Thromboinflammation in therapeutic medicine

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Thromboinflammation

Clot

Inflammation

Proliferation

Remodelling

Sowa et al, J Biomed Opt, 2017
The cascade systems of the blood

Markiewski, Trends Immunol, 2007
Coagulation
Complement

Classical pathway
- Ag–Ab complexes
- IgG, IgM, and pentraxins
- Polymers

Lectin pathway
- Microbial molecules
- Mannose
- Polymers

MBL, ficolins or collectins

Alternative pathway
- Microbial molecules
- Polysaccharides

C1q
- C1r
- C1s

MBL, ficolins or collectins

C3(H₂O)

C3a

C4bC2a
- C3
- C3bBbP

C3

C3b

C4bC2aC3b
- C3b
- C3bBbP

C5
- C5a
- sC5b–C9
- MAC
C3 deficiency leads to prolonged bleeding time

Bauer et al., PlosOne, 2011

Gushiken et al., J Thromb Haemost, 2009
C3 KO mice have attenuated platelet aggregation and prolonged bleeding time

Formation of PMN (CD16+)-platelet (CD42a+) complexes in whole blood from a C3 deficient person

Hamad, Thrombosis Haemostasis, 2015
Thrombo-inflammation

Classical pathway: C1q, CRP
Alternative pathway: Properdin → C3a, C5a
Lectin pathway: MBL, Ficolins
Contact system: FXII → Thrombin
Tissue factor pathway: TF-FVII → Thrombin

Immune complexes → platelets → Thrombin
Ischemia → Cell stress
Thromboinflammation triggered by complement dysregulation

Immune complexes

C3a, C5a

Endothelium

Platelet

Complement dysregulation

sC5b-9, sC5b-9

MAC, MAC
1. Complement activation triggered by C1q

Hamad et al., JTH, 2009
Binding of complement components to activated platelets

Hamad et al., J Immunol, 2010
Blocking at the C1q and C3 levels or activation in the presence of EDTA, EGTA did not affect the binding.
2. C3H₂O acts as a ligand for leukocyte receptors CR1 and CR3

C3H₂O acts as a ligand for:

1. CR1 (CD35)
2. CR3 (CD11b/CD18)

on PMNs and monocytes
The specificity of MASP-1 and -2
Generation of MASP-AT/C1INH Complexes by TRAP activated platelets

Kozarcanin et al., JTH, 2016
MASP-1,-2/serpin complexes

Activation of PPP by glas

Kozarcanin et al., JTH, 2016
3. Platelet- and fibrin-mediated lectin pathway activation

Kozarcanin et al., JTH, 2016
Thromboinflammation is an important pathophysiological mechanism in several clinical conditions and treatments

1. DIC

2. Thrombotic events such as cardiac infarction, stroke and other cardiovascular conditions

3. Biomaterials implants (joint replacements, scaffolds for tissue engineering etc), extracorporeal treatments (hemodialysis, cardiopulmonary bypass)

4. Pharmacological delivery systems e.g. lipid micelles, polymers, virus vectors etc.

5. Microangiopathies- dysregulation (aHUS)

6. Cell and cell cluster transplantation and therapies.

7. Whole organ transplantation

1. Rheumatic disease (scleroderma, SLE, antiphospholipid syndrome).
1. Thromboinflammation in hemodialysis

Ekdahl et al., Nature Rev Neph, 2017
2. Thromboinflammation in transplantation

**Characterized by:**
- Coagulation and complement activation
- Platelet consumption
- Leukocyte activation and infiltration
- Cytokine generation
- Cell death
- Graft loss / delayed graft function

**Mechanism:**
- Antibodies and lectins
- Lack of cell associated regulators e.g. DAF, MCP, AT, C1-INH
- Cell stress- cyto/chemokines, TF expression
Ischemia/reperfusion injury

Normoxia

Ischemia/Reperfusion Injury

Non-activated Platelets
PMNs

Glycocalyx bound regulators
HS fragments

Clustering of activated platelets
Interaction of platelets and PMNs

EC
Subendothelial layer

EPCR
TM
EC-Selectin
VCAM1
ICAM1
W-P body

AT
TFPI
C1-INH
PC
HS
Conjugation of peptides or macromolecules phospholipid via a PEG-phospholipid linker

Hydrophobic interaction

Short peptide-PEG-lipid

Heparin conjugates

Cell membrane

Heparin-coating

Asif, Acta Biomaterialia, 2015
A. Regulation of the complement system

Recruited factor H

| Inflammation, cytolysis (C5a, C3a) |

B. Regulation of platelet activation (and coagulation system)

Immobilized apyrase (CD39)

| Platelet aggregation |

Teramura, Biomaterials, 2013
Slide chamber model experiment

Porcine aortic endothelial cells

Microscope slide glass
Factor H on PAEC after exposure with whole blood

5% fH-bp-PEG-lipid/PAEC

Control PAEC
Whole blood experiment on Apyrase/5C6-immobilized surface

(a) Platelet consumption (%)

(b) TAT (μg/L)

(c) C3a (ng/mL)

(d) sCSA-9 (AU/mL)

Nilsson, Biomaterials, 2013
Protection of transplanted kidneys against I/R in two models

En bloc, short-term
- Donors: N=7
- Recipient: not related to donor (n=7)

Long-term, allogeneic
- Donors: n=6
- Recipient A: not related to donor (n=6)
- Recipient B: not related to donor (n=6)
Binding of PEG-phospholipid
Complement deposition

- C3b deposition in kidney
  - Control
  - Treated

- C4d deposition in kidney
  - Control
  - Treated

- MAC deposition in kidney
  - Control
  - Treated
Coagulation and Complement activation

**Graphs showing TAT, C3a, and sC5b-9 levels over time post reperfusion for control and treated groups.**
Diuresis

Total diuresis (ml) during 360 min post tx

Control

PEG-phospholipid construct

n=6

*
Cytokines

Inflammatory markers

IL-1β (pg/ml)

Control (n=3)
PEG-lipid (n=7)

Time (hour) post reperfusion

IL-6 (pg/ml)

Control (n=3)
PEG-lipid (n=7)

Time (hour) post reperfusion

MCP-1 (pg/ml)

Time (hour) post reperfusion

TNFα (pg/ml)

Time (hour) post reperfusion

* Indicates statistical significance.
Plasma Creatinine
Kidney function marker

![Plasma Creatinine graph showing time (hour) post reperfusion and plasma creatinine levels for Control and PEG lipid groups.](image-url)
Summary

1. PEG/phospholipid-linked regulators are efficient regulators of these reactions \textit{in vitro}

2. Also the PEG/phospholipid linker alone has a significant effect on I/R injury \textit{in vivo}
Tack!