Intravenous Immunoglobulin: A tale of two ends of the molecule

John W. Semple, PhD
Toronto Platelet Immunobiology Group, St. Michael’s Hospital.
Outline of Talk

• Immunoglobulin G.
• IVIg preparation/Utilization.
• IVIg and anti-D mechanisms of action:
  1) Fc Receptor mediated effects.
  2) Idiotypic-mediated effects on the immune response.
• Conclusions
The principal mechanisms of innate and adaptive immunity.

**Innate immunity**
- Microbe
- Epithelial barriers
- Phagocytes
- Complement
- NK cells

**Adaptive immunity**
- B lymphocytes
- T lymphocytes
- Antibodies
- Effector T cells

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<th>Time after infection</th>
<th>Hours</th>
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2 Heavy and 2 Light chains
2 Fab and 1 Fc fragment
4 Subclasses (IgG1, IgG2, IgG3, IgG4)
Mol. Wt. 150,000
~70-75% of serum immunoglobulin.
The major antibody of the secondary immune response
Change in affinity with time
(Somatic Mutation)
Intravenous Immunoglobulin (IVIg):

A relatively pure collection of polyclonal gammaglobulins (IgG) derived from the pooled plasma of thousands of blood donors.
### Types of IVIg:

<table>
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<tr>
<th>Polyclonals</th>
<th>Hyperimmunes</th>
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<tr>
<td>IVIg (IVIg)</td>
<td>Anti-Rh (D)</td>
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<td>(WinRhO)</td>
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<td>Anti-Tetanus</td>
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<td>Anti-Varicella</td>
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<td>Anti-etc.</td>
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INDICATED Uses of IVIg (RCT):

**Immune Replacement**
- **Primary Immunodeficiencies** (Congenital agammaglobulinemia (X-linked), Hypogammaglobulinemia, Common variable immunodeficiency, X-linked immunodeficiency with hyper IgM, Severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome.

**Immune Modulation**
- Immune Thrombocytopenic purpura (ITP)
- Kawasaki Syndrome
- Allogeneic Bone Marrow Transplantation
- B-Cell Chronic Lymphocytic Leukemia
- Pediatric HIV infection
Pharmacokinetics of IVIg and AntiD:

Kinetics are complex and variable.
- IVIg has a biphasic elimination:
  Distribution: \((\alpha \text{ phase})\) 3-5 days
  Elimination: \((\beta \text{ phase})\) 3-4 weeks (avg. 23 days)
- Anti-D has similar kinetics in D- individuals.

Dosage:

**IVIg (Pediatric)**:
- **PID**: Severe; 400 mg/kg, Partial, 100-200 mg/kg once monthly (x3 months).
- **ITP**: Higher doses (1-2 g/kg x 2 days).

**Anti-D (Pediatric)**:
- **ITP**: 50-75 ug/kg once (Concentration based on WHO activity units; still infusing mg amts of protein).
IVIg Manufacturing Processes:

- **Primary cold ethanol (Cohn-Oncley) fractionation.**
- **Secondary fractionation may include:**
  - Chemical modification
  - Incubation at pH 4.0 with or without pepsin
  - PEG precipitation
  - Ion-exchange chromatography
  - Enzymatic cleavage
  - Solvent detergent treatment
  - Diafiltration and ultrafiltration
## Canadian IVIg Brands:

<table>
<thead>
<tr>
<th></th>
<th>Gamunex™</th>
<th>CBSIVIg/Gamimune®</th>
<th>Gammagard S/D</th>
<th>Iveegam</th>
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<tbody>
<tr>
<td><strong>Manufacturer</strong></td>
<td>Bayer</td>
<td>Bayer</td>
<td>Baxter</td>
<td>Immuno</td>
</tr>
<tr>
<td><strong>IgA Content (ug/ml)</strong></td>
<td>&lt;1</td>
<td>&lt;270</td>
<td>&lt;3.7</td>
<td>&lt;2</td>
</tr>
<tr>
<td><strong>Process</strong></td>
<td>Chromatography</td>
<td>Cohn</td>
<td>Cohn</td>
<td>Cohn</td>
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<tr>
<td><strong>IgG%</strong></td>
<td>&gt;98</td>
<td>&gt;98</td>
<td>&gt;90</td>
<td>&gt;98</td>
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<tr>
<td><strong>Half life</strong></td>
<td>&gt;21 d</td>
<td>&gt;21 d</td>
<td>37 d</td>
<td>23-29 d</td>
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<tr>
<td><strong>Sugar stabilizer</strong></td>
<td>No sugar</td>
<td>Maltose (9-11%)</td>
<td>Glucose (2%)</td>
<td>Glucose (5%)</td>
</tr>
<tr>
<td><strong>Sodium</strong></td>
<td>Not given</td>
<td>Not given</td>
<td>8.5mg/ml</td>
<td>3mg/ml</td>
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<tr>
<td><strong>Form</strong></td>
<td>Liquid</td>
<td>Liquid</td>
<td>Lyophilized</td>
<td>Lyophilized</td>
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<tr>
<td><strong>Administration</strong></td>
<td>10% soln</td>
<td>5-10% soln</td>
<td>5-10% soln</td>
<td>5% soln</td>
</tr>
<tr>
<td><strong>Shelf life</strong></td>
<td>18 mo</td>
<td>36 mo</td>
<td>27 m</td>
<td>24 mo</td>
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<tr>
<td><strong>Storage</strong></td>
<td>RT</td>
<td>2-8°C</td>
<td>RT</td>
<td>2-8°C</td>
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<tr>
<td><strong>Viral Inactivation</strong></td>
<td>Caprylate</td>
<td>Solvent detergent</td>
<td>Solvent detergent</td>
<td>Solvent detergent</td>
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Antibody content in IVIg:

Antibodies against bacterial-, viral-, fungal- and auto-
antigens and can be found in IVIg preparations. **Antiidiotypic antibodies are also found.**

Ab titers vary substantially:

e.g.    Anti-E. Coli J5 LPS (<5-140)
        Anti-VZV (100-1920)
        Anti-thyroglobulin (2-40)
        Anti-GPIIbIIIa
        Anti-Factor VIII

These specific antibodies may be responsible for some of IVIg’s benefits and mechanism(s) of action.
IVIg Utilization:

**IVIg Usage (Canada)**

<table>
<thead>
<tr>
<th>Year</th>
<th>IVIg Issued (Kg)</th>
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<tbody>
<tr>
<td>1992</td>
<td>0.025</td>
</tr>
<tr>
<td>1993</td>
<td>0.050</td>
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<tr>
<td>1994</td>
<td>0.075</td>
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<td>1995</td>
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<td>1997</td>
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<td>0.175</td>
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<td>1999</td>
<td>0.200</td>
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<td>2000</td>
<td>0.225</td>
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<tr>
<td>2001</td>
<td>0.250</td>
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<tr>
<td>2002</td>
<td>0.275</td>
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</tbody>
</table>

**IVIg use per capita**

<table>
<thead>
<tr>
<th>Country</th>
<th>Grams/capita</th>
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<tbody>
<tr>
<td>Australia</td>
<td>0.025</td>
</tr>
<tr>
<td>Japan</td>
<td>0.025</td>
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<tr>
<td>Sweden</td>
<td>0.025</td>
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<td>Germany</td>
<td>0.025</td>
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<tr>
<td>US</td>
<td>0.025</td>
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<tr>
<td>Canada</td>
<td>0.275</td>
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</tbody>
</table>

**2002**

Worldwide usage: ≈40,000 kg
IVIg Shortages:

• Inappropriate usage
• Production problems
• Manufacturers schedules
• Product recalls (e.g. worldwide CJD recall, 1999)
**INDICATED**

**Immune Replacement**

- **Primary Immunodeficiencies** (Congenital agammaglobulinemia (X-linked), Hypogammaglobulinemia, Common variable immunodeficiency, X-linked immunodeficiency with hyper IgM, Severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome.

**Immune Modulation**

- Allogeneic Bone Marrow Transplantation
- B-Cell Chronic Lymphocytic Leukemia
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**OFF LABEL**

(Potentially Indicated)

- Acute Guillain-Barré Syndrome
- AIDS-Related Complex (ARC)
- Anemia (autoimmune hemolytic, aplastic, Diamond Blackfan)
- Dermatomyositis
- Group A streptococcus infection
- Lymphoid leukemia
- Multiple myeloma
- Myasthenia gravis
- Necrotizing fasciitis
- Pediatric Immunodeficiency Syndrome
- Polyneuropathy (CIDP)
- Polymyositis
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Inappropriate Use

IVIg Use in BC (Apr.-Dec. 1999)

- Labelled: 59%
- Unlabelled - Potentially Indicated: 19%
- Unlabelled - Not Indicated: 19%
- Indication Not Specified: 3%
IVIg, Mechanisms of action:

How do IVIg and anti-D preparations increase platelet counts in patients with ITP?
Pathogenesis of immune platelet disorders:

- Transfusion
- Spontaneous

Autoantibodies

Alloantibodies

Platelet

RES
IVIg, Mechanisms of action:

**Theory 1:**
Blockade of Fc receptors

**Theory 2:**
FcγRIIB-dependent monocyte inactivation

- Platelet
- Mono

Competitive.

*Fehr et al, 1982*

*FcγRIII or IIA*

*FcγRIIB inhibitory signal*

*Samuelsson et al, 2001*
### The Fc receptor system:

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Fcγ RI (CD64)</th>
<th>Fcγ RII-A (CD32)</th>
<th>Fcγ RII-B2 (CD32)</th>
<th>Fcγ RII-B1 (CD32)</th>
<th>Fcγ RIII (CD16)</th>
<th>FcεRI</th>
<th>FcαRI (CD89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>α 72 kDa</td>
<td>α 40 kDa</td>
<td>γ-like domain</td>
<td>ITIM</td>
<td>α 50–70 kDa</td>
<td>α 45 kDa</td>
<td>α 55–75 kDa</td>
</tr>
<tr>
<td>Binding</td>
<td>IgG1 10^8 M^-1 1) IgG1=IgG3 2) IgG4 3) IgG2</td>
<td>IgG1 2 x 10^5 M^-1 1) IgG1 2) IgG3=IgG2* 3) IgG4</td>
<td>IgG1 2 x 10^5 M^-1 1) IgG1=IgG3 2) IgG4 3) IgG2</td>
<td>IgG1 2 x 10^6 M^-1 1) IgG1=IgG3 2) IgG4 3) IgG2</td>
<td>IgG1 5 x 10^5 M^-1 1) IgG1=IgG3</td>
<td>IgE 10^-14 M^-1</td>
<td>IgA1, IgA2 10^7 M^-1</td>
</tr>
<tr>
<td>Order of affinity</td>
<td>Macrophages, Neutrophils, Eosinophils†, Dendritic cells</td>
<td>Macrophages, Neutrophils, Eosinophils Platelets, Langerhans' cells</td>
<td>Macrophages, Neutrophils, Eosinophils</td>
<td>B cells, Mast cells</td>
<td>NK cells, Eosinophils, Macrophages, Neutrophils, Mast cells</td>
<td>Mast cells, Eosinophils†, Basophils</td>
<td>Macrophages, Eosinophils†, Neutrophils</td>
</tr>
<tr>
<td>Cell type</td>
<td>Uptake, Stimulation, Activation of respiratory burst, Induction of killing</td>
<td>Uptake, Granule release (eosinophils)</td>
<td>Uptake, Inhibition of stimulation</td>
<td>No uptake, Inhibition of stimulation</td>
<td>Induction of killing (NK cells)</td>
<td>Secretion of granules</td>
<td>Uptake, Induction of killing</td>
</tr>
</tbody>
</table>

Fig 9.30 part 1 of 2 © 2001 Garland Science
A murine model of Passive ITP:

- Mice injected with monoclonal anti-GPIIb or anti-GPIIIa.
- Platelet concentration assessed by flow cytometry.
- Thrombocytopenia
- IVIg protects

Samuelsson et al, 2001
Teeling et al, 2001
Crow et al, 2001
IVlg does not reverse immune thrombocytopenia in FcγRIIB Knock Out Mice:

Crow et al, 2001
Summary:

1. IVIg therapy appears to mediate its effects via interaction of the Fc inhibitory receptor and reduces platelet destruction by the RES.

How does anti-D work (by the same mechanism?)
Anti-D (WinRho™) Treatment of Children With Chronic Autoimmune Thrombocytopenic Purpura Stimulates Transient Cytokine/Chemokine Production


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3The Toronto Platelet Immunobiology Group, Toronto, Ontario, Canada
4Division of Hematology, Hôpital Ste-Justine, Montreal, Quebec, Canada
5The Cangene Corporation, Winnipeg, Manitoba, Canada
Cytokines: Post Anti-D

**IL8** (★)

**IL10** (★)

**IL6** (★★)

**GM-CSF** (★★)

**MIP-1-α** (★★)

**MCP-1** (★★)

**TNF-α** (★★)

**IL1RA** (★★)

Concentration (pg/ml)
To understand anti-D’s mechanism of action:

Can we mimic these results in vitro?

Test early events: 1 minute to 4 hours after anti-D treatment:

- Reactive Oxygen Species (ROS)
- Phagocytosis of opsonized RBC
- Cytokine expression
Phagocytosis Assay:

CMFDA (CellTracker Green)-labelled and opsonized (or not) RBC (Anti-D) or platelets (mouse; anti-MHC, human; W6/32)

RT°C

THP1 (4°C)

4°C control

37°C test (DMEM media)

2 hrs

Quench

wash

Flow cytometric analysis
Anti-D-RBC Phagocytosis

Coopamah et al Blood 2003

FL2 fluorescence

Cell Number

Forward Scatter

Side Scatter

Monocytes

Granulocytes

0°C

37°C
IL1ra Expression (4 hours):

Monocytes

Granulocytes

RBC to WBC ratio

(+anti-D)

(-anti-D)
Effect of IL1ra on Phagocytosis:

Erythrophagocytosis in the presence of IL-1ra (2 hours)

Fold change in phagocytosis

Opsonized RBC 1:1000

Control

IL-1ra 100 ng/ml

Monocytes

Granulocytes

p=0.02

p=0.01
Time course of Events:

- **ROS**
- **Phagocytosis**
- **IL1ra**

Fold Change vs. Time (hours)
THP 1:

Human monocytic leukaemia

Derived from the peripheral blood of a 1 year old male with acute monocytic leukaemia.

Properties:

Receptors: FcRII/III, C3b, lack surface Ig.
Positive for alpha-naphthyl butyrate esterase.
Produce lysozymes.
Phagocytic (both latex beads and sensitised erythrocytes,
(show increased CO2 production on phagocytosis ).
Can restore the response of purified T lymphocytes to Con A
Can differentiate into macrophage-like cells using DMSO.
THP1 Phagocytosis of platelets:

IgG bound Plts  F(\text{ab}’)\text{2} bound Plts

CM Green Fluorescence
THP1 Phagocytosis and IVIg:

Control (No IVIg)

IVIg (1.4 mg/ml; 10 fold less than 1g/kg)
(Inhibition titrates to 0.0014 mgs/ml; 10,000 fold less)

CM Green
THP1 Phagocytosis and Anti-D:

Cell Counts

CM Green Fluorescence

- Control (No Anti-D)
- Non-opsonized or Anti-D opsonized RBC (RBC:THP)

- Non-ops RBC
- Anti-D coated RBC

Percent Inhibition

RBC:WBC Ratio
Summary:

1. Anti-D mediated effects are Fc dependent but appear to additionally require the production of anti-inflammatory cytokines in order to inhibitory platelet phagocytosis.

2. Phagocytic cell lines such as THP-1 can be used to study the biochemical mechanisms of anti-D-mediated platelet rescue from phagocytosis.
And now for something completely different!
Theory 3: Idiotypic Effects.

\[ F(\text{ab}')_2 \]

\[ \text{Fc} \]
Theory 3, Antiidiotypic antibodies:

- IVIg contains anti-idiotype antibodies which:
  - Neutralize the auto-antibodies.
  - Form antibody dimers which block the RES

*Sultan et al, 1984*
Jerne’s Hypothesis:

Antigen

Antiidiotype (Ab2)

Antibody (Ab1)

Ab3
Anti-HLA antiidiotypes:

Anti-HLA antibodies can induce the production of antiidiotypes (e.g. kidney transplant recipients).

Anti paternal HLA antibodies induced by pregnancy induce the production of antiidiotypes.
Clinical observation:

Although IVIg has benefit for patients with autoimmune thrombocytopenic purpura.

It has little or no benefit in patients with alloimmune platelet refractoriness.
Immune pathogenesis:

- Autoantibodies
- Alloantibodies
- Platelet
- Transfusion
- Spontaneous
- RES
Possibilities:

Perhaps the nature of the antibodies (e.g. antiidiotypes) contained within commercial IVIg cannot neutralize or inhibit anti-HLA.
Hypothesis:

Multiparous IVIg (MP IVIg) can significantly inhibit alloimmunity in vivo.
SCID MICE:

- Severe combined immune deficiency.
- Chromosome 16 point mutation.
- Inability to repair double-stranded DNA breaks.
- T and B lymphocytes are ablated.
- Can accept xenografts and human Ab responses can be examined.
Basic protocol:

- Engraft SCID mice with lymphocytes from HLA-sensitized donors.
- Induce human anti-HLA immunity by challenges with allogeneic cells.
- Test various IgG and F(\text{ab}')_2 preparations for the ability to modulate anti-HLA.
Donor Characteristics:

Volunteer multiparous women (> one yr post-partum, N=48) screened for anti-HLA in 30 cell LCT panel.

Two anti-HLA+ donors found:

1. Anti-HLA-B7+
2. Anti-HLA-A3+
SCID mouse protocol:

- Human PBMC Engraftment
- Twiceweekly Allogeneic PBMC Challenge
- Serum anti-HLA reactivity
- Twice weekly IVIg treatment (0.5 - 1 g/kg)
Anti-HLA production:

Anti-HLA (MCF)

PBMC Challenge Week
SCID mouse protocol:

Human PBMC Engraftment

Bi-weekly Allogeneic PBMC Challenge

Serum anti-HLA reactivity

Biweekly IVIg treatment (0.5 - 1 g/kg)
Anti-HLA inhibition in SCID mice:

A.

Percent of baseline anti-HLA reactivity

F(ab')_2

B.

Percent of baseline anti-HLA reactivity

IgG

Week of treatment

Semple et al Blood 2002
Conclusions:

• IVIg and Anti-D preparations mediate many of their effects via Fc-dependent actions.

• Anti-D preparation mediate their effects via anti-inflammatory cytokine actions.

• Antiidiotypic actions of IVIg can be clearly observed when donor selection is implemented (e.g. multiparous sera).

• None of the current theories of the mechanism of action of IVIg can be eliminated.

• It will be necessary to exploit the major mechanisms of any particular IVIg preparation.
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