Staph Protein A, Immune Complexes, Cryoglobulins, and the Treatment of Rheumatoid Arthritis:

Immunomodulation, not Immunosuppression

Written by Craig Wiesenhutter, M.D.

January 2016

This paper has been published (posted) on Dr. Wiesenhutter's website. CDAarthritis.com. All copyright (rights) are reserved and are not to be used without Dr. Craig Wiesenhutter's permission.

The Humoral Immune Response

The acquired immune system consists of two arms; cellular immunity, and the humoral immune response. Humoral immunity is mediated by antibodies, also called immune globulins, which are produced by cells of the B lymphocyte lineage. Antibodies combine to antigens forming immune complexes (IC). (Figure 1).

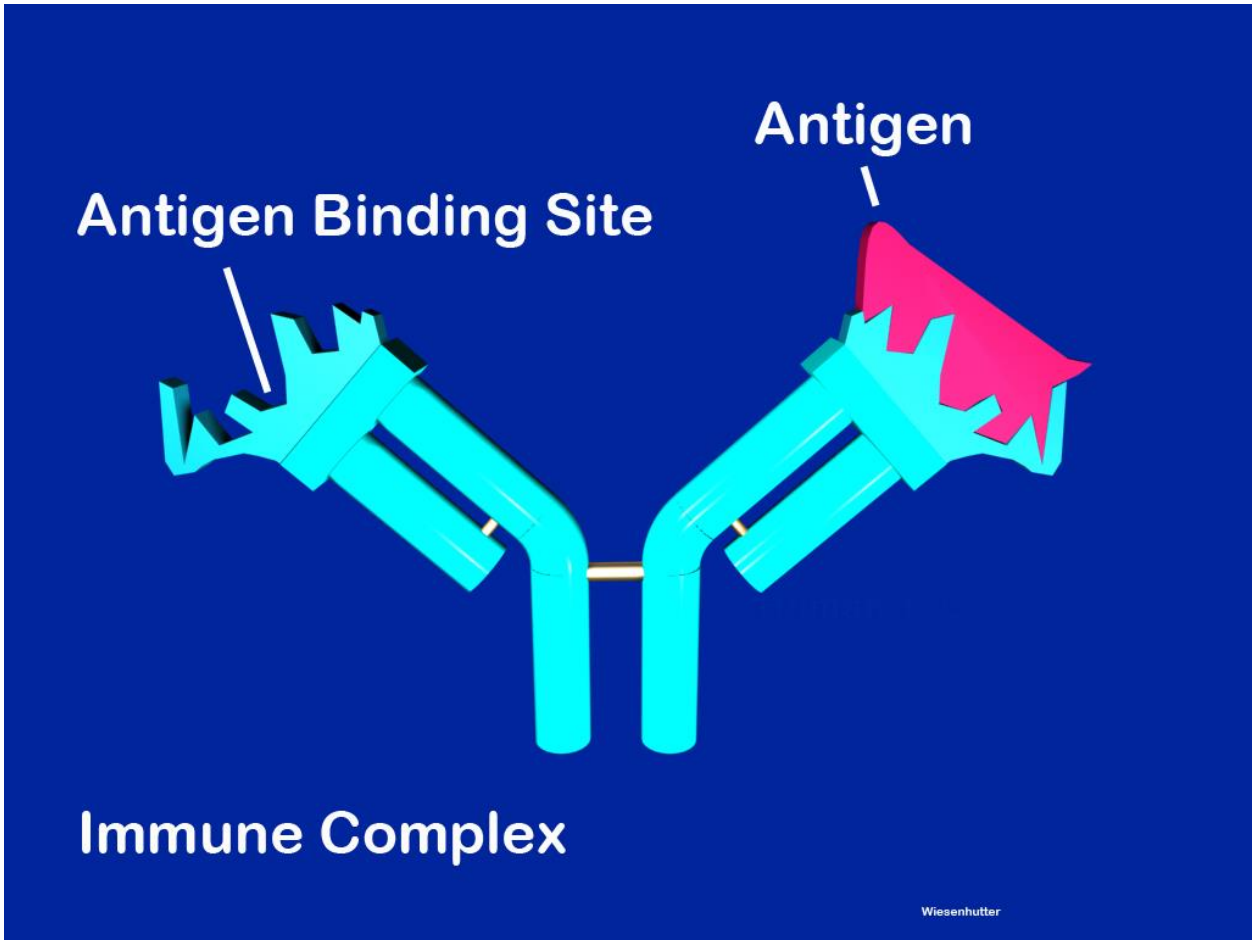


Figure 1. An antibody binds to its antigen forming an immune complex.

Generally, the formation of an immune complex benefits the host. If the antigen is a toxin, then binding to antibody can neutralize it. In many cases, the formation of an immune complex also facilitates the disposal of the antigen.

The Fc portion of the antibody mediates biological functions, such as phagocytosis, and intra cellular signaling and several other effector functions. (Figure 2).

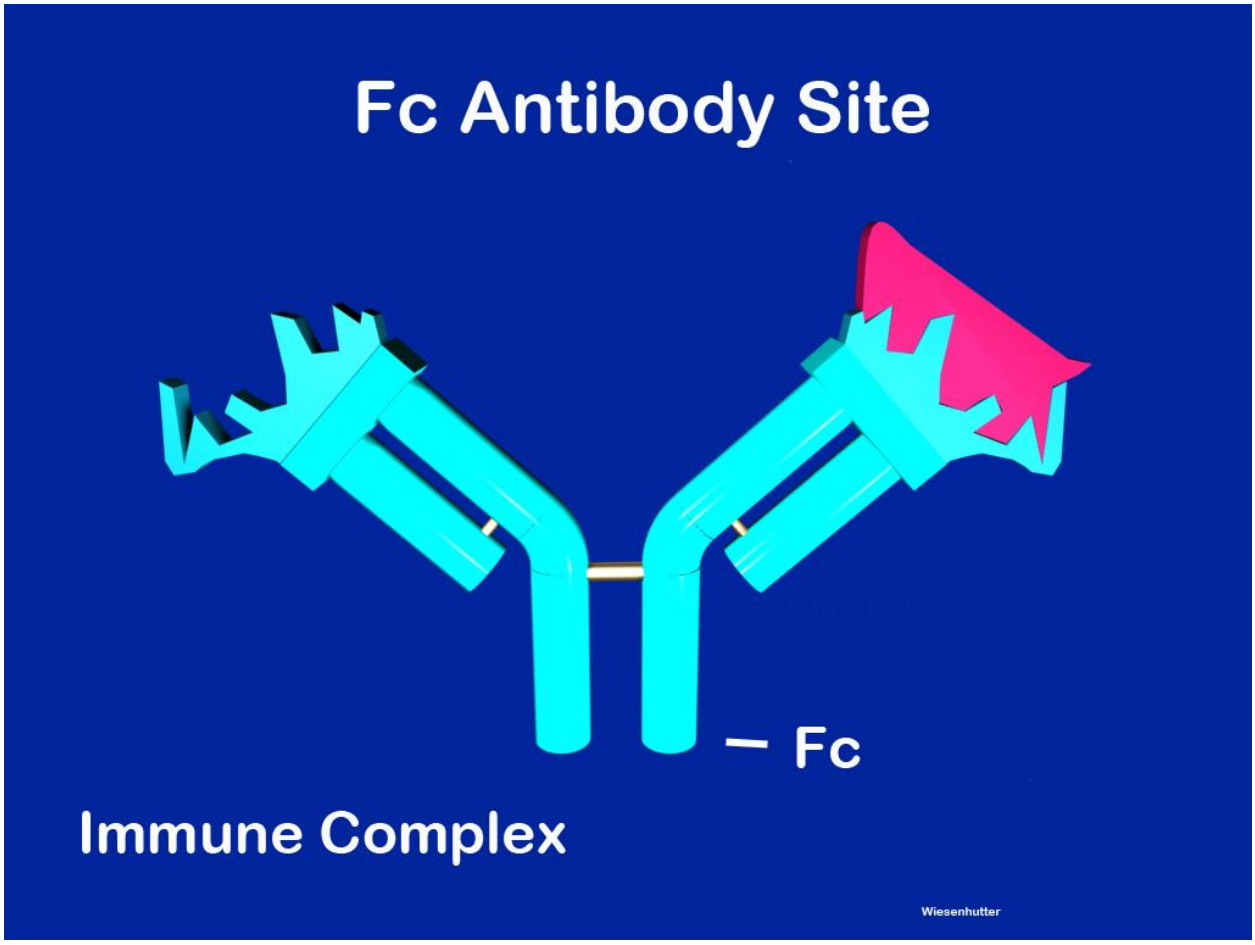


Figure 2. The Fc portion of the antibody interacts with Fc receptors which mediate numerous effector functions. The Fc site of an antibody is shown.

