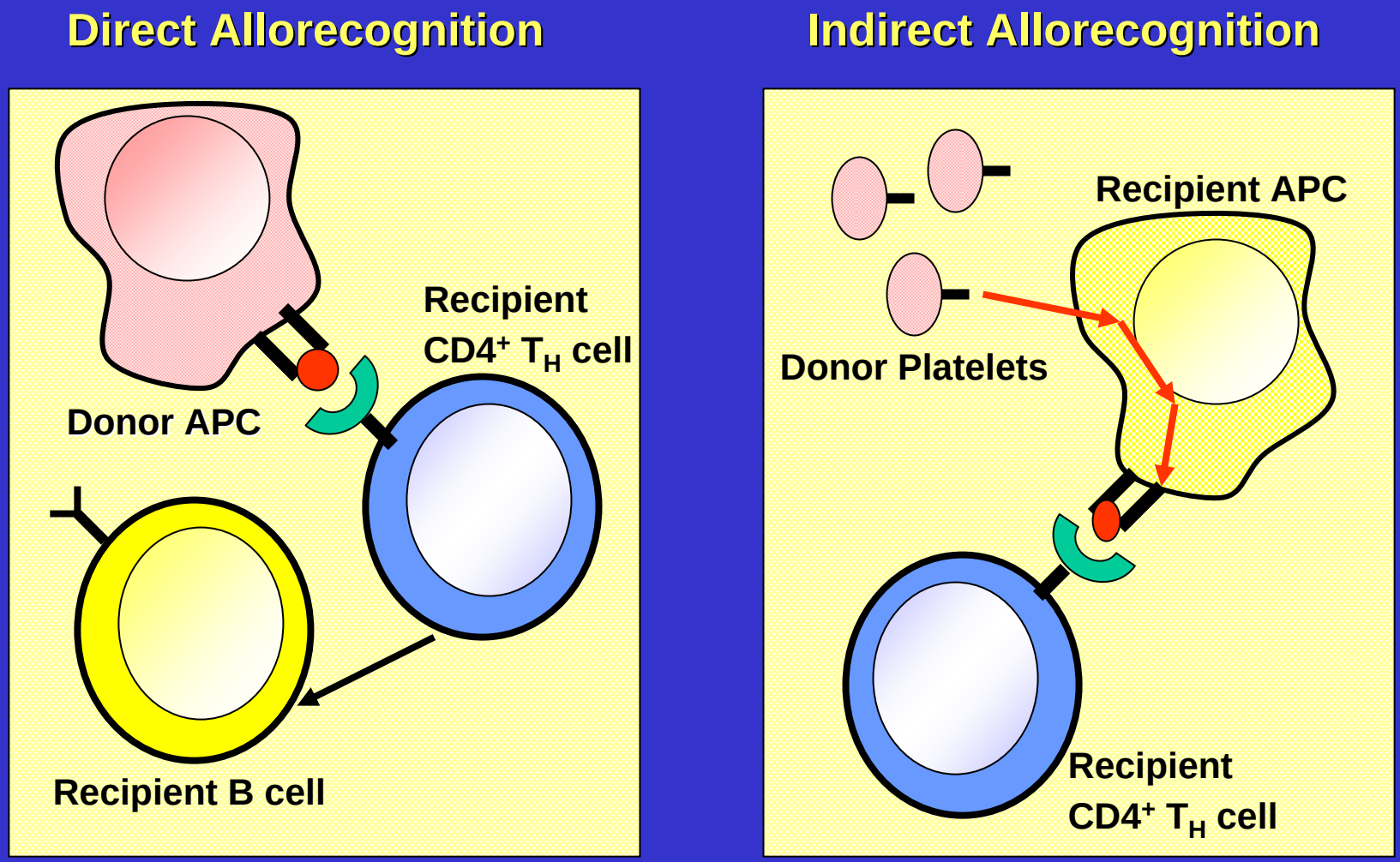


Platelets and Transfusions:

Adverse reactions/effects:

- Infectious disease transmission:
  - e.g. Hepatitis C, CMV, Trypanosomes (Chagas) etc.
- Febrile nonhemolytic transfusion reactions
  - fever, chills, rigors, nausea etc.
- Alloimmunization and platelet refractoriness.
  - antibody-mediated platelet destruction.

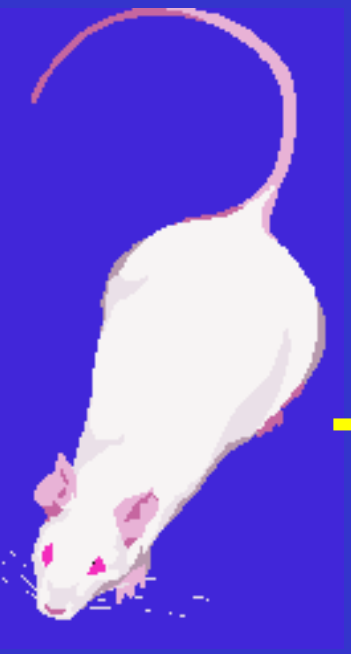
T cell Pathways Leading to Alloimmunization:



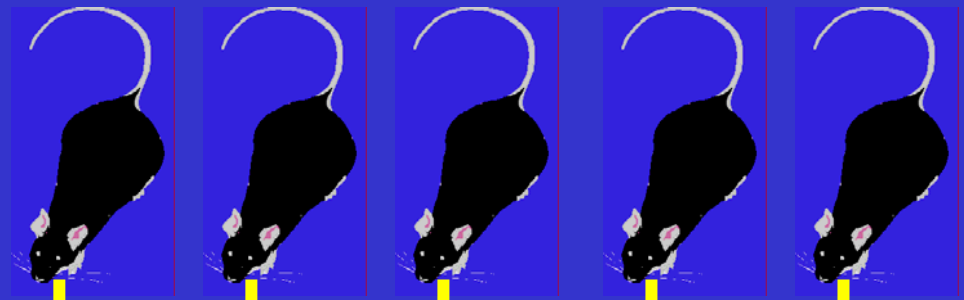
3. How platelets are seen by innate immune responses.

Transfusion protocol:

BALB/c
(H-2^d)



10^8 C57BL/6 (H-2^b) platelets weekly



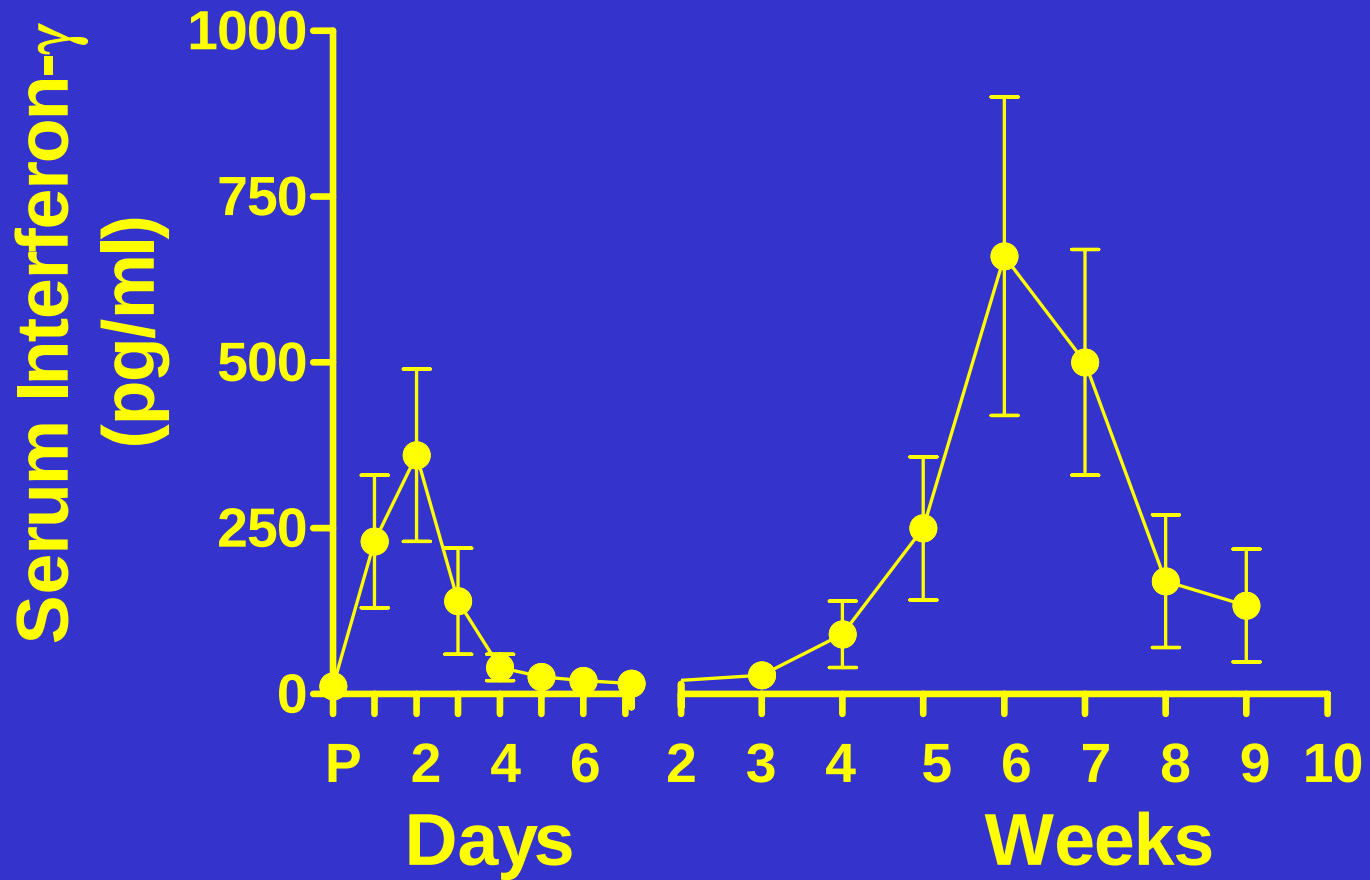
t

Serum Collection/Spleens

Pre-
bleed

Anti-donor antibodies
Serum Cytokines

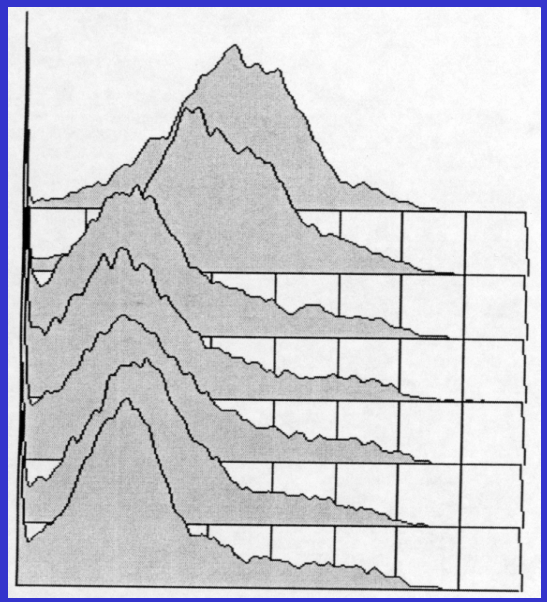
Platelet-induced Interferon levels:

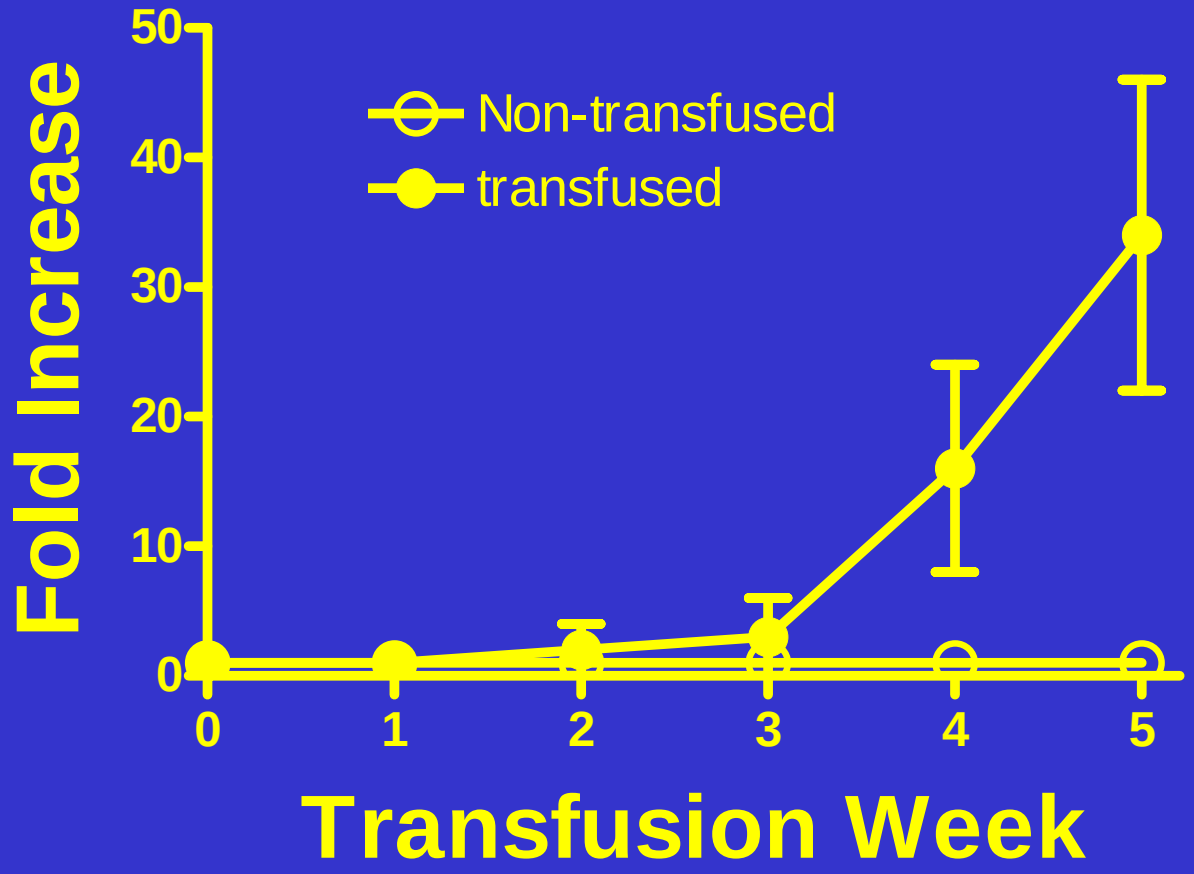


Bang et al, 1996

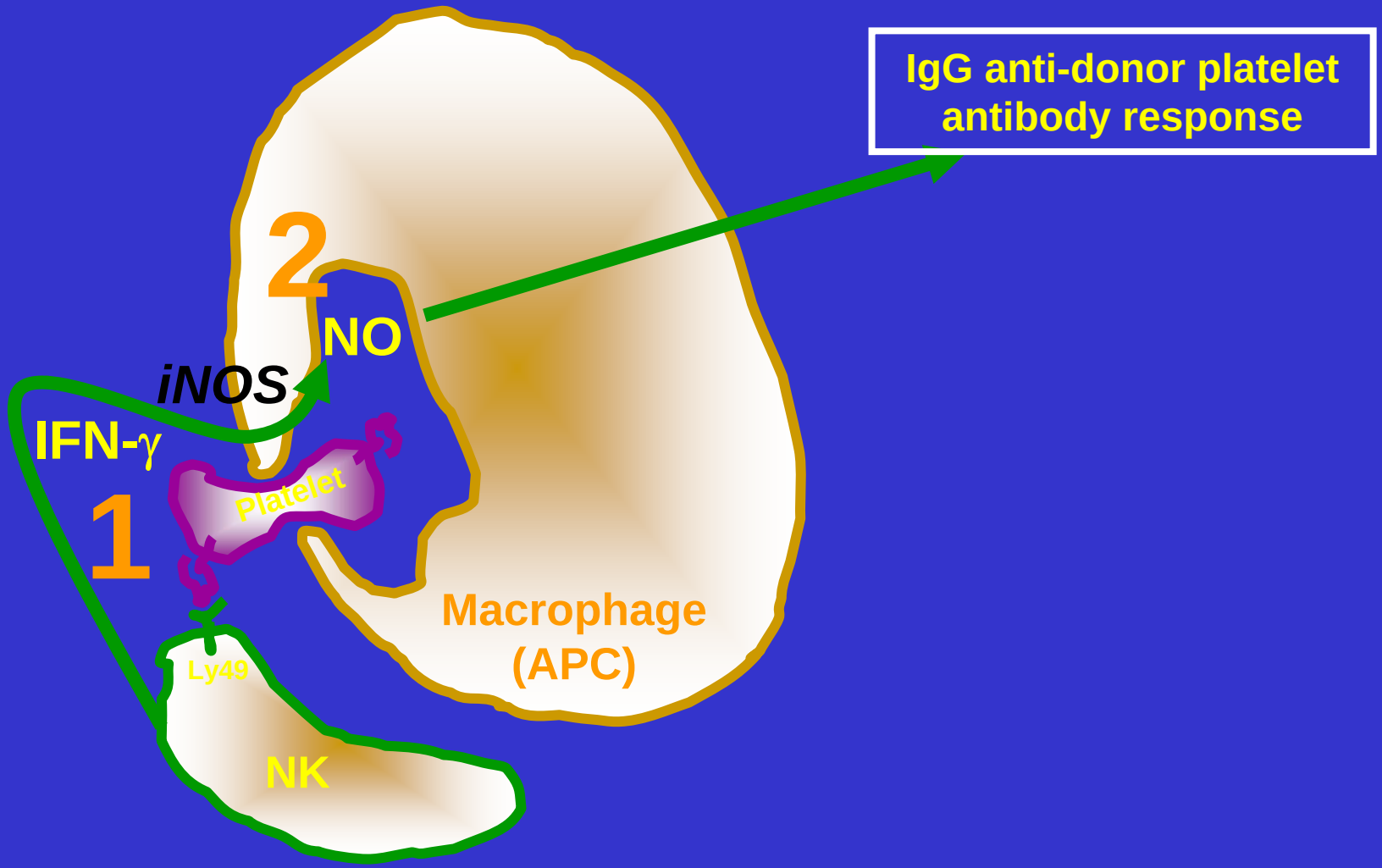
IgG anti-donor response against transfused platelets:

Individual mouse



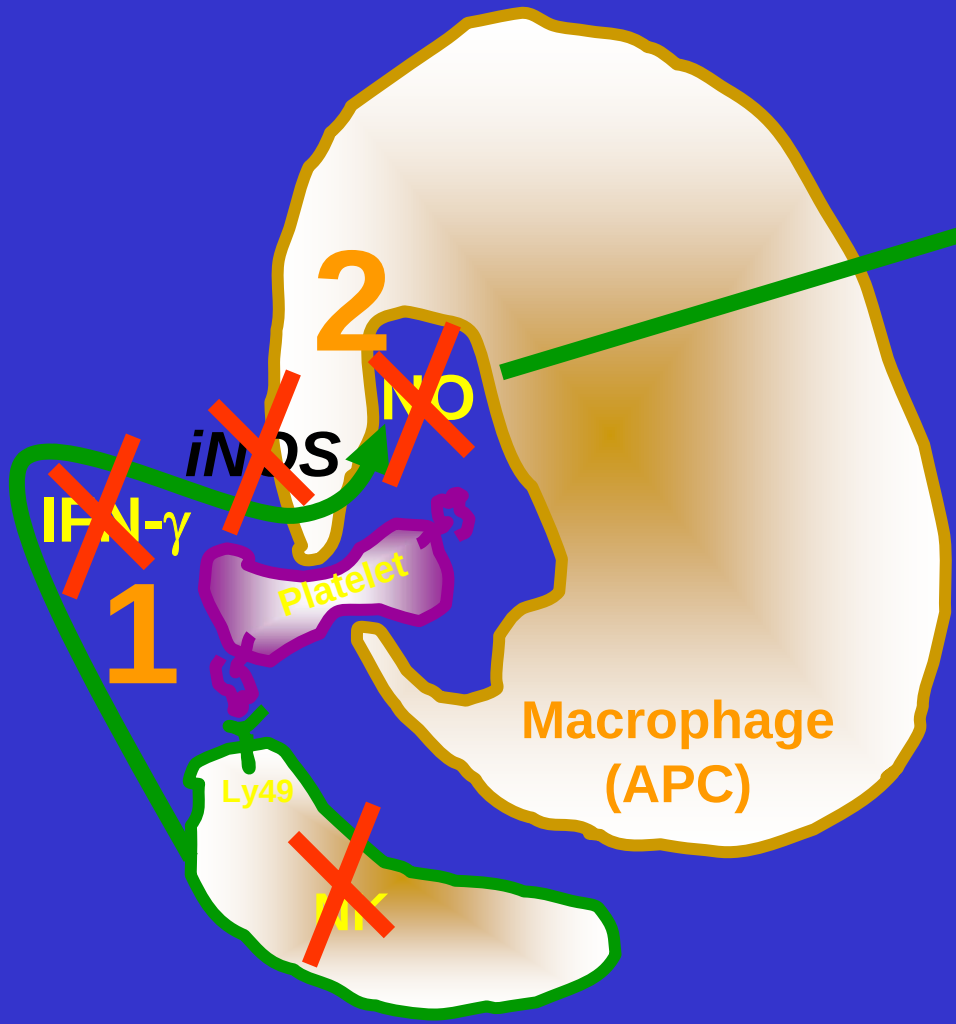


Semple et al, 1995



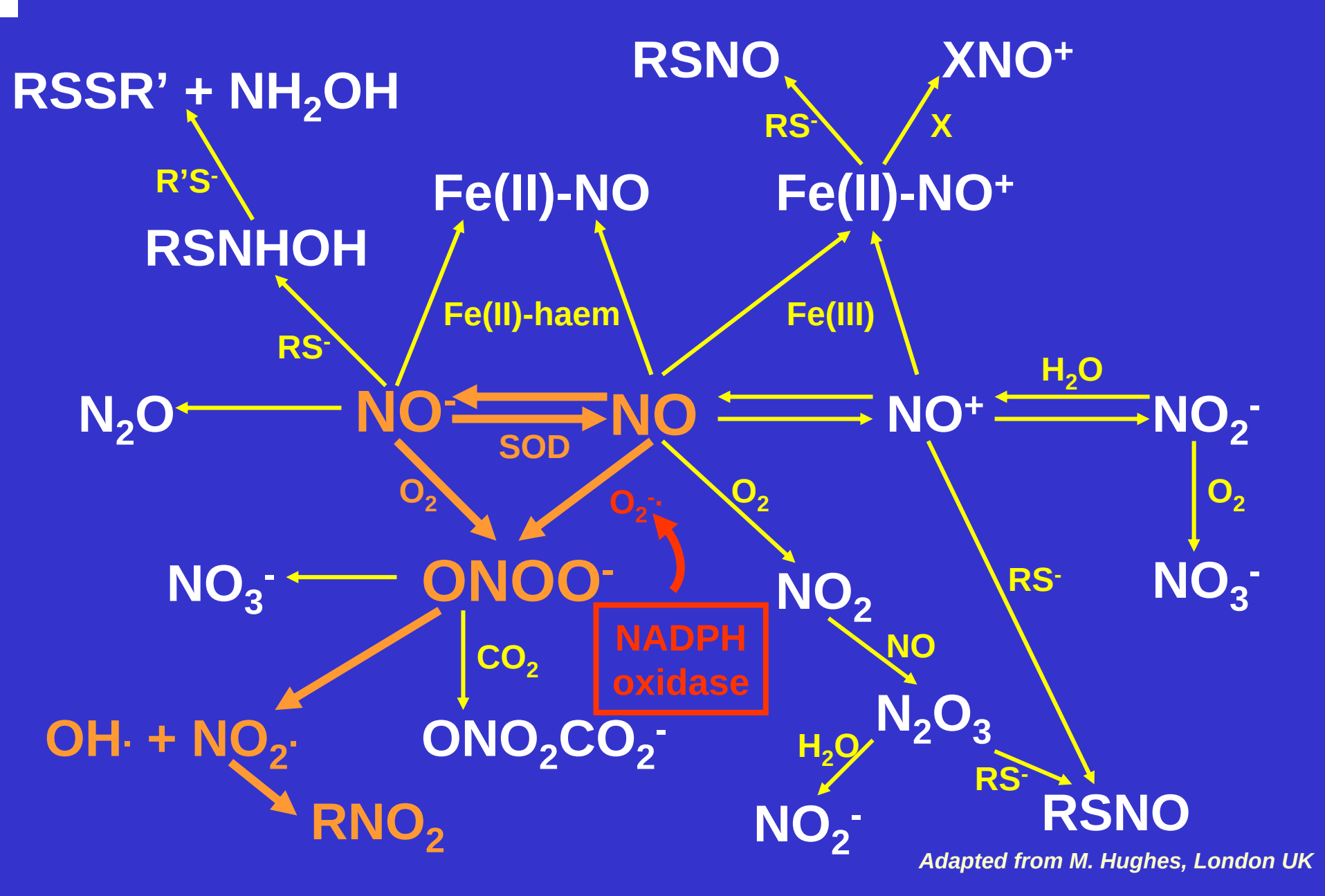
Early recipient innate immune responses involving NK cells, IFN and NO production are essential for IgG immunity against donor platelets to proceed.

Bang et al, 1996, 2000
Sayeh et al 2004



How does NO production affect platelet immunity?

Bang et al, 1996, 2000
Sayeh et al 2004

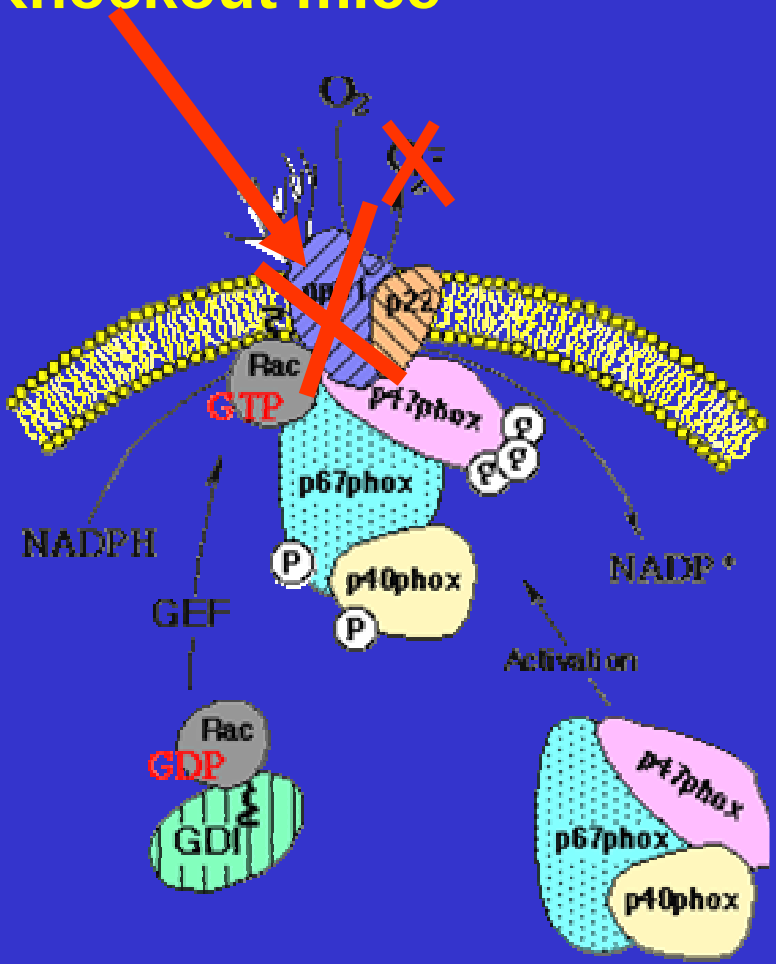


Adapted from M. Hughes, London UK

What role does ONOO- play in platelet immunity?

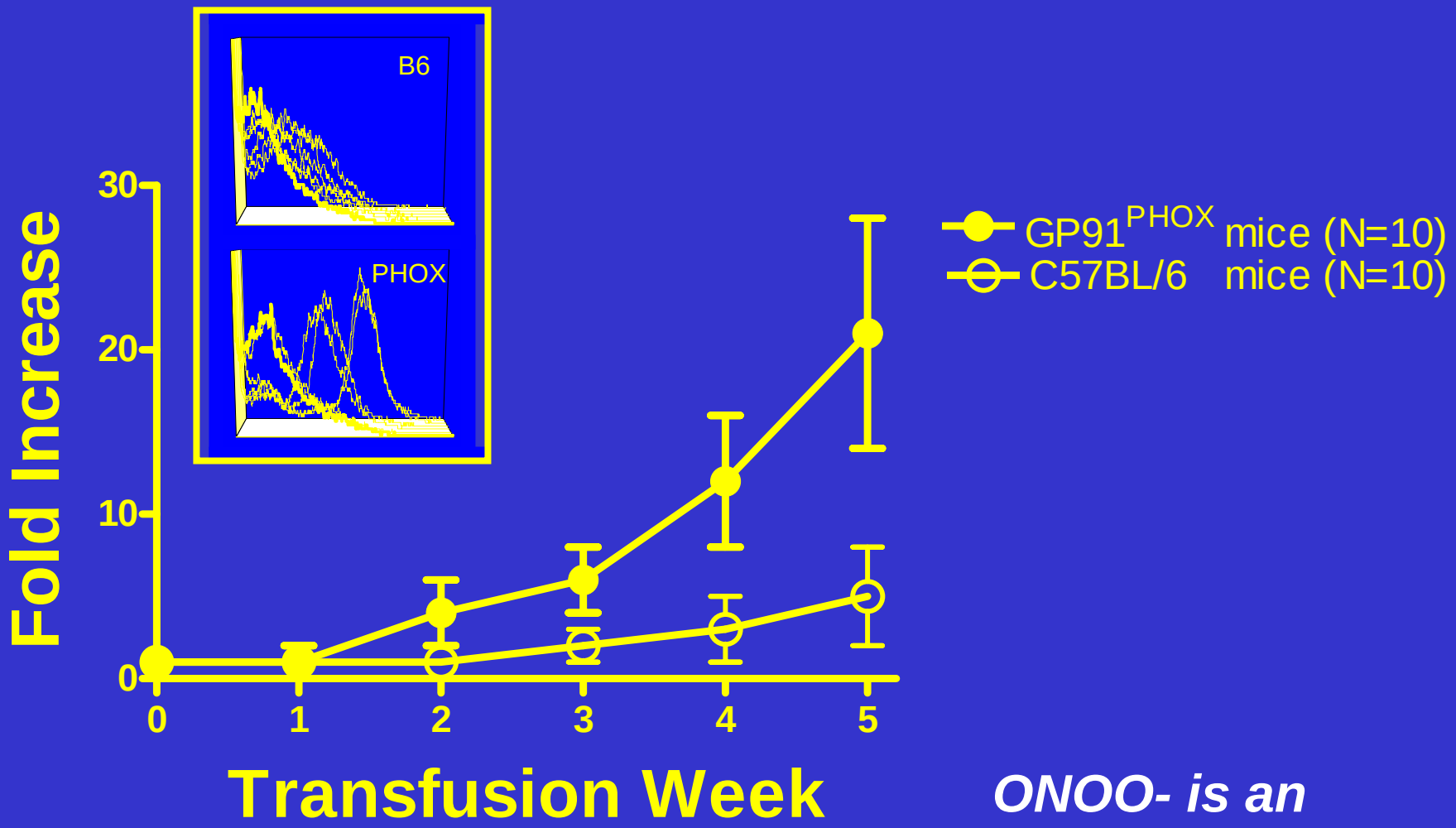
Transfuse mice that cannot produce ONOO-:

GP91^{PHOX} Knockout mice



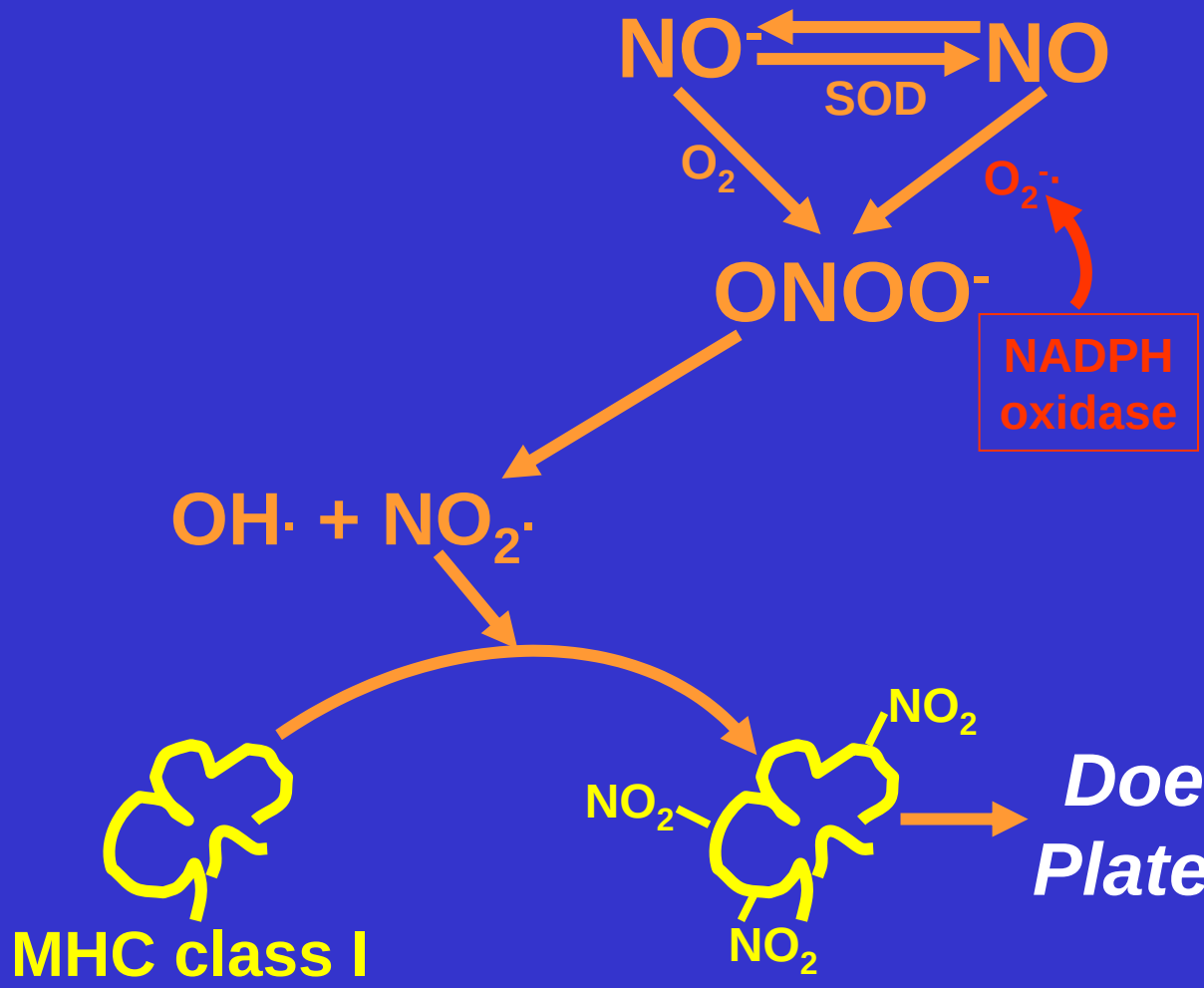
Unable to generate O_2^- and ONOO-

IgG anti-donor response against platelets is enhanced in GP91^{PHOX} mice:



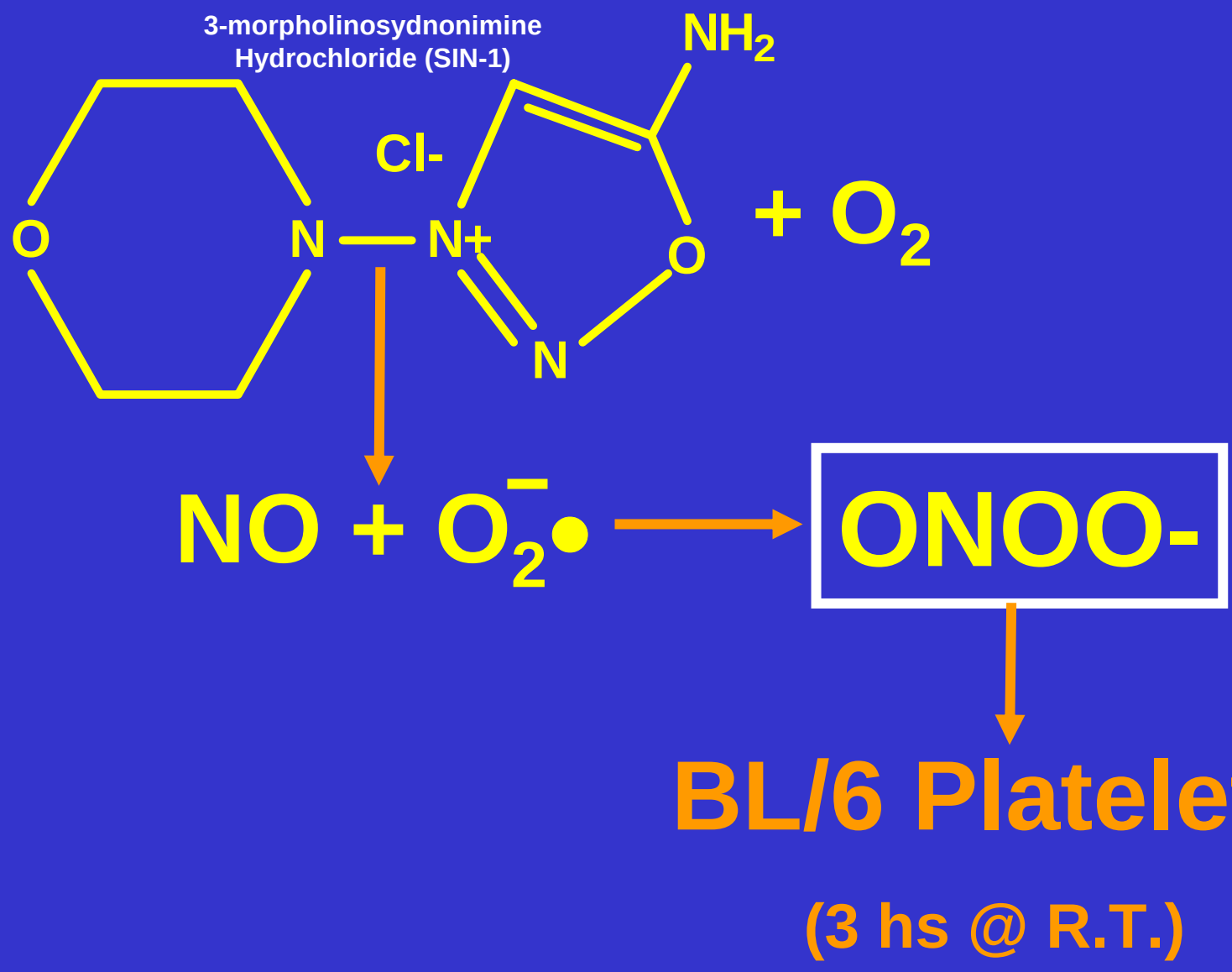
ONOO- is an inhibitory molecule

Does platelet MHC Class I Nitrosylation play a role in immunity?:

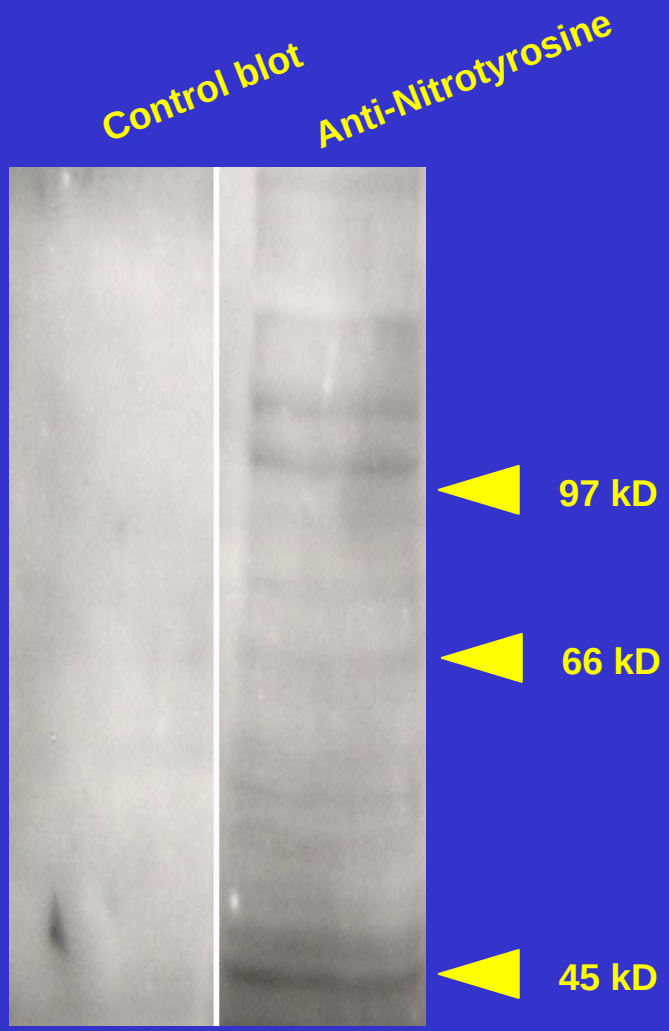


Does this inhibit Platelet immunity?

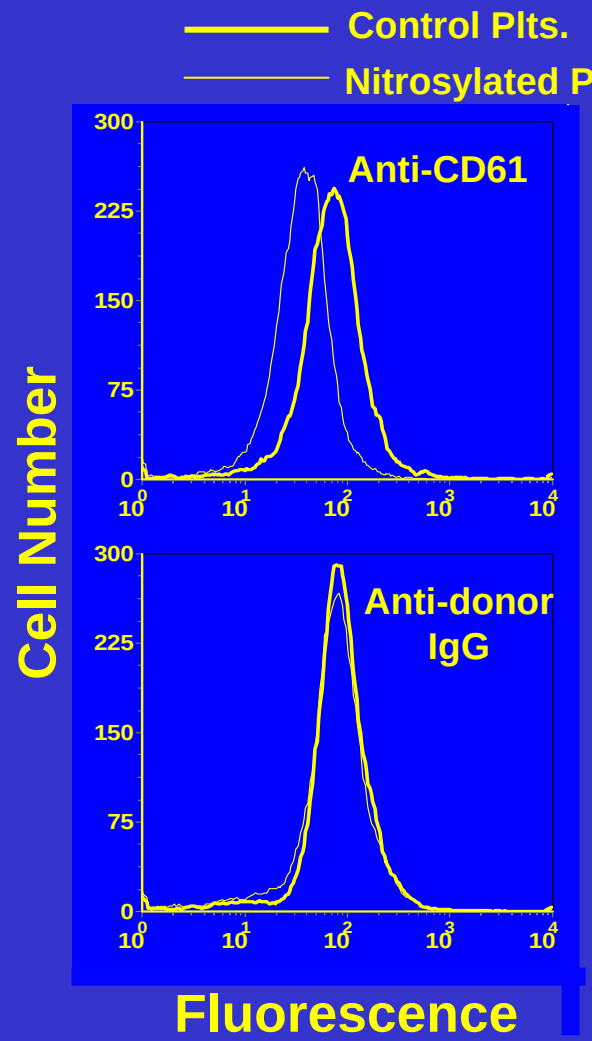
Nitrosylated platelet antigens:



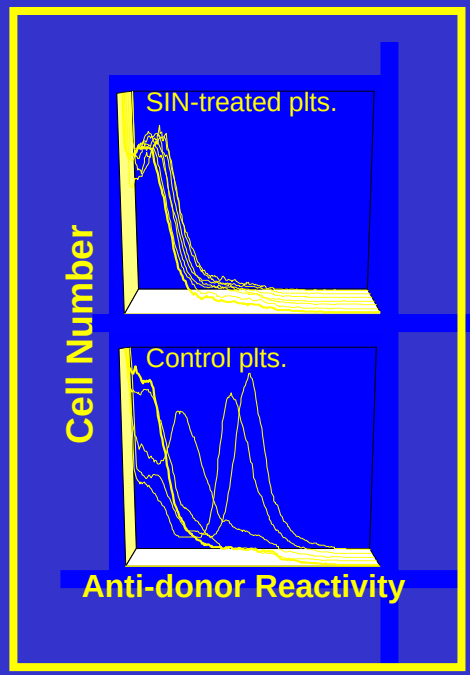
Nitrosylated platelets DO NOT lose antigenicity:



8% SDS-PAGE transferred to nitrocellulose and blotted with ECL-anti-Nitrotyrosine antibodies



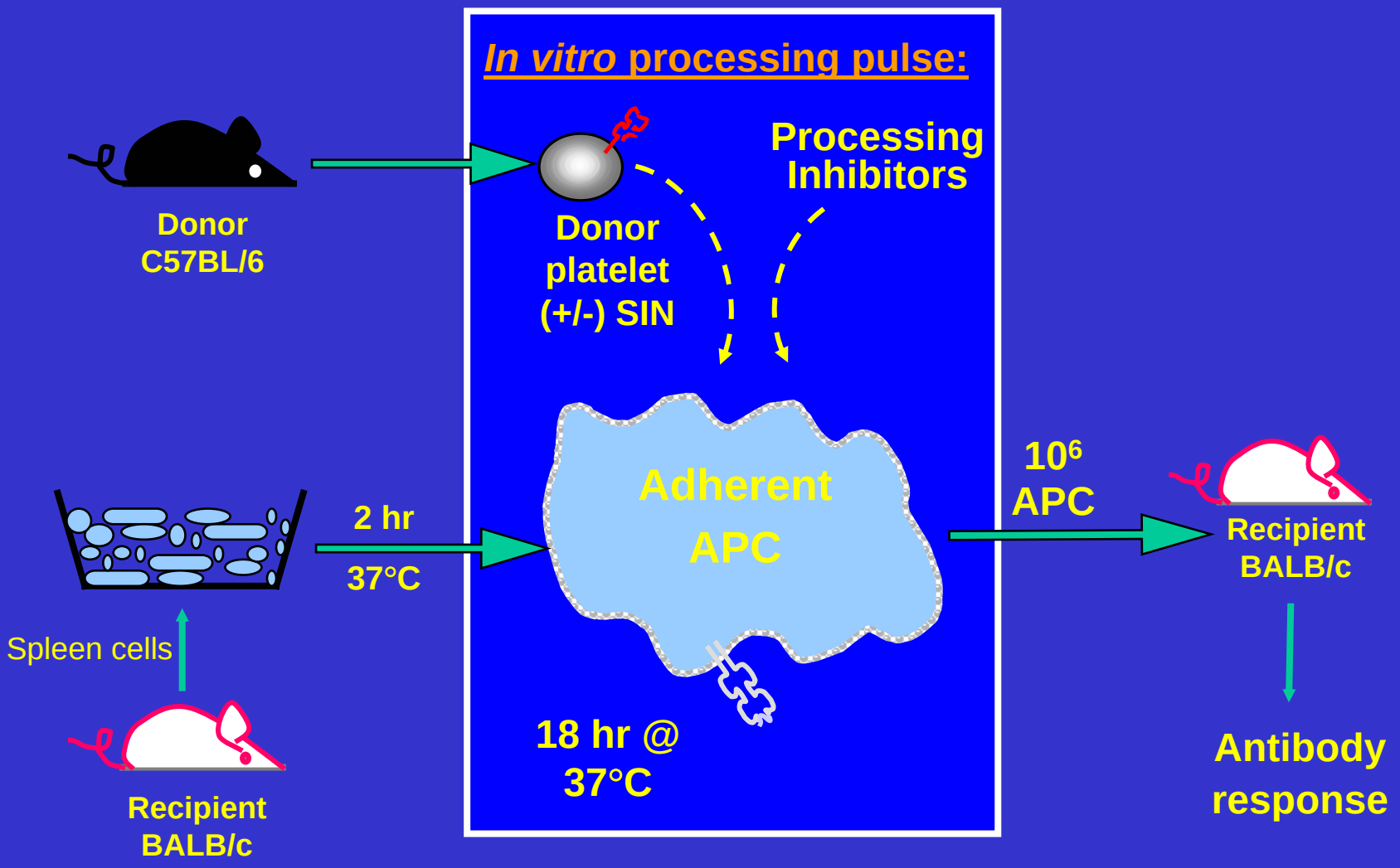
Nitrosylated Platelets are NOT immunogenic in vivo:



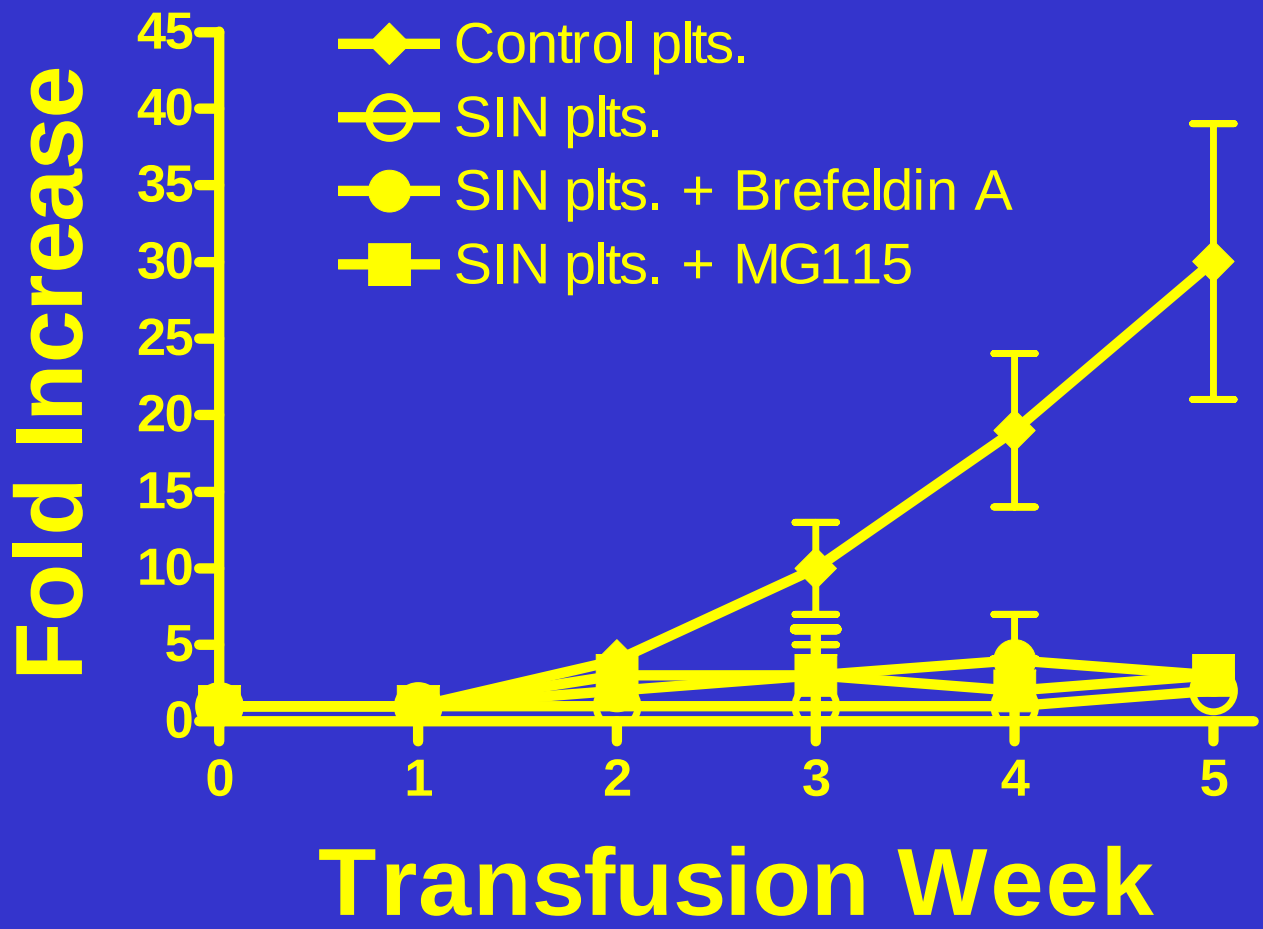
Control Plts.
SIN-treated Plts.

Does ONOO- have a role in antigen processing?

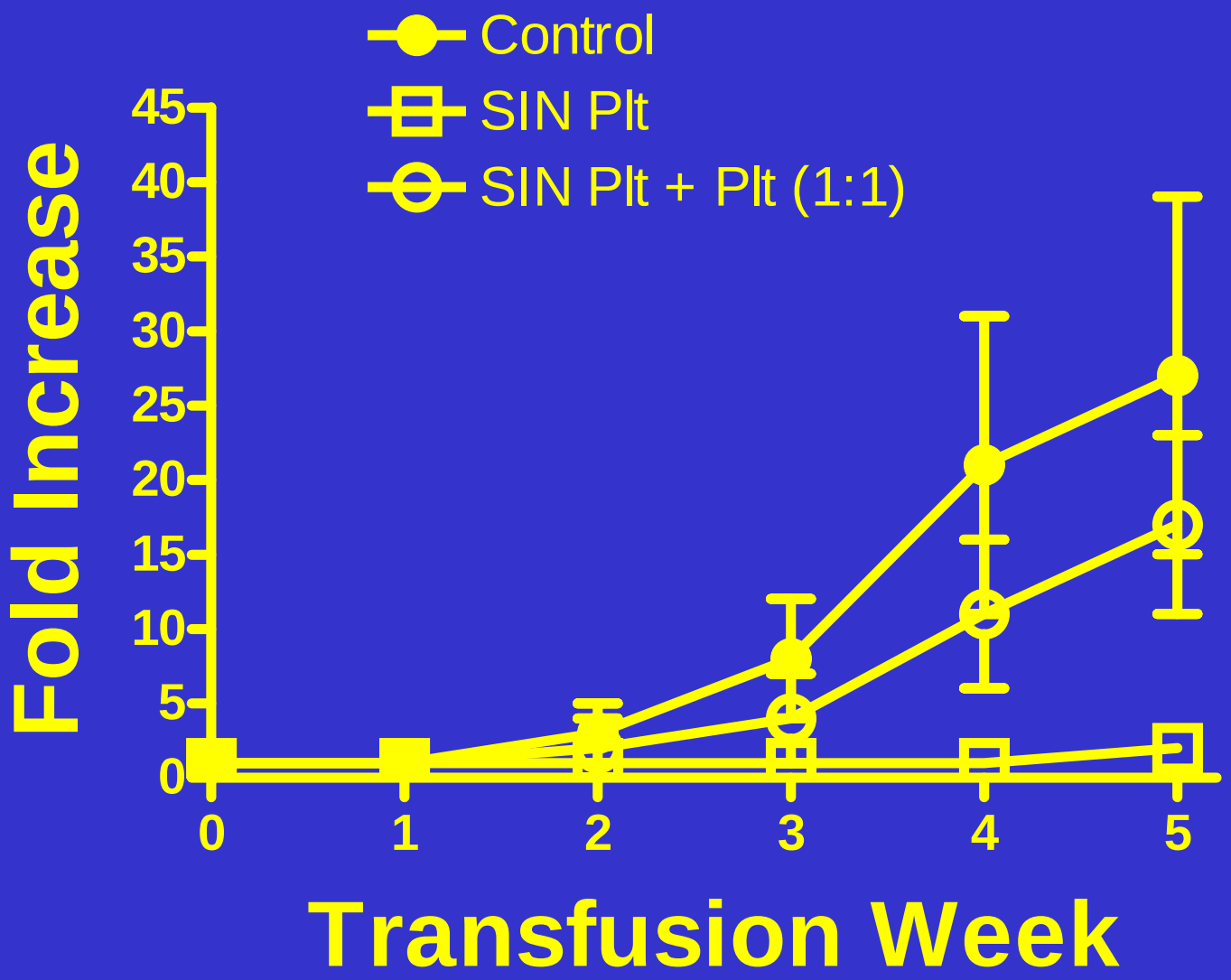
Donor platelet (PLT) - pulsed APC:



Inhibiting MHC class I processing does not rescue nitrosylated platelet immunity:



Adding normal platelets rescues nitrosylated platelet immunity:



Platelet transfusions:

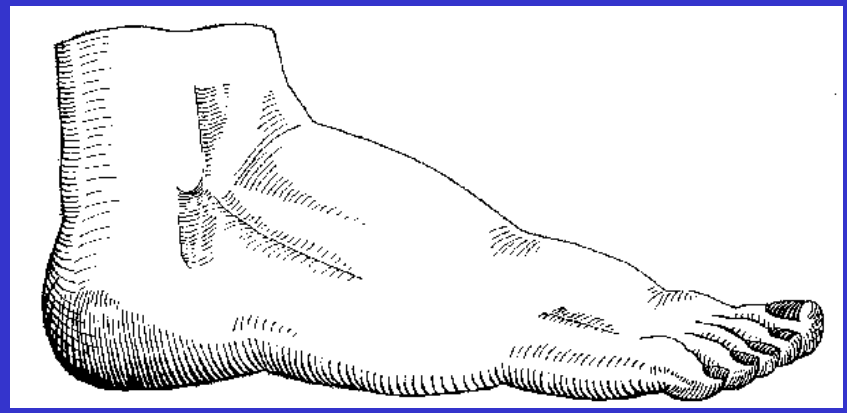
1. Stimulate NK cell-derived IFN production that activates iNOS and NO production.

2. NO production is essential for the generation of IgG anti-donor antibodies (Aminoguanidine sensitive).

3. Peroxynitrite (ONOO-) generation in the recipient is associated with suppressed platelet immunity.

Thus, nitric oxide plays a dual role in platelet immunity:

- NO-induced stimulation of antibody production via unknown pathway.
- ONOO- mediated immunosuppression via inhibition of membrane movements within the APC.

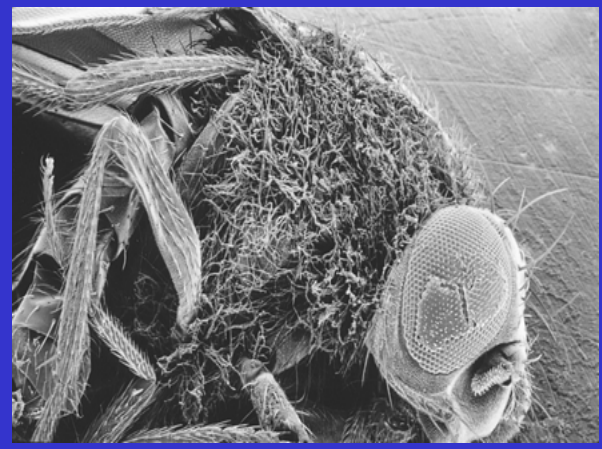


And now for something completely different!

4. Platelets also mediate innate immunity.

Cell, Vol. 86, 973–983, September 20, 1996, Copyright ©1996 by Cell Press

The Dorsoventral Regulatory Gene Cassette *spätzle/Toll/cactus* Controls the Potent Antifungal Response in Drosophila Adults

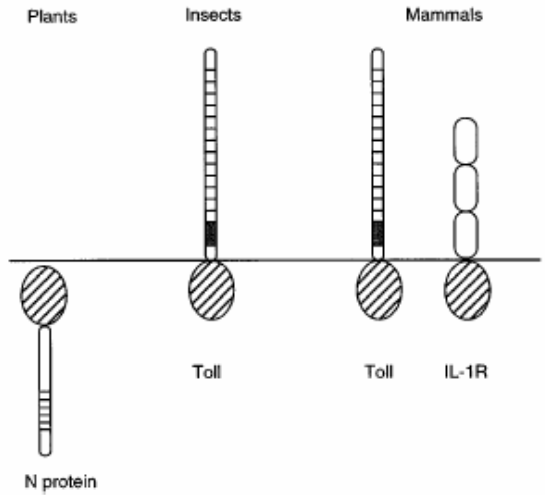


letters to nature

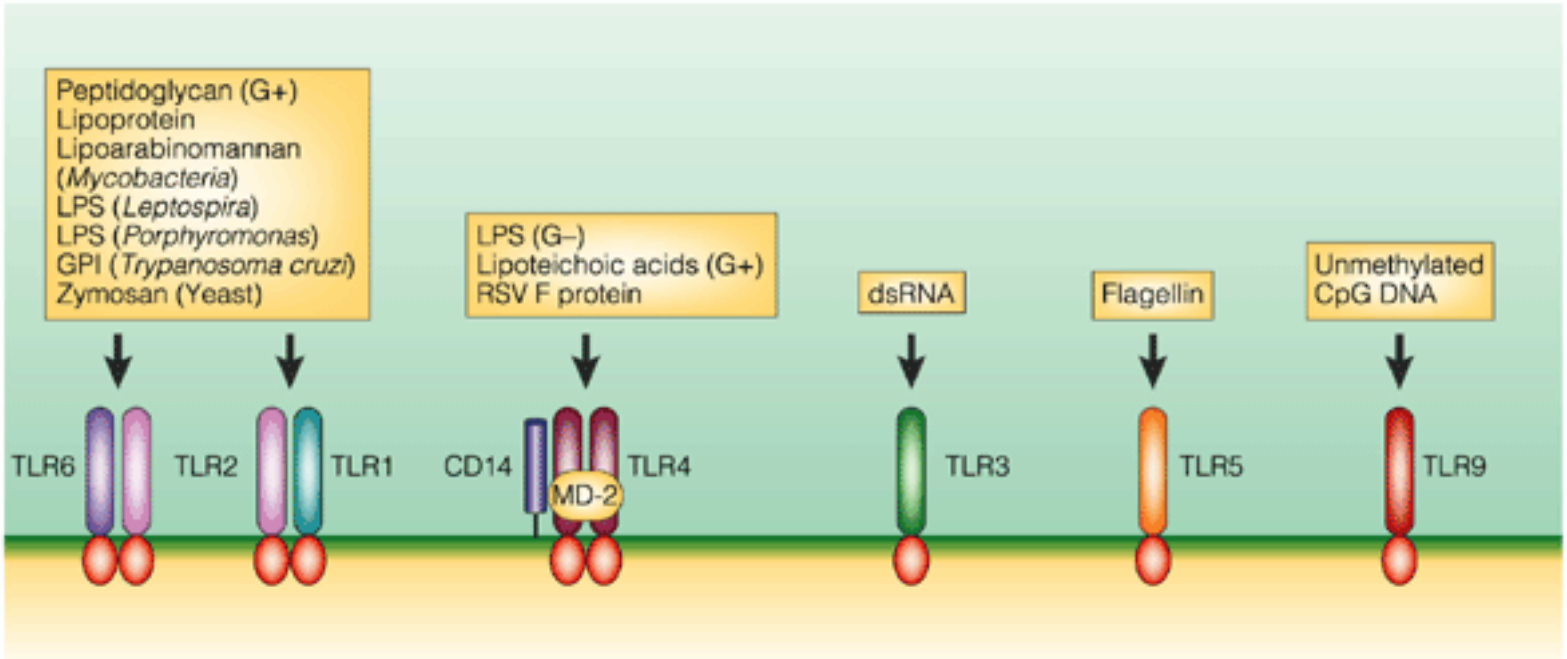
A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity

Ruslan Medzhitov*, Paula Preston-Hurlburt & Charles A. Janeway Jr*

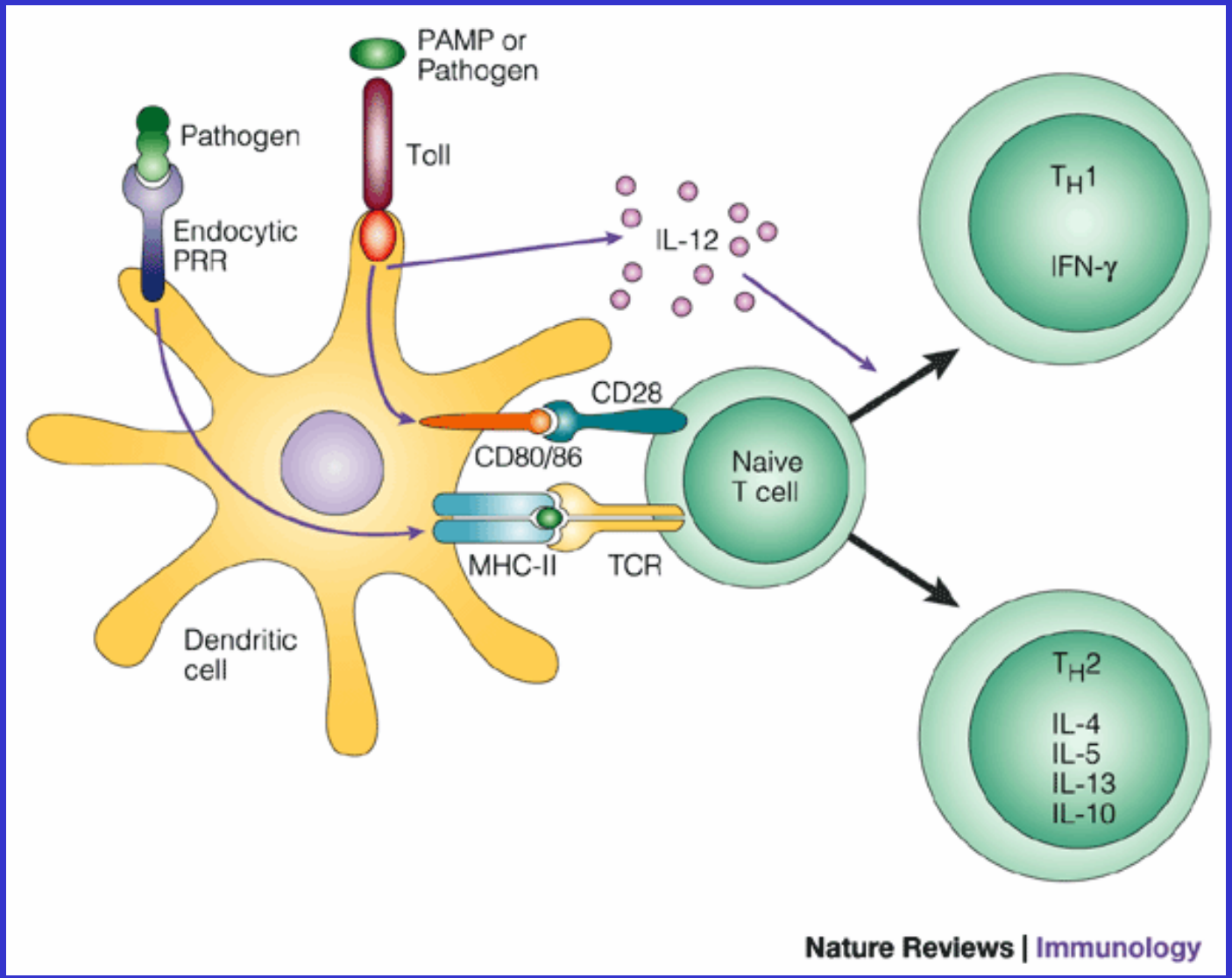
*Section of Immunobiology, Yale University School of Medicine, and * Howard Hughes Medical Institute, New Haven, Connecticut 06520-8011, USA*



TLR Ligands:


Peptidoglycan (G+)
Lipoprotein
Lipoarabinomannan
(Mycobacteria)
LPS (Leptospira)
LPS (Porphyromonas)
GPI (Trypanosoma cruzi)
Zymosan (Yeast)
LPS (G–)
Lipoteichoic acids (G+)
RSV F protein
dsRNA
Flagellin
Unmethylated
CpG DNA
TLR6
TLR2
TLR1
CD14
MD-2
TLR4
TLR3
TLR5
TLR9
Nature Reviews | Immunology

Toll-like receptors link innate and adaptive immunity:



Host response to LPS:

- Mediated by macrophages as demonstrated by experiments with C3H mice:
- HeN mice: macrophages have normal LPS receptors ⇒ mice killed by LPS
- HeJ mice: macrophages have non-functional LPS receptors ⇒ no response of mice to lipid A
- HeJ mice transplanted with HeN macrophages ⇒ mice killed by LPS
- HeJ mice transplanted with HeN macrophages pre-treated with LPS ⇒ mice die of septic shock without further exposure to LPS

Mode of Action of TNF:

- TNFa (tumour necrosis factor alpha) – most important cytokine produced in response to LPS stimulation.

- Rapid induction of TNFa synthesis by LPS such that TNFa represents 1-2% total secreted protein.

- High circulating TNFa levels :
  - pyrogenic, fever response
  - alters metabolic processes ⇒ muscle wasting, destruction of fat cells (cachexia)
  - causes tissue injury (including necrosis of tumours)
  - induces production of other cytokines, including interleukin 6 (IL6), IL8, IL1 and platelet activating factor (PAF)

- Normal role is to stimulate responses to combat infection
  - activates vascular endothelium, increases vascular permeability:
  - increases release of plasma proteins
  - increases phagocyte and lymphocyte migration
  - increases blood clotting
  - prevents localised infection becoming systemic

- Injection of TNFa into laboratory animals
  - reduced ability to contain infection ⇒ toxic shock.

Platelets and LPS:

Shibazaki et al. Biphasic, Organ-Specific, and Strain-Specific Accumulation of Platelets Induced in Mice by a Lipopolysaccharide from Escherichia coli and Its Possible Involvement in Shock. Infect. Immun. 64:5290–5294, 1996.

Sheu et al. Mechanism involved in the antiplatelet activity of Escherichia coli lipopolysaccharide in human platelets. Brit J Haematol. 103:29-38,1998.

Montrucchio et al. Mechanisms of the priming effect of low doses of lipopolysaccharides on leukocyte-dependent platelet aggregation in whole blood. Thromb Haemostasis. 90: 872-881,2003.

Ohba et al. Platelet responses and anaphylaxis-like shock induced in mice by intravenous injection of whole cells of oral streptococci. Oral Micro Immunol. 19:26-30,2004.

An academic lesson for graduate students on what not to do

or…

you can cure cancer in retrospect:

Semple et al. Murine **platelets** express **Toll like receptor 2**: A potential regulator of innate and adaptive immunity. Platelets. 15:267,2004. (Rust Austria). But its only phenotype…who cares….Simon Panzer's revelation….."you know John, I reviewed a paper for Thrombosis Research last month and it was just like your stuff" ….great….

Shiraki et al. Expression of **Toll-like receptors** on human **platelets**. Thromb. Res. 113:379-385, 2004. Auuggghhhh…Simon was telling the truth…oh well, its only phenotype…..who cares….they looked at 1 and 6…we looked at 2, 4 an 9….

Coppinger et al. Characterization of the proteins released from activated **platelets** leads to localization of **novel platelet proteins** in human atherosclerotic lesions. Blood. 103:2096-2104, 2004. TLR5; 2nd on the list…oh well, of course high flowthru proteomics will show you anything….(hey, these guys were at the Rust meeting)

Yu et al. Critical roles of **platelets** in **lipopolysaccharide**-induced lethality: effects of glycyrrhizin and possible strategy for acute respiratory distress syndrome. Int. Immunopharmacol. 5:571–580, 2005. hey, they did the same thing as us…

Cognasse et al. Evidence of **Toll-like receptor** molecules on human **platelets**. Immunol. Cell Biol. 83:196-198, 2005. More phenotype…..(hey, these guys were at the Rust meeting also).

Ward et al. Agonists of **toll-like receptor (TLR)2 and TLR4** are unable to modulate **platelet** activation by adenosine diphosphate and platelet activating factor. Thromb. Haemost. 94:831–8, 2005. And more phenotype……what's the function?? (hey, these guys were at the Rust meeting too).

John goes to Calgary……

Andonegui et al. **Platelets** express functional **Toll-Like Receptor-4** (TLR4). Blood. 106:2417-2423, 2005. Ahhhh….function at last…..(I should have kept my mouth shut in Calgary).

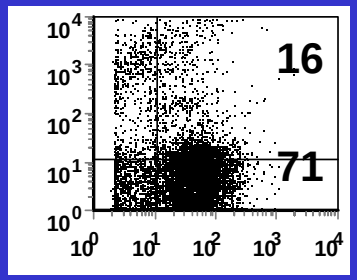
Aslam et al. **Platelet Toll-like receptor** expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-production in vivo. Blood. 107:637-641, 2006. Finally, us again….Extreme function…platelets do everything…

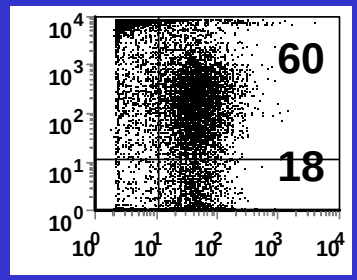
Patrignani et al. Reduced **thromboxane** biosynthesis in carriers of **Toll-Like Receptor 4** polymorphisms in vivo. Blood First Edition Paper, prepublished online January 5, 2006; DOI 10.1182/blood-2005-12-4811. More function….

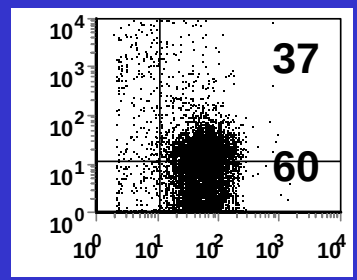
Ståhl et al. Lipopolysaccharide from enterohemorrhagic Escherichia coli binds to **platelets** via **TLR4** and CD62 and is detected on circulating platelets in patients with hemolytic uremic syndrome. Blood First Edition Paper, prepublished online March 2, 2006; DOI 10.1182/blood-2005-08-3219. And more function…where will it end??

TLR2 TLR4 TLR9

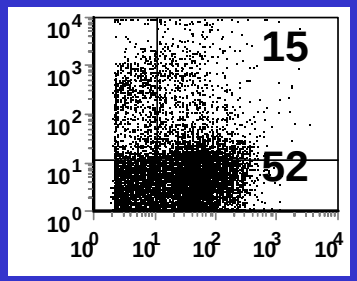
Resting Human Platelets

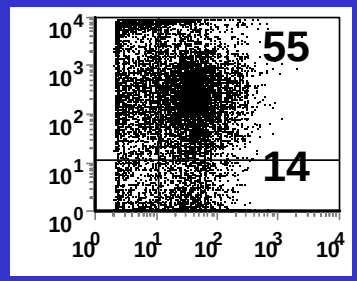


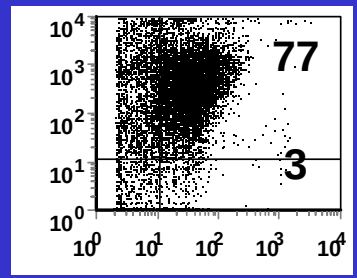




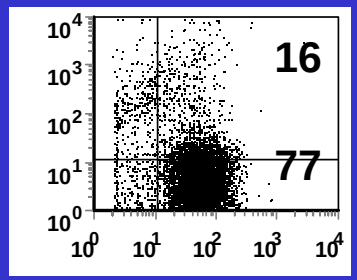
Thrombin-activated Human Platelets

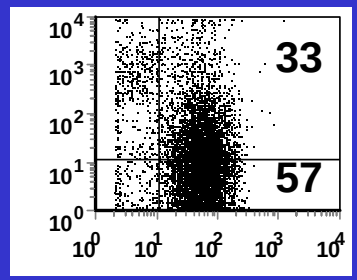


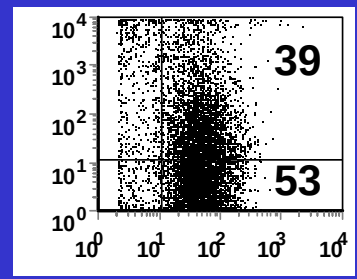




LPS-stimulated Human Platelets



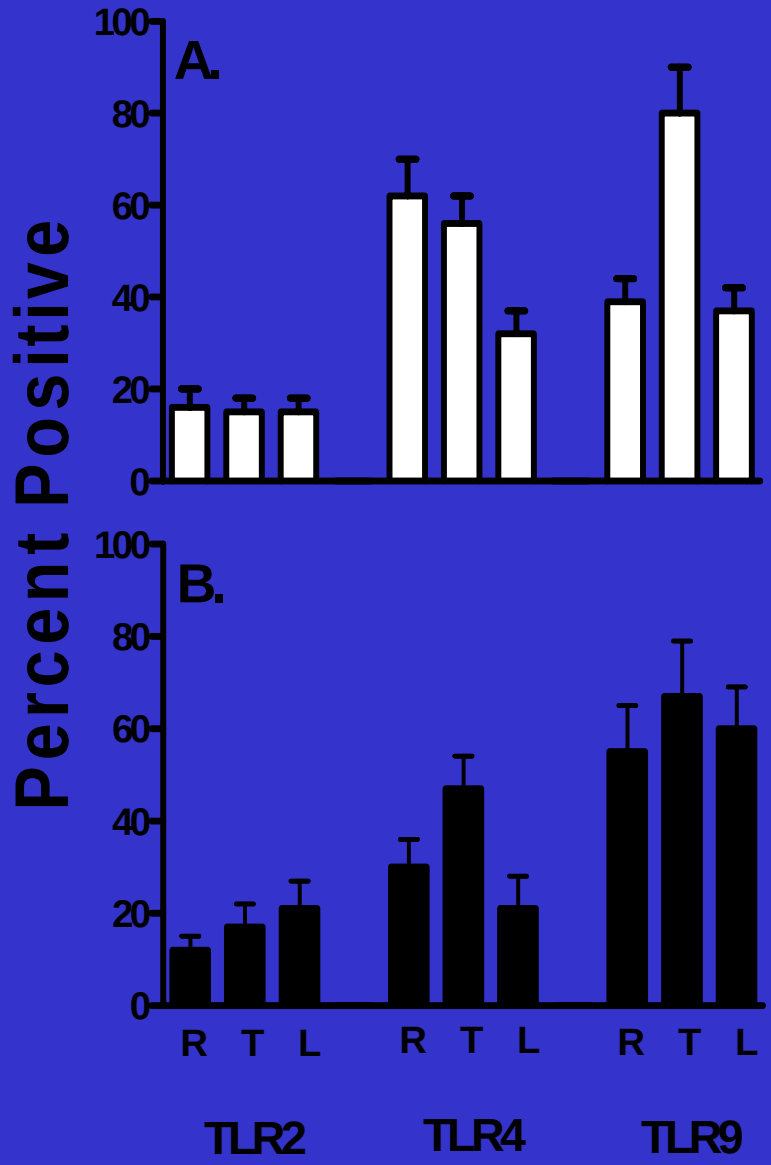




Cell Count

Forward Scatter

Aslam et al, 2006

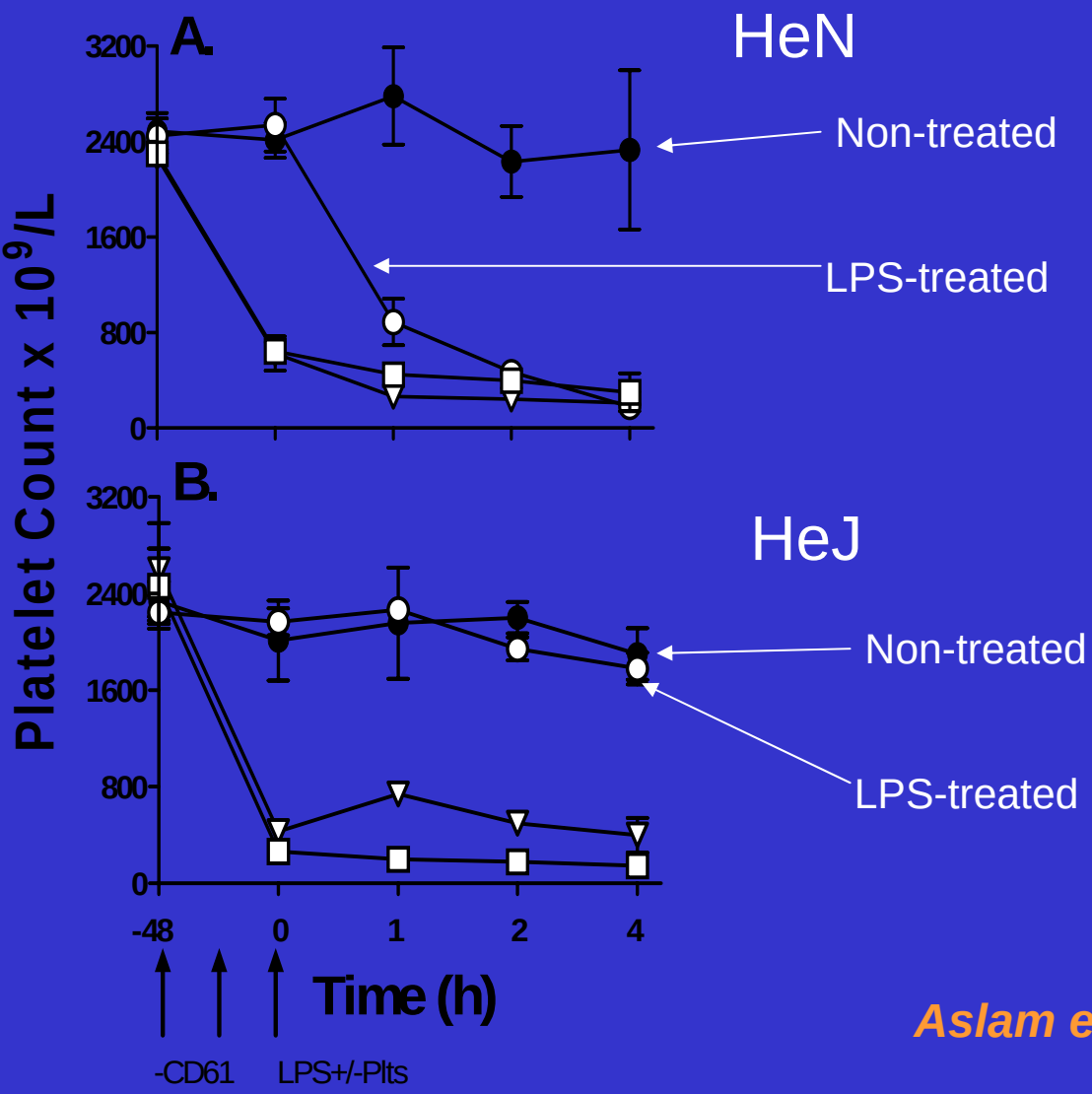


Human

Mouse

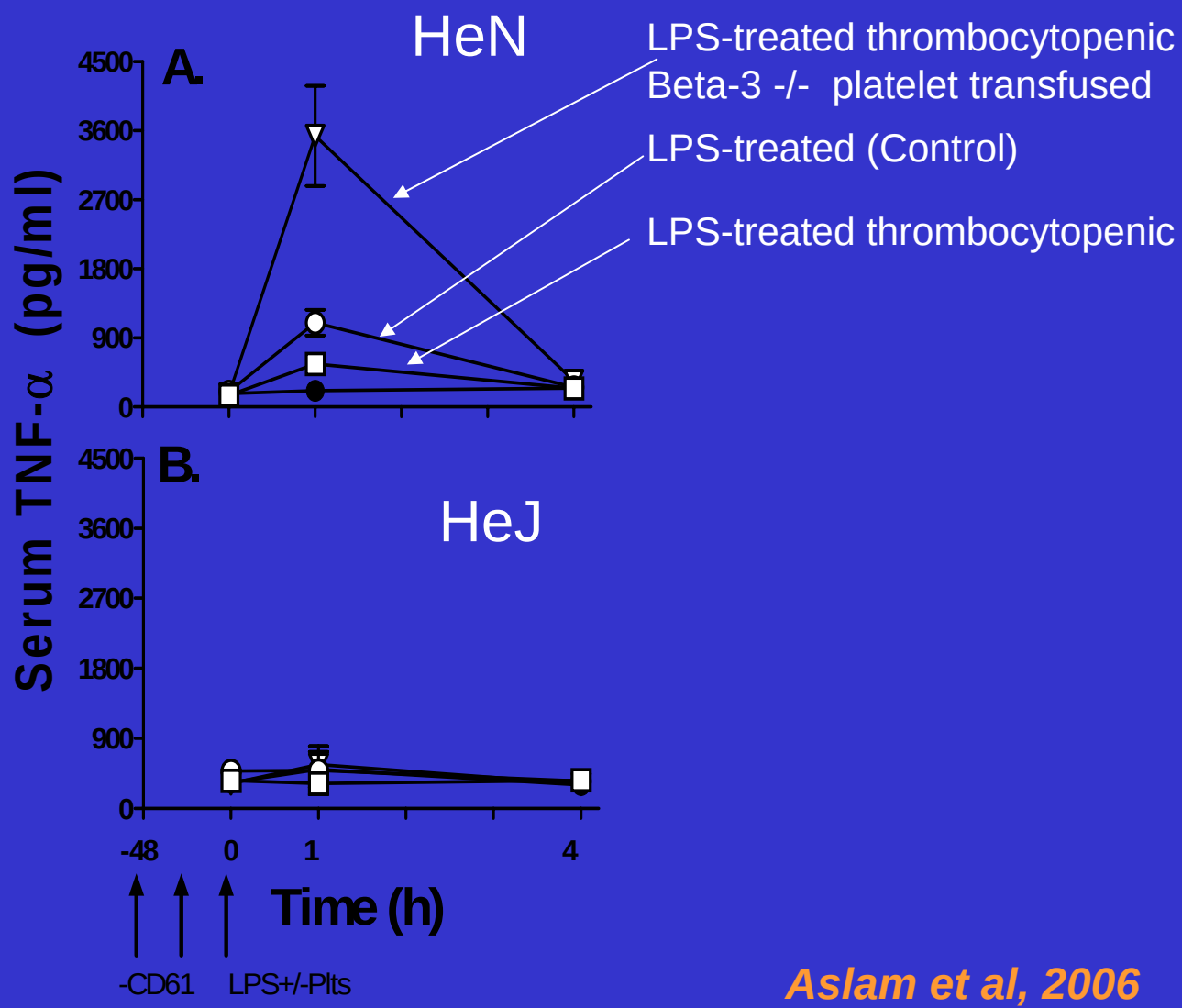
Aslam et al, 2006

Platelet counts in LPS treated mice:



Aslam et al, 2006

Serum TNF Production:



Aslam et al, 2006

CONCLUSIONS:

TLR 2, 4, and 9 can be detected on murine and human platelets.

Functional platelet TLR4 expression is required for LPS-induced thrombocytopenia.

Functional platelet TLR4 expression is required for LPS-induced TNF-a production.

These findings suggest that platelets have a critical role in mediating LPS-induced innate immunity.

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Questions?