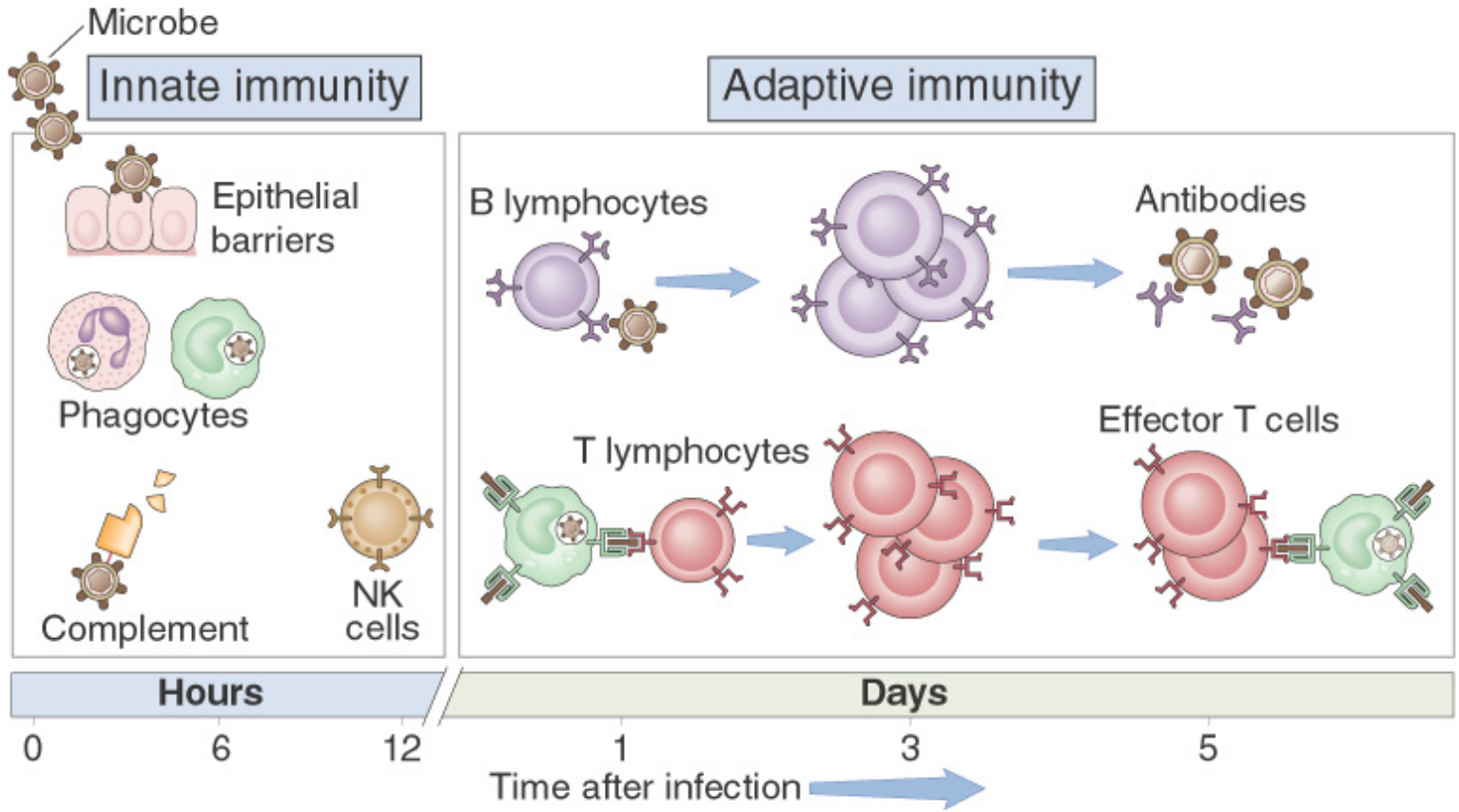
Intravenous Immunoglobulin: A tale of two ends of the molecule

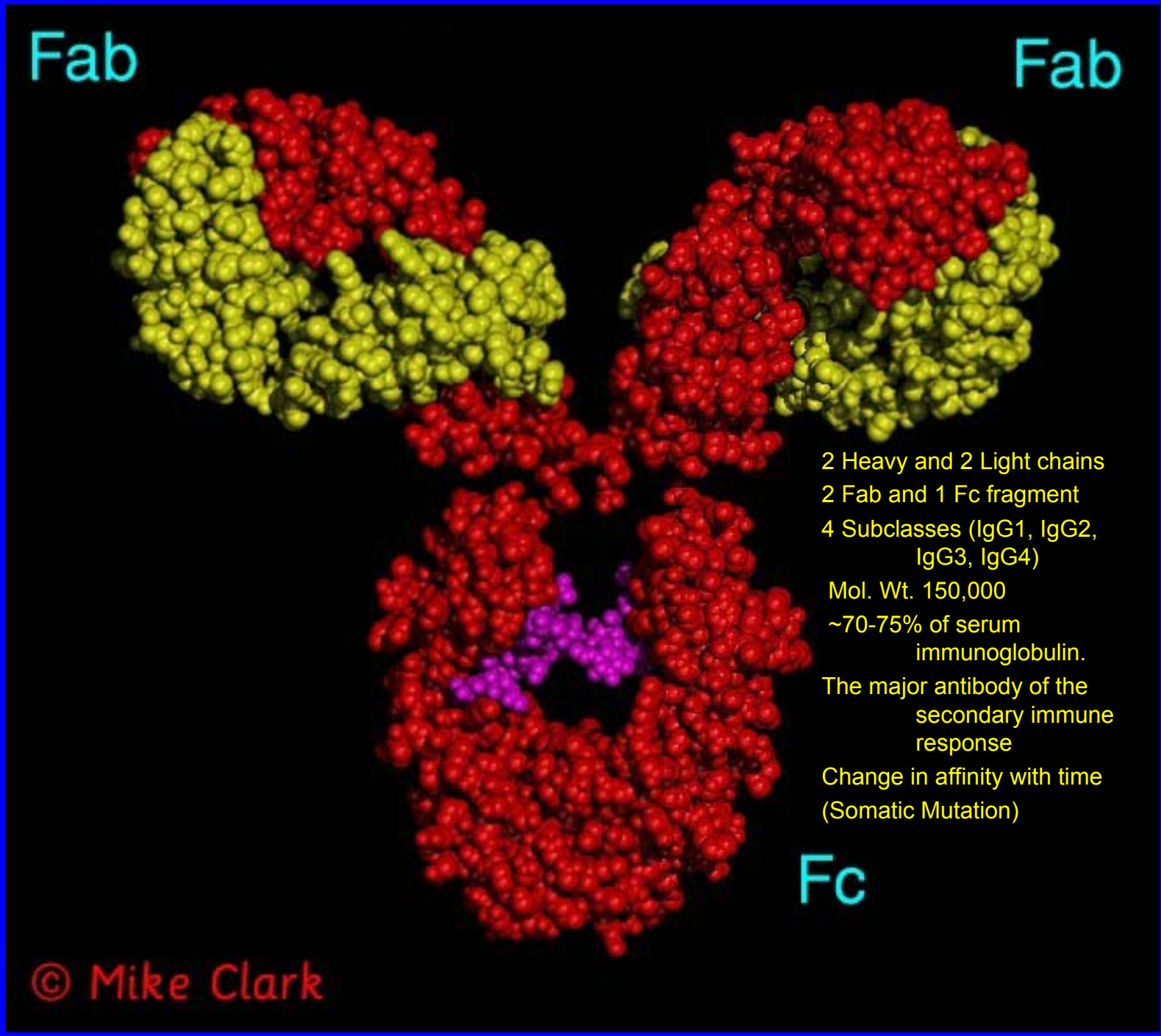
John W. Semple, PhD
Toronto Platelet Immunobiology Group,
St. Michael's Hospital.

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.




Fab
Fab
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)
Fc
© Mike Clark

Intravenous Immunoglobulin (IVIg):

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

Types of IVIg:

Polyclonals

- IVIg (IVIg)

Hyperimmunes

- Anti-Rh (D) (WinRhO)
- Anti-Tetanus
- Anti-Varicella
- Anti-etc.

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: (α phase) 3-5 days**
  - **Elimination: (β phase) 3-4 weeks (avg. 23 days)**
- **Anti-D has similar kinetics in D- individuals.**

Dosage:

IVIg (Pediatric)i:

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:

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

Antibody content in IVIg:

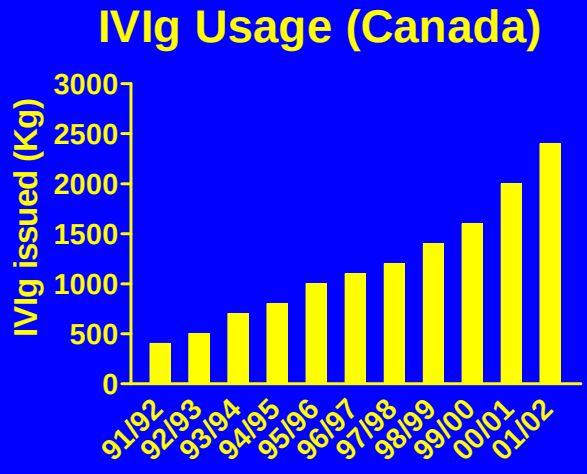
Antibodies against bacterial-, viral-, fungal- and auto-antigens and can be found in IVIg preparations. **Antiidiotypic antibodies are also found.**

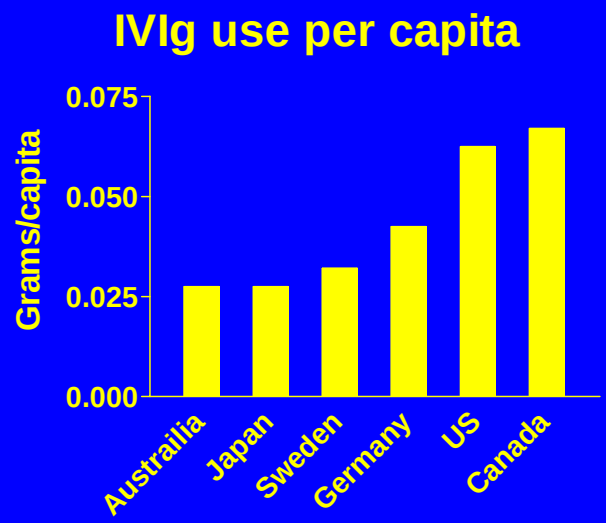
Ab titers vary substantially:

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:





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**
•**Immune Thrombocytopenic purpura (ITP)**
•**Kawasaki Syndrome**
•**Pediatric HIV infection**

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**
•**Immune Thrombocytopenic purpura (ITP)**
•**Kawasaki Syndrome**
•**Pediatric HIV infection**

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**

INDICATED

- Primary Immunodeficiencies
- Allogeneic Bone Marrow Transplantation
- B-Cell Chronic Lymphocytic Leukemia
- Immune Thrombocytopenic purpura (ITP)
- Kawasaki Syndrome
- Pediatric HIV infection

OFF LABEL

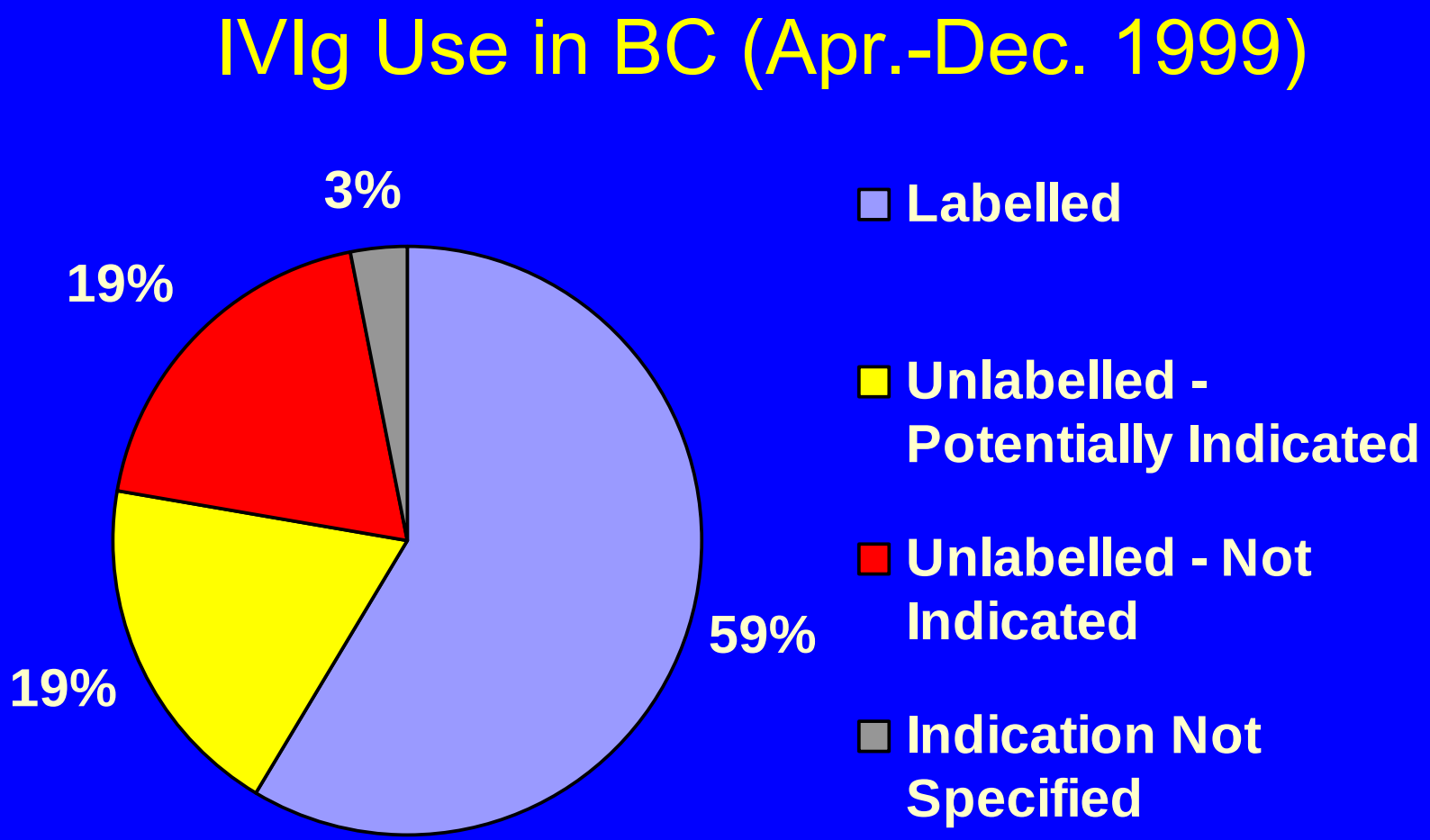
(Potentially indicated)

- 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

NOT INDICATED

- Acquired Factor VII inhibitors
- Acute Lymphoblastic Leukemia
- acute renal failure
- Acquired Von Willebrand's Syndrome
- Adrenoleukodystrophy
- Aplasia, Pure Red Cell
- Asthma/Inflammatory Chest Disease
- Autism
- Behcet's Syndrome
- Chronic Fatique Syndrome
- Clostridium (C.) Difficile Toxin
- Congenital heart block
- Cystic Fibrosis
- Diabetes mellitus
- Endotoxemia
- Epilepsy
- Hemophagocytic syndrome
- Hyper IgE syndrome
- Intractable Pediatric Epilepsy
- Juvenile Arthritis
- Myositis (inclusion body myositis, polymyositis)
- Immunoproliferative Neoplasms
- Motor Neuron Syndromes
- Multiple sclerosis
- Myelopathy associated with Human T-cell
- Leukemia/Lymphoma Virus-I (HTLC-I)
- Nephrotic Syndrome
- Neuropathy (membranous, paraproteinemic)
- Euthyroid Opthalmopathy
- Recurrent Otitis Media
- Pemphigus (pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus)
- Progressive Lumbosacral Plexopathy
- Post Transfusion Purpura
- Recurrent Fetal Loss
- Renal Failure
- Rheumatoid Arthritis
- Spontaneous abortion
- Systemic Lupus Erythematosus (SLE) related (cytopenia, nephritis, CNS involvement, vasculitis, pericarditis, or pleural effusion)
- Systemic Vasculitic Syndromes
- Thrombocytopenia (refractory to platelet transfusion, thrombotic thrombocytopenic purpura, nonimmune thrombocytopenia, neonatal alloimmune thrombocytopenia (pre- and postnatal), septic thrombocytopenia, quinine induced thrombocytopenia
- Transfusion reactions
- Trauma
- Uveitis

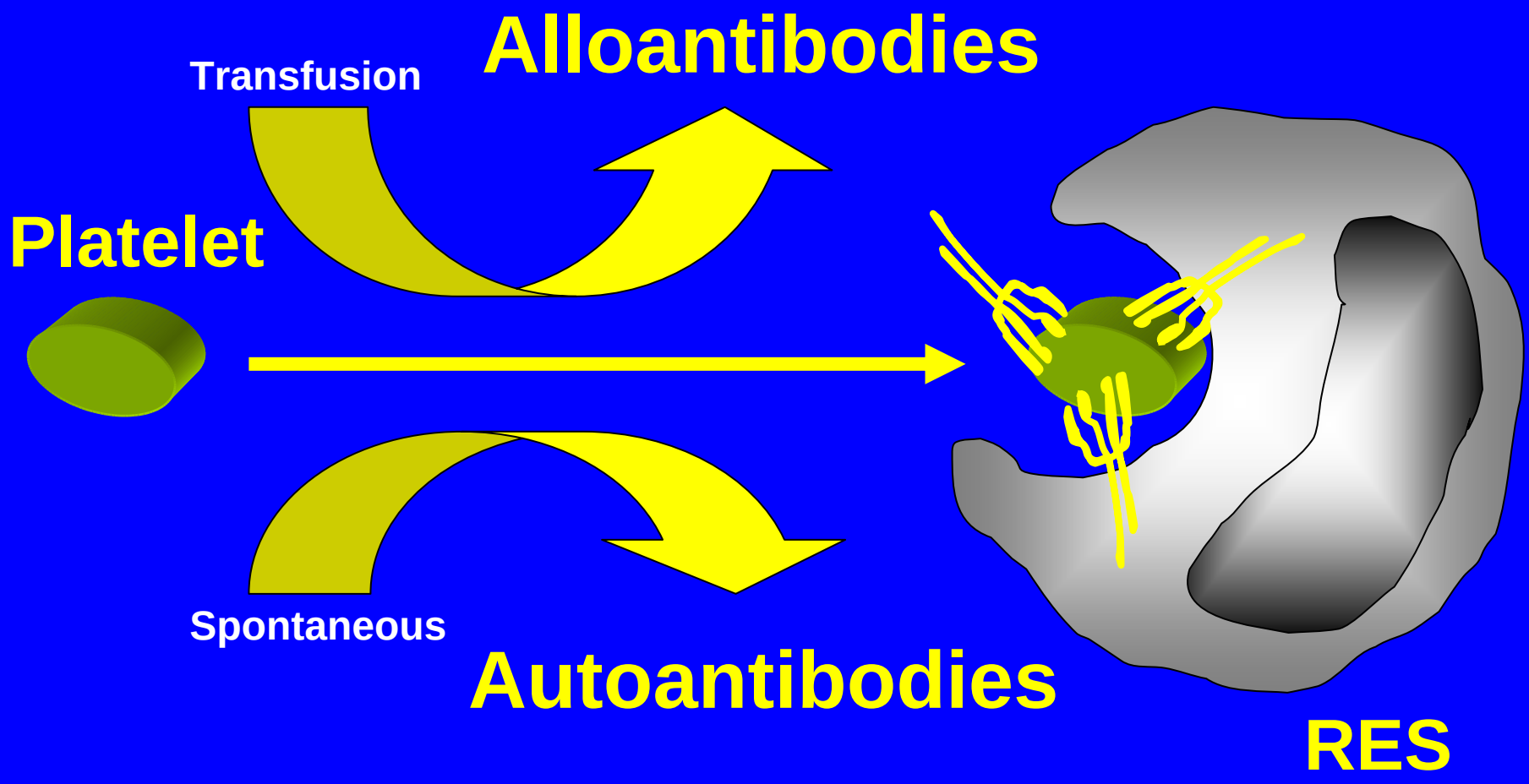
Inappropriate Use



IVIg, Mechanisms of action:

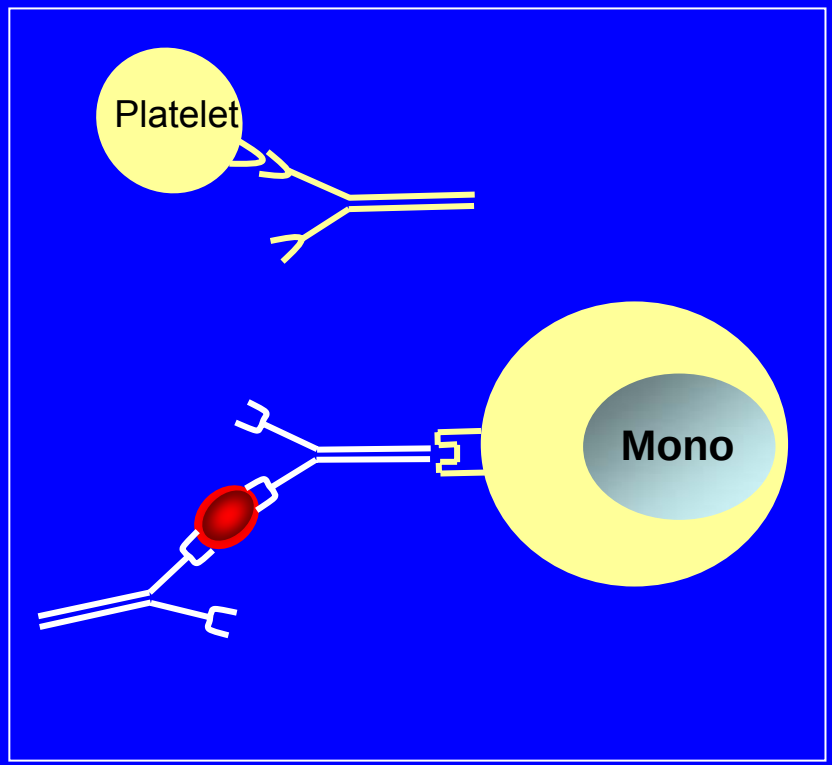
How do IVIg and anti-D preparations increase platelet counts in patients with ITP?

Pathogenesis of immune platelet disorders:



IVIg, Mechanisms of action:

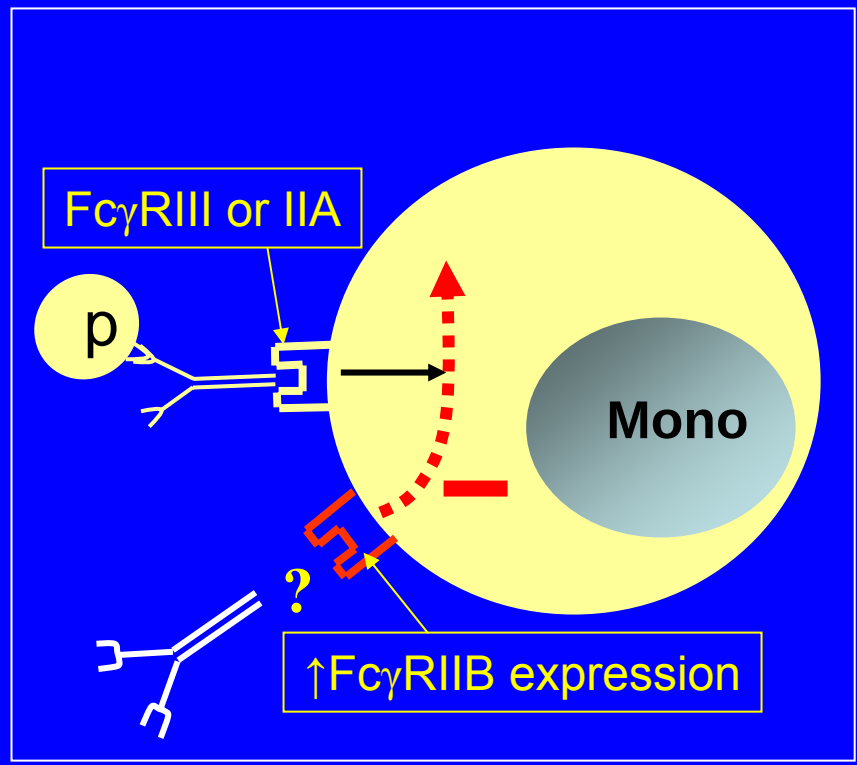
Theory 1:
Blockade of Fc receptors



Competitive.

Fehr et al, 1982

Theory 2:
FcγRIIB-dependent monocyte inactivation



FcγRIIB inhibitory signal.

Samuelsson et al, 2001

The Fc receptor system:

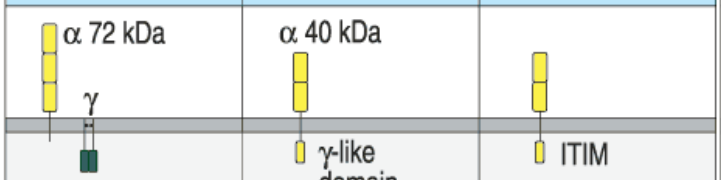
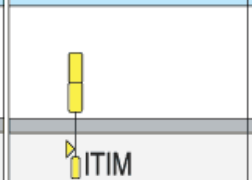
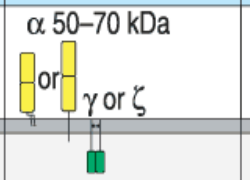
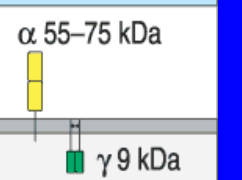
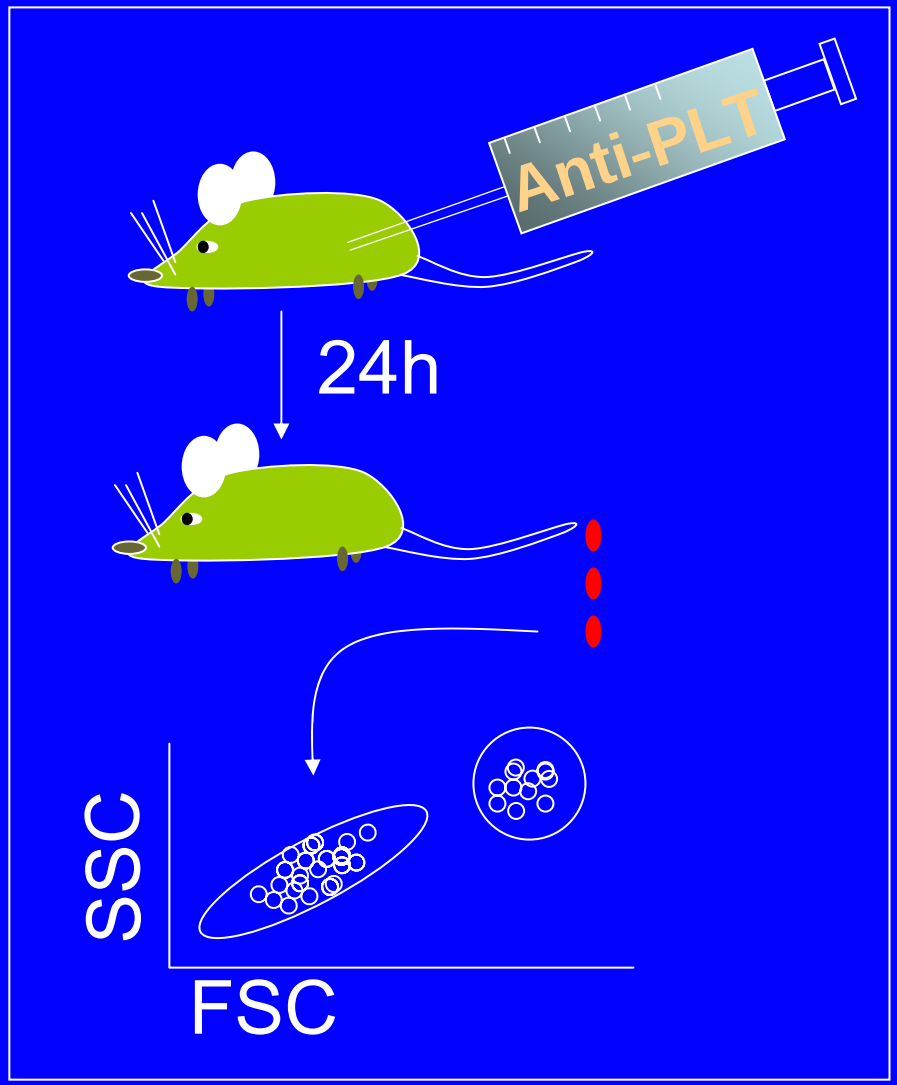
Receptor	FcγRI (CD64)	FcγRII-A (CD32)	FcγRII-B2 (CD32)	FcγRII-B1 (CD32)	FcγRIII (CD16)	FcεRI	FcαRI (CD89)
Structure							
Binding Order of affinity	IgG1 10^8 M^{-1} 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 2×10^6 M^{-1} 1) IgG1 2) IgG3=IgG2* 3) IgG4	IgG1 2×10^6 M^{-1} 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 2×10^6 M^{-1} 1) IgG1=IgG3 2) IgG4 3) IgG2	IgG1 5×10^5 M^{-1} IgG1=IgG3	IgE 10^{10} M^{-1}	IgA1, IgA2 10^7 M^{-1} IgA1=IgA2
Cell type	Macrophages Neutrophils† Eosinophils† Dendritic cells	Macrophages Neutrophils Eosinophils Platelets Langerhans' cells	Macrophages Neutrophils Eosinophils	B cells Mast cells	NK cells Eosinophils Macrophages Neutrophils Mast cells	Mast cells Eosinophils† Basophils	Macrophages Eosinophils‡ Neutrophils
Effect of ligation	Uptake Stimulation Activation of respiratory burst Induction of killing	Uptake Granule release (eosinophils)	Uptake Inhibition of stimulation	No uptake Inhibition of stimulation	Induction of killing (NK cells)	Secretion of granules	Uptake Induction of killing

Fig 9.30 part 1 of 2 © 2001 Garland Science

2 of 2 © 2001 Garland Science

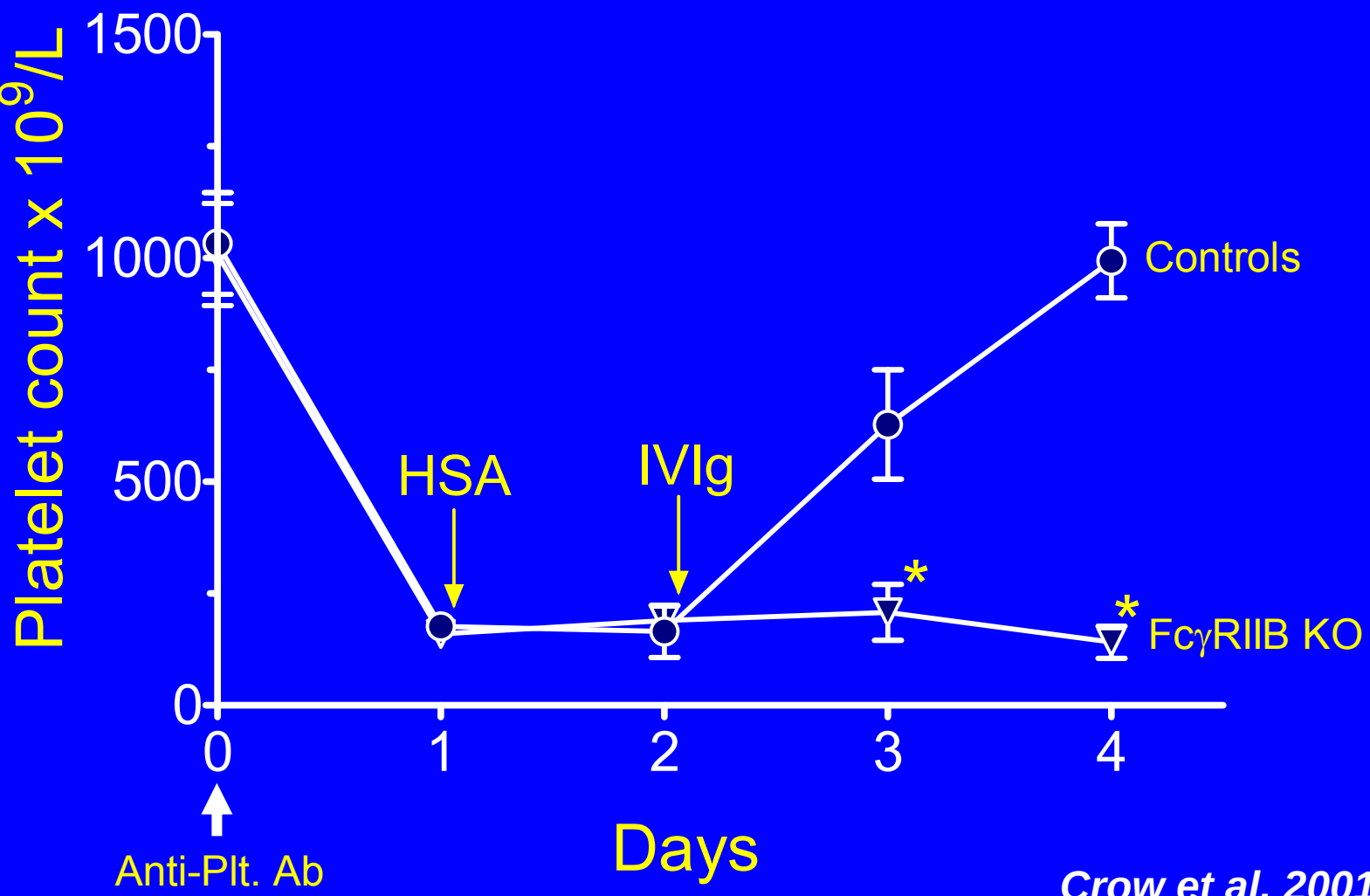
A murine model of Passive ITP:



- 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

IVIg 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?)

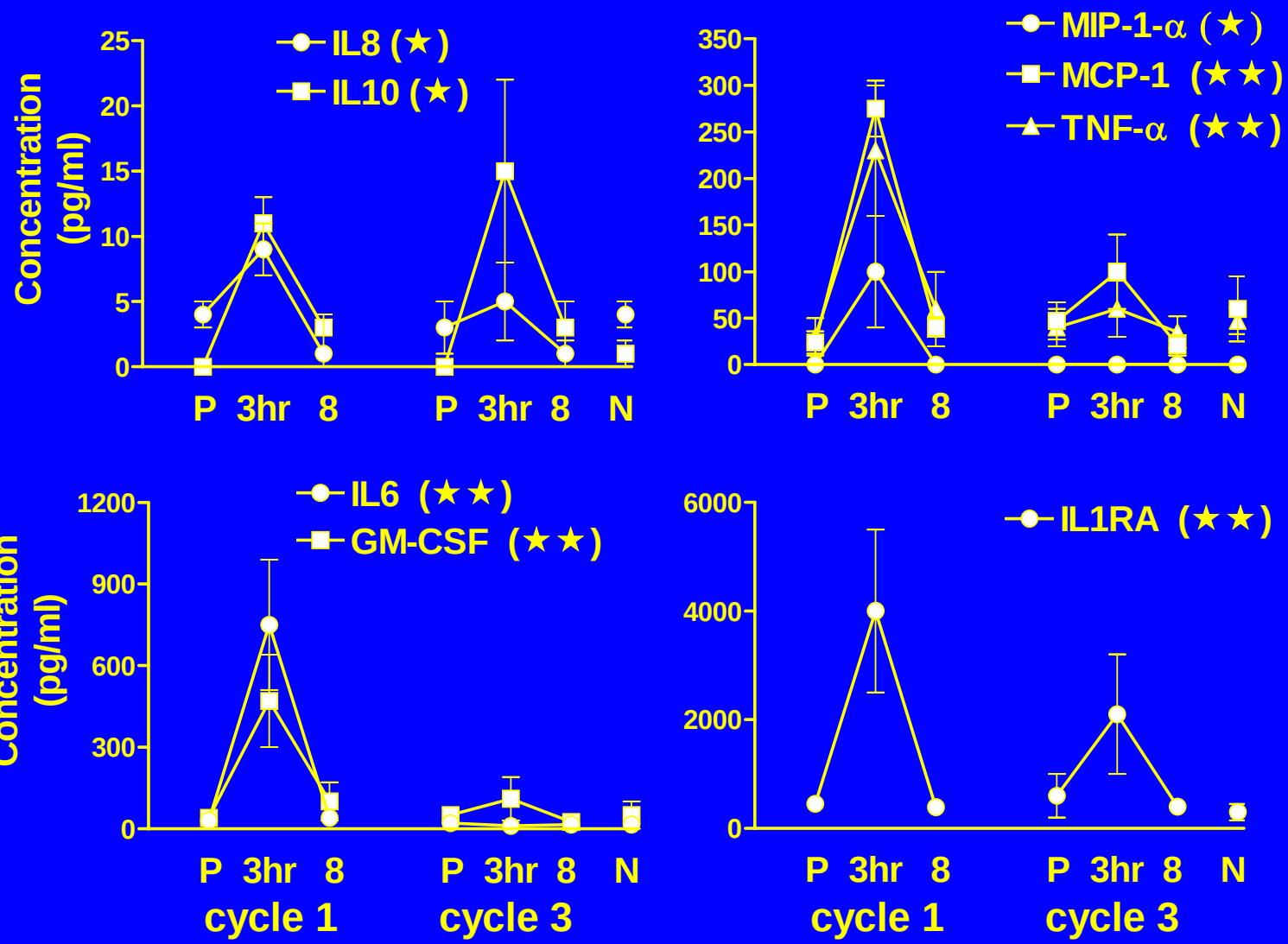
American Journal of Hematology 69:225-227 (2002)
DOI 10.1002/ajh.10065

Anti-D (WinRho™) Treatment of Children With Chronic Autoimmune Thrombocytopenic Purpura Stimulates Transient Cytokine/Chemokine Production

J.W. Semple,[1,3] D. Allen,[1] M. Rutherford,[1] M. Woloski,[5] M. David,[4] C. Wakefield,[2] S. Butchart,[2] J. Freedman,[1,3] V. Blanchette,[2] and the Canadian Children's Platelet Study Group (CCPSG)

[1]Department of Laboratory Medicine and Pathobiology, St. Michael's Hospital, Toronto, Ontario, Canada
[2]Division of Hematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada
[3]The Toronto Platelet Immunobiology Group, Toronto, Ontario, Canada
[4]Division of Hematology, Hôpital Ste-Justine, Montreal, Quebec, Canada
[5]The Cangene Corporation, Winnipeg, Manitoba, Canada

Cytokines: Post Anti-D



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)

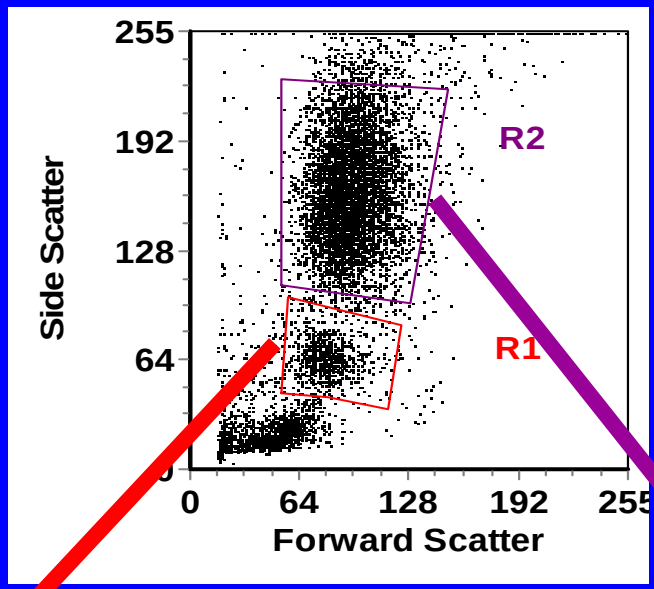
4°C control

37°C test (DMEM media)

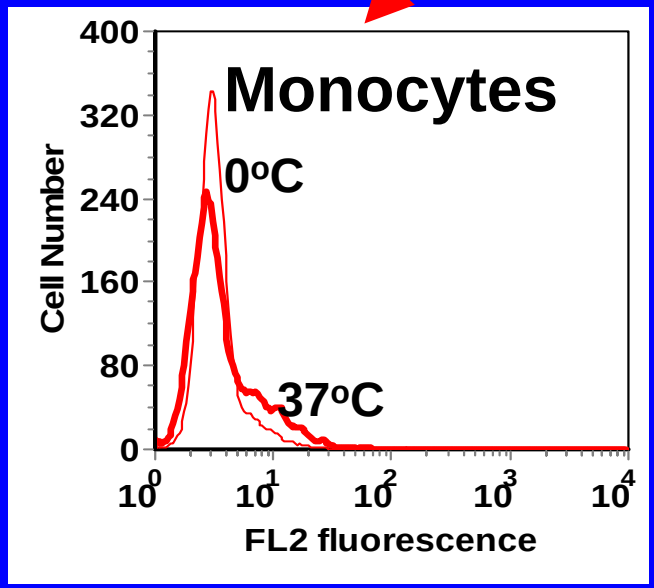
2 hrs
Quench
wash

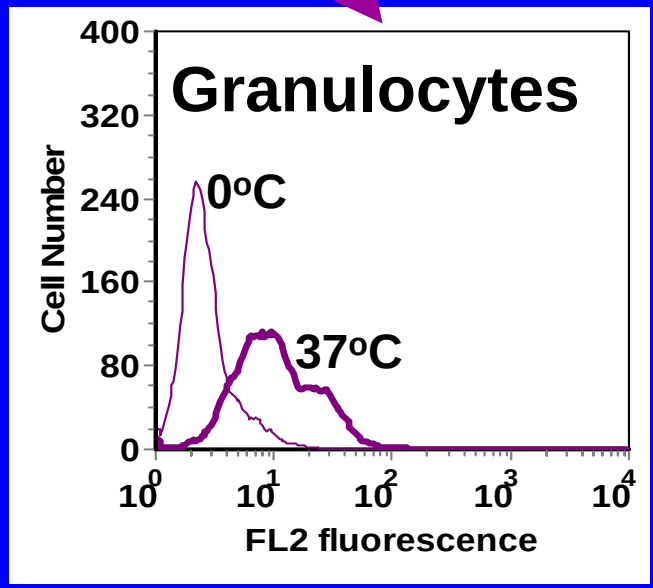
Flow cytometric analysis

Anti-D-RBC Phagocytosis

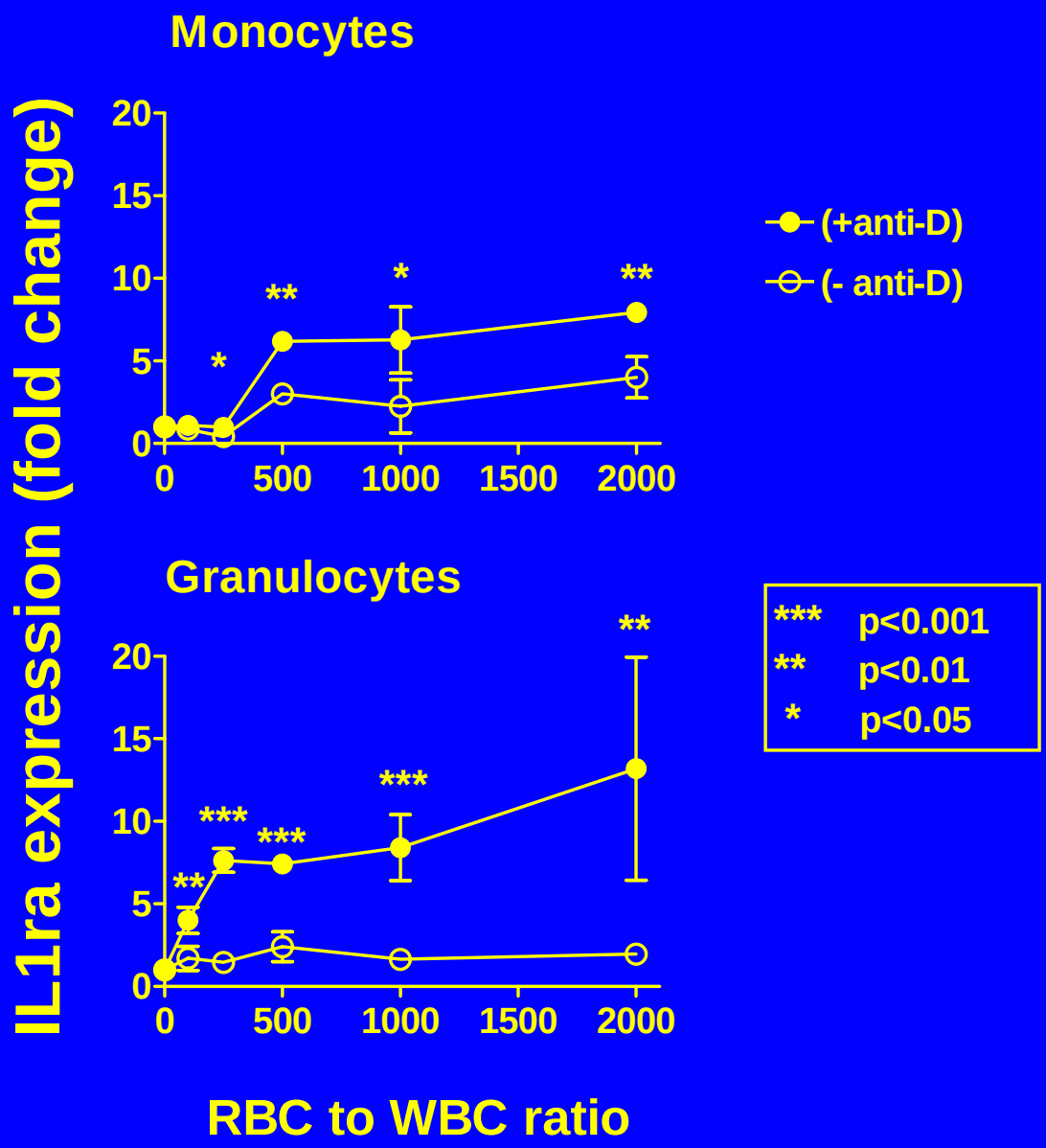


Coopamah et al Blood 2003



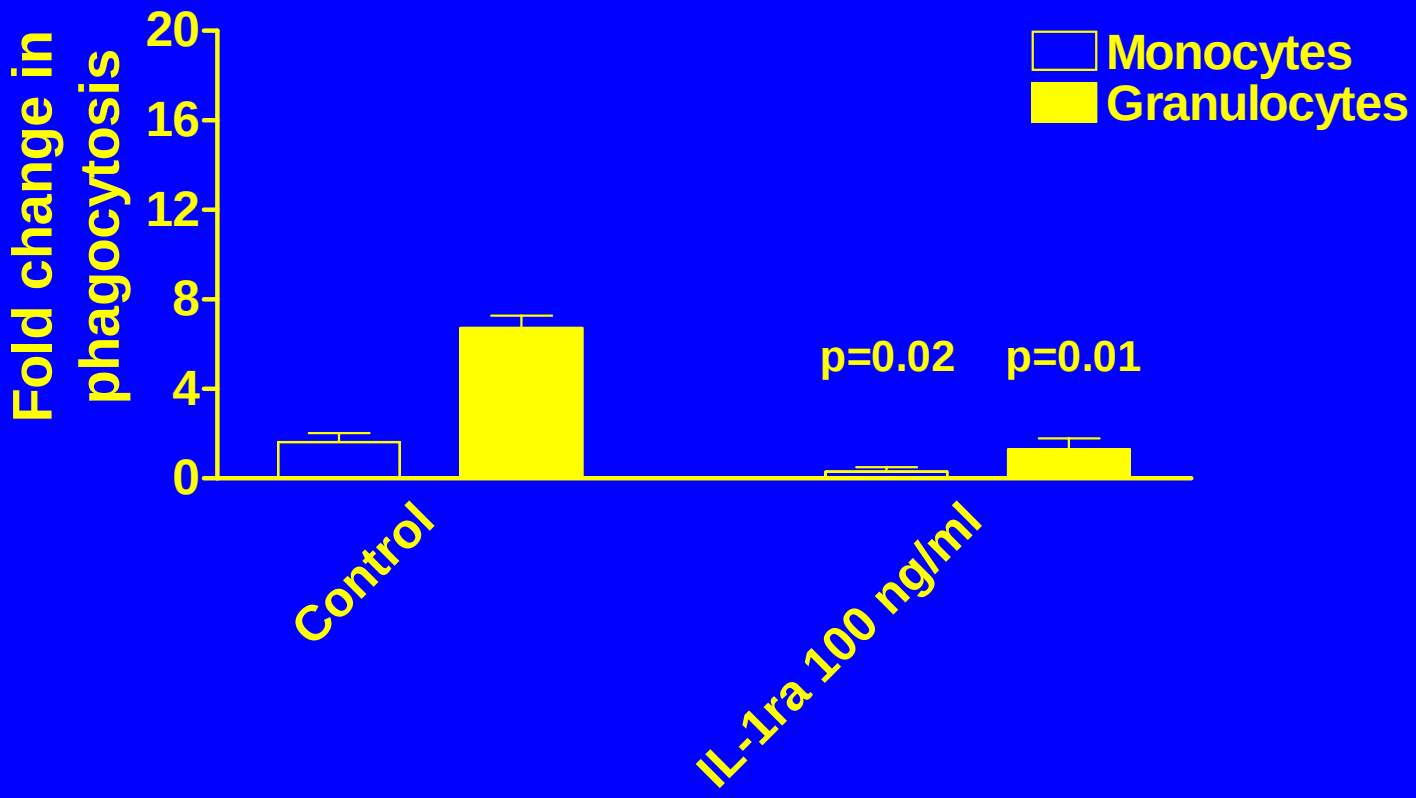


IL1ra Expression (4 hours):



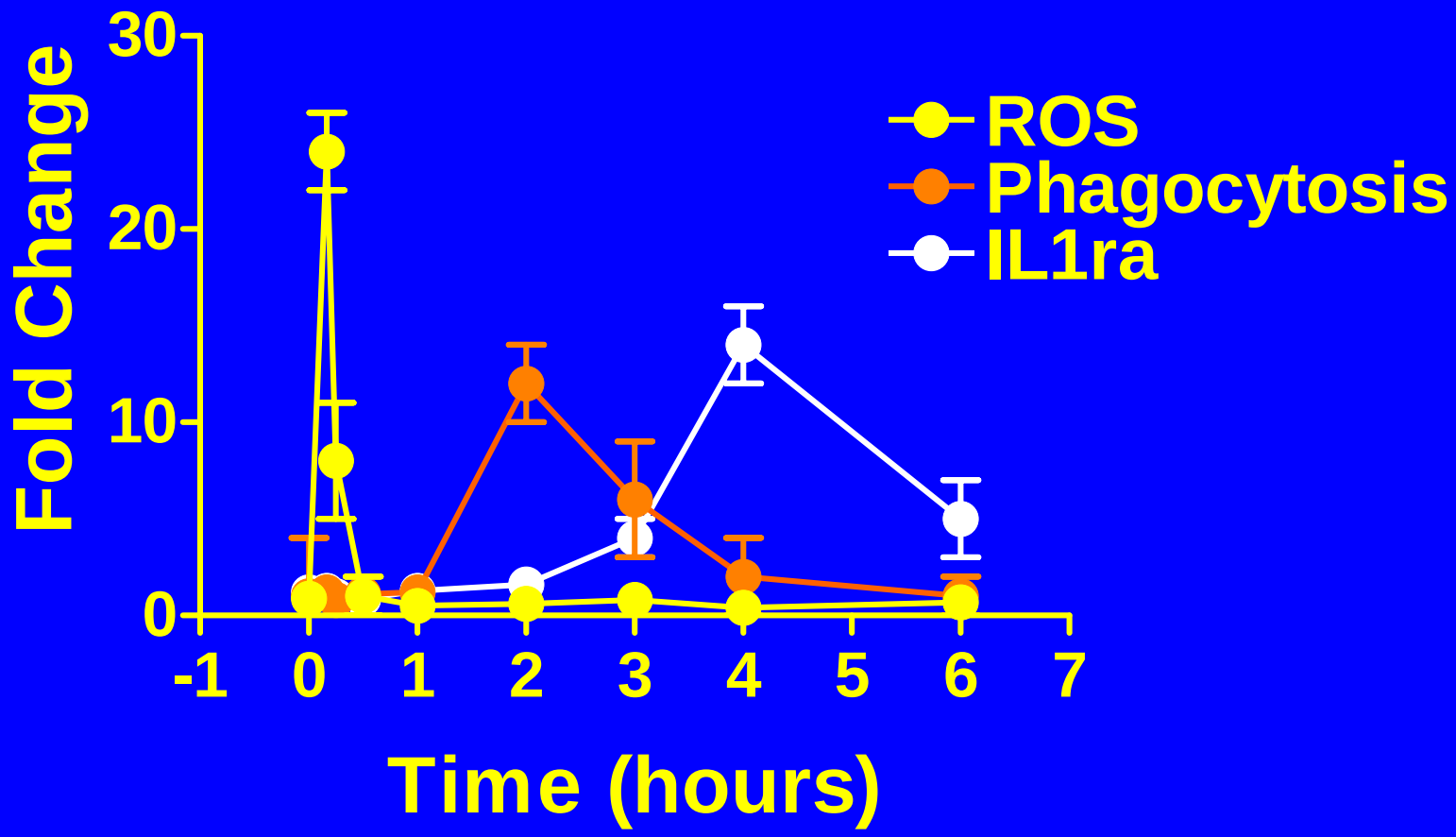
Effect of IL1ra on Phagocytosis:

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



Opsonized RBC 1:1000

Time course of Events:



THP 1:

Human monocytic leukaemia

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

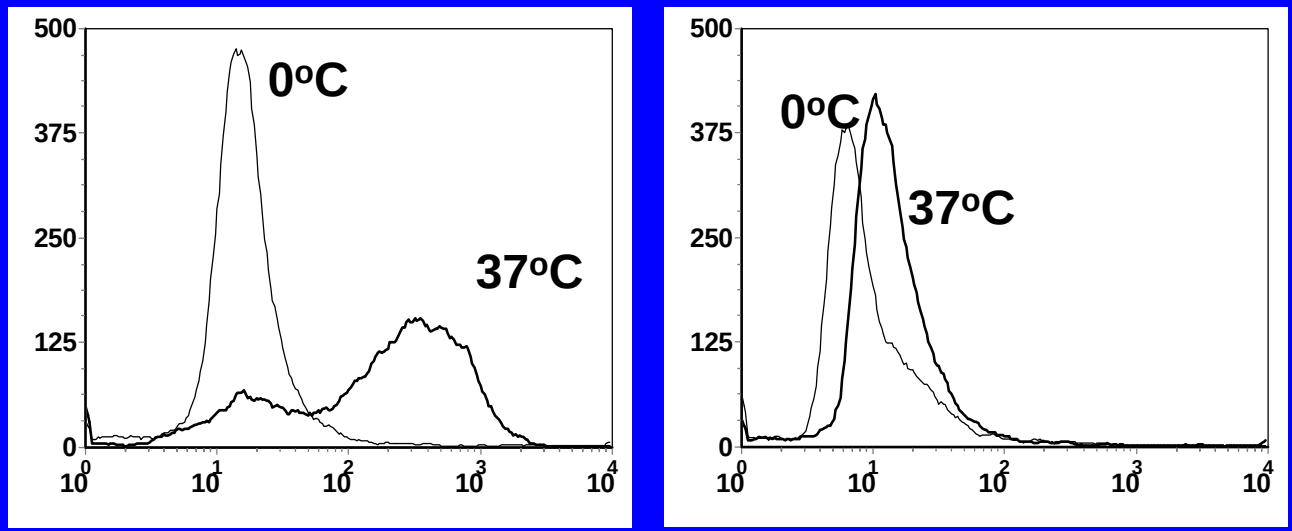
Int J Cancer 1980;26:171; Cancer Res 1982;42:1530; J Immunol 1983;131:1882

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 CO_2 production on phagocytosis).
Can restore the response of purified T lymphocytes to Con A
Can differentiate into macrophage-like cells using DMSO.

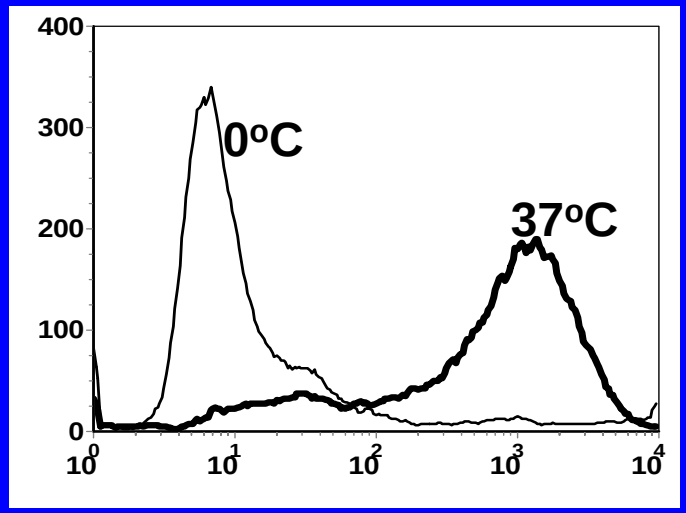
THP1 Phagocytosis of platelets:

IgG bound Plts **F(ab')$_2$ bound Plts**

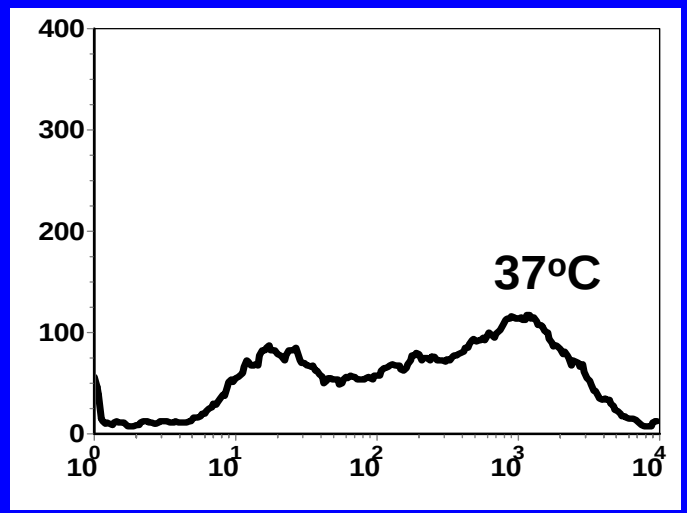


CM Green Fluorescence

THP1 Phagocytosis and IVIg:



Control (No IVIg)



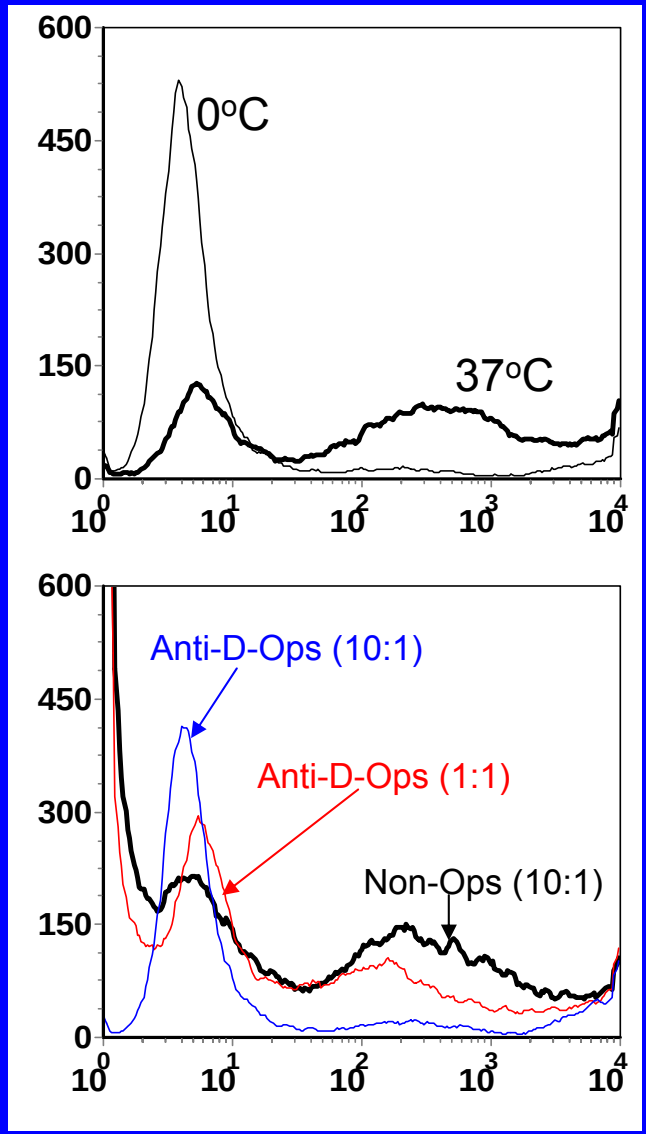
IVIg (1.4 mg/ml; 10 fold less than 1g/kg)

(Inhibition titrates to 0.0014 mgs/ml; 10,000 fold less)

Events

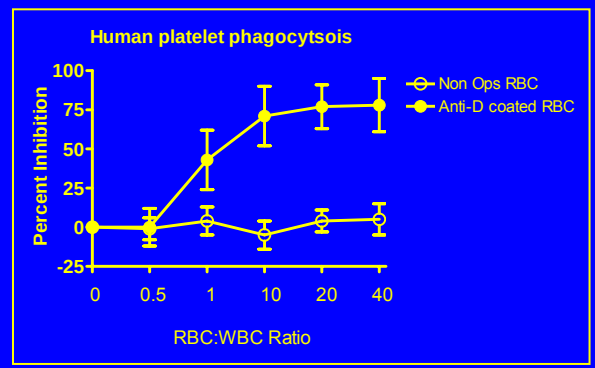
CM Green

THP1 Phagocytosis and Anti-D:



Control (No Anti-D)

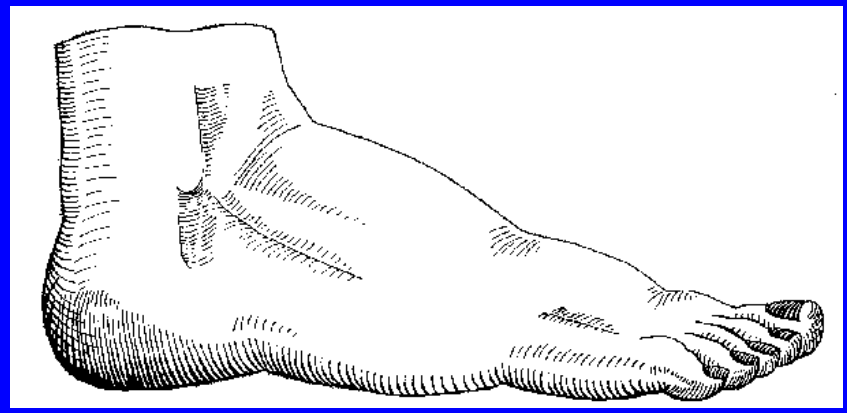
Non-opsonized or Anti-D opsonized RBC (RBC:THP)



Summary:

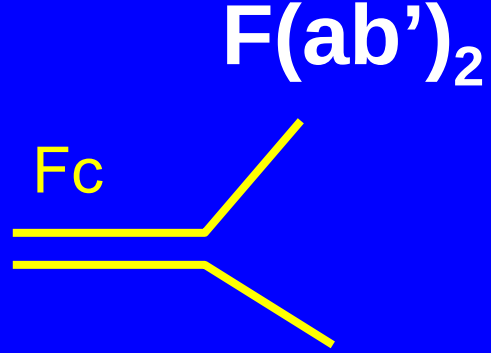
1. Anti-D mediated effects are Fc dependent but appear to additionally require the production of anti-inflammatory cytokines in order to inhibitory platelet phagocytosis.

2. Phagocytic cell lines such as THP-1 can be used to study the biochemical mechanisms of anti-D-mediated platelet rescue from phagocytosis.

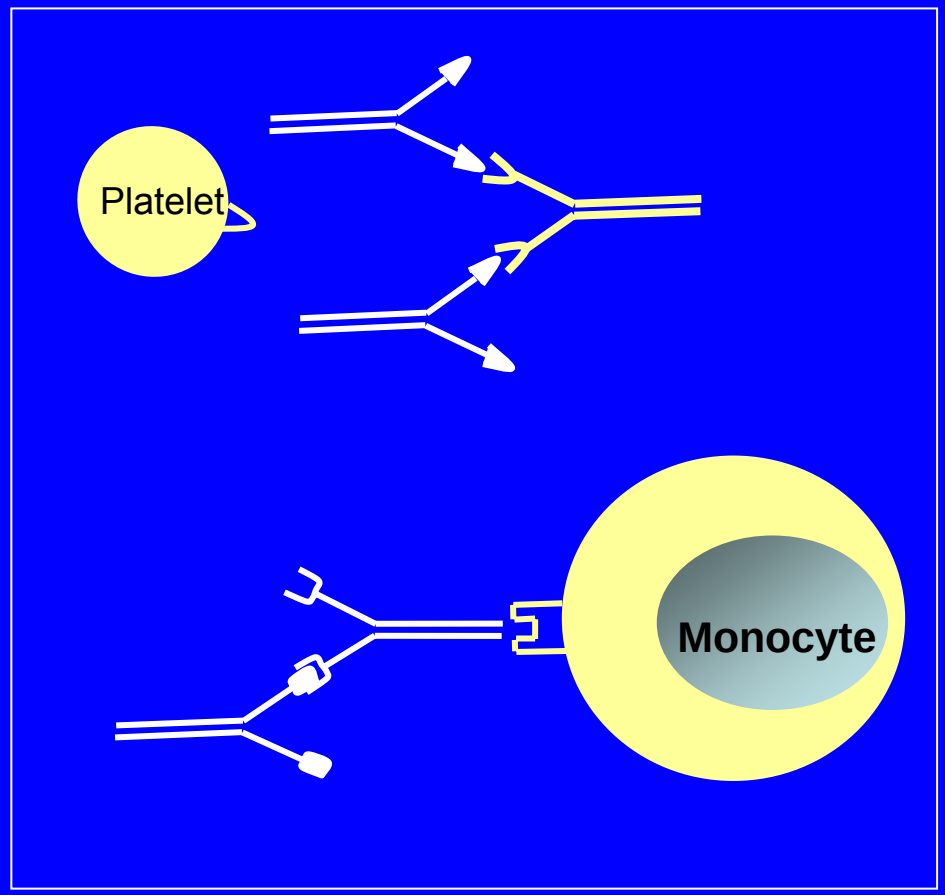


And now for something completely different!

Theory 3: Idiotypic Effects.



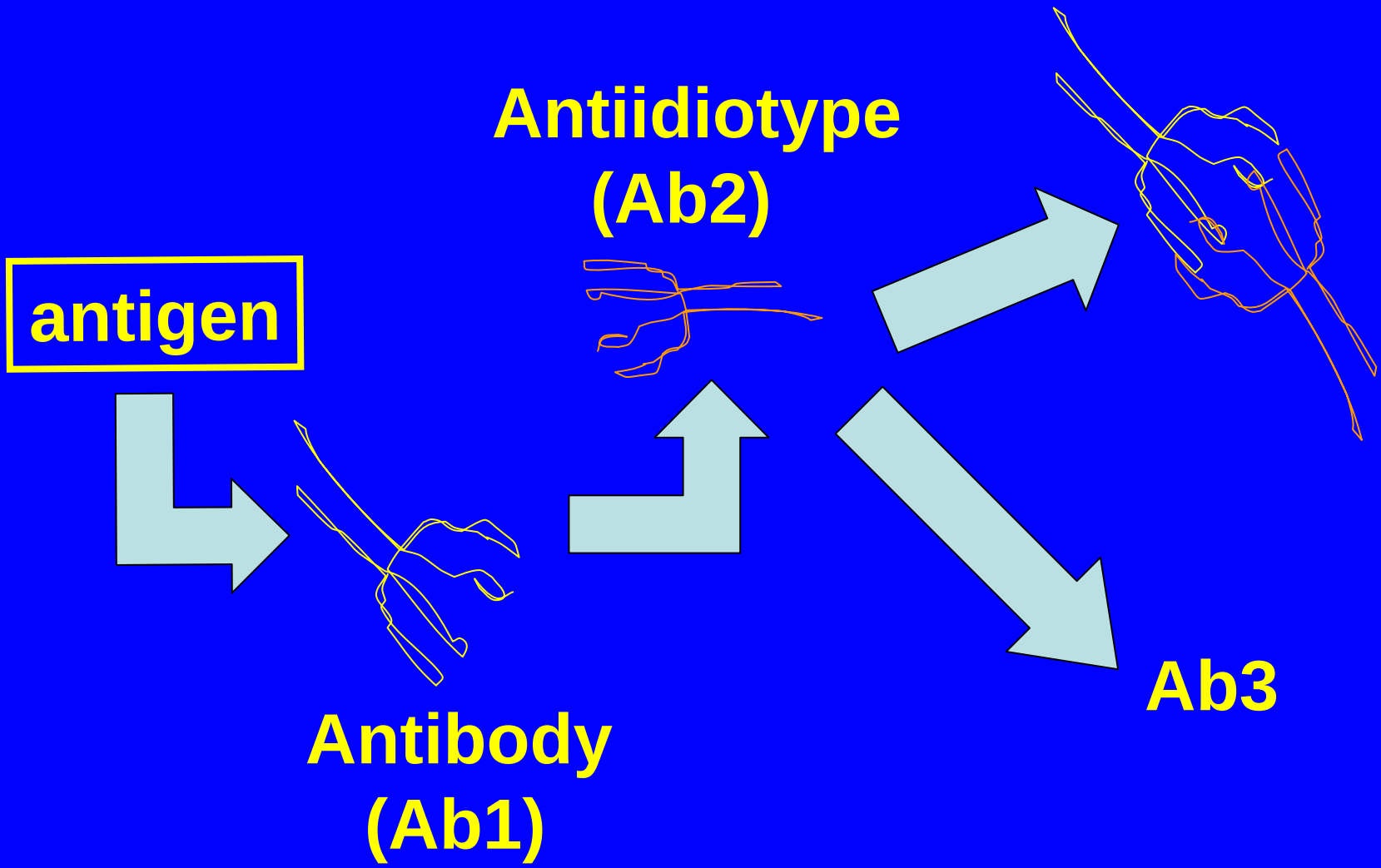
Theory 3, Antiidiotypic antibodies:



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

Sultan et al, 1984

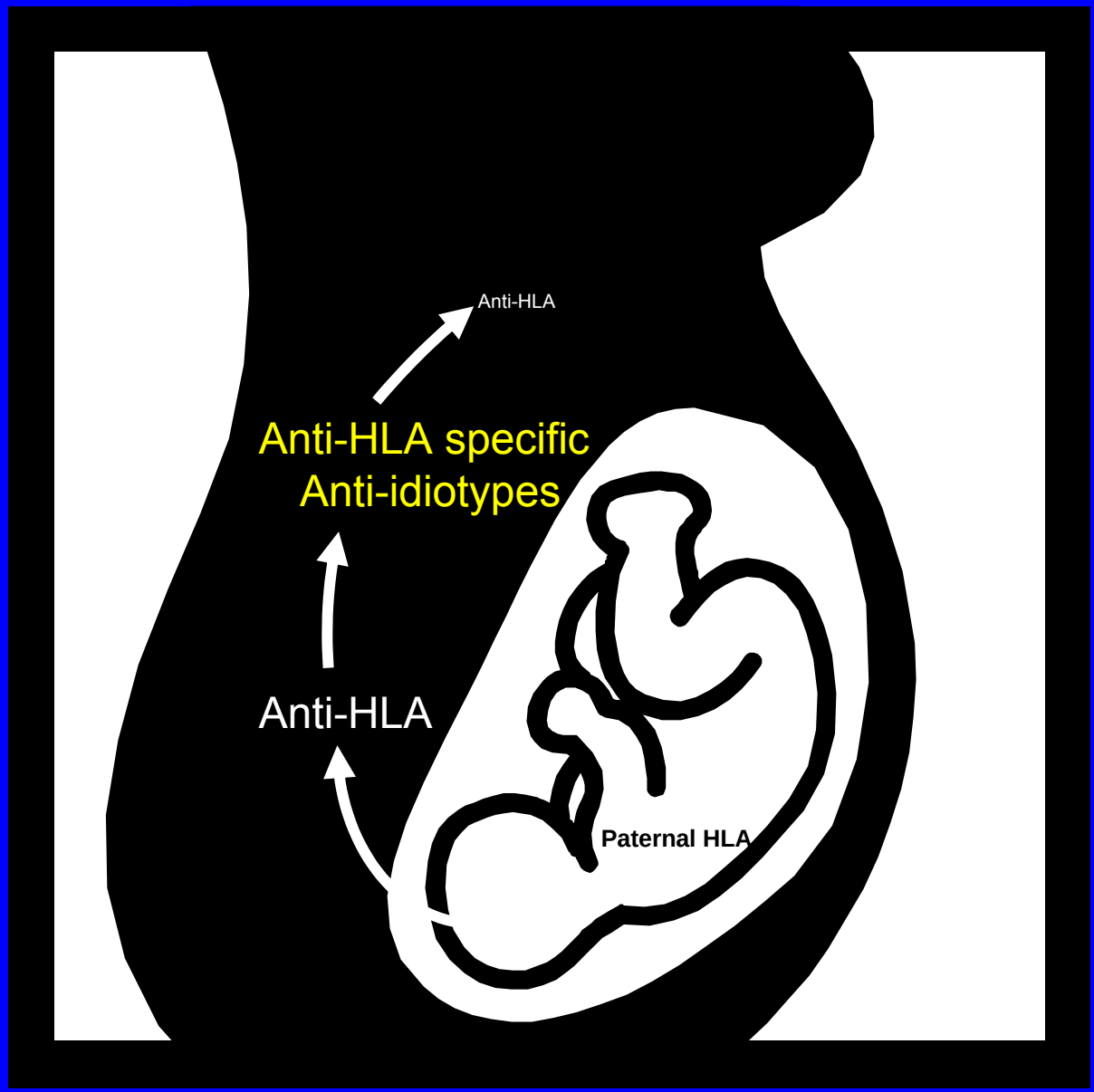
Jerne's Hypothesis:



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.



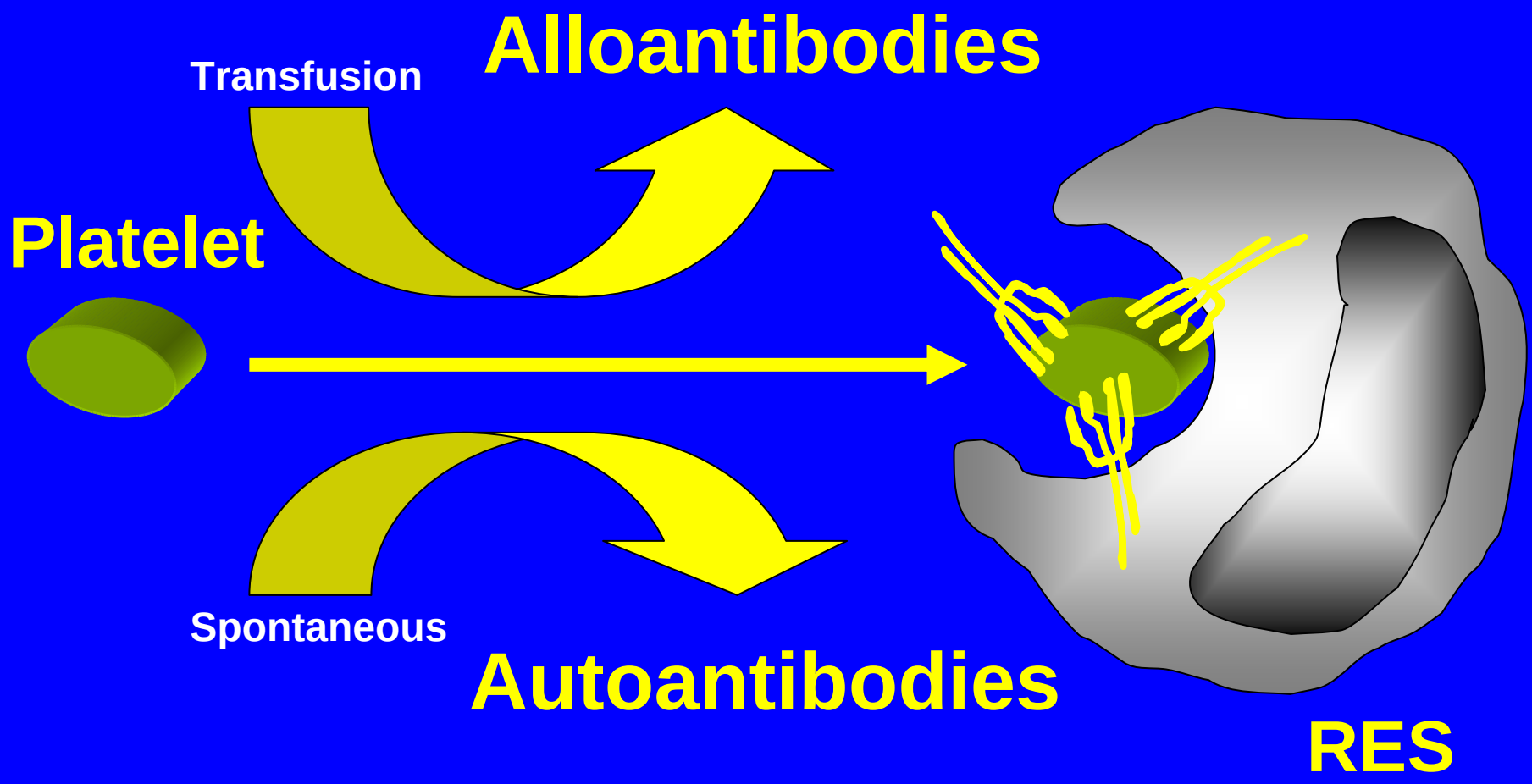
Suciu-Foca et al PNAS, 1983
Singal et al Trans Proc, 1991
Atlas et al Blood, 1993
Semple et al, Blood, 2002

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:



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 $F(ab')_2$ preparations for the ability to modulate anti-HLA.

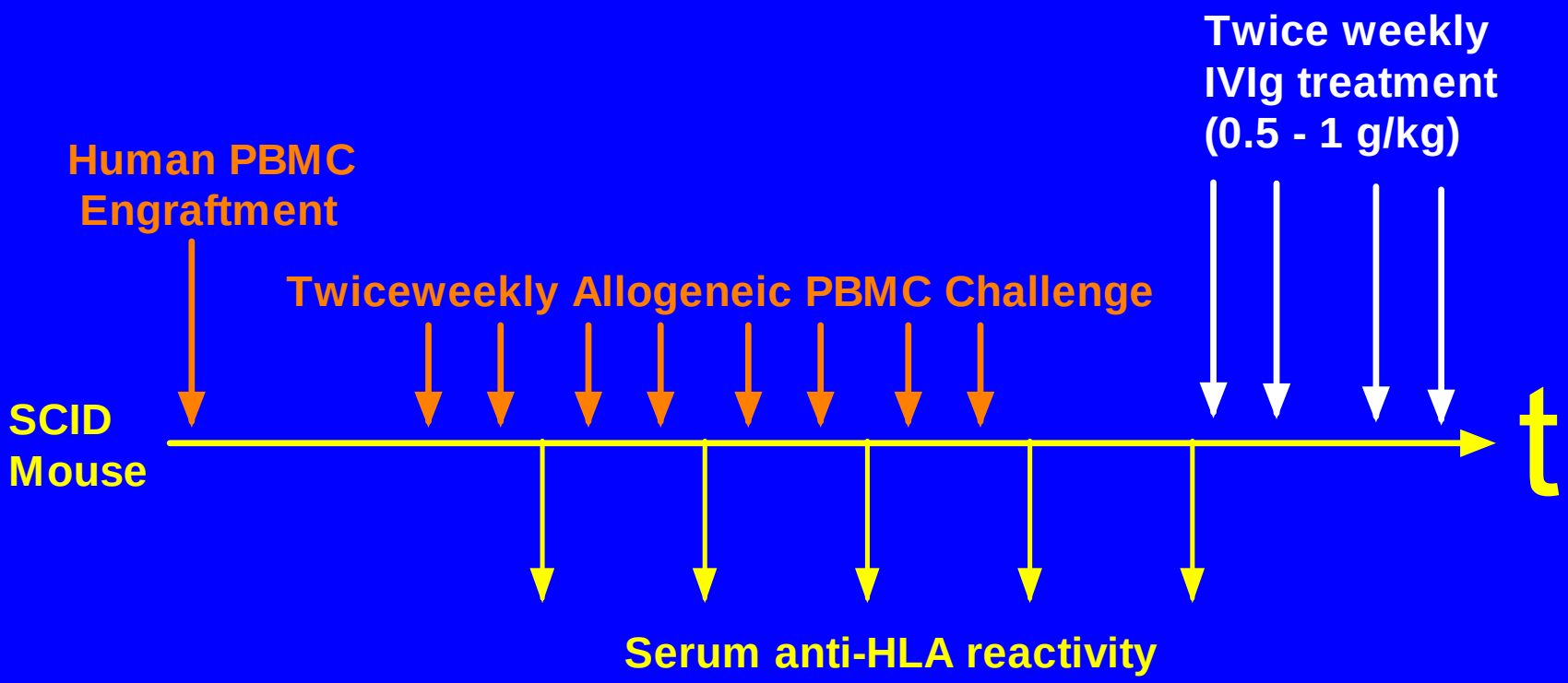
Donor Characteristics:

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

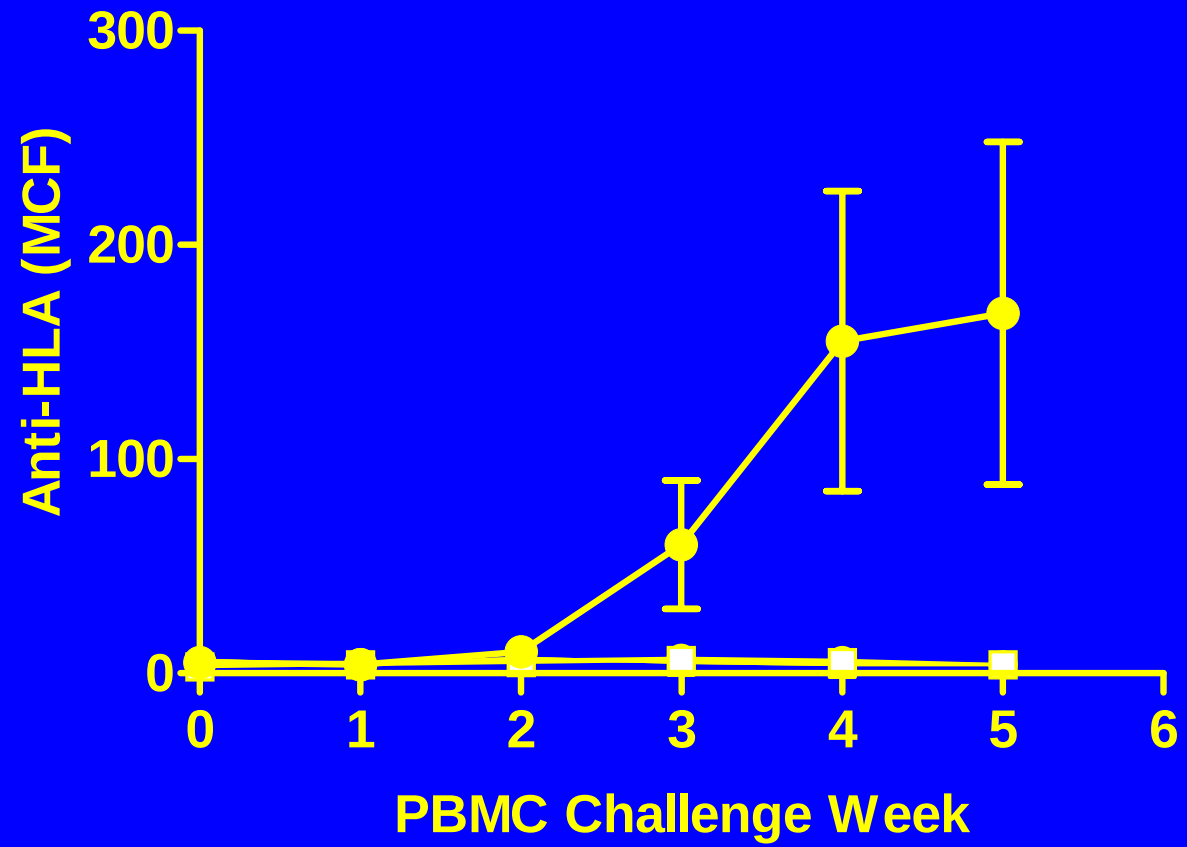
Two anti-HLA+ donors found:

1. Anti-HLA-B7+
2. Anti-HLA-A3+

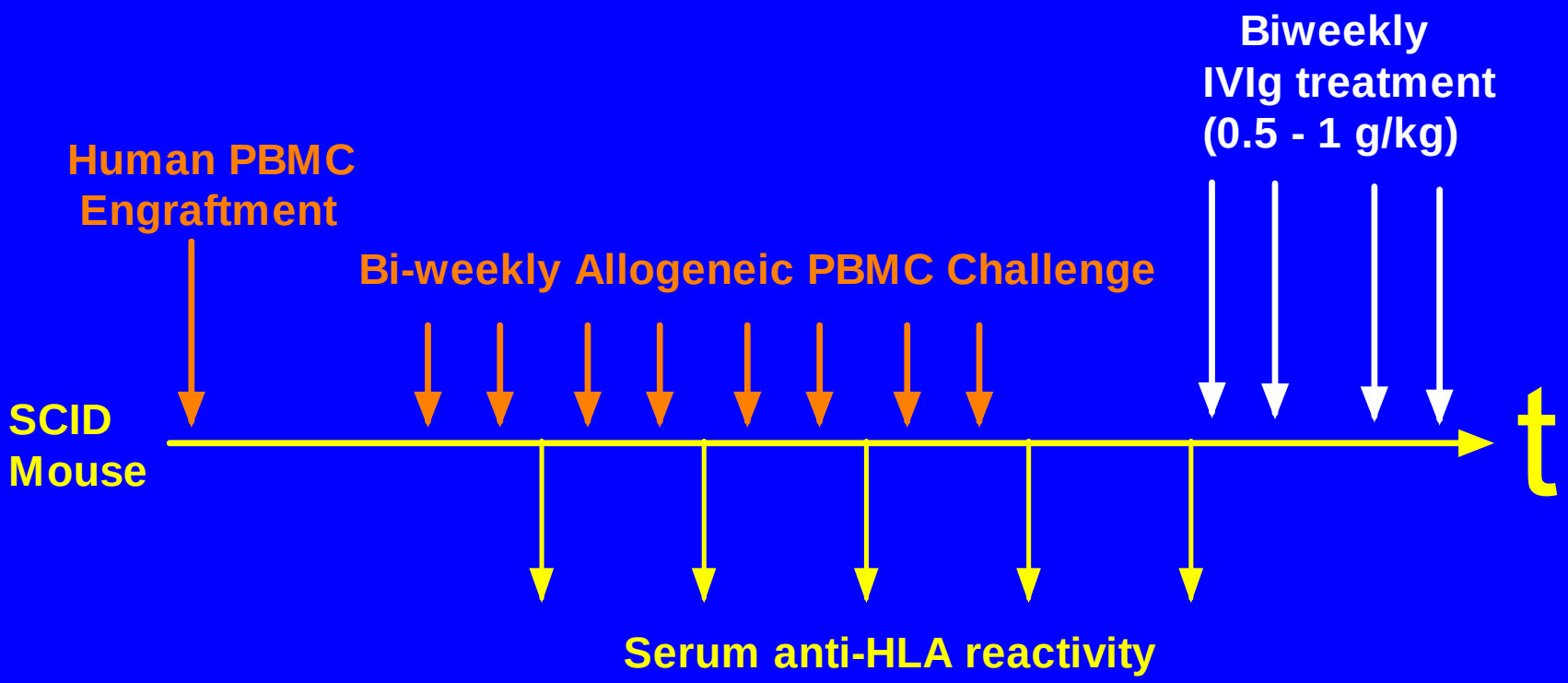
SCID mouse protocol:



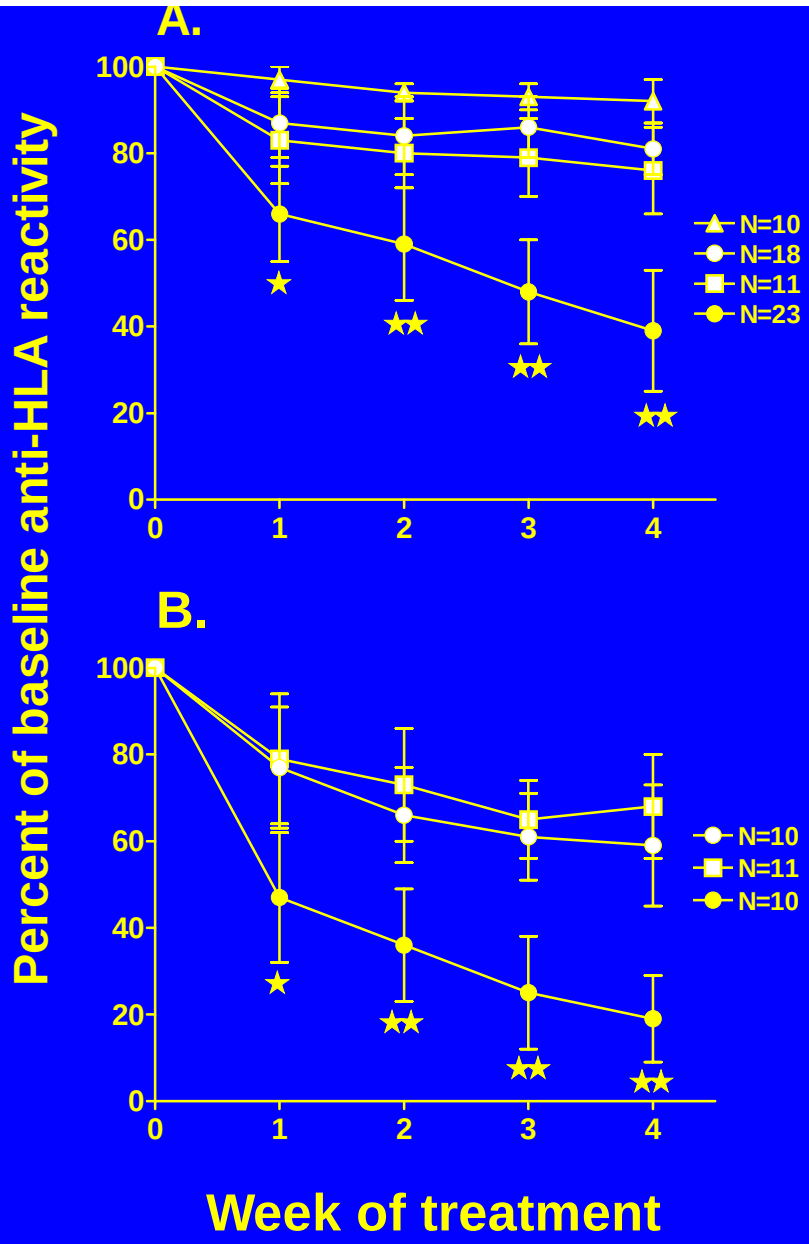
Anti-HLA production:



SCID mouse protocol:



Anti-HLA inhibition in SCID mice:



$F(ab')_2$

IgG

Semple et al Blood 2002

Conclusions:

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

- Anti-D prpearation mediate their effects via anti-inflammatory cytokine actions

- Antiidiotypic actions of IVIg can be clearly observed when donor selection is implemented (e.g. mutiparous 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.

Acknowledgements:

THEPIG,
St. Michael's Hospital, Toronto,
Toronto, ON

Michael Kim,
Rukhsana Aslam,
David Allen,
Mary Speck,
Ella Atlas,
Jeff Robson,
John Freedman

IRNS
Paris, France

Michel Kazatchkine
Shrini Kaveri

Cangene Corporation,
Mississauga, ON

Don Stewart
Erik Weirsma
Libby McCardy-Hall

Bayer Biologics Division,
Raleigh, NC

Peter Radke

Supported by:
Canadian Blood Services
Bayer Blood Partnership Fund
Cangene Corporation