I. Complement Activation and the Disposal of Immune Complexes

The activation of the complement system, by IC formation, is also pivotal to the events leading up to IC disposal.

In Figure 3, the formation of an immune complex is depicted. If molecular arms from the first complement component, C1Q, binds to two Fc receptors, the activation of complement ensues. (Figure 4).

Figure 5a and Figure 5b shows the binding of complement C4 to C1Q, initiating the complement cascade.

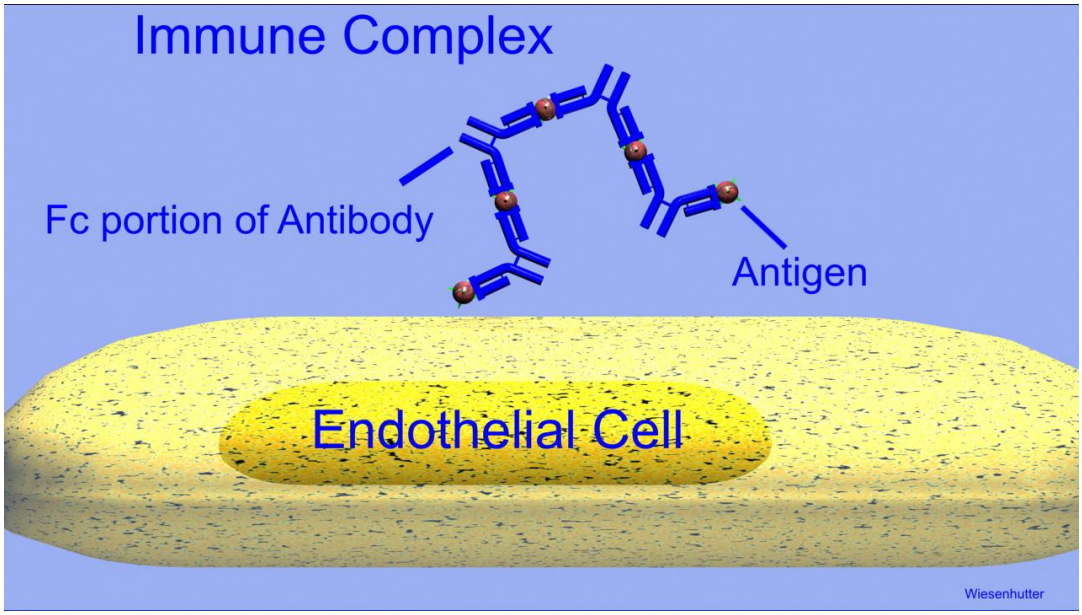


Figure 3. An immune complex is formed in the blood vessel.

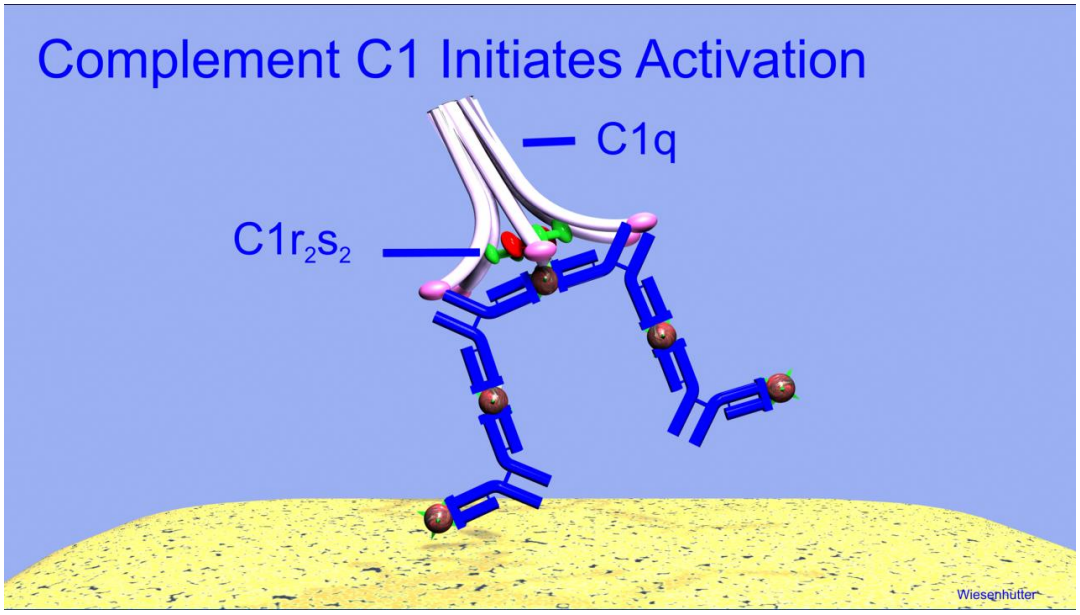


Figure 4. The first component of complement is shown combining to two antibody Fc receptors.

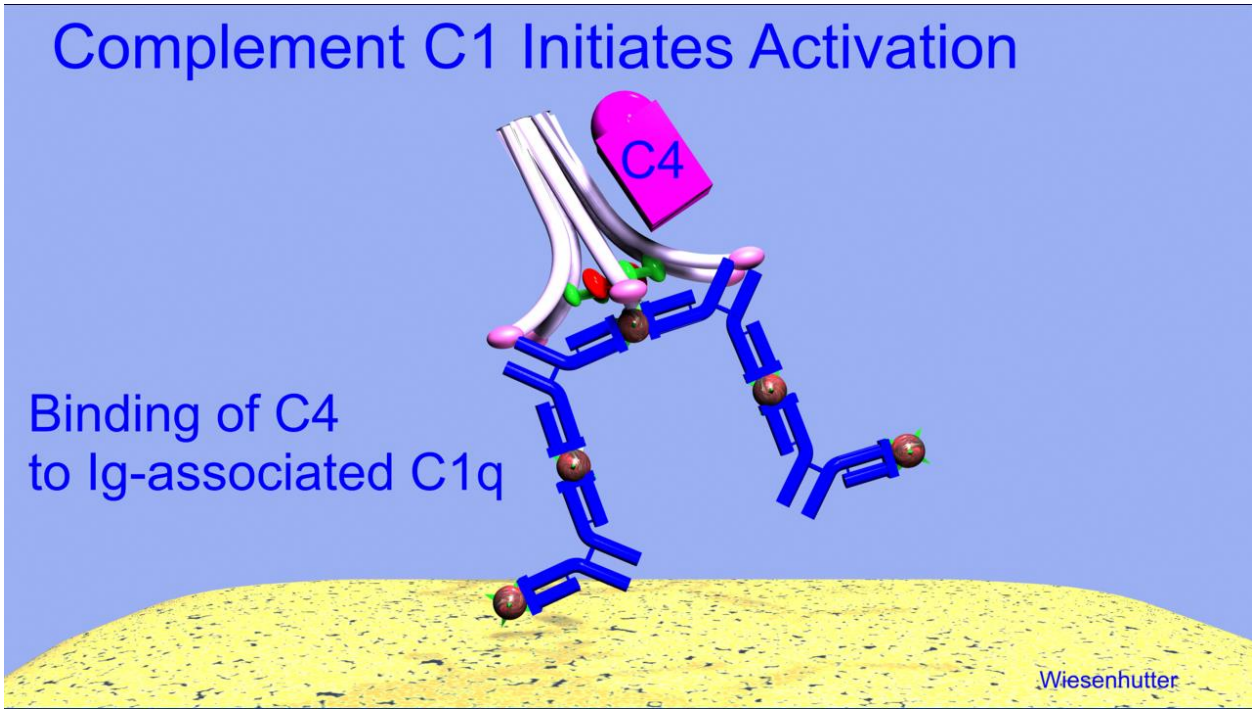


Figure 5a. In the next step in complement activation, C4 binds to the complex, as shown above.

The cascade of events, which leads through several incremental chemical steps to cleavage of C3 component of complement, results in the binding of the C3b fragment to the immune complex, as shown in Figure 6.

II. C3b Receptors and the Red Blood Cell Shuttle System for Immune Complex Disposal

Most immune complexes are cleared by the shuttle system in which the complement receptor type I (CRI) on red blood cells (RBCs) binds to the immune complex via the C3b fragment. The immune complex is thus carried to the liver and/or spleen where it is stripped off the RBC and destroyed. The RBC returns to the circulation.

