Biologics

Health Canada broadly defines biological drugs as products derived from living sources. This include a broad range of active ingredients such as blood products, cells and tissues, gene therapies, vaccines, etc. Biologics are created through biological processes rather than being synthesized chemically as is it for most conventional drugs. Biologics are often considered as a relatively new category of medicinal drugs, though blood transfusion was first completed in 1665 and Frederic Banting first injected insulin into dogs to regulate their blood glucose levels in 1921.

This section starts by summarizing the distinctive features of biologics including their complexity and production systems. Specific biologics will then be reviewed to illustrate some of the numerous uses of biologics.

1 General Properties of Biologics

1.1 Complexity of Biologics

Biologics involve either large biomolecules or living cells or organs, and are therefore more prone to be altered than conventional small molecule drugs. The simpler biologic drugs are biomolecules and these are much larger in size and have more complex molecular structures than small molecule drugs (see figure below). Even the simpler biologics are far too large to be prepared effectively by organic synthesis. ``As a result, millions of different chemical forms of active ingredient can be present in a biologic drug product. For example, the 527-amino acid tissue plasminogen activator protein, with its 17 disulphide bridges and 3 glycosylation sites, may contain more than 1 billion chemically distinct active ingredient molecules in the final drug product. Also, the interactions between small molecules and their binding targets cover a much smaller surface area than those of biologic products and their receptors, highlighting the importance of proper three-dimensional structure and surface characteristics of biologic drugs.`` (Biologicals (2012) 40: 517-527). Biologics are therefore more complex and more difficult to characterize than conventional, small-molecule drugs. For instance, ``biologics are often glycoproteins — proteins that are modified by the addition of sugar side chains. These chains can differ from one glycoprotein molecule to the next, despite the protein portion itself remaining unchanged.`` CPF 2010, 143:4

FIGURE 1 **Molecular size comparison of a small molecule drug (Aspirin) to 3 different classes of biologics***

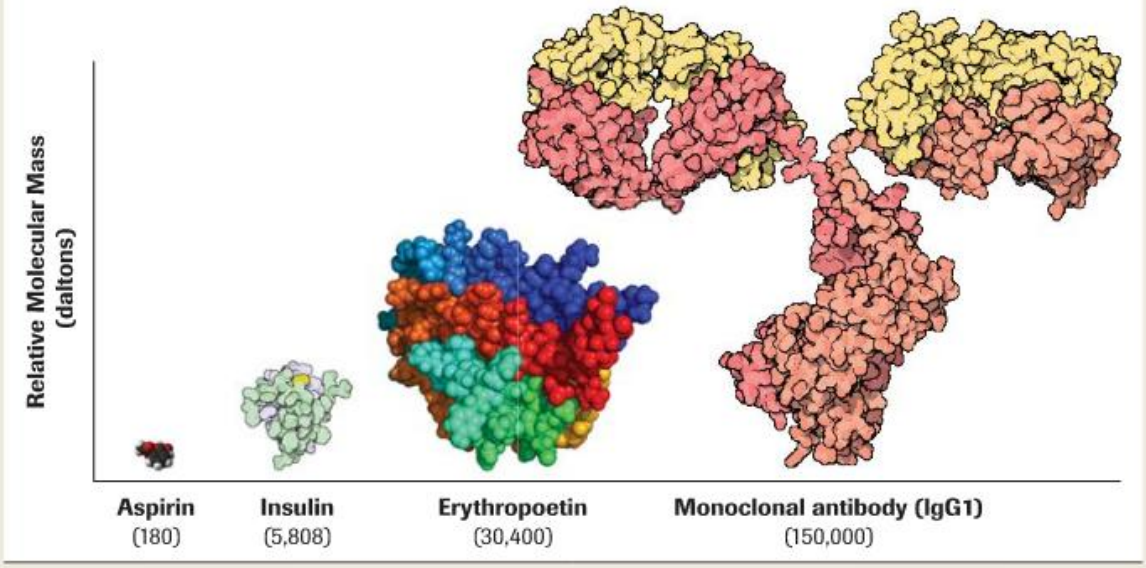


Figure from CPJ 2010: 143(4)

1.2 Manufacturing of Biologics

Manufacturing, storage and administration of drugs need to be regulated to ensure high quality standards, and this is particularly important for biologics given their complexity. Changes in raw materials, manufacturing unit operations, critical process parameters or manufacturing sites are all susceptible to alter the final biologic products. For instance, any subtle change to an expression system (host cell type, species, clonal isolate), a cloning vector, a cell growth system (media, vessel, environmental conditions) a purification process (chromatography, resins, buffers) or storage (container-closures system, storage conditions) may result in potential differences. For instance, most, if not all, biologics are required to be shipped chilled in the form of powder that must be reconstituted with sterile buffer, which is water with specific ionic composition and pH, and the resulting drug solution must be used within a 24-hour time frame. (CPF 2010, 143:4)

