Cytokines / molécules apparentées et événements indésirables receveurs liés aux transfusions de concentrés plaquettaires

22/11/2018 - Auditorium Chateaubriand
11:00-12:30 : Session 13 - SFVTT/SFTS - Du produit au malade (2)

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Platelet a great immunomodulatory cell

• Brief Overview of Platelet Functions

The Non-Hemostatic Aspects of Transfused Platelets

• Blood platelets are important reservoirs of soluble mediators
• Machine learning and inflammatory aspects of transfused platelets
• Interaction between transfused platelets and endothelial cells

CONCLUSION : Getting the right product to the right patient!
Platelets as autonomous drones for hemostatic and immune surveillance

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A Hemostasis
1. Detection of vascular breach
2. Response to endothelial alarms
3. Pathogen binding and/or degranulation
4. Regulation of vessel permeability
5. Sealing of vascular breaches during leukocyte transmigration
6. Blood-lymph separation

B Immunity
1. Direct pathogen detection
2. Response to leukocyte alarms (e.g., NETs)
3. Leukocyte recruitment and activation
4. Leukocyte degranulation
5. Physical interaction with leukocytes and delivery/exchange of activation signals
6. Neutrophil

Major platelet tasks in hemostasis and immunity.

Platelets circulate in blood, surveying the vasculature for

(A) hemostatic and
(B) immune stress
Platelet receptors. List of receptors in human platelets categorized by their major functional types.
Platelet payloads. List of bioactive mediators released by human platelets categorized by their major functional roles.

**Microbicidal effectors (Immune payload)**
- C3 precursor
- C4 precursor
- Complement factor D
- CXCL7-derived peptides (PBP, TAP-III, thromboxin-1 and 2, thromboglobulin)
- IgG
- MMP-1, 2 and 9
- Thromosin-β4
- Cathepsin D and E

**Coagulation factors (Thrombotic payload)**
- α2-antiplasmin
- Factor II/prothrombin
- Factor V
- Factor X
- Factor XIII
- Fibrinogen
- Fibronectin
- HMW kininogens
- PAI-1
- Vitronectin
- VWF
- Glutamate

**Signaling factors (communication)**
- P-selectin (CD62P)
- TGF-β
- ADP
- ATP
- Calcium
- Epinephrine
- Histamine
- Polyphosphate
- Pyrophosphate
- Serotonin
- Acid phosphatase
- IL-1β
- Thromboxane A2

**Chemokines (calling reinforcements)**
- CCL2
- CCL3
- CCL5
- CXCL1
- CXCL12
- CXCL4/PF4
- CXCL5
- CXCL8
- NAP2 (CXCL7)

**Anti-microbial factors (Immune regulation)**
- C1 inhibitor
- Complement factor H
- TIMP-1 and 4

**Anti-coagulative factors (Thrombotic regulation)**
- α2-macroglobulin
- Antithrombin
- Plasmin
- Plasminogen
- Protein S
- TFPI

**Growth/angiogenic regulators (support and delivery)**
- Angiopoietin-1
- BDNF
- bFGF
- BMP-2,4 and 6
- CTGF
- Thrombospondin
- EGF
- Endostatin
- HGF
- IGF-1
- PDGF
- VEGF
- β-glucosaminidase
- 5′-arabinosidase
- β-galactosidase
- β-glucuronidase
- RNA (mRNA, miRNA etc.)

**Secretory package**
- α granules
- Dense granules
- Lysosomes
- Microparticles or other
All patients received transfusions with high levels of platelet-derived mediators.
Blood transfusion and inflammation: Platelet components associated with acute transfusion reactions

Main product transformation:
- Automated cell separation
- Centrifugation
- Leucoreduction
- Platelet additive solutions
- Occasionally pathogen or Reduction or inactivation technology
- Irradiation
- Deplasmatization/washing
- Cryopreservation
- Volume reduction

Increase:
- Activation (release of granular contents)
- Proteolysis
- Platelet aggregates
- Volume and density heterogeneity
- Procoagulant activity
- Platelet apoptosis
- pCO2
- Lactate production
- Glucose consumption

Decrease:
- pH
- pO2
- glucose
- mean platelet volume
- Calcium ion flux
- Mitochondrial oxidative respiration
- Fibrinogen binding

Released/increased factor:
- EGF, ENA-78, Gro-α, IL-1β, IL-6, IL-7, IL-8, IL-27,
- Lyso-PCs, sOX40L, PAI-1, PDGF-β, PF4, RANTES,
- sCD40L, TGF-β, TNF-α, VEGF, α-TG, Microvesicles,
- Mitochondrial DNA

Storage lesion:
- Shape changes from discoid to spheroid
- Altered platelet surface receptor expression
- ATP/ADP ratio change

Typical storage duration for platelets is 5-7 days

Platelet concentrates storage and biological response modifier release. (Front Med (Lausanne). 2018 Feb 27;5:42)
CD40/CD40L, is well known for its roles of bridging between innate and adaptive immunity.

Hypothetical mechanisms by which platelet membrane CD40/CD40L and soluble CD40L might regulate interactions between immune cells.

Platelets can interact with numerous immune cells such as
- B cells,
- T cells,
- Neutrophils,
- Macrophages,
- Endothelial cells,
- Natural killer (NK) cells
- Dendritic cells (DC)

- Activation
- Cytokine secretion
- Anti-tumoral activity
- Activation
- Leukocyte adhesion
- Proliferation
- Differentiation into Dendritic cells
- Activation
- BRM secretion
- Activation
- Proliferation
- Cytokine secretion
- Isotypic commutation
- Cytokine secretion
- Proliferation/cell survival
- Activation
- Proliferation
- Cytokine secretion

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Increased levels of sCD40L in transfused blood are associated with transfusion-related Adverse Events (AEs)
Bioactivity of IL-27 and sOX40L-rich pathologic PLT supernatant

- IL-27 bioactivity was tested on isolated, purified blood B lymphocytes
- sOX40L bioactivity was tested on isolated, purified, activated blood T lymphocyte
• Levels of such cytokine-like factors increased significantly during storage.

• but no significant difference was detected between PRT- and control PCs.
This study clearly showed that sCD40L levels are not fully predictive of SARs, but leaves open the possibility of

- The comorbidities of the recipient,
- Genetic susceptibility (high affinity binding of sCD40L by off target receptors),
- Or a causal disease condition,
- Or all three.
Soluble CD40L and CD62P levels differ in single donor apheresis platelet concentrates and Buffy-coat-derived pooled platelet concentrates – *Transfusion in press – Sut et al.*

- SDA-PCs appeared more activated than PPCs at the end of the production step (i.e., prior to storage);
- However, pro-inflammatory soluble factors increases in PPCs than in SDA-PCs during storage.

In SDA-PCs, PAS-D (65%) led to reduced secretion of sCD62P, but favored secretion of sCD40L, compared with the alternative PAS-E.

**Conclusion:** These data stress the importance of the production (processing) steps of PC manufacture, and of storage.
Extracellular mitochondria, produced by platelets, at the midpoint of a potent mechanism leading to inflammatory responses

Extracellular mitochondria are present in various situations where platelets are known to be activated

1) Extracellular mitochondria (as detected by mtDNA quantification) are found at higher concentration in PFP of platelet storage bags that have cause adverse transfusion reaction to the recipient (no adverse reaction group \( n = 61 \) vs adverse reaction group \( n = 74 \) matched in term of storage duration).

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We investigated a large series of AEs after platelet component transfusions reported.

We used a combination of clinical observations, ex vivo and in vitro investigations, and mathematical modeling systems.

We calculated the statistical association of a large variety (n = 17) of cytokines, chemokines with acute inflammatory potential in patients presenting with transfusion-related Adverse Events.
Decision-tree learning: febrile nonhemolytic transfusion reactions (FNHTRs), atypical allergic transfusion reactions (AATRs), Acute transfusions reaction (ATRs)

A. Assays without IL13 (among 16 assays, the success rate of the sCD40L model was the highest, 78%)

B. Assays with IL13 (among 17 assays, the success rate of the IL13 model was the highest, 82%).

- **Decision-tree learning**: that proved Acute transfusions reaction (ATRs) are dependent on the level (amount) of a given cytokine-like platelet product.

- We further modelled the risk of the patient presenting either a FNHTRs or an AATRs, depending on the amount of the chemokine MIP-1α.
Platelet components associated with adverse reactions: predictive value of mitochondrial DNA relative to biological response modifiers

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Decision tree. Assays from a set of **101 training samples with 18 attributes** (age of the blood sample; age of the donor; PLT count; and levels of Gro-a, sCD40L, 6-Ckine, CXCL9, IL-23, MIP-1a, IL-13, IFN-c, IL-15, MDC, IL-33, CCL19, CD62P, RANTES, and mtDNA).

The success rate of the IL-13 model was the highest: **83%**
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Platelets (and Transfused Platelets) are critical in maintenance of endothelial barrier function.

- **A**: when platelet numbers are sufficient, semipermeable endothelial barriers that restrict transfer of water and proteins out of systemic and alveolar capillaries are maintained and protected.

- Release of stabilizing factors by platelets is one mechanism for endothelial barrier maintenance.
Platelets (and Transfused Platelets) are critical in maintenance of endothelial barrier function but also induce increased endothelial permeability in inflammation. (Physiol Rev 96: 1211–1259, 2016)

- **C:** activated platelets can induce or amplify increased permeability of alveolar and systemic endothelial barriers in inflammation.

- Several mechanisms have been proposed or demonstrated in experimental models, including release of platelet factors that disrupt endothelial barriers, signaling of endothelial cells, and interaction with PMNs and monocytes, leading to disruption of endothelial bonds and leak of fluid, proteins, and RBC.
Endocan/ESM-1 is a proteoglycan secreted by endothelial cells under the control of proinflammatory cytokines.

We aimed to measure endocan activity (EA.hy926 endothelial cells) in supernatants of PLT components (PCs), implicated in serious adverse reactions (SARs) or not (no.AR), sampled at different stages during storage.

PLT activation does not induce endocan release
We next investigated the bioactivity of the no.AR and SAR PC supernatants aged 0 to 3 or 3 to 5 days on EA.hy926 cells by measuring IL-6 and endocan secretion after 6- or 24-hour exposure to the supernatant.

IL-6 and endocan secretion were significantly higher for cells stimulated with SAR than those stimulated with the no.AR PC supernatants, regardless of the time in storage.

There was a significant correlation between IL-6 and endocan secretion after EA.hy926 cell activation.
How can non-nucleated platelets be so smart?

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Transfusion of platelets is generally safe and largely beneficial to patients.

On rare occasions, SARs, occur with clinical presentation of acute inflammation.

In all cases investigated to date, either based on clinical observations or tested experimentally, Biological response modifiers (BRMs) (such as sCD40L) are found to be in close association.

CONCLUSION: Getting the right product to the right patient!

Transfusion-linked inflammation is likely the result of a combination of factors related to the Donor, the BC, and the Recipient.

Unfortunately the main factor that can be targeted at present is the BC

Transfusion medicine may become one of the first medical specialties where personalized medicine comes into effect: “How can a given patient be given the BC most suited to his or her condition”?
Platelet Inflammation Response to Stress: Team PIRS / GIMAP EA3064

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INFLAMMATION

Acute stress, Sepsis,
Infectious stress, Sepsis,
Acute Lung Injury

In vitro models
Animal models
Clinical Approach

INJURY

Platelets as a stress markers

IMMUNITY

Acute transfusion reactions,
Related Acute Lung Injury

FINANCIAL SUPPORT

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