The C3b which is attached to the immune complex, is enzymatically altered to iC3b and C3dg. Mononuclear Phagocytes have receptors for these molecules, CR3 and CR4, which facilitates the attachment to and subsequent phagocytosis of the IC. (Figure 9). Fc receptors on the mononuclear phagocytes also play a role in binding to and destroying the ICs.

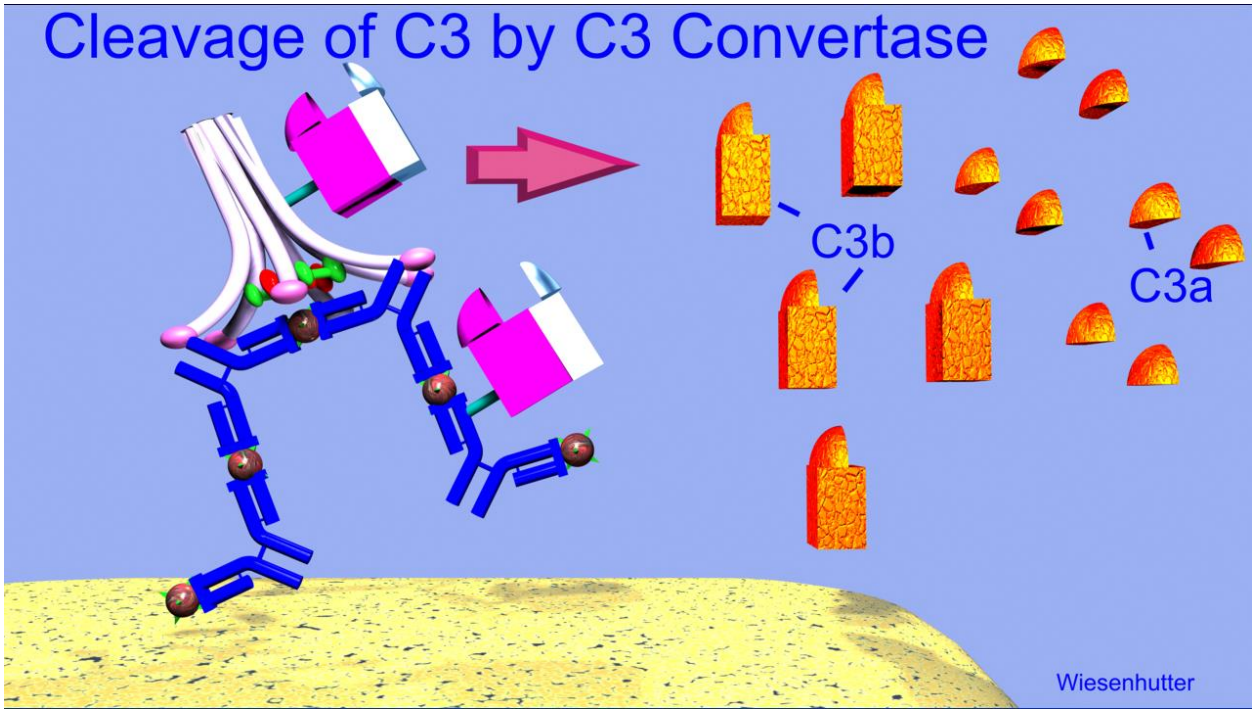


Figure 5b. Important aspects of the early activation of the complement system is shown.

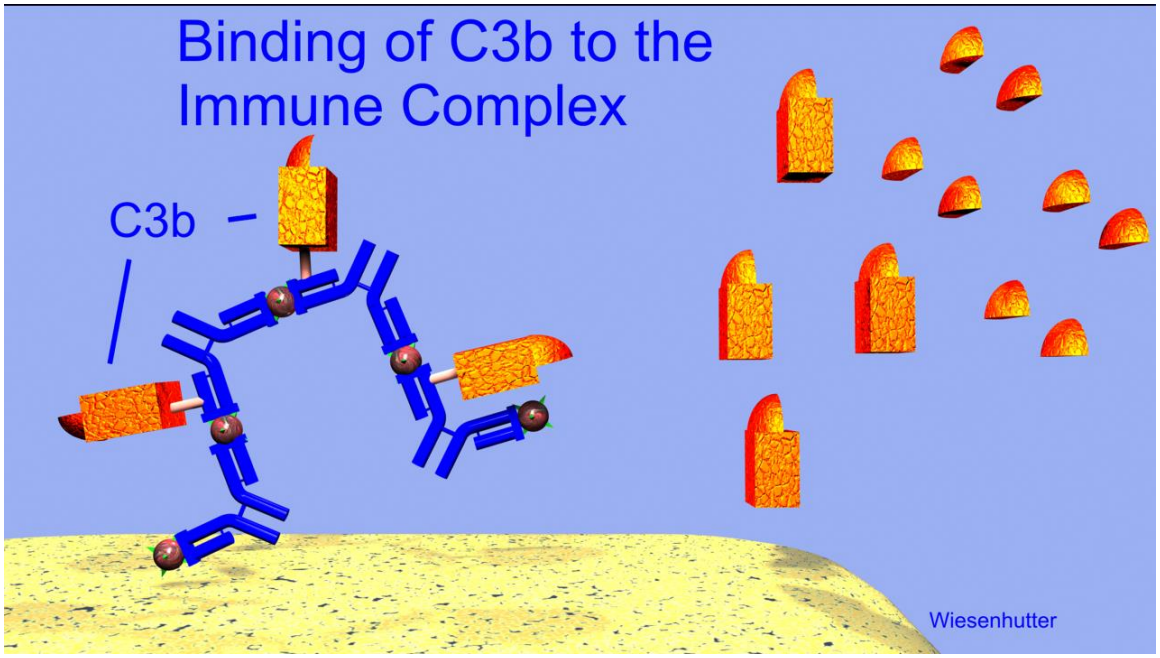


Figure 6. The binding of C3b to the immune complex is depicted.

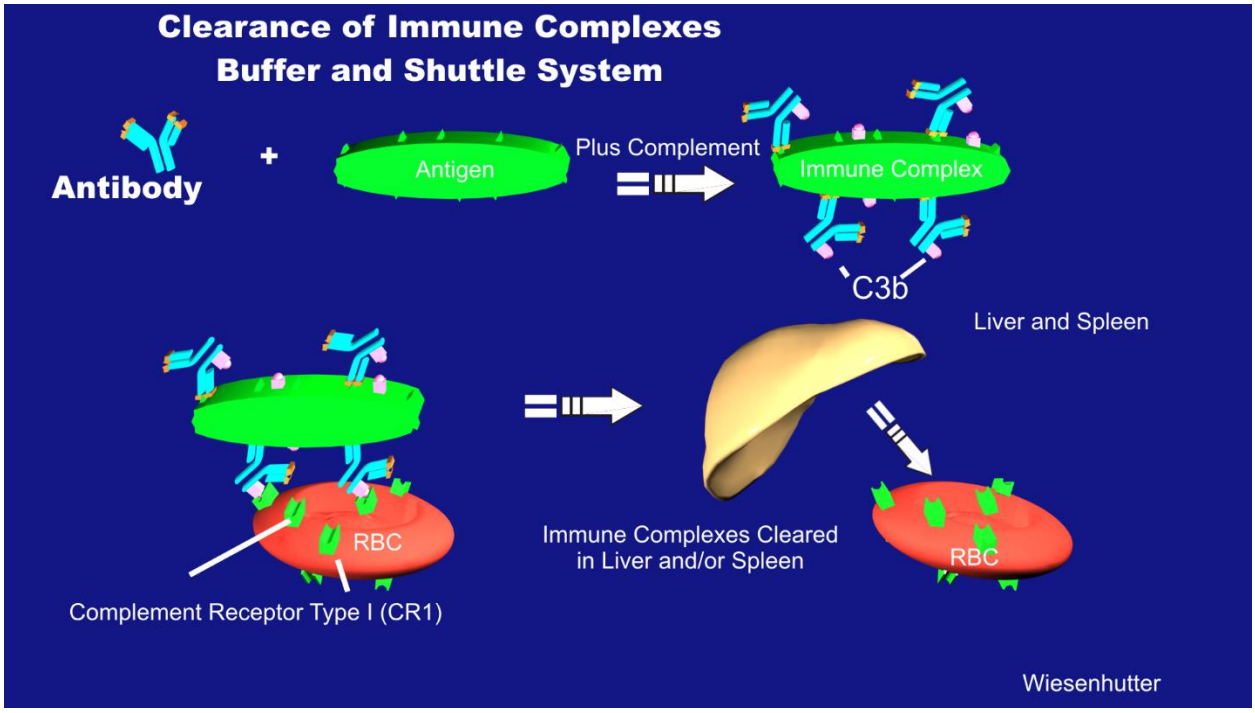


Figure 7. Immune complexes which have C3b fragments attached are taken up by RBCs via binding to the CR1 receptor and then shuttled to the liver or spleen.

