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

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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.

**Toll-Like Receptors**

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

![Toll-like Receptors Diagram]

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

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**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 NFκB, MAPK, PI3K/Akt
- Results in the release of various inflammatory cytokines, including TNFα, IL6, and IFN
- Expressed on monocytes, macrophages, dendritic cells, B cells, neutrophil

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**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 Express TLRs

TLR2  TLR1  TLR6
The biogenesis of platelets from megakaryocyte proplatelets
Sunita R. Patel, John H. Hartwig, Joseph E. Italiano
Published in Volume 115, Issue 12
J Clin Invest. 2005; 115(12):3348 doi:10.1172/JCI26891
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 NFκB pathways;
- COX2 through NFκB pathways;
- TLR2 through PI3K/Akt, ERK-MAPK, and NFκB pathways;
- NFκB1 through NFκB pathways.
1) Platelet Adhesion  2) Platelet Activation/Secretion  3) Platelet Aggregation
TLR2 Activates the Inflammatory Function of Platelets

TLR2 Activates the Inflammatory Function in Platelets
Platelet Granules

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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