

Toll-Like Receptors, Thrombosis and the Relationship to Gulf War Illness

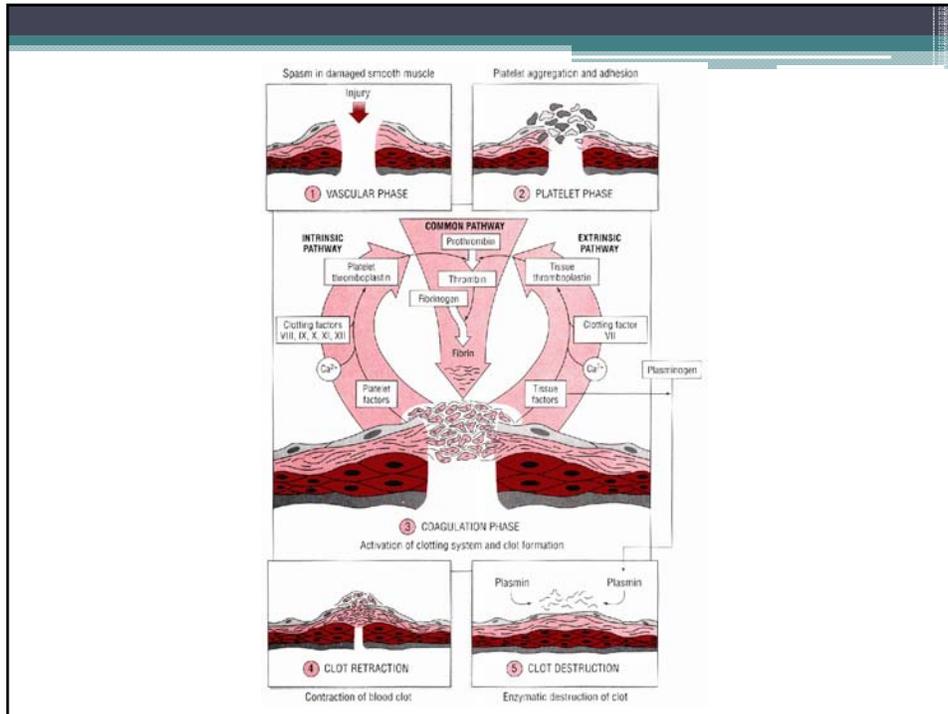
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Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses
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Gulf War Illness

- Chronically ill with symptoms similar to Chronic Fatigue Syndrome and Fibromyalgia
- Chronic fatigue, joint inflammation, neurocognitive disorders, sleep disturbances, IBS, depression, skin disorders, and so on
- Etiology and pathogenesis are unknown



Gulf War Illness and Coagulation

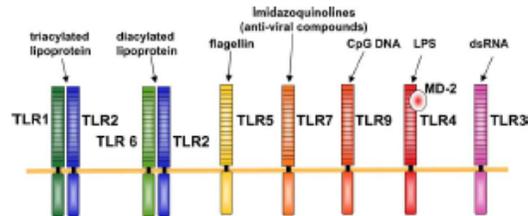
Clinical studies have shown activation of the coagulation system in Gulf War Veterans -

- Increased platelet tissue factor activity,
- Increased thrombin-antithrombin complex,
- Activation of anticoagulation pathways,
- Increased soluble fibrin monomers and cleavage products.

(Bach, RR et al. Abstract ISTH 2009; Hannan, KL et al. 2000 Blood Coagulation and Fibrinolysis 11:673)

Toll-Like Receptors

Immune Cells distinguish between a pathogen and self through signals obtained from Toll-like Receptors (TLRs).



Each TLR recognizes a specific Pathogen-Associated Molecular Pattern (PAMP).

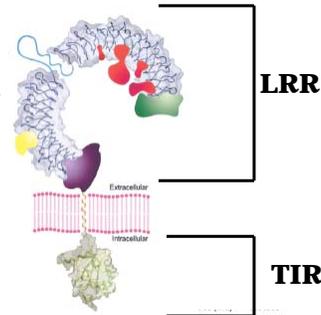
Gulf War Illness and Toll-Like Receptors

Increase inflammatory response in Gulf War Illness:

- Increase in circulating antibodies (IgA);
- Increased regulatory T cells
- Increased circulating mycoplasma (recognized by TLR2);
- Increased vaccine adjuvants (recognized by TLRs);
- Increased circulating opiates (recognized by TLR4).

Toll-Like Receptor (TLR) 2

- Type 1 transmembrane protein characterized by extracellular Leucine-Rich Repeats (LRRs) and intracellular Toll-Interleukin-1 Receptor (TIR) domain
- Recognizes peptidoglycans, lipoteichoic acid, and lipopeptides of Gram-positive bacteria, zymosan of fungi, lipoarabinomannan of mycobacteria, LPS of non-enterobacteria, such as *P. gingivalis*
- Heterodimers with TLR1 (triacylated lipopeptides) or TLR6 (diacylated lipopeptides)
- Activate multiple signaling pathways including NF κ B, MAPK, PI3K/Akt
- Results in the release of various inflammatory cytokines, including TNF α , IL6, and IFN
- Expressed on monocytes, macrophages, dendritic cells, B cells, neutrophil



Texereau, J, et al. 2005 Clinical Infectious Disease 41:S408-15.

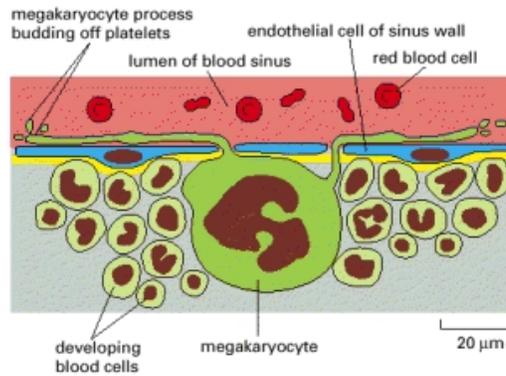
TLR2, Megakaryocyte Maturation, and Platelet Activation

Hypothesis: Through TLR2, Megakaryocytes are triggered to mature and produce Platelets which would increase the number of circulating Platelets and create platelets that are pro-inflammatory and pro-coagulant. In addition, Platelets can be activated through TLR2 to have a role in thrombosis and inflammation.

Megakaryocytes

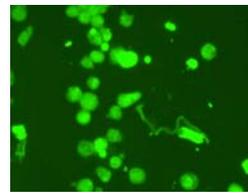
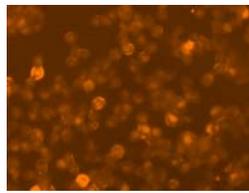
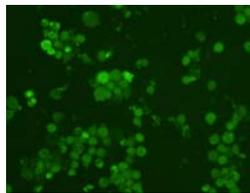


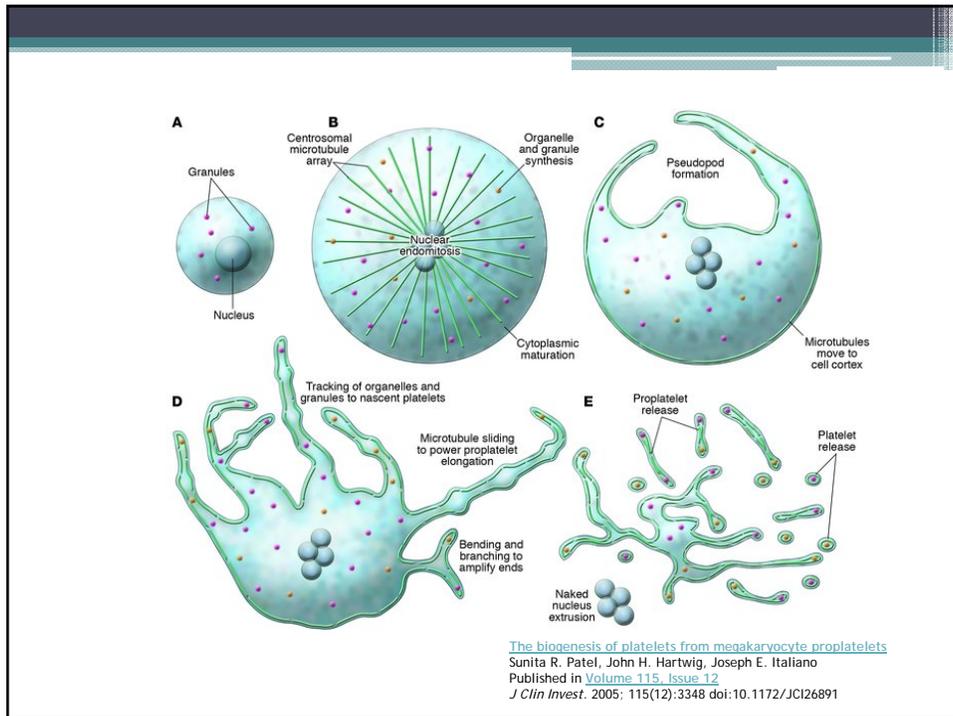
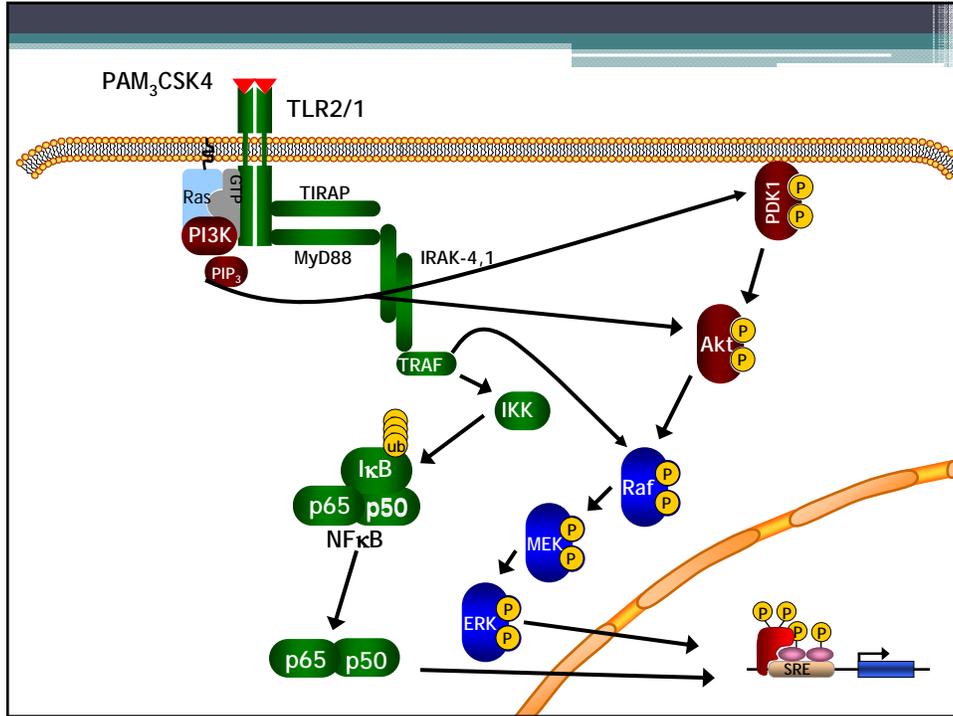
Travlos, GS. (2006) Normal Structure, Function, and Histology of the Bone Marrow. *Toxicologic Pathology*, 34:548 - 565



Molecular Biology of the Cell, 4th edition
Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter

Megakaryocytes Express TLRs





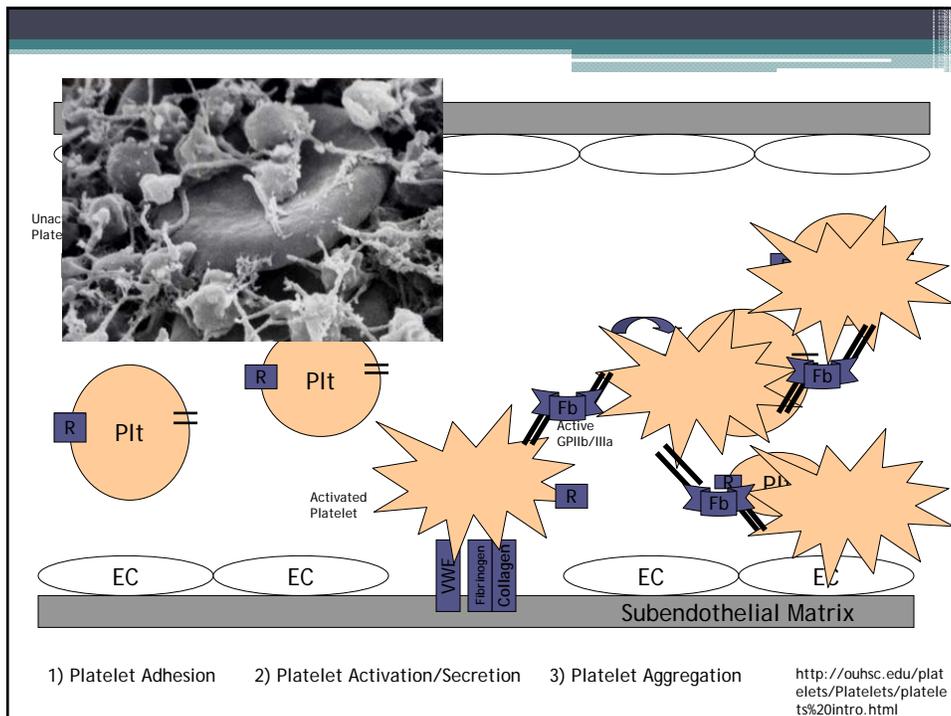
TLR2 Affects Megakaryocyte Gene Expression

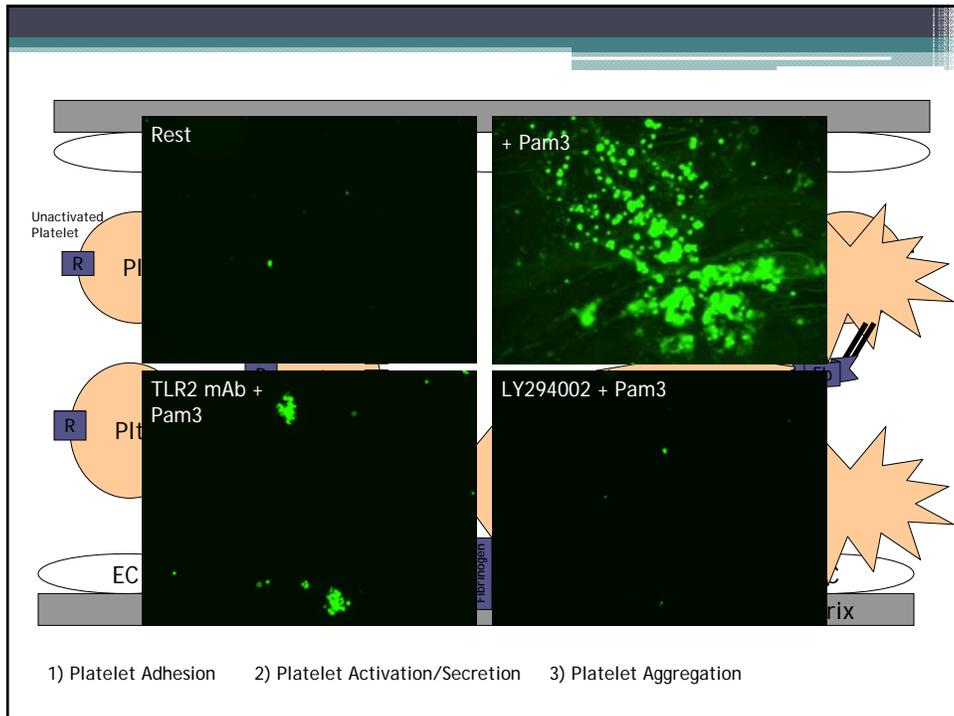
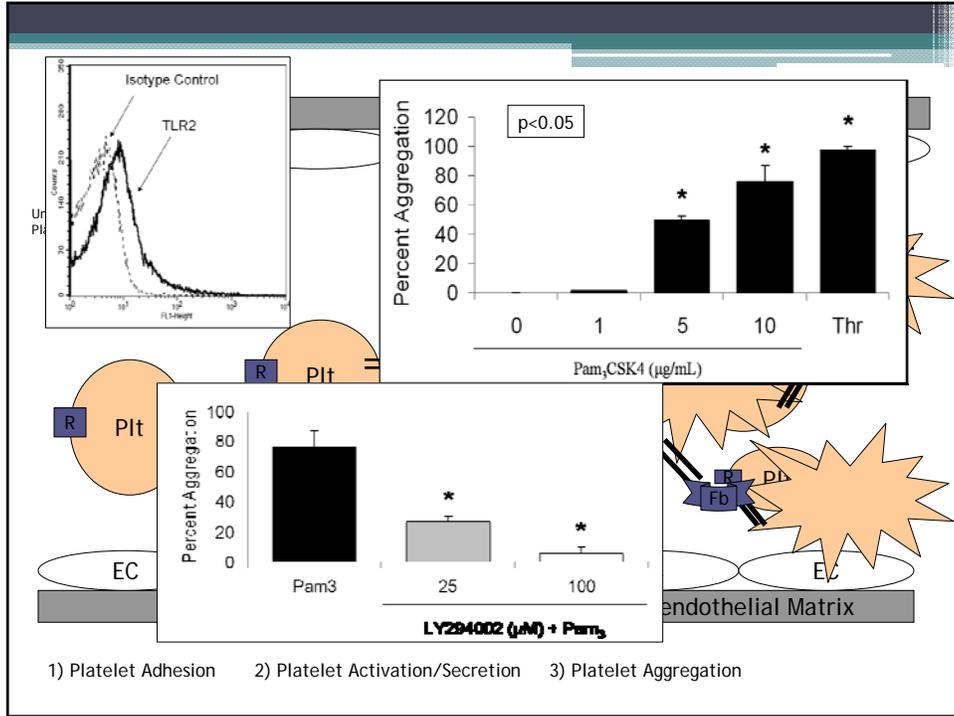
Increases in Thrombotic Related Genes:

- GP1b through PI3K/Akt and ERK-MAPK pathways;
- CD41 through PI3K/Akt and ERK-MAPK pathways;

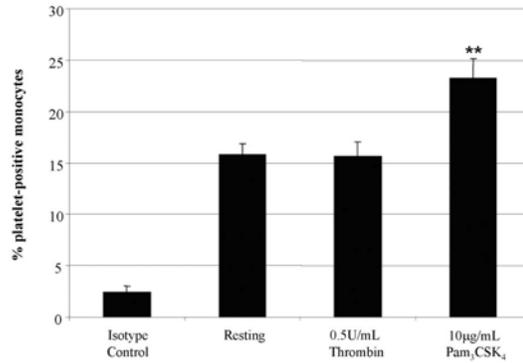
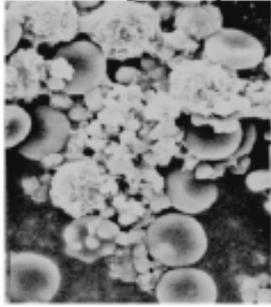
Increases in Inflammatory Related Genes:

- MCP-1 through PI3K/Akt, ERK-MAPK, and NF κ B pathways;
- COX2 through NF κ B pathways;
- TLR2 through PI3K/Akt, ERK-MAPK, and NF κ B pathways;
- NF κ B1 through NF κ B pathways.

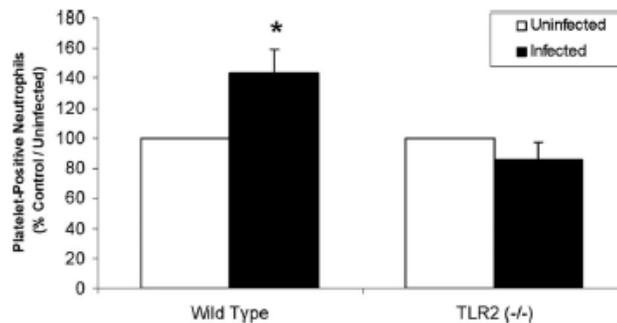




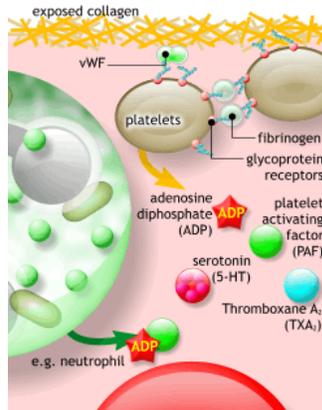
TLR2 Activates the Inflammatory Function of Platelets



TLR2 Activates the Inflammatory Function in Platelets



Platelet Granules



www.blobs.org/science/cells/platelet_plug1.gif

Conclusions

- Megakaryocytes and Platelets express functional TLR2;
- Upon activation, TLR2 will activate megkaryocytes and increase maturation;
- Upon activation, TLR2 will activate platelets and cause clot formation and interactions with immune cells;

Through TLR2, inflammation can regulate thrombosis and could be a link between the coagulopathy and inflammation in Gulf War Illness.

Future Direction

- Test platelet function in Individuals with Gulf War Illness - including platelet aggregation, adhesion, heterotypic aggregate formation, TLR expression;
- Test for activation of coagulation - including thrombin generation, TAT complex, fibrin monomer;
- Determine if there are alterations in gene expression in platelets and immune cells- microarray, real-time PCR for specific genes

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