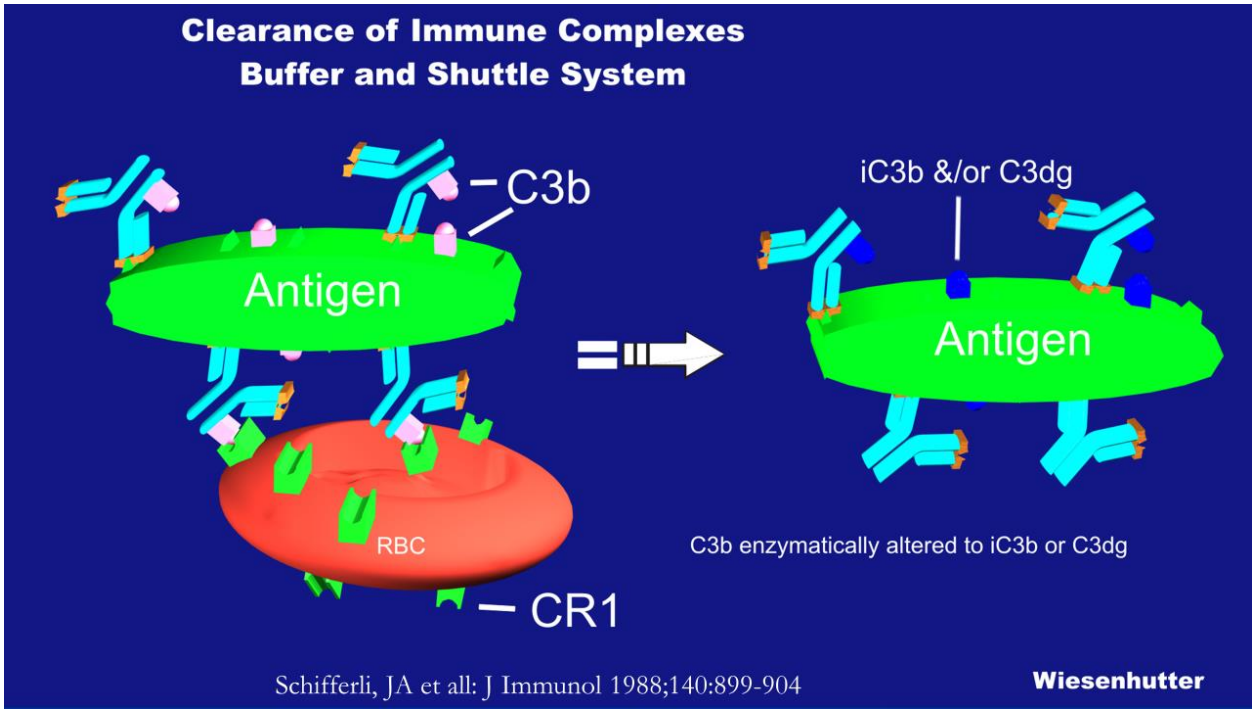


Figure 8. C3b is enzymatically altered to iC3b and C3dg.

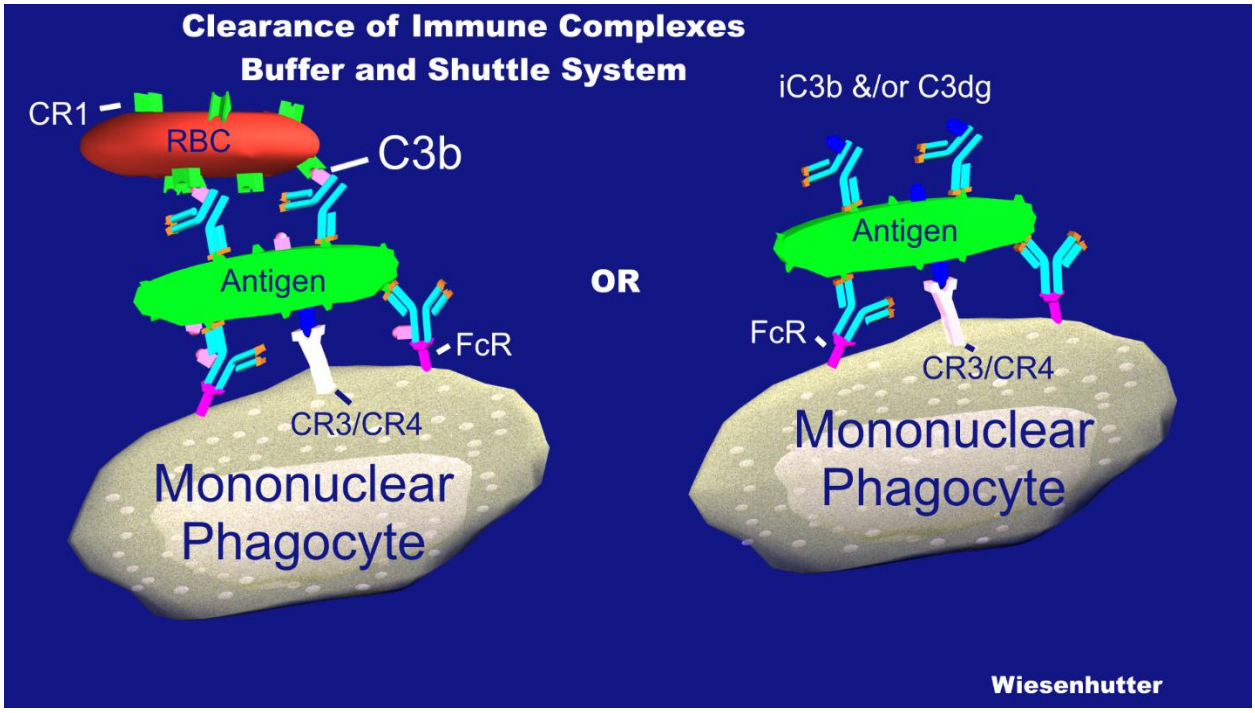


Figure 9. Mononuclear Phagocytes have receptors, CR3 and CR4, for the C3b breakdown products, iC3b and C3dg. Fc receptors on the mononuclear phagocytes also play a role in binding to and destroying the ICs.

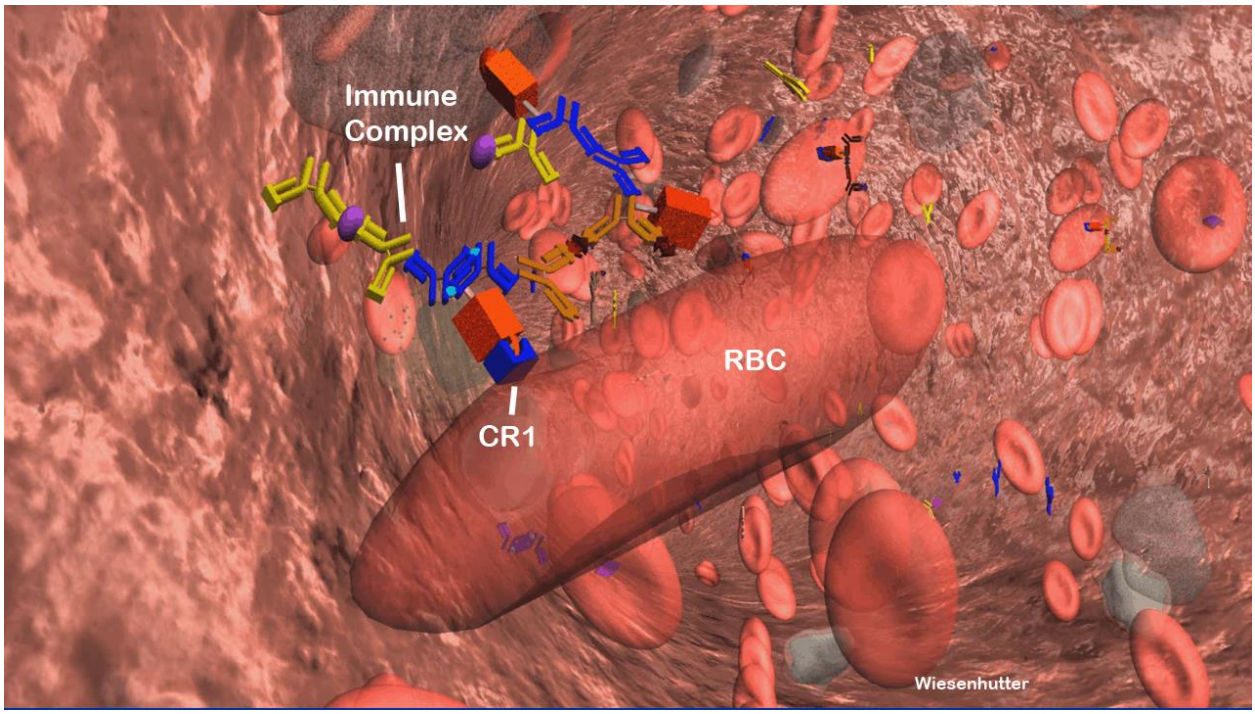


Figure 10. A RBC with IC attached via a CR1 receptor within a blood vessel. Not to scale.

