The immunomodulatory effects of blood transfusions and haemoderivatives

Relevance of soluble HLA class I, soluble Fas Ligand and TGFβ

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Effect of blood transfusions on subsequent kidney transplants.

Opelz G, Sengar DP, Mickey MR, Terasaki PI.

Tolerogenic effect of pre-transplant blood transfusions in humans.
Immunomodulation and blood transfusion.

Blajchman MA.

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Over the past three decades, evidence from a variety of sources has suggested that allogeneic blood transfusions can induce clinically significant immunosuppression in recipients. This clinical syndrome is referred to in the transfusion medicine literature as transfusion-associated immunomodulation (TRIM) and has been linked to an improved clinical outcome in the setting of renal transplantation. Possible deleterious TRIM-associated effects include increased prevalence of cancer recurrence and postoperative bacterial infections. The recognition that TRIM can increase morbidity and mortality in allogeneically transfused individuals has become a major concern for those involved in transfusion medicine. Whether TRIM predisposes recipients to increased risk for cancer recurrence and/or bacterial infections is still not proven, however. In contrast to the available clinical data, studies in experimental animal models suggest that TRIM is an immunologically mediated biologic effect associated with the infusion of allogeneic leukocytes, which can be ameliorated by prestorage leukoreduction. Although considerable data have been accumulated in an attempt to unravel the clinically adverse effects of TRIM, the precise mechanism of TRIM has yet to be elucidated. Further studies, both basic and applied, to establish the clinically relevant manifestations of TRIM as well as the mechanism(s) are urgently required.

Publication Types:
- Review
## Immunomodulation by Allogeneic Blood Transfusion

<table>
<thead>
<tr>
<th>Beneficial Effects</th>
<th>Adverse Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improvement of allograft survival</td>
<td>Elicit anti-HLA antibodies</td>
</tr>
<tr>
<td>Reduced recurrence rate of Crohn’s disease</td>
<td>Promote tumor growth</td>
</tr>
<tr>
<td>Effective treatment of recurrent abortion</td>
<td>Increase postoperative infections</td>
</tr>
</tbody>
</table>

Are dependent on the presence of leukocytes and/or plasma factors in haemocomponent transfused. Are reduced by prestorage leukodepletion but not by poststorage leukodepletion.
IMMUNOMODULATION BY ALLOGENEIC BLOOD TRANSFUSION
Factors involved

- Number of units transfused
- Leukocyte number in each unit
- Leukodepletion procedure
- Storage conditions
- Trial design
- Type of surgery / anaesthesia
- Immune system of patients
Leukoreduction, as a way to reduce complications and costs of cardiac surgery, is still being debated and in need of a general consensus. Recommended by an advisory committee of the US Food and Drug Administration in 1998, it has not been implemented on a large scale. The direct costs involved are estimated in the range of $25-$35 per transfusion.
IMMUNOMODULATION BY ALLOGENEIC BLOOD TRANSFUSION

Current questions

• Which constituent induces the effect:
  T cells, B cells, dendritic cells, stem cells
  Anti-idiotypes, cytokines, soluble factors

• Does the effect result from:
  Thymic or peripheral tolerance
  Clonal deletion or induction of anergy
  Active immune suppression
  Microchimerism

• What are the requirements for donor-recipient matching

  Does a unifying hypothesis account for transplantation
tolerance, susceptibility to bacterial infections and breakdown
of tumor surveillance
IMMUNOMODULATION BY CLOTTING FACTOR CONCENTRATES

Numerous *in vitro* and *ex vivo* studies show that clotting factor concentrates (CFCs) may exert immunomodulatory activities. In fact, it has been shown that they inhibit PHA-stimulated lymphocyte proliferation (1) and the activity and secretion of several cytokines, (2, 3), trigger lymphocyte apoptosis (4), downregulate Fc receptors expression and function in monocytes (5), and modify the expression of cell-surface molecules involved in T-B lymphocyte interactions (6, 7). The results of several studies confirmed that many haemophiliac patients with no evidence of viral infection had abnormalities in their immune systems, particularly with regard to cell mediated immunity.

And IVIG or apheresis?

Could this "unifying hypothesis" have the same role for haemoderivatives immunomodulation?

Linfocita T CD8⁺ attivato
TGFβ
IMMUNOMODULATION BY ALLOGENEIC BLOOD TRANSFUSION...

Aims

A. To determine sHLA-I, sFasL and TGFβ1 in blood components and haemoderivatives by immunoenzymatic assays in blood components’ supernatant (after centrifugation at 12,000 g for 2 min)

B. To perform their immunochemical analysis by SDS-PAGE and Western Blot

C. To assess their immunosuppressive activity in vitro by antigen-specific allorestricted CTL activity, mixed lymphocyte response and apoptosis induction in Fas+ cells
ALLOGENEIC and/or AUTOLOGOUS BLOOD COMPONENTS and HAEMODERIVATIVES

1) Washed packed red blood cells (not buffy-coat depleted) (W-RBC)
2) Prestorage leukodepleted RBC stored 30 days (LD-RBC)
3) RBC stored for 5 days (RBC-5)
4) RBC stored for 30 days (RBC-30)
5) Random Donor Platelets (platelet-rich plasma) (PLT)
6) Fresh frozen plasma (FFP)
7) Blood donors’ sera
8) Clotting factors concentrates (purified from plasma and recombinant)
9) Intravenous immunoglobulin preparations (different batches, from different manufacturers)
Concentrations of sHLA-I (A) and sFasL (B) in blood components
### Concentration of sHLA-I, sFasL and TGFβ1 molecules in clotting factors (ng/ml)

<table>
<thead>
<tr>
<th>No</th>
<th>Sample</th>
<th>Batch</th>
<th>sFasL</th>
<th>sHLA-I</th>
<th>TGFβ1</th>
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<tr>
<td>1</td>
<td>ReFacto 500</td>
<td>11315A51</td>
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<td>n.d.</td>
<td>n.d.</td>
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<td>3</td>
<td>Hyland Recombinate 500</td>
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<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>4</td>
<td>Benefix 500</td>
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<td>5</td>
<td>Novoseven</td>
<td>JU61083</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
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<td>6</td>
<td>Kogenate 1000</td>
<td>DAGUHI</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
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<tr>
<td>7</td>
<td>Fibrinogeno TIM 3</td>
<td>040197B</td>
<td>3959.71</td>
<td>930</td>
<td>n.d.</td>
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<td>8</td>
<td>Kryobulin TIM 2 1000</td>
<td>09M3886115</td>
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<td>2440</td>
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<tr>
<td>9</td>
<td>Kryobulin SP 500</td>
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<td>25.79</td>
<td>740</td>
<td>4.994</td>
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<td>10</td>
<td>Fandhi</td>
<td>N-008</td>
<td>3.71</td>
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<td>11</td>
<td>Emoclot DI 1000</td>
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<td>12</td>
<td>Haemate P 500</td>
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<td>13</td>
<td>Feiba TIM 3 1000</td>
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<td>n.d.</td>
<td>n.d.</td>
<td>1.153</td>
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<td>14</td>
<td>Hemofil M 1000</td>
<td>99J22B20</td>
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<td>n.d.</td>
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<td>15</td>
<td>Protromplex TIM 3 500</td>
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<td>16</td>
<td>Proventin UM TIM 3 500</td>
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<td>Uman Complex DI 500</td>
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</table>
## Concentration of sHLA-I and sFasL in commercial IVIG preparations

<table>
<thead>
<tr>
<th>Type of IVIG process</th>
<th>Lot #</th>
<th>sHLA-I (µg/ml)</th>
<th>sFas-L (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepsin treatment, pH 4</td>
<td>1</td>
<td>2.9</td>
<td>47.8</td>
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<tr>
<td></td>
<td>2</td>
<td>3.2</td>
<td>44.2</td>
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<tr>
<td>ß-Propiolacton</td>
<td>1</td>
<td>1.3</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.3</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.4</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.5</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.0</td>
<td>18.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.6</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>7</td>
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<td>1.5</td>
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<td>9</td>
<td>0.6</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Trypsin, PEG, DEAE</td>
<td>1</td>
<td>&gt;6.0</td>
<td>67.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;6.0</td>
<td>-</td>
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<tr>
<td>PEG, DEAE, Heat</td>
<td>1</td>
<td>&gt;6.0</td>
<td>166.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&gt;6.0</td>
<td>47.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>&gt;6.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>&gt;6.0</td>
<td>156.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>&gt;6.0</td>
<td>131.3</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>&gt;6.0</td>
<td>112.8</td>
</tr>
<tr>
<td>Diafiltration, pH 4.25</td>
<td>1</td>
<td>2.4</td>
<td>62.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.2</td>
<td>57.6</td>
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<tr>
<td></td>
<td>3</td>
<td>1.4</td>
<td>52.6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.0</td>
<td>66.7</td>
</tr>
</tbody>
</table>
Western blot analysis of blood components with TP25.99 mAb

1: PBL
2: RBC stored 30 days
3: Platelets
4: Washed RBC
5: Fresh frozen plasma
6: RBC stored 5 days
Western blot analysis of blood components with anti-FasL G247.4 mAb

1: THP1 cells  4: Washed RBC
2: RBC-30    5: RBC-5
3: PLT        6: FFP

40 kD
Effect of allogeneic blood components on MLR response
Effect of autologous blood components on MLR response
Effect of clotting factors concentrates on MLR response

CFC

CTRL
CFC
CFC-sHLA-I
CFC-sFasL
CFC-sHLA-I/sFasL

rH-CFC

CTRL
rH-CFC
rH-CFC-sHLA-I
rH-CFC-sFasL
rH-CFC-sHLA-I/sFasL
Effect of IVIG on MLR response

Days: 2, 3, 4, 5, 6, 7, 8, 9, 10

c.p.m.: 0, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000

- Controls
- IVIG
- -sHLA-I
- -sFasL
- -sHLA-I + sFasL
Effect of blood components on CTL activity (1)
Effect of clotting factors concentrates on CTL activity

% Target cell lysis

- E:T 30:1
- E:T 10:1
- E:T 3:1
- CFC
- CFC - sHLA-I
- CFC - sFasL
- CFC - sHLA-I / sFasL
- rH-CFC
Effect of IVIG components on CTL activity

- % Target cell lysis
- Contr, IVIG, IVIG - sHLA-I, IVIG - sFasL
- 30:1, 10:1, 3:1
Apoptosis-inducing capacity of blood components on Jurkat cells (propidium iodide staining)

<table>
<thead>
<tr>
<th>Culture Medium</th>
<th>Anti-Fas</th>
<th>CH11 mAb</th>
<th>RBC-30</th>
<th>RBC-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>W-RBC</td>
<td>0.5%</td>
<td>75%</td>
<td>89%</td>
<td>11%</td>
</tr>
<tr>
<td>LD-RBC</td>
<td>12%</td>
<td>2%</td>
<td>31%</td>
<td>10%</td>
</tr>
<tr>
<td>PLT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
sFasL Concentration and Apoptotic Activity of IVIG
Apoptosis-inducing capacity of blood components on resting PBL (2)
Apoptosis-inducing capacity of autologous blood components on activated PBL (3)
Apoptosis-inducing capacity of blood components on activated PBL (72h) (4)
Modified Blind Well Boyden Chamber
Chemotaxis Assay

Upper chamber with cells

Nitrocellulose filter

Lower chamber with chemoattractant
Upper chamber with cells

Nitrocellulose filter

Lower chamber with RBC
Dose-dependent induction of neutrophil migration by various dilutions of RBC supernatant.
Upper chamber with cells and RBC

Nitrocellulose filter

Lower chamber with chemoattractant
Dose-dependent inhibition of FMLP-induced neutrophil migration by various dilutions of RBC supernatant
RBC supernatant

- Stimulation of migration
- Inhibition of migratory response to FMLP

Chemotactic and cross-desensitizer factor in RBC supernatant (?)

TGF-β₁
Effect of RBC transfusion on plasma modulation of FMLP-triggered chemotaxis.
Time-course of percent inhibition of FMLP-triggered chemotaxis by plasma from three transfused patients
Cumulative data from transfused patients
A novel family of Ig-like receptors for HLA class I molecules that modulate function of lymphoid and myeloid cells

Marco Colonna, Hideo Nakajima, Francisco Navarro,* and Miguel López-Botet*

Basel Institute for Immunology, Switzerland; and *Hospital de la Princesa, Madrid, Spain

Fig. 1. ILT/LIR/MIR receptor family. The receptors are classified by differing transmembrane and cytoplasmic domains into three groups: inhibitory receptors (cytoplasmic ITIMs), activating receptors (associated with FcγR), and soluble receptors (no transmembrane and cytoplasmic domains).
ILT-1 is expressed on peripheral blood granulocyte

**Cutting Edge: Human Myeloid Cells Express an Activating ILT Receptor (ILT1) That Associates with Fc Receptor γ-Chain**

Hideo Nakajima, Jacqueline Samaridis, Lena Angman, and Marco Colonna

*The Journal of Immunology, 1999, 162: 5–8.*
Analogamente si riscontra l’up-regulation del TGF-β1 (mRNA) all’analisi citofluorimetrica intracitoplasmatica dei neutrofili dei pazienti trasfusi.
• Dati assolutamente sovrapponibili circa l’effetto immunomodulante delle trasfusioni sulla popolazione NK e l’attività LAK

• Dati assolutamente sovrapponibili circa l’effetto immunomodulante delle IVIG sulle varie popolazioni circolanti nel ricevente
Considerazioni finali

• sHLA-I, sFasL e TGFβ “contaminanti” gli emocomponenti e gli emoderivati agiscono in maniera sinergica sulle varie popolazioni cellulari del ricevente. La loro azione risulta prevalentemente immunosoppressiva/tollerogena.

• La situazione clinica, lo stato immunitario del paziente nonché della terapia farmacologica condizionano pesantemente la suscettibilità a tale “immunomodulazione”

• Se da un lato tali dati indicano un uso più prudenziale della terapia trasfusionale, dall’altro (es: IVIG) possono “rappresentare uno strumento terapeutico a tutti gli effetti”
We are here