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Effect of Ferric Citrate on Cell Growth and Monoclonal Antibodies.

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Introduction

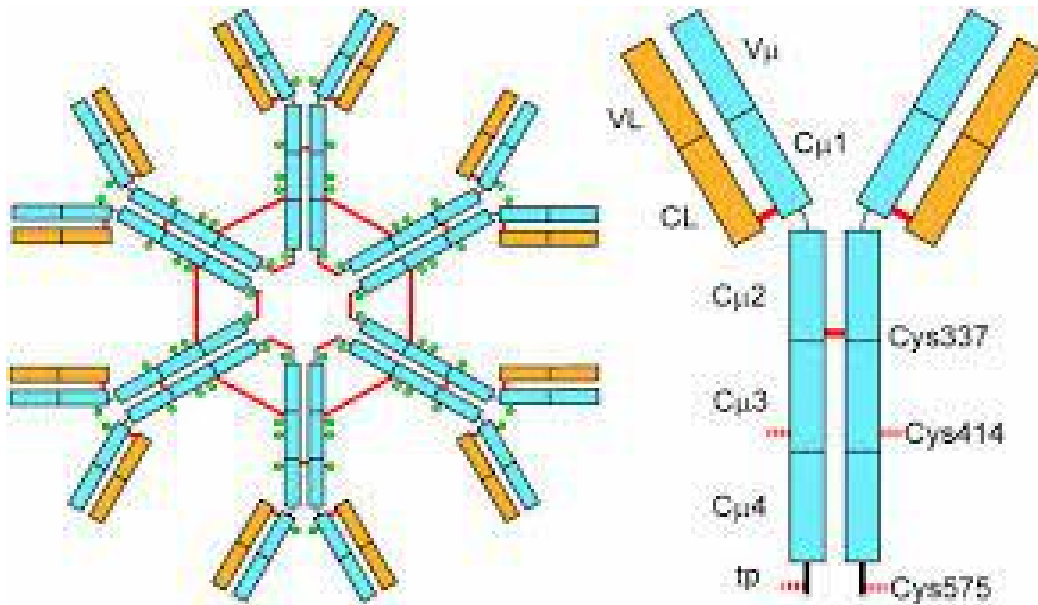
- Nobel Laureate Karl Landsteiner was discovered both the ABO blood group (1901) and Rh blood group (1937). There are more than 20 genetically determined blood group systems known today.
- These antigens may be Proteins, Carbohydrates, Glycoprotein's, or Glycolipids, depending on the blood group system.

Rh (D)

- In 1940, Landsteiner and Wiener reported another human blood group factor called 'Rhesus' or 'Rh'.
- Clinically the Human Rh (D) antigen is the most important red blood cell (RBC) membrane protein in transfusion medicine.
- The Alloimmune response against Rh (D) produces high affinity IgG antibodies that cause Hemolytic transfusion reactions and Hemolytic Disease of New born (HDN).
- The prophylactic use Rh (D) immunoglobulin in pregnant Rh (D) negative women has been a major advance in the prevention HDN

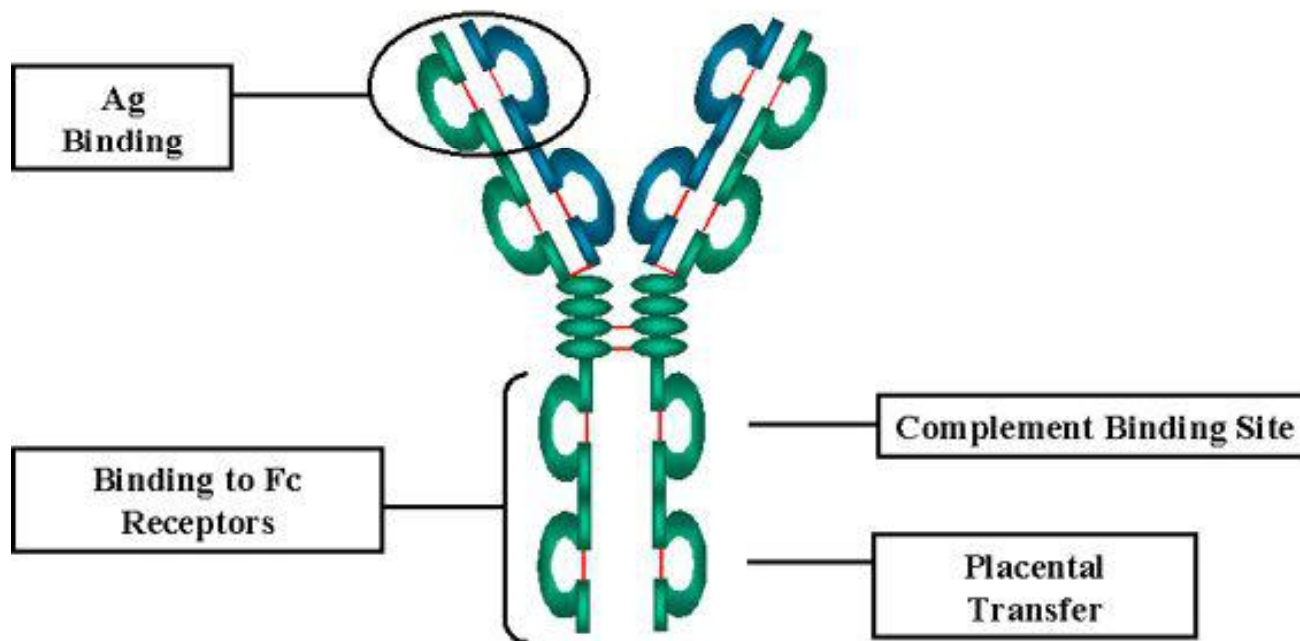
IgM

First immunoglobulin to be produced after the immune response takes place. IgM is the predominant isotype in the primary response. The role of the IgM is essential as that of the first immunoglobulin to be produced in the humoral response.



IgG

Immunoglobulin Fragments: Structure/Function Relationships



- IgG, a monomer, is the predominant Ig classes present in human serum. Produced as part of the secondary immune response to an antigen, this class of immunoglobulin constitutes approximately 75% of total serum Ig.
- IgG is the only class of Ig that can cross the placenta in humans, and it is largely responsible for protection of the newborn during the first months of life.

Monoclonal antibodies (mAbs):

- A monoclonal antibodies (mAbs) is a pure antibody of one molecular type derived from a single clone of B lymphoid cells which hybridized with myeloma cells, invented by Kohler and Milstein in 1975.
- Influence of different abiotic factors like, medium composition, cultivation conditions and medium osmolarity on mAbs secretion is a matter of interest for many scientists and industries (Chua *et al.*, 1994)



Fetal Calf Serum :

- Serum is non- physiological fluid for the cells, provides undefined low molecular weight nutrients
- Generally, in cell culture 5% to 15% serum is used as supplement in the standard basal media in addition to salts and amino acids for healthy cell growth (Babcock *et al.*, 2007).

Ferric citrate:

- Iron is required by cells and iron is an essential part of any serum-free media formulation.
- Cells must maintain proper intracellular iron levels. Without iron, cells usually fail to divide after a few divisions.
- One problem that arises from iron in media is that iron possesses oxidant properties that damages cells (Garcia, et al., 1996). Nature's solution to this problem is transferrin, an iron binding protein that prevents the toxic effects of iron and also maintains proper cellular iron homeostasis (Conrad, et al., 1999: Elliott, et al., 1993).

Objectives

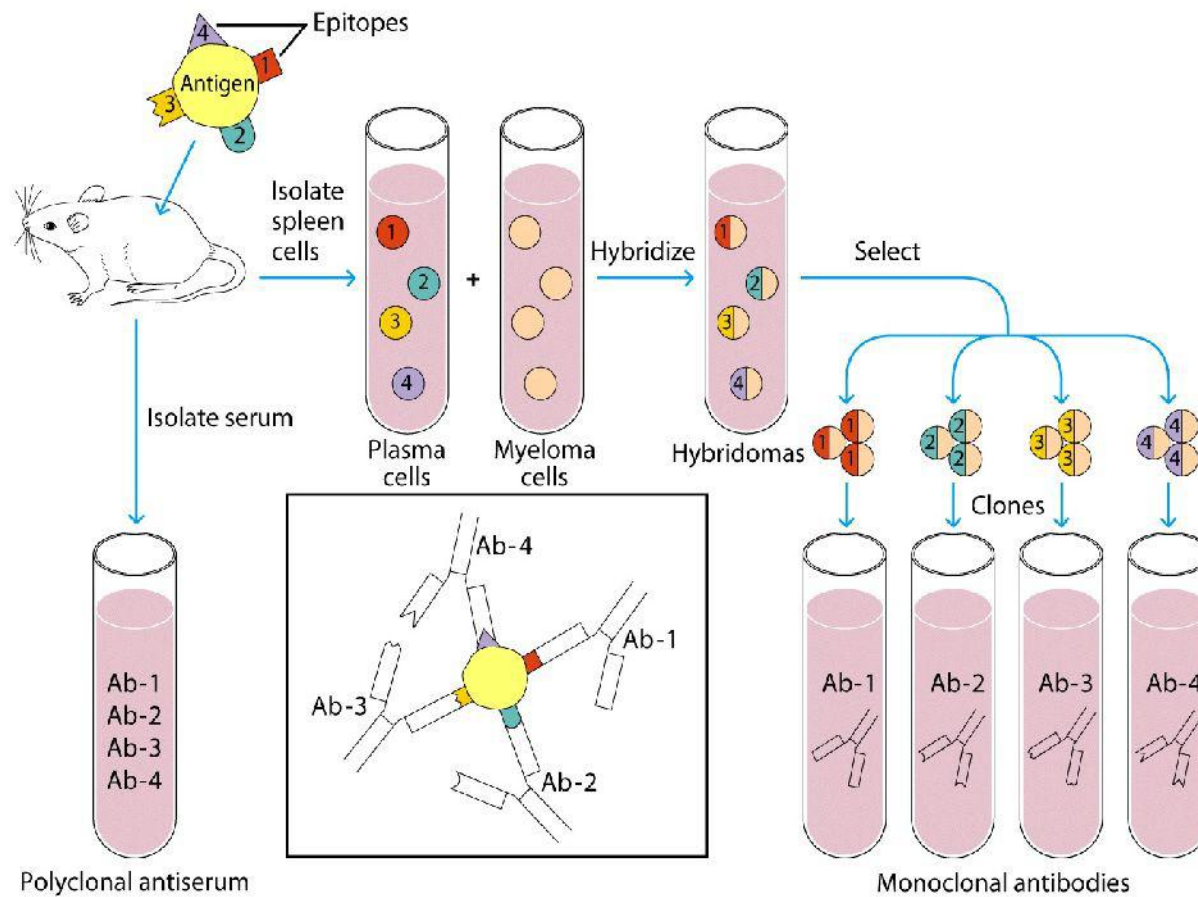
- Develop Hybridoma clone for Anti-D (Rh (D))
- To increase the mAbs secretion by Ferric citrate
- To produce quality mAbs, in-house technology & Cost effective technology

Methodology

- Avidity
- Titer Analysis
- Total Protein Estimation (Lowry, et al., 1951)
- Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) (Laemmli, 1970)

Experimental Setting

- Immunization
- Hybridoma Technology



Normal Cells:

- For Monoclonal Antibodies
 - Animal Is Immunized With Antigen 5% RBC +ve Blood group
- Intraperitoneal Immunization
 - Balb/c Mouse
 - Day 0: 50 μg of Ag In Complete Freund's Adjuvant
 - Day 14: 25 μg of Ag In Incomplete Freund's Adjuvant
 - Day 28: 25 μg of Ag In D-PBSA
 - Bleed Animal Test Reactivity To Antigen
 - Serum Is Diluted 1:30
 - Final Boost of 10 μg Antigen i.v or i.p 3 days before fusion
- Aseptically Isolate Spleen Cells



Myeloma Cells:

- This Cell Line Is Deficient In HGPRT (hypoxanthine guanine phosphoribosyl transferase)
- Alternatively TK (thymidine kinase deficient)
- Cell Line Cannot Survive In Selection Medium
 - Aminopterin Inhibits “*De novo* Pathway”, “Salvage Pathway” Is Not Possible Due To HGPRT or TK Deficiency
- It Is Also Ig Deficient
 - It can not secret any Immunoglobulins
- Aminopterin (folic acid antagonist) Blocks *De novo* Pathway
 - SP2O cells die in the presence of Aminopterin
 - They cannot utilize the “salvage pathway” because they are HGPRT deficient



Hybridoma Cell Lines:

- Normal Cells are Fused with a Cancerous Cell Line myeloma SP2/0
- Fusion Is Accomplished with PEG (polyethylene glycol)
- The new Hybrid Cell Exhibits Properties of both Cell Types
 - Unlimited growth
 - Secretes monoclonal antibodies
 - Or Secretes cytokines

Cell Culture Development:

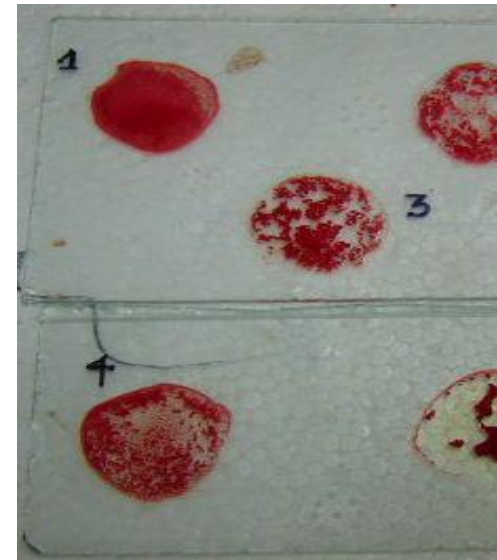
- 96 well plate
- 24 well plate
- T25 flask
- T75 flask
- T125 flask
- Roller bottle culture

Results and Discussion

Avidity:

- 5 – Commercial; 1-Control
- 2- 0.25mM Ferric citrate+5% FBS+RPMI
- **3 -0.5mM Ferric citrate+5% FBS+RPMI**
- 4 – 1.0 mM Ferric citrate+5% FBS+RPMI

Fig. 1. Antigen antibody agglutination test.



Results and Discussion

Titer Analysis



- 1. Control (RPMI-1640+5% FBS)
- 2. 0.25 mM Ferric citrate+5% FBS+RPMI
- 3 -**0.5 mM Ferric citrate+5% FBS+RPMI**
- 4 – 1.0 mM Ferric citrate+5% FBS+RPMI
- 5-1.0 mM Ferric citrate+10% FBS+RPMI

Fig. 2. Antigen and Antibody button formation.

	1:2;	1:4;	1:8;	1:16;	1: 32;	1:64;	1:128;	1:256;	1:512;	1:1024
1.	4+;	4+;	4+;	4 +;	4+;	4+;	3+;	2+;	+	+
2.	4+;	4+;	4+;	4+;	4+;	3+;	3+;	2+;	2+;	2+
3.	4+;	4+;	4+;	4+;	4+;	4+;	4+;	4+;	3+;	2+
4.	4+;	4+;	4+;	4+;	4+;	4+;	4+;	3+;	+	+
5.	4+;	4+;	4+;	4+;	4+;	4+;	4+;	3+;	3+;	2+

Table. 1. Titer analysis

Total Protein Estimation

- Control (RPMI-1640+5% FBS); 4.2 ± 0.2 mg/ml
- 0.25 mM Ferric citrate + RPMI 5%FBS; 2.5 ± 0.3 mg/ml
- **0.5 mM Ferric citrate + RPMI 5%FBS; 5.2 ± 0.2 mg/ml**
- 1.0 mM Ferric citrate + RPMI 5%FBS; 3.4 ± 0.3 mg/ml
- 1.0 mM Ferric citrate + RPMI 10%FBS; 4.2 ± 0.4 mg/ml

Table. 2. Total protein estimation analysis. $p < 0.001$

SDS-PAGE Analysis

Lane1- BSA;

Lane2- Control RPMI-1640 + 5% FBS;

Lane3-0.25mM Ferric citrate +5% FBS

Lane4- 0.5 mM Ferric citrate +5% FBS

Lane5- 1.0 mM Ferric citrate +5% FBS

Lane6- 1.0 Mm Ferric citrate +10% FBS

Lane7-RPMI-1640+5% FBS;

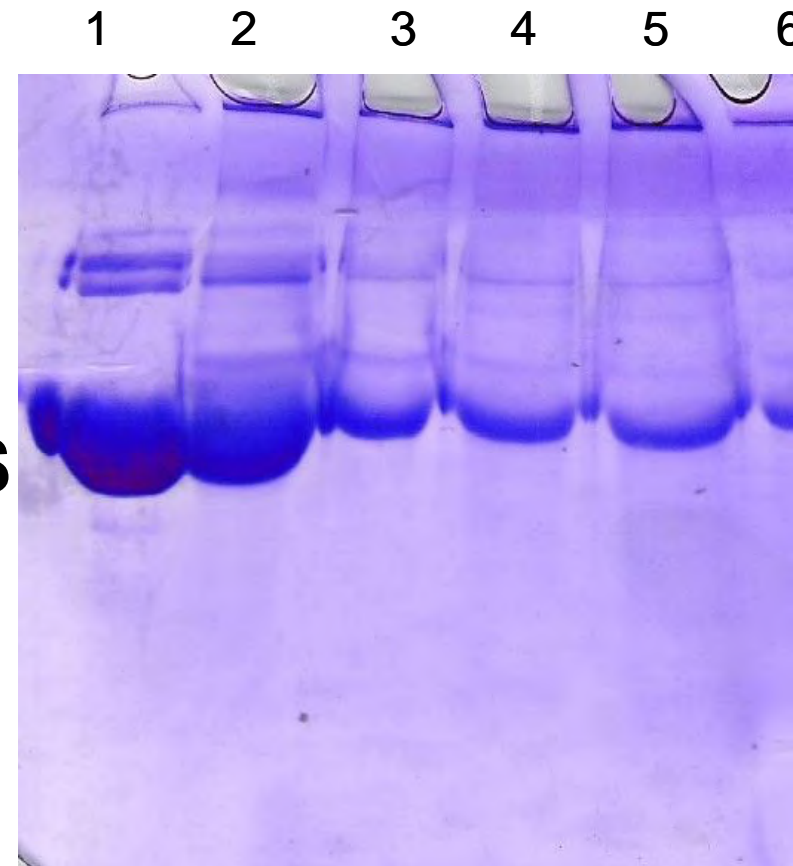


Fig. 3. SDS-PAGE profile of IgM, IgG and BSA

Conclusions & Recommendations

- Avidity: The 0.5 mM Ferric citrate + % FBS culture samples shown good agglutination in 2-3 seconds.
- Titer analysis, 0.5 mM Ferric citrate + % FBS culture sample has shown the 4+ value up to 1:128 (7th tube) dilution.
- Total protein: 0.5 mM Ferric citrate + % FBS culture sample has shown highest total protein (5.2 ± 0.4 mg/ml).
- SDS-PAGE: performed using 8% separating gel to confirm the high secretion of IgM antibody by comparing with the control.

- Concludes that yield of mAbs is maximized by addition of Ferric citrate cell culture samples.
- Anti-D mAbs can be used for Diagnostic USE.
- In-house Technology Facilitate the quality and Economy

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THANK YOU ALL