III. Staph Protein A: Introduction

Staphylococcal Protein A (SpA) is a virulence factor produced by most strains of Staphylococcal aureus. SpA is initially formed as a membrane bound protein, but with an increasing rate of proliferation of the bacterium, it is also secreted into the surrounding media/environment (Figure 11). SpA has five homologous domains, with each domain capable of simultaneously binding Human IgG via the Fc portion of the molecule, and about 50% of all human immunoglobulin (Ig), which is VHIII positive, via a Fab framework location (Figure 12). These binding sites bind with high affinity.

Highly purified SpA has been used in the treatment of human RA and immune mediated thrombocytopenic purpura (ITP). A ribbon model of SpA is depicted in Figure 13.

IV. Staph Protein A and Immune Complexes

The different isotypes of antibodies are shown in Figure 14. A simple immune aggregate, a type of IC, is shown in Figure 15. An important part of our calculations concerning the impact of SpA on forming immune complexes, is influenced by the role of rheumatoid factors. Antibodies that bind to the Fc portion of immunoglobulins are called rheumatoid factors (RF), which are shown in Figure 16.

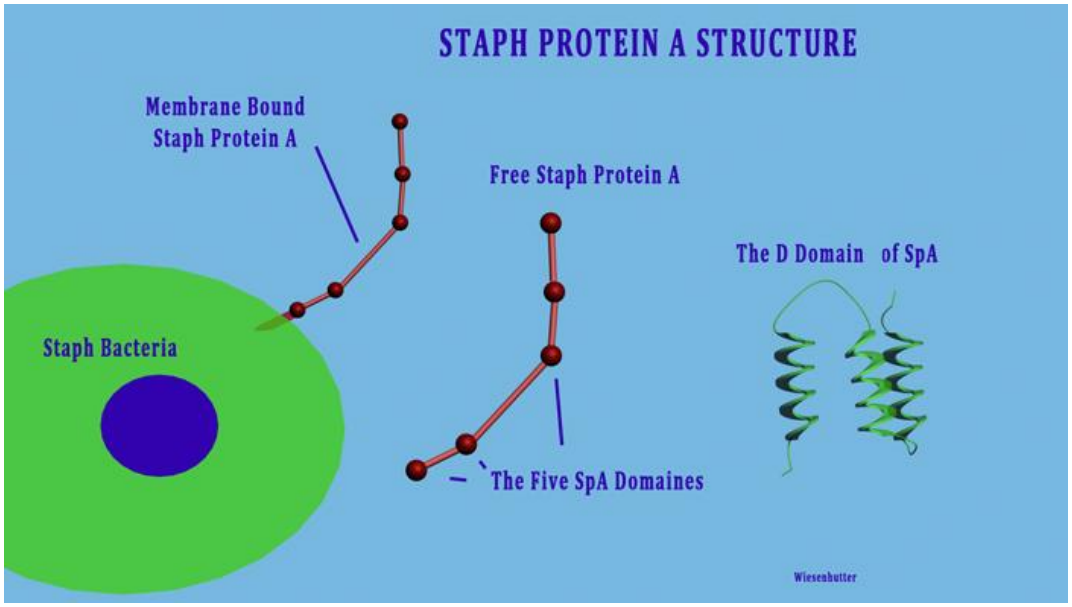


Figure 11. SpA is shown as a Staph bacteria membrane bound molecule and as a molecule secreted into the milieu. One of the five homologous domains, Domain D, is shown as a ribbon model.

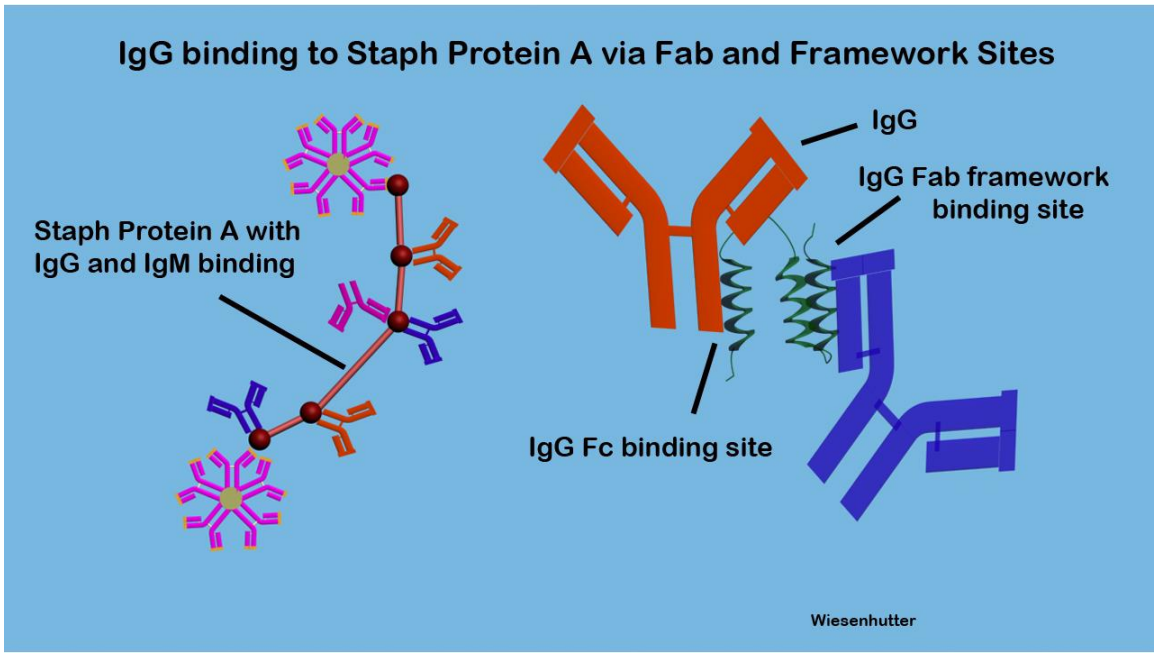


Figure 12. The five SpA domains can each bind two immunoglobulin molecules, either via the Ig Fc fragment, or via a unique antibody binding site framework location, as shown.