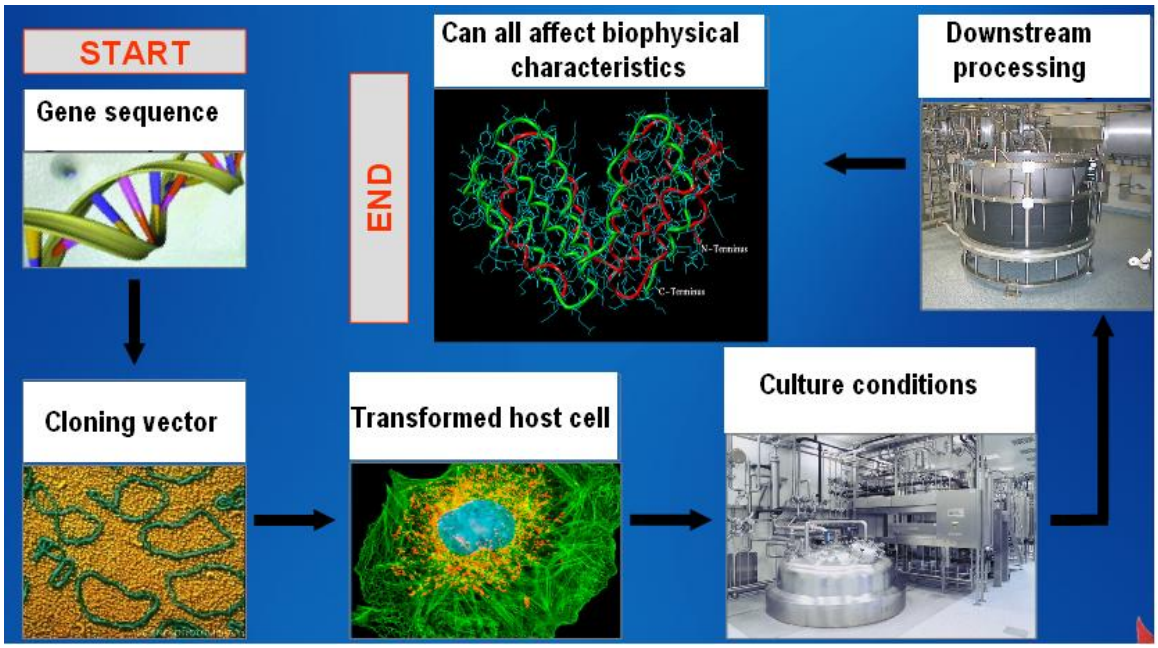


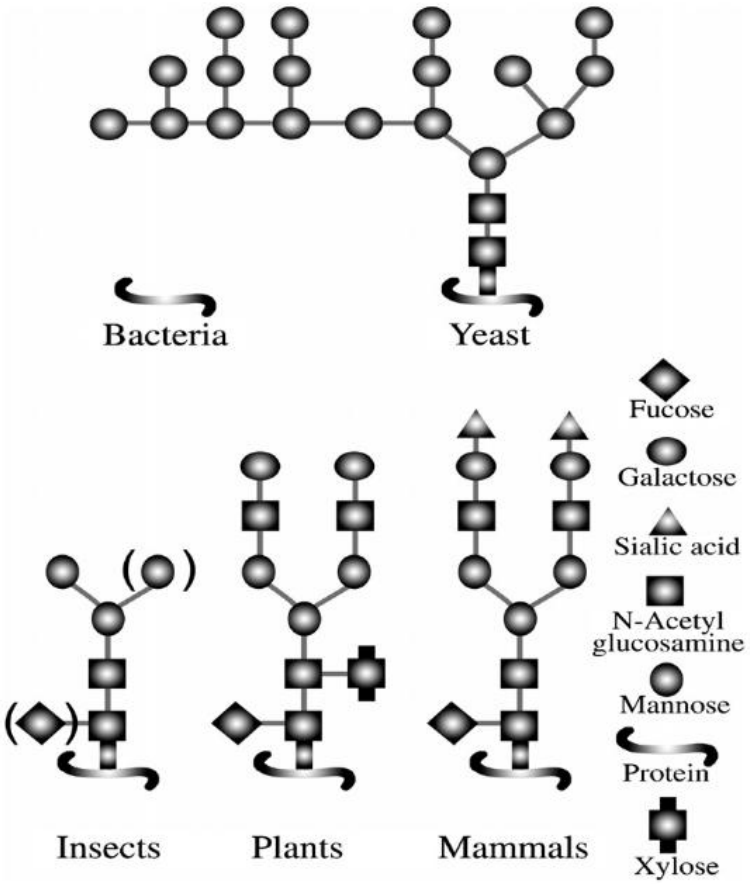
Figure modifiée à partir de BIOTECanada

Minor changes to biologics can result into real clinical differences such as described in the following case involving the human recombinant erythropoietin.

Erythropoietin (EPO) is a glycoprotein hormone controlling formation of erythrocytes or red blood cells into the bone marrow. EPO is often used as a performance-enhancing drug in sports, but it is also used for treating patients with anemia who have low red blood cell levels. Patients having experienced chemotherapy or a major surgery may also be treated with EPO to facilitate their recovery. Recombinant human EPO (rhEPO) has been commercialized since 1988. During the first 10 years of therapy, only three cases of immune reaction to rhEPO, which means that the body produced antigens against the administered drug, were reported. These three patients were shown to produce antibodies against rhEPO and were diagnosed with pure red cell aplasia, a disease characterized by the cessation of erythrocyte production into the bone marrow. However, a significant increase in the number of cases of rhEPO-induced pure red cell aplasia has been noticed between January 1998 and July 2003. A total of 195 confirmed cases of pure red cell anemia were confirmed during this period, including 177 patients who were exposed to the newly introduced brand of rhEPO (Eprex) alone and 18 other cases having been treated with Eprex and another rhEPO. During more or less the same period of time, eight cases of EPO-induced PRCA have been observed with the rhEPO produced by Amgen and marketed in the United States. The reasons for this difference are unclear, but differences in storage and handling may account for these differences in incidence of EPO-induced pure red cell aplasia. None of the companies producing rhEPO released information, but occurrence of pure red cell anemia has dropped to its baseline level since 2002. (full story in J Am Soc Nephrol, 2005)

1.2.1 Benefits and Limitations of Different Production Systems

Several simple protein biologic drugs can be prepared from bacteria such as *Escherichia coli*. But the use of bacterial cells entails some limitations as bacteria cannot synthesize complex proteins such as monoclonal antibodies which must be matured by post-translational modifications to be active or stable in vivo. These modifications include mainly folding, cleavage, subunit association, carboxylation and glycosylation of some target amino acids.



Comparison of glycosylation patterns in recombinant proteins produced in different host cell systems. Comp Immunol Micriobiol Infect Dis 2009, 32(2)

To minimize immunogenicity, which is the ability of a molecule to trigger an immune response, the recombinant protein drugs should be humanized or modified to make them similar human proteins. Posttranslational modifications of biologic proteins can be humanized by simply producing biologics into mammalian cells which can be cultured in fermenters at an industrial scale or produced in living animals. Several transgenic animal species can produce recombinant proteins. Milk from transgenic farm mammals has been studied for 20 years and in 2006 human antithrombin III received the agreement from European Agency for the Evaluation of Medicinal Products to be put on the market. Another system is chicken egg white which recently became more attractive after essential improvement of the methods used to generate transgenic birds. Two monoclonal antibodies and human interferon could be recovered from

chicken egg white. The different systems to produce recombinant proteins are summarized below.

Comparison of the different systems to produce recombinant pharmaceutical proteins

Points to consider	Production systems					
	Bacteria	Yeast	Insect cells + baculovirus	Animal cells (CHO cells	Transgenic plants	Transgenic animals
Theoretical production level	+++++	+++++	+++	+	+++++	+++++
Practical production level	++ (+)	++ (+)	+	+	++	++++
Investment cost	+++++	+++++	++	+	++++	+++
Production cost	+++++	+++++	++	++	+++++	++++
Flexibility	+++++	+++++	++	+	+++++	++++
Line conservation	+++++	+++++	+++	+++	+++++	+++++
Line stability	+++++	+++++	++++	+++	+++++	+++++
Delay for the first production	+++++	+++++	+++	+++++	++++	+++ (+)
Scaling up	+++++	+++++	++	+	+++++	++++
Collection	+++++	+++++	+++++	+++++	+++++	++++
Effect on organism	+++ (+)	+++ (+)	+++ (+)	+++ (+)	+++ (+)	+++
Post-translational modifications	+	++	+++	++++	+++	++++
Glycosylation	+	++	+++	++++	++	++++
Stability of product	+++++	+++++	+++	+++	++++	++++
Purification	+++	+++	+++	++++	+++	+++
Contaminant pathogens	+++++	+++++	+++++	++++	+++++	++++
Intellectual property	++++	+++	+++	++	+++	+++
Products on the market	++++	+++	+++	+++++	+	+++

Table from Comp Immunol Micriobiol Infect Dis 2009, 32(2)

EPO has been illegally used by some athletes to improve their physical performance – higher levels of red blood cells means better oxygen transport through the body and better performance. But athletes injecting themselves with rhEPO are more at risk to be caught. This is because the posttranslational modifications of rhEPO differ between what happens within the human body versus a cell-based system (Drug Test Anal 2012: 4(11)). EPO gene doping can be detected too because the vectors available to transfer a gene into human cells cannot handle very large DNA fragments and only the coding sequence or exons can be transferred. PCR assays targetting sequences within the transgene DNA corresponding to exon/exon junctions, which are absent in the endogenous gene due to their interruption by introns, allow detection of trace amounts of a transgene coding for rhEPO (Gene Ther 2010 :17(8)). There is a doping controlling laboratory accredited by the World Anti-Doping Agency in Montreal that is affiliated with the Armand-Frappier Institute.

Dr. Illimar Altoosar is a researcher at the University of Ottawa who seek to develop transgenic plants for the large-scale production of recombinant proteins. Production cost of biologics through plants has been ``estimated to be 2–10% the cost of microbial platforms, and up to 1,000-fold more cost effective than mammalian platforms.`` (Methods Mol Biol 2013, 956)

Dr. Altoosar is particularly interested in overexpressing biologics into the rice seed which he considers as an ideal system due to its low protease activity (better preservation of the recombinant biologic protein), low water content, stable protein storage environment, relatively high biomass, and the molecular tools available for manipulation.

Medicago is a great success story of a company having succeeded in using a plant-based platform to produce vaccines. The technology developed by Medicago allows rapid, flexible, high yielding and a robust vaccine and antibody production system. Within one month, Medicago was indeed able to produce a first lot of anti-porcine H1N1 vaccine during the outbreak which occurred in 2009-2010 (see figure below). This one month delay compares advantageously with the conventional egg-based manufacturing of vaccines which requires a minimum of 3 months. Medicago`s technology also offers high yield production capacity with a current estimated production of 10 million doses of vaccine per month – the company used to be based in Quebec city, but it was recently bought by two private companies, one being an affiliate of Philip Morris International Inc that is a large cigarette maker company with total annual revenues of 140 billion. This yield is significantly higher than the observed 3 million doses produced within 5 months during the pH1N1 epidemic through egg-based manufacturing.

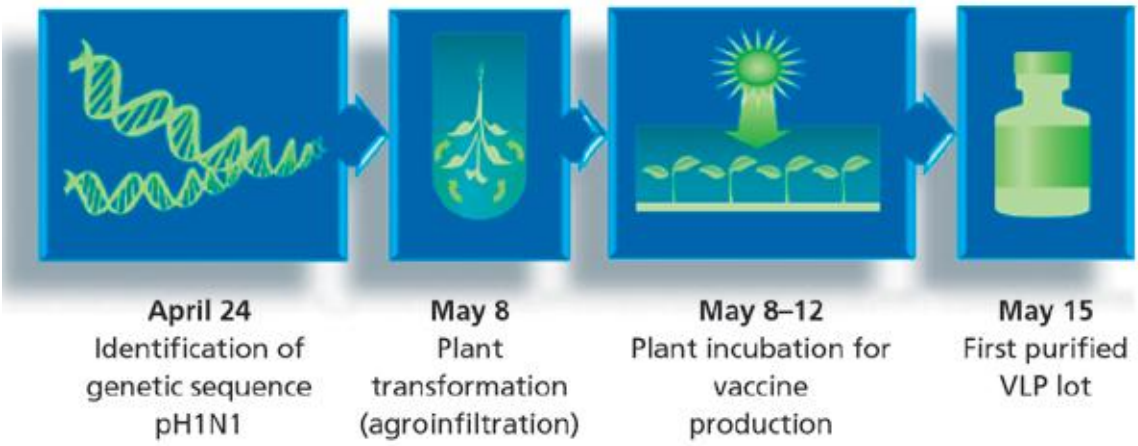


Figure from BioPharm (2011) 24(5)

The Medicago technology is quite time effective as it did not involve the production and growth of transgenic plants. It is rather based of a transient expression system that uses the capacity of a bacterium, *Agrobacterium tumefaciens*, to infect plant cells and transfer essential DNA in the form of a mobile DNA fragments to the nucleus of the host plant cells. This is considered a transient expression system as the mobile DNA only remains viable for only a few days and is not permanently integrated in the plant genome. Despite the transient nature of the Medicago technology, high yields can be achieved. Again, it is the transient expression of the Medicago technology that makes it possible to quickly produce vaccines as whole plants already in place are used – the growth of the host plant is therefore not a limiting step.

The underlying principle of Medicago`s technology is quite intriguing. The influenza virus is an enveloped particle budding from the plama membrane of host cells (see A on the figure below). Hemagglutinin (green) and neuramidase (orange) are the two major viral proteins protruding outside the viral envelope. Research performed at Medicago has established two essential facts. First, it is the mainly the hemagglutinin protein that triggers immunogenicity and production of antiviral antibodies. It was also shown that out of the different peptides encoded by influenza viruses only the matrix protein is essential for the cell membrane budding. Based

on these two important results, Medicago was able to produce immunogenic virus like particles (VLPs) by selectively overexpressing only the hemagglutinin and matrix proteins of a given influenza virus. Both the VLPs and the true viral particles purified from tobacco plants yield similar immune responses when injected into mice.

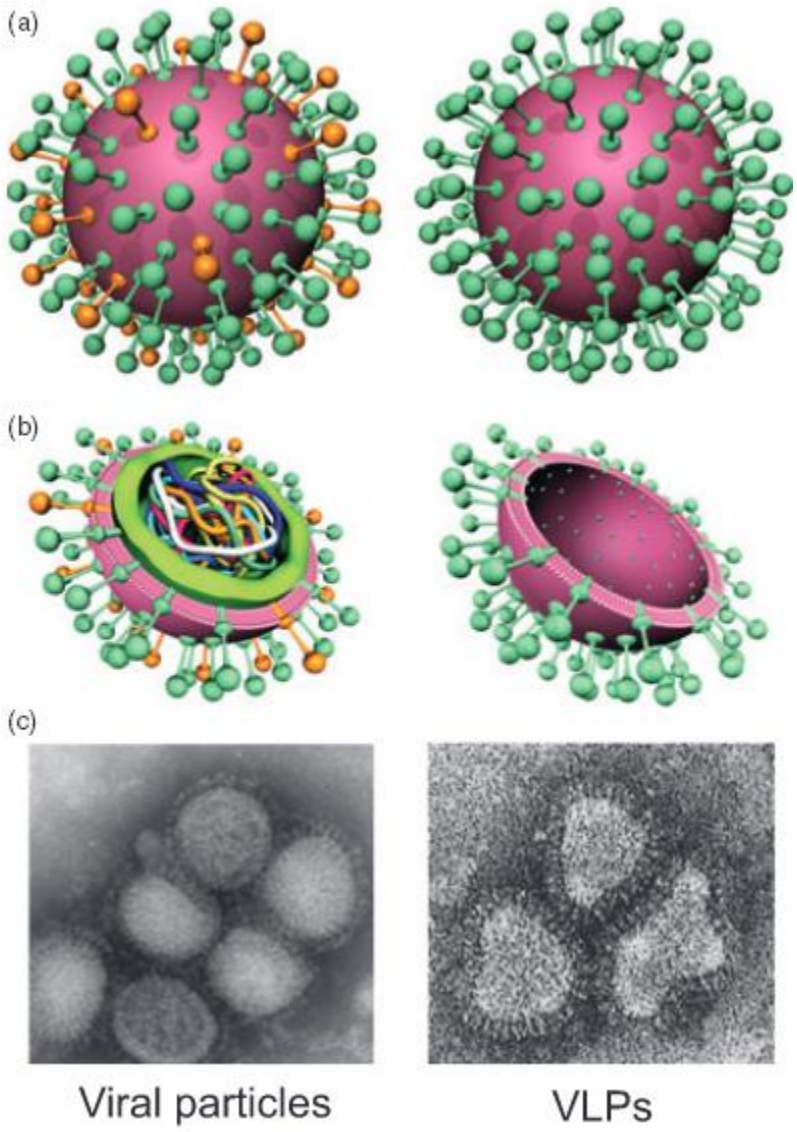


Figure from Plant Biotechnology journal (2010) 8

1.3 Some Examples of Biologics

1.3.1 Canadian Blood Services: Business of Blood and Blood-Derived Products

``Canadian Blood Services is a not-for-profit, charitable organization whose mission is to manage the blood and blood products supply for Canadians. To this end, Canadian Blood Services:

- Collects approximately 850,000 units of blood annually and processes it into the components and products that are administered to thousands of patients each year.
- Manages the OneMatch Stem Cell and Marrow Network whose mission is to secure, in an expeditious way, donors for Canadian bone marrow transplant patients and for patients abroad.
- Screens every donor and tests each unit of blood or blood product collected for a variety of transmissible diseases.
- Ensures that Canadian transfusion medicine research and development remains at the cutting edge.`` (Canadian Blood Services)

Blood is a complex bodily fluid containing both cellular and non-cellular components. Blood`s main constituents are red cells or erythrocytes, white cells or leukocytes and the plasma which contains coagulation factors and albumin.

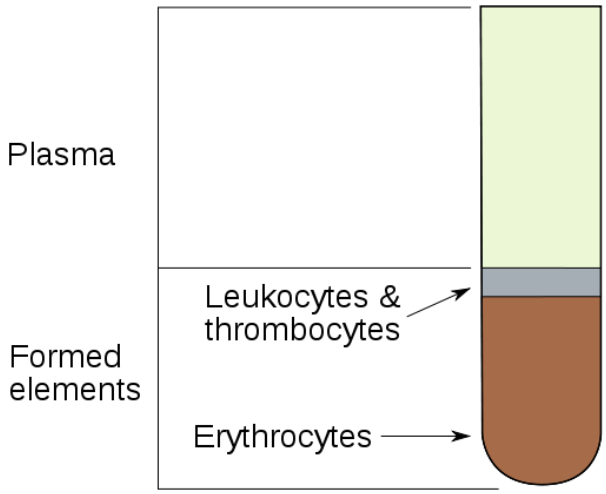
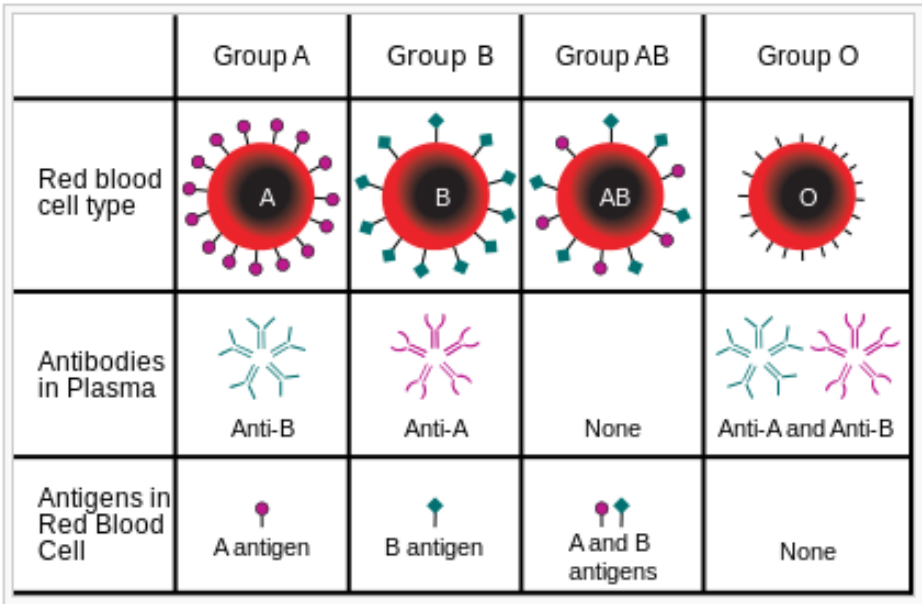


Figure from Wikipedia.

CBS recently launched – September 26, 2013 – its Project Recovery, a world first initiative aiming at recovering precious proteins left over from the manufacture of plasma products (Project Recovery). ``Factor VIII, a protein essential to blood clotting, is contained in cryoprecipitate, one of the components of plasma. Not all of the cryoprecipitate contained in plasma from CBS donors is needed to make factor VIII for Canadian patients and until now the excess was discarded. With project Recovery, the cryoprecipitate will be harvested … and manufactured. This finished pharmaceutical product will be manufactured and released under the BIOTEST license and trademarked Haemoctin, a high purity … factor VIII product for the treatment of hemophilia A.`` It is estimated that the Project Recovery will transform surplus cryoprecipitate to treat an extra 5000 patients affected by anemia. These surplus factor VIII samples will be distributed in different countries through the Humanitarian Aid Program of the World Federation of Hemophilia.

CBS is a major player in the sector of biologics. In addition to coordinating the collection of blood and blood products – it is possible to give only plasma or clotting factors – CBS is also coordinating the production and distribution of most blood and blood products throughout the Canadian hospital network. CBS coordinates the matching program for stem cell and marrow

transplant (see below for detail about stem cells and their medicinal uses). Compatibility testing between donors and receivers should be carefully assessed prior to a blood transfusion and stem cell transplant. ``Many patients have died of blood transfusion and it was not until 1901, when the Austrian Karl Landsteiner discovered human blood groups, that blood transfusions became safer. … Nobel Laureate Karl Landsteiner was involved in the discovery of both the AB0 blood group (in 1901) and Rh blood group (in 1937). `` (NobelPrize.org) There are many other blood typing systems including the human lukocyte antigen (HLA) system that refers to a group of genes coding for cell-surface antigen-presenting proteins. HLA factors are important elements for immune function and are the major cause of organ transplant rejections. CBS has played an important role for developing the technology and protocols for typing HLA factors by serology (use of specific antibodies able to recognize specific HLA factors) and PCR (HLA genes are amplified and sequenced to determine their types) (CBS Typing Services). There are six major HLA genes and, for these six genes, more than six thousand HLA variants have been characterized (HLA Database).



The blood type is determined, in part, by the ABO blood group antigens present on red blood cells. Figure from Wikipedia.

What are stem cells?

Stem cells are immature cells that can become either:

- red blood cells (which carry oxygen),
- white blood cells (which fight infection) or
- platelets (which help to stop bleeding).

What is a stem cell transplant?

In a stem cell transplant, a patient's diseased bone marrow is replaced with healthy stem cells from a donor. To prepare for the transplant, the recipient is usually given high doses of radiation and/or chemotherapy to destroy the diseased marrow. At this point, stripped of the ability to manufacture life-giving blood cells, the recipient is extremely vulnerable. They will not survive unless the donor proceeds with the donation. Once the healthy stem cells are collected from the donor, it is given intravenously to the recipient as soon as possible.

What diseases are treated with stem cell transplants?

A variety of diseases and disorders are treated with stem cell transplants including blood-related diseases such as leukemia, aplastic anemia and inherited immune system and metabolic disorders.

What do you mean by a "match"?

Donors and patients are matched according to the compatibility of inherited genetic markers called Human Leukocyte Antigens (HLA). These antigens are inherited from your parents. Up to 12 antigens are considered important in the matching process.

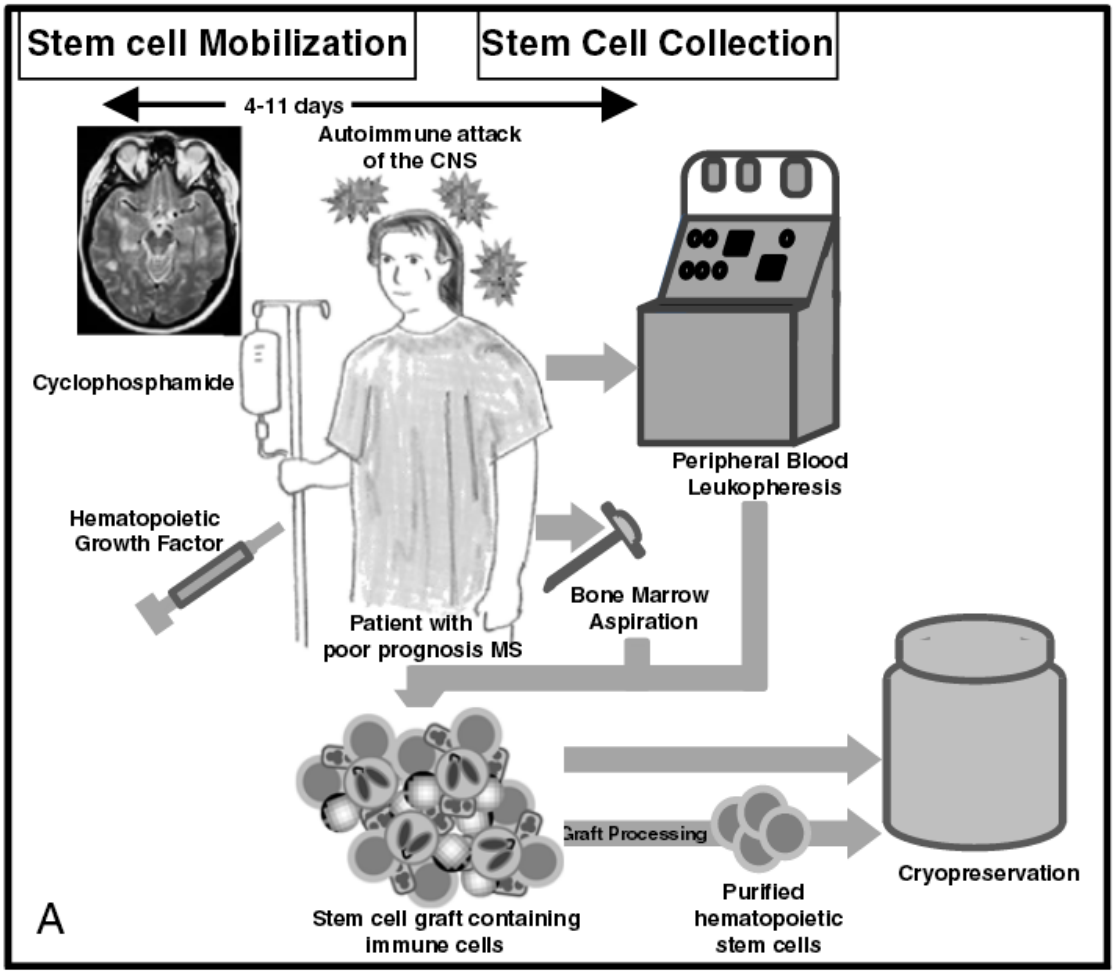
CBS` OneMatch Program

CBS also coordinates the Umbilical Cord Blood Program (UCB). ``Umbilical cord blood is blood that remains in the placenta and in the attached umbilical cord after childbirth. Cord blood is collected because it contains stem cells, which could be used to treat hematopoietic and genetic disorders.`` (Wikipedia) The UCB Program is a necessary complement to the Bone Marrow Transplant Program as less than 30 percent of potential hematopoietic stem cell transplant recipients can find a matched related donor. UCB has gained in popularity and this has resulted in an increase in the cord blood banking. UCB transplantation is advantageous as it requires less stringent matching parameters compare with the bone marrow transplant; it is therefore easier to find a match based on umbilical blood transplantation than through bone marrow transplant.

Information released by CBS researchers about the UCB Program nicely illustrates the complexity of working with living cells, which is a specific case of biologics. UCB must be frozen and thawed whenever needed, but this process is relatively stressful and only 50 to 80 percent of cells are recovered prior to infusion at time of transplant (Int J Hematol, 2011: 93-1). Lower cell survival necessarily reduces the success rate of UCB transplantation and contributes to delayed engraftment and transplant-related mortality. Researchers at CBS and the Ottawa

Hospital Research Institute have recently reviewed the processing, cryopreservation, and thawing of UCB samples to improve cell survival rate. Their results suggests that an increase by 10 to 30 percent in the faction of surviving cells may be possible, and such increase could increase the success rate of UCB transplantation from 12 out of 21 to 19 out of 21 patients (Stem Cells International 2013). A more specific list of biochemical markers influencing cell survival rate in banked UCB can be obtained from Stem Cells International 2013 – this article was also written by CBS and OHRI researchers.

Another approach is to harvest the patient`s own stem cells that can be stored temporarily until being transplanted. This is a common practice with patients suffering from multiple sclerosis, an autoimmune disease resulting in central nervous system demyelination and axonal damage. Hematopoietic stem cells are first collected, then the patient is treated with high doses of chemotherapy to destroy the auto-destructive immune cells. The collected hematopoietic cells can then be infused into the patient. The process illustrated below will be further discussed in class.



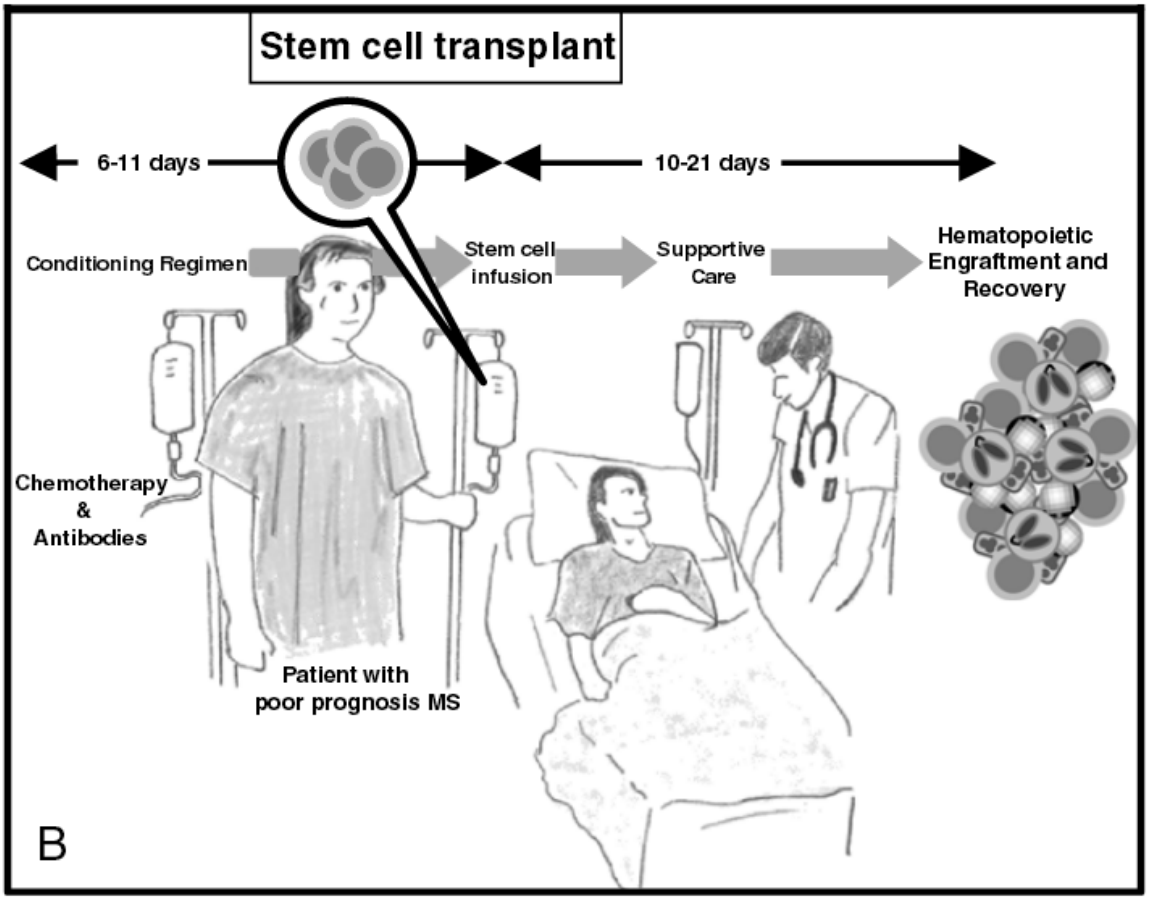


Figure from Neurotherapeutics (2013) 10:68

1.3.2 Ottawa-Based Cell and Gene Therapy Research

Access the Stem Cell Network for a list of ongoing research projects related to research and clinical uses of stem cell therapy in Ottawa and other Canadian cities.

Pioneering heart attack stem cell trial treats 1st patient

The first patient has been treated in a groundbreaking medical trial in Ottawa that could lead to a new way to repair damaged tissues following a heart attack.

Researchers announced Thursday that Harriet Garrow of Cornwall, Ont., who suffered a severe heart attack in July, was their first test subject. Her heart had stopped beating before she was resuscitated, causing major damage to her cardiac muscle.

The hope is that a new form of combined gene and stem cell therapy will be able to better repair her heart and those of potentially millions of other heart attack patients.

The therapy involves injecting a patient's own stem cells into their heart to help fix areas that become damaged in a heart attack. Stem cells are a fertile regenerative tissue that can replicate into millions of new, healthy cells.

But the Ottawa study, led by cardiologist Duncan Stewart of the Ottawa Hospital Research Institute, takes the technique one step further, combining the stem-cell treatment with gene therapy — which the researchers say is novel.

"Stem cells are stimulating the repair. That's what they're there to do," Stewart said in an interview. "But what we've learned is that the regenerative activity of the stem cells in these patients with heart disease is very low, compared to younger, healthy patients."

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To try to restore some of that regenerative capacity, Stewart and his colleagues will supply the stem cells with extra copies of a gene. The gene makes the cells produce more of an enzyme called endothelial nitric oxide synthase, which helps the damaged heart build up new blood vessels and heal itself.

"That, we think, is the key element," he said. We really think it's the genetically enhanced cells that will provide the advantage."

Funding for the Ottawa study comes from the Canadian Institutes of Health Research, the Ottawa Hospital foundation and the St. Michael's Hospital foundation in Toronto. Some equipment was donated by drug and medical products company Abbott.

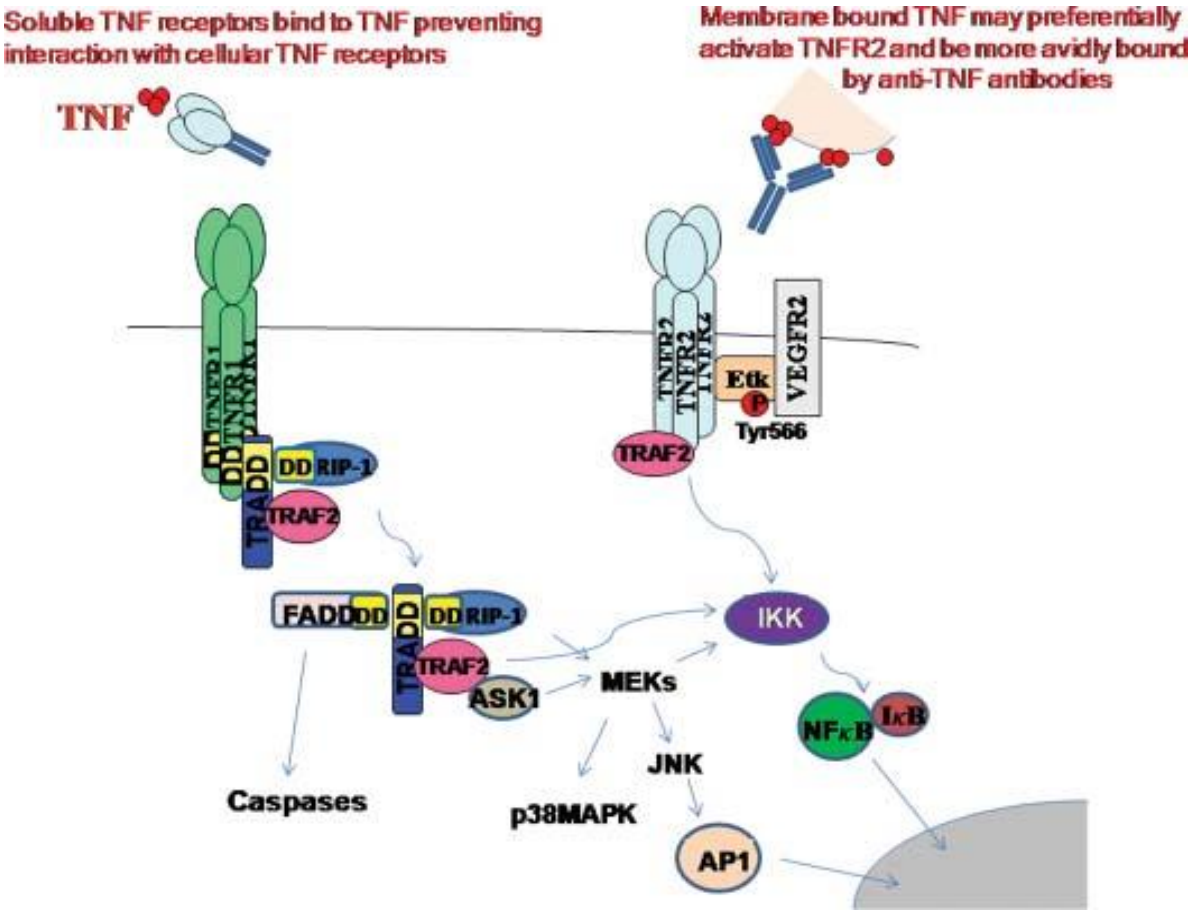
CBC News, September 5, 2013

1.3.3 Vaccines and Use of Antibody Drugs

Vaccines have shown to be cost-effective tools for treating different diseases including measles, polio and smallpox. ``Smallpox was officially declared eradicated in 1980 and is the first disease to have been fought on a global scale`` (WHO) owing to a concertated international vaccination campaign. In Ontario, the recommended immunization schedule involved vaccination against diphteria, tetanus, whooping cough, polio, *Haemophilius influenza* type B, pneumococcal disease, rubella, and chicken pox (Public Health Canada). This section reviews some of the limitations and challenges related to the development of vaccines.

1.3.3.1 Clinical Studies for Rheumatoid Arthritis Vaccines

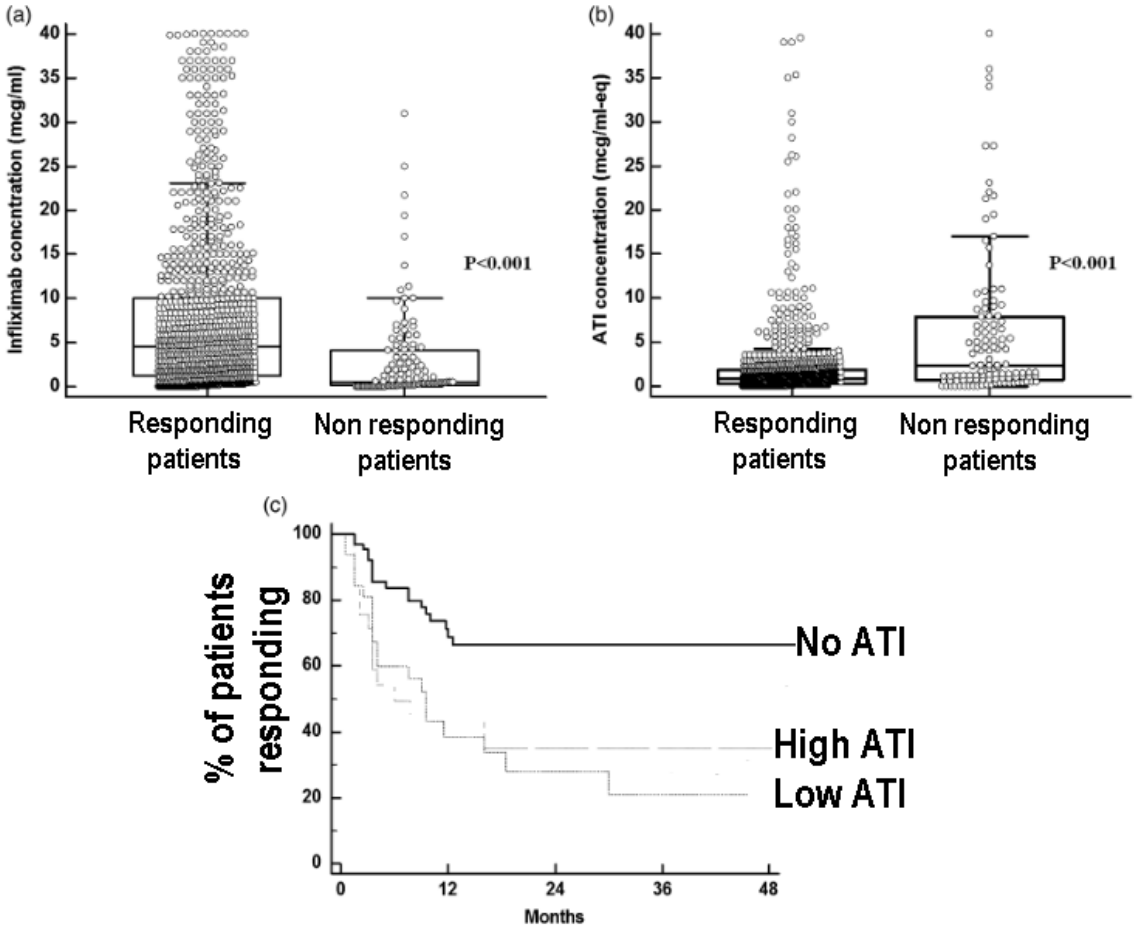
There are numerous diseases related to inflammation, including rheumatoid arthritis, atherosclerosis, psoriasis and Crohn`s disease. The pathogenesis of all these diseases relies on a common regulator of the inflammatory response, the tumor necrosis factor alpha (TNFa) (J Pathol 2008, 214, 2). One therapeutic strategy to treat inflammatory diseases is to prevent the extracellular pool of TNFa to bind onto its usual receptors, TNFR1 and TNFR2, which are differentially regulated on various cell types in normal and diseased tissue. The figure below illustrates two possible strategies to bind with the free pool of TNFa in the extracellular environment and therefore prevents the activation of inflammation.



Signalling pathways leading to the main cellular responses of TNF. Soluble TNF receptors or monoclonal anti-TNF antibodies, which prevent TNF interacting with its receptors and activating these pathways, can be used to treat inflammatory disease. Figure from J Pathol 2008, 214, 2.

There are currently three antibody drugs that have been approved in the USA for treating patients with rheumatoid arthritis and other inflammatory diseases. The efficacy of these anti-TNFa drugs tends to be high during the firsts few months, but is then lowered with time. This reduction of efficacy results from an immune response that appears in some

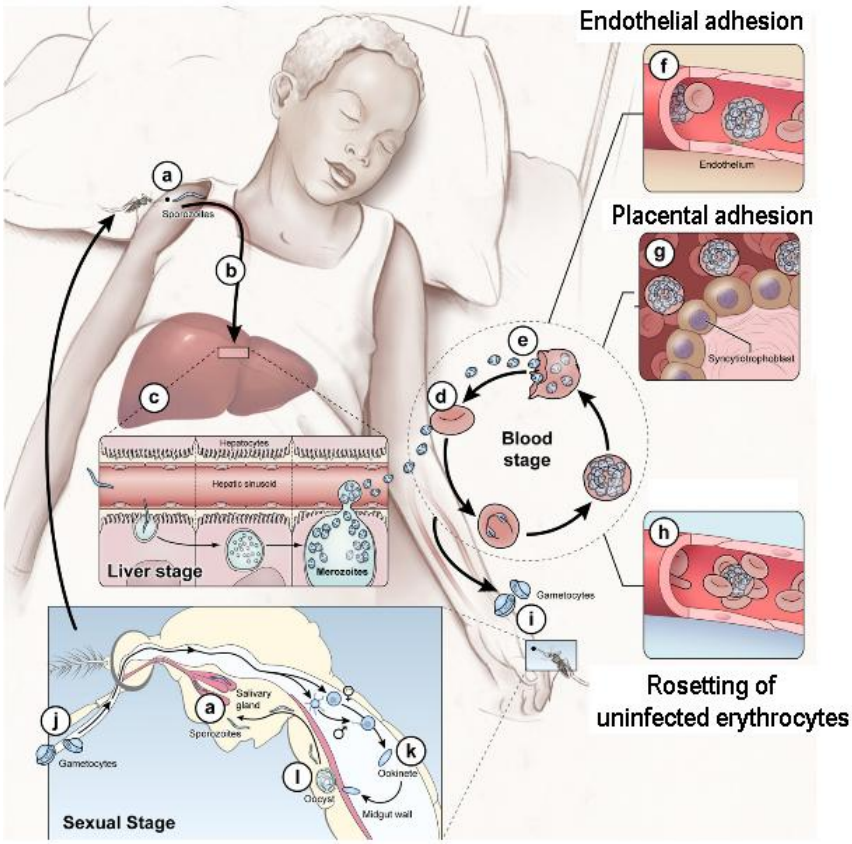
individuals who produce antibodies against the antibody drugs. The patients become immune to the antibody drug which cannot bind TNFa and prevent inflammation. Patients treated with Infliximab, for instance, produce antibodies against this drug in 10–76.7% of rheumatoid arthritis patients (Expert Rev Clin Immunol 2008, 4). Another study reported that 42% of patients treated with Infliximab remained free of anti-Infliximab antibodies by 4 years of treatment and most (90%) of the patients who developed antibodies did so within the first 12 months of therapy (Gut 2013). One approach to delay the appearance of antidrug antibodies in patients is to use a therapy combining an anti-TNFa drug with an immunosupressor that reduces the activation or efficacy of the immune system (see results below). A common side-effect of many immunosuppressive drugs is immunodeficiency resulting in increased susceptibility to infections and cancer.



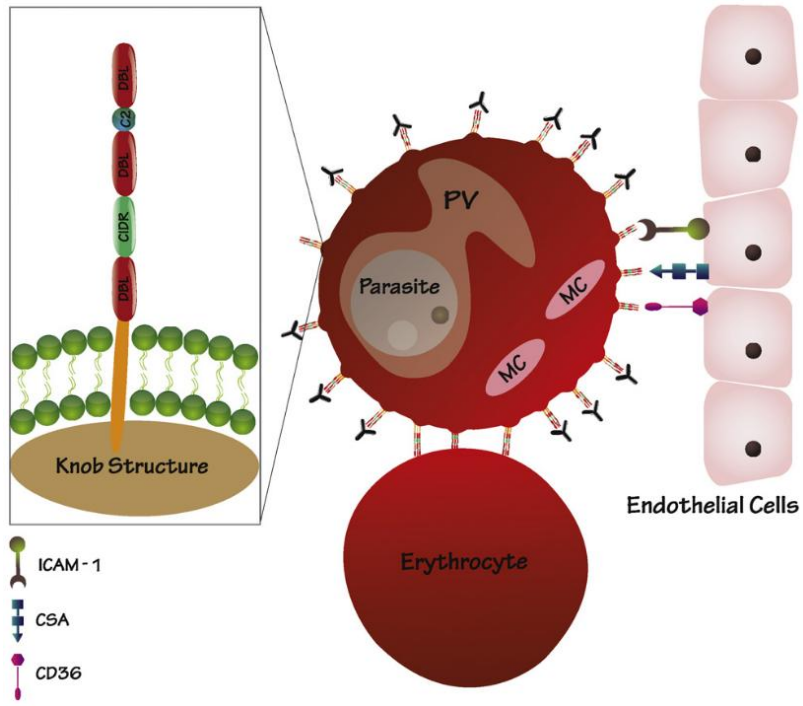
(A) Infliximab levels in responding vs non responding patients. Each dot represents an individual time point of infliximab levels. (B) Anti-Infliximab antibodies (ATI) at time points in responding vs non responding patients. (C) ATI levels in responding patients. Figure modified from GUT, 2013.

1.3.3.2 Malaria Vaccines

Dr. Durst has mentioned that there is no licensed malaria vaccine. Malaria is caused by mosquito-borne *Plasmodium falciparum* whose complex lifecycle is illustrated below. Only the blood stage of infection is associated with disease.



Pathogenesis of malaria involves adhesion of infected red blood cells to the internal surface of blood vessels allowing the parasite to sequester in capillaries and prevent destruction in the spleen. This adhesion is mediated through a *P. falciparum* protein protruding through the membrane of infected red blood cells, which is called the PfEMP1 (*P. Falciparum* erythrocyte membrane protein 1).



PfEMP1 mediates adhesion of the infected red blood cells. PfEMP1 is expressed by the malaria parasite *P. falciparum* on the knobs formed on the surface of infected erythrocytes. The variable extracellular regions DBLs and CIDR mediate adhesion through binding to several endothelial receptors. In addition, PfEMP1 mediates adhesion to uninfected erythrocytes forming rosettes. Figure from Int J Biochem Cell Biol 2009.

The PfEMP1 protein is a target of choice for vaccines given its accessibility relative accessibility to drug antibodies – antibodies are part of the humoral or extracellular immunity and cannot directly reach intracellular antigens. Some preliminary attempts to develop malaria vaccines based on antibodies directed against PfEMP1 have led to minimum protection efficacy. This might be due to the highly polymorphic nature of PfEMP1 : `` These polymorphic proteins are encoded by a multi-copy gene family called *var*. Each individual parasite expresses a single *var* gene at a time, maintaining the remaining ~60 *var* genes found in its genome in a transcriptionally silent state. As the antibody response against the single expressed PfEMP1 develops, small sub-populations of parasites switch expression to alternative forms of PfEMP1 and re-establish the infection. Therefore, PfEMP1 is considered a key player in the pathogenicity of *P. falciparum*.`` (Int J Biochem Cell Biol 2009) The polymorphism is even greater when taking into consideration that ``The Plasmodium parasite has a complex life cycle with multiple stages and stage-specific expression of ~5300 putative proteins.`` (Int J Parasitol 2011, 41:1)

Sequestration of *P. falciparum* within the infected host cells – first into liver cells then into red blood cells – is therefore a first line of protection against vaccines or antibody drugs. The clonal variation of the PfEMP1 antigen – an antigen is an exogenous molecule found on a pathogen and that triggers immunity or production of antibodies – among different *P. falciparum* isolates represents another additional complexity to the production of malaria vaccines. A third limiting aspect, the faltering of B lymphocytes, which normally produce antibodies, is explained below.

Antibodies are produced by B lymphocytes that, in general, are long-lived cells. ``Estimates of half-lives of antibodies responses range from 11 year for tetanus vaccines to >300 years for measles vaccines. In sharp contrast, the half-lives of *P. falciparum*-specific antibodies responses generally appear to be much shorter, particular in young children. For example, in a 12-week study of Kenyan children following acute malaria, the half-lives of antibodies responses specific for five surface antigens were estimated to vary between 6.1 and 9.8 days.`` (J Immunol 2013, 190:7). It is still unknown why and how *P. falciparum* can result into lower survival of B lymphocytes and, consequently, less effective vaccines.

It is hoped that the recent availability of comprehensive genomic, proteomic and transcriptomic datasets from human and other species will provide a foundation to move from empirical development of vaccines, which essentially relies on trial and error, to a more effective approach based on rational design. The figure below illustrates a state of the art high-throughput approach for design of vaccines.

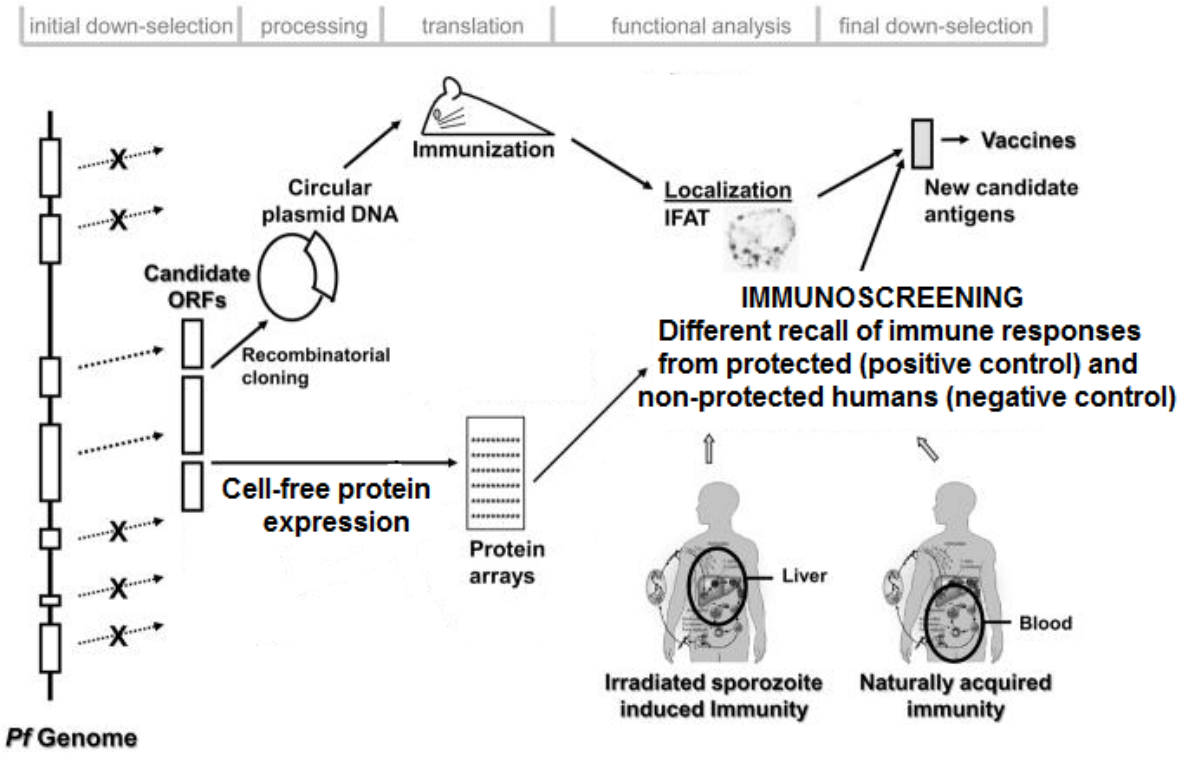


Figure adapted from Int J Parasitol 2011, 41:1.

Get ready for the final exam

It`s been impossible so far to produce commercial malaria vaccines. Identify and briefly explain three reasons that likely explain the difficulty of producing malaria vaccines.

Identify one major *P. falciparum* antigen that researchers have targeted for the production of antibodies. Explain (1) why researchers chose that specific antigen in their attempt of producing malaria vaccines and (2) why this approach failed or, at best, partially succeeded?

What is an antibody drug? How is it that the efficacy of antibody drugs is diminished in some patients who`ve been treated for a long period of time? If you were a medical doctor, what could you do to minimize or at least delay the inefficacy of antibody drugs?

What is the physiological role of TNFa? Describe the mode of action of anti-TNFa drugs.

1.3.4 Recombinant Human Insulin

`` The isolation of the first-ever biologic, the hormone insulin, was achieved by Frederick Banting and Charles Best in Toronto in 1921. Insulin (from the Latin insula) is named for the islets of Langerhans in the pancreas, which secrete the hormone. Banting and Best isolated the substance from the pancreatic ducts of dogs and later used it to correct the inborn insulin deficiency in type 1 diabetic patients. Several decades later, in 1955, it was identified by Frederick Sanger as a protein composed of 51 amino acids, with an MW of 5808. The molecule is an archetype for the so-called replacement therapy category of biologics, in that it is introduced into the body to correct a physiological deficiency. While extraction of insulin from the pancreas of cattle and pigs quickly became a commercial success, it was soon apparent that there were allergic complications in some people, because insulin is large enough to be monitored by the immune system. In these cases, the subtle difference between the human and animal forms of insulin caused the latter to be recognized as foreign.

The real breakthrough in insulin production came with the advent of recombinant DNA technology, first described by Herbert Boyer and Stanley Cohen and colleagues in 1973. After hearing about Boyer and Cohen's breakthrough, a venture capitalist by the name of Robert Swanson placed a call to Boyer and requested a meeting. Famously, Boyer agreed to give Swanson 10 minutes of his time. Swanson's enthusiasm for the technology and his faith in its commercial viability was contagious, and the meeting extended from 10 minutes to 3 hours; by its conclusion, the world's first biotechnology company, Genentech, was born. Using Boyer and Cohen's techniques, a team at Genentech was able to introduce the human insulin gene into bacteria, enabling the isolation of biosynthetic insulin that was indistinguishable from human insulin. Though Swanson and Boyer faced skepticism from both the academic and business communities, by 1982, in partnership with pharmaceutical giant Eli Lilly, they had succeeded in obtaining the FDA's approval for the drug, which has been marketed successfully ever since under the trade name Humulin. In 2008, Lilly reported collective sales of US$2.8 billion for its family of recombinant insulin products.`` CPJ 2010: 143(4)

1.3.5 Botox

BOTOX Cosmetic, which is a preparation of *Clostridium botunilum* neurotoxin, is a prescription medicine that is injected into muscles and used to improve the look of moderate to severe frown lines between the eyebrows in adults for a short period of time (temporary). It is also injected into the area around the side of the eyes to improve the look of moderate to severe crow`s feet lines in adult (BOTOX Cosmetics).

The active ingredient of botox is the botulinum neurotoxin that destabilizes the SNARE complex that is essential for the attachment of acetylcholine vesicles onto the presynaptic membrane. In presence of the botulinum toxin, acetylcholine cannot be released from the presynaptic neuron into the synaptic cleft, and aceylcholine-dependent neurotransmission is prevented or lowered. The botulinum toxin has indeed been used for treating different pathologies related to an excessive activity of the sympathetic nervous, including overactive bladder (incontinence), muscle spasticity, and excessive sweating.

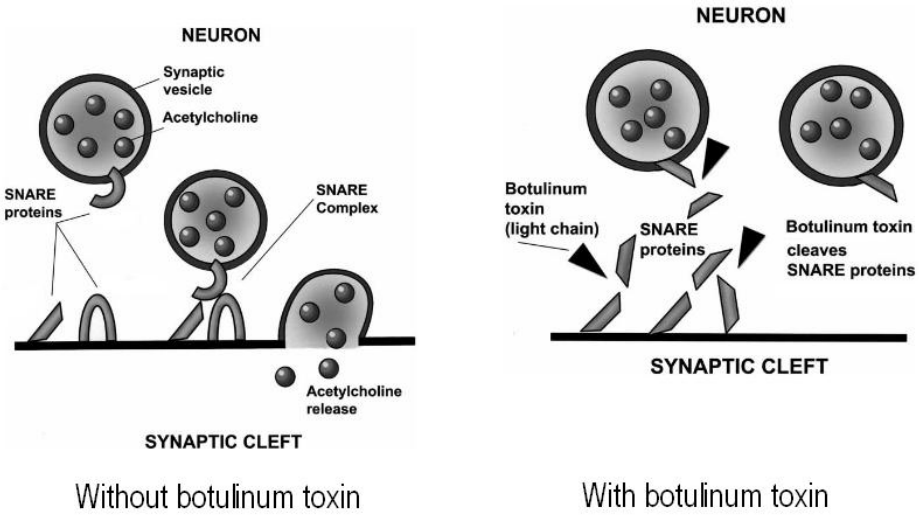


Figure from J Spinal Cord Medication, 2013.

The botulinum toxin is considered as a biologic drug as a protein produced by *Clostridium botulinum*. It is interesting that it is ``the most acutely toxic substance known, with an estimated human median lethal dose of 1.3–2.1 ng/kg when intravenously or intramuscularly injected`` (Wikipedia). Health Canada has released some information on foodborne botulism, a disease caused by consuming food contaminated with *C. botulinum,* such as improperly sealed canned jars.

Get ready for the final exam

What specific pathologic symptoms would you expect in a person suffering from botulism ? Why ?