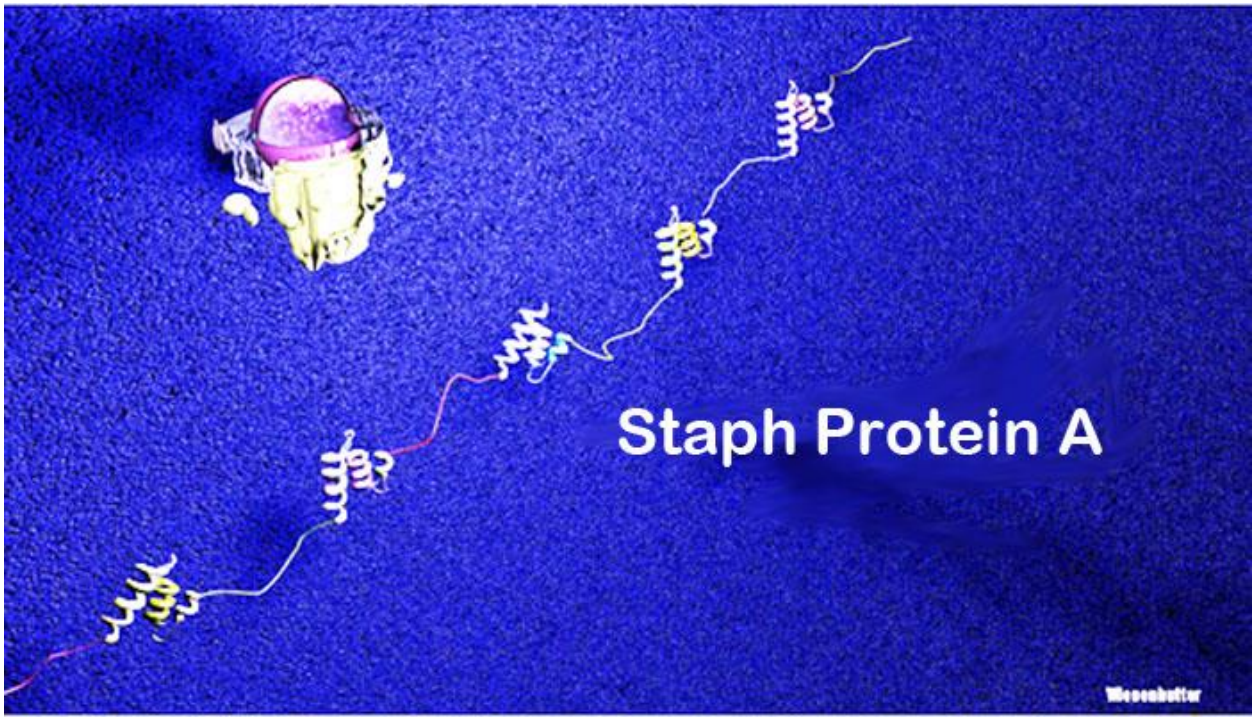


Figure 13. A ribbon model of the secreted SpA molecule, with its five domains, interconnected by alpha helix, is depicted.

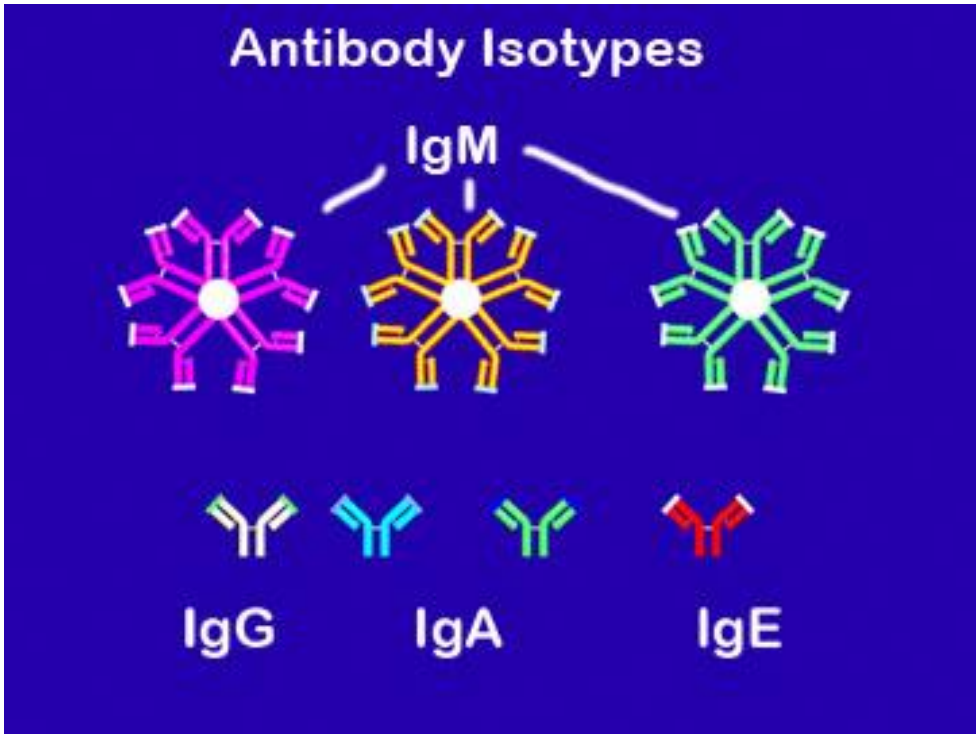


Figure 14. The main antibody isotypes are shown

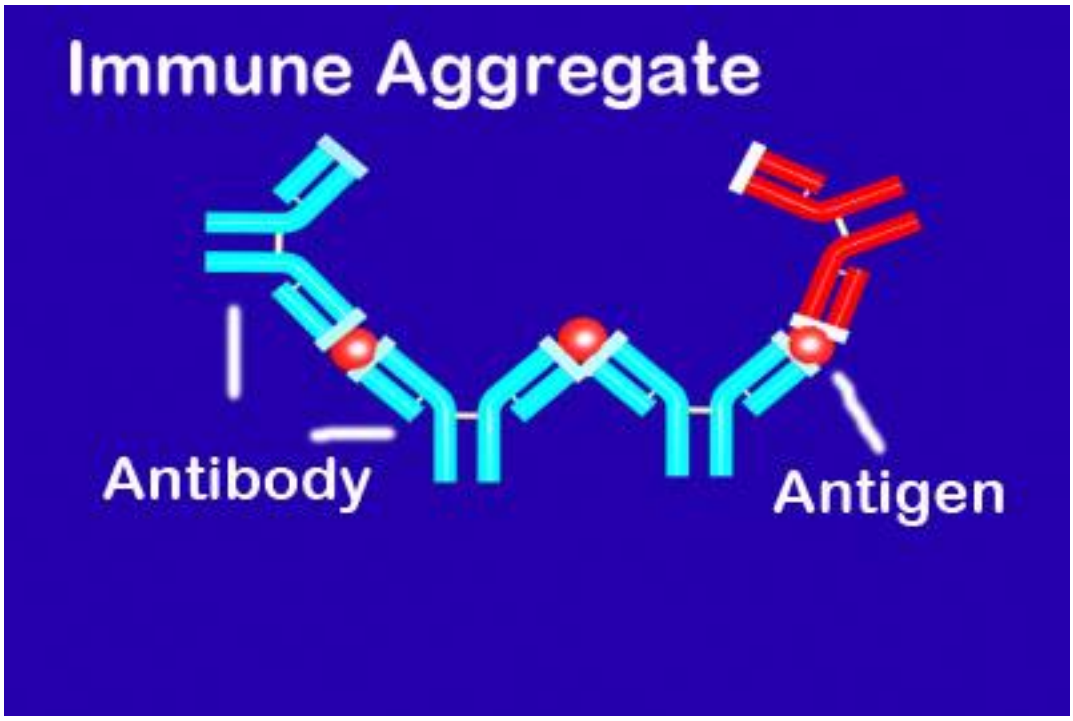


Figure 15. A simple immune aggregate, a type of IC, is shown with two IgG clones, and one antigen.

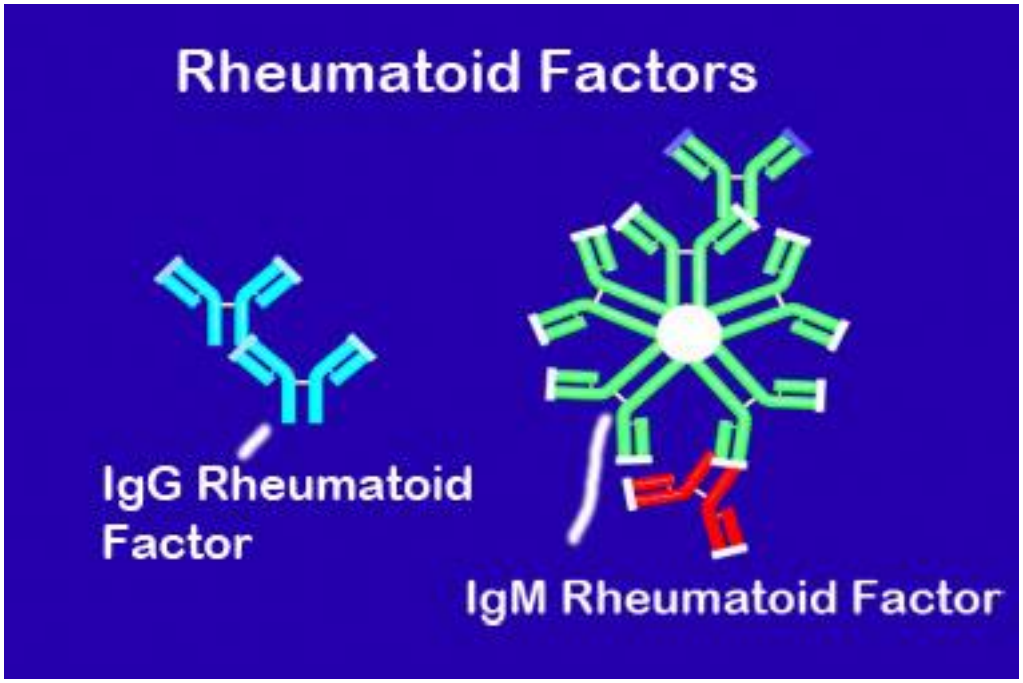


Figure 16. Rheumatoid factors are antibodies that bind to the Fc portion of other immunoglobulins

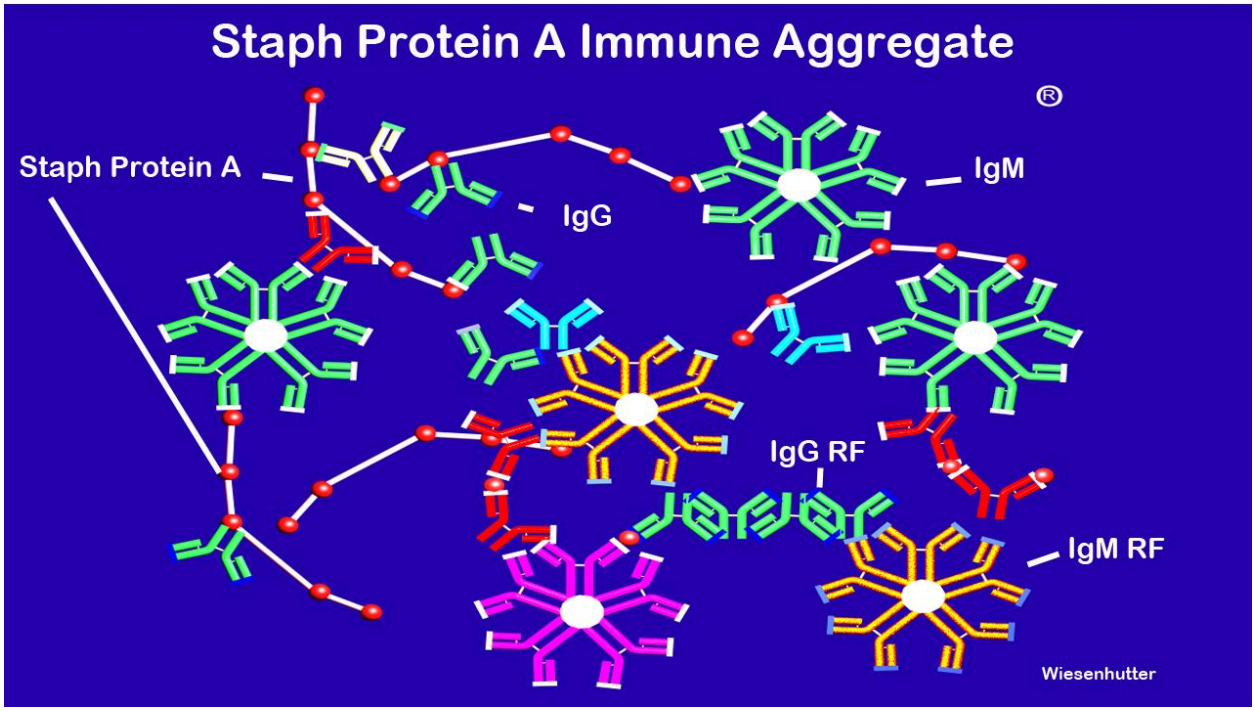


Figure 17. It would be anticipated, that once SpA entered the human blood stream, that the binding between molecules would be similar to the model shown above. This model has left out "B cells" which I will add shortly.

Given what we know about SpA, and RF and IC in general, it is more likely than not that such an immune aggregate, as depicted in Figure 17, can manifest.

V. Measurement of Immune Complexes

In clinical practice and in clinical studies, the measurement of IC is problematic. In most cases the relevant pathogenic antigens within the ICs varies with each given disease entity, and may differ when comparing two patients with the same disease. Even with an individual patient, the most relevant pathogenic antigens may change over time. Therefore, there is no standard measurement for IC at this time, and ICs are rarely used for diagnostic purposes, or for even following responses to a patient with a known IC associated disease. The most commonly employed IC assays utilized in clinical medicine include the RAJI assay, C1Q assay, and Peg Assay, all of which are expensive, tedious to perform, requires special reagents and trained experienced technicians, and the results are difficult to interpret.

VI. Cryoglobulins as a Surrogate Immune Complex Assay

Cryoglobulins (Cryos) are immunoglobulins (IGs) that precipitate or gel out of serum at low temperatures and re-solubilize on warming. Cryos are usually ICs. A cryo which measures at 1% by the cryocrit method is shown is figure 18.

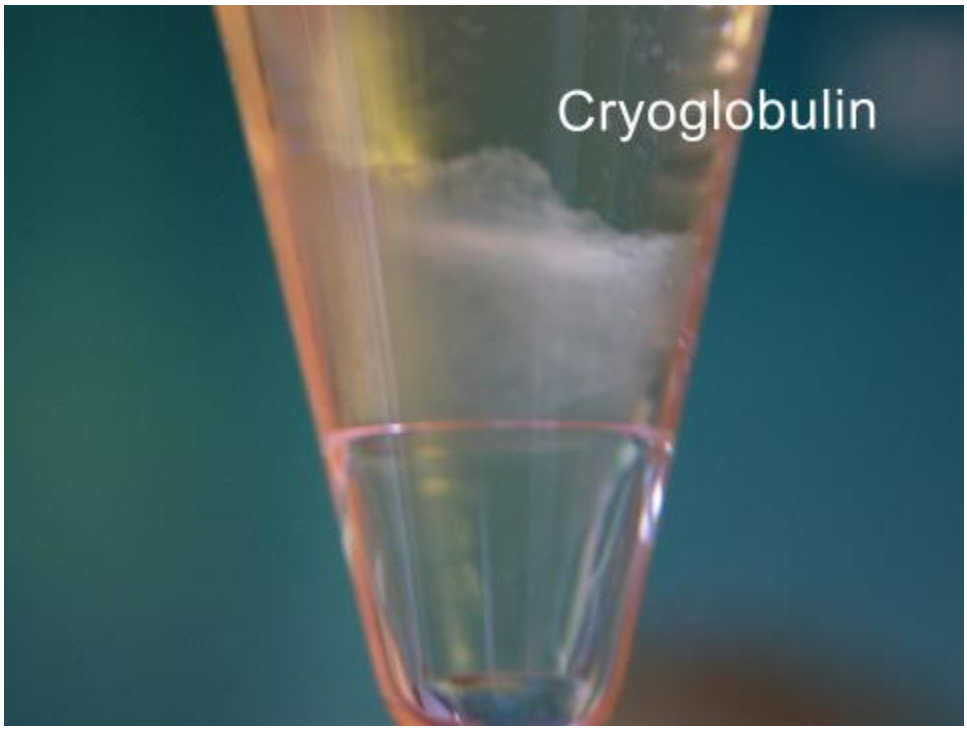


Figure 18. The precitate in this tube is a cryoblobulin which measures at 1% by the cryocrit method, and “melts” when warmed.

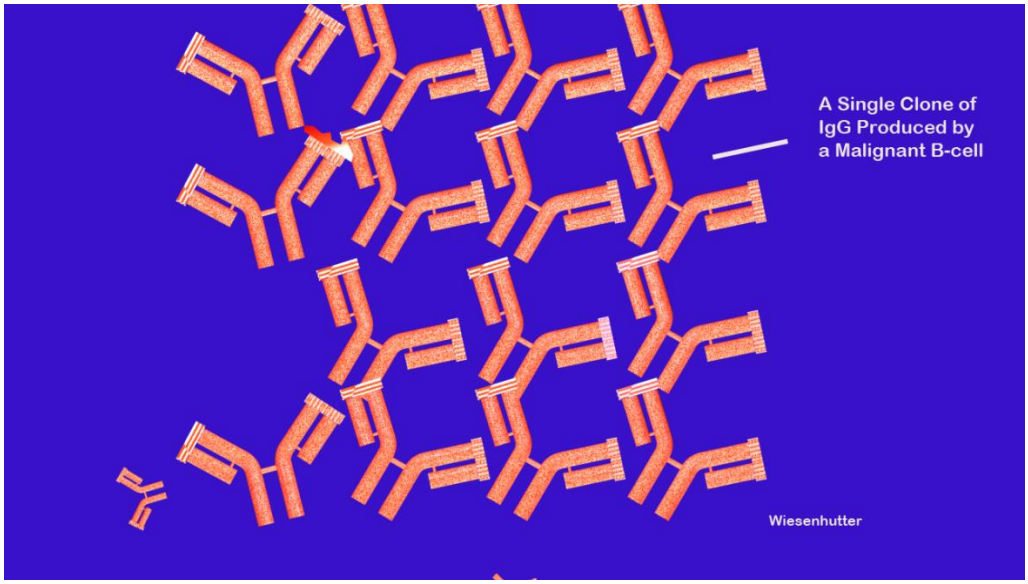


Figure 19. A type I cryo is shown. Type I cryos are usually a result of a monoclonal antibody produced by a lymphoma

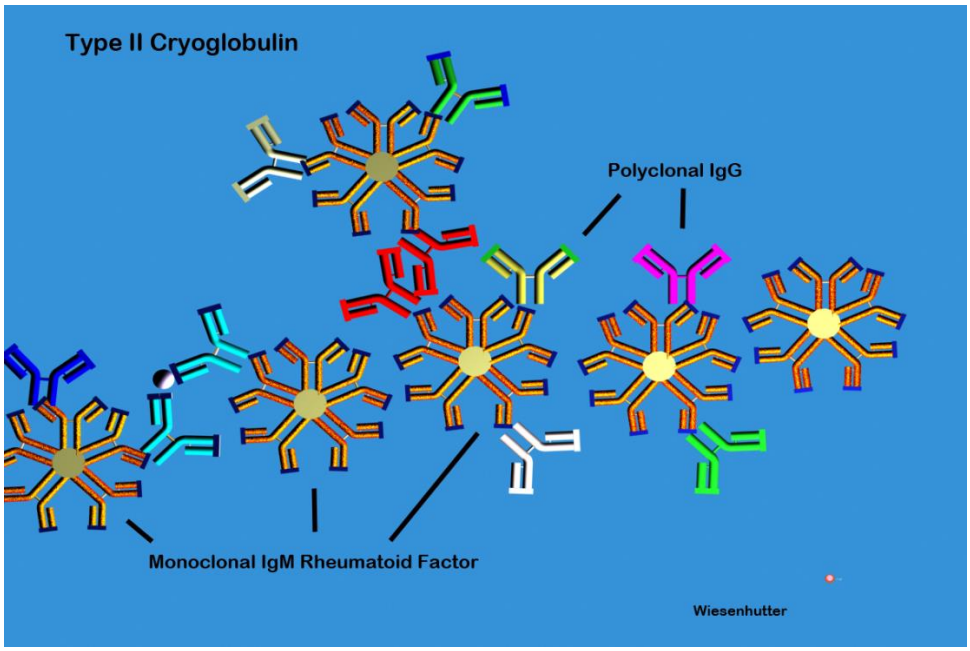


Figure 20. A type II cryo is shown. Type II cryos are the most common type of cryos and are generally a result of an infection with hepatitis C virus.

There are three types of cryos. Type I cryos (10%-15% of cases) consist of monoclonal IGs, generally secondary to lymphomas, such as multiple myeloma or macroglobulinemia of Waldenstrom, and which do not represent ICs. An example of

the structure of a Type I cryo is shown in figure 19. Such a cryo would form in multiple myeloma.

A Type II cryo (50-60%) consists of a mixture of IG of different clones (polyclonal IgG). There is at least one monoclonal antibody, usually an IgM, directed to the Fc portion of normal IgG. An example of such a cryo is shown in figure 20.

Type III (25%-30%) cryos are commonly associated with autoimmune disease, and consist of polyclonal IgM directed to the Fc component (the IgM's are rheumatoid factors) of polyclonal IgG. An example of a Type III cryoglobulin is shown in figure 21.

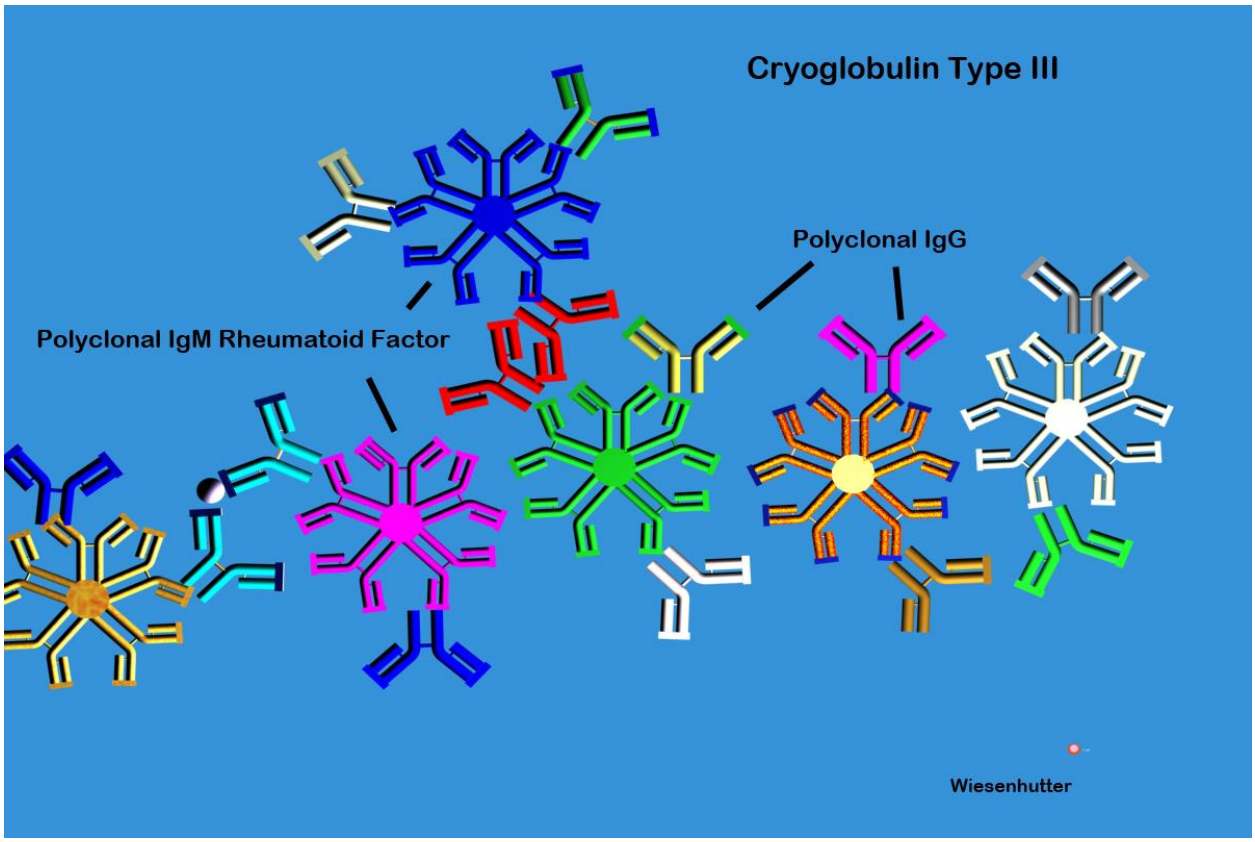


Figure 21. A type III cryo is shown. Type III cryos are the most commonly associated with autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus.

About 40% of seropositive RA patients (patients who are rheumatoid factor positive) will have positive Type III cryoglobulins either constantly, or intermittently. Type III cryoglobulinemia are often asymptomatic, and not associated with any tissue injury.

VII. Staph Protein A Infusions and the Formation of Immune complexes

Spa readily binds to serum immunoglobulin with high affinity. The five domains of SpA can each bind, simultaneously, IgG via the Fc component of the Ig and the antigen binding framework of the Ig. It would therefore be highly likely that IC rapidly form when SpA is infused into the human vasculature. The formation of IC on SpA exposure to plasma has been demonstrated in numerous non-human and human trials. Also, this formation of IC would be expected to lead to complement consumption, and complement consumption has also been shown on numerous occasions.

The most fascinating report to date concerning SpA and IC formation, was published by MacLellan, L et all ("Co-Opting Endogenous Immunoglobulin for the Regulation of Inflammation and Osteoclastogenesis in Humans and Mice", A and R, Vol 63 No 12, December 2011 pp 3897-3907) This riveting paper, published by one of the most respected immunology/rheumatology labs in the world, purport to show that IC formed by SpA **"interact with and modulate cells of the monocytic lineage…leading to Fc gamma recptor type I – dependent polarization of macrophages leading to a regulatory phenotype, rendering them unresponsive to activators such as interferon gamma. The anti-inflammatory complexes also had the capacity to directly inhibit differentiation of preosteoclasts into osteoclasts in humans. Moreover, administration of SpA in the early stages of disease substantially alleviated the clinical and histologic erosive features of CIA in mice."**

In this paper, the assumption was made that a rather "simple" IC was formed, described as $(IgG_2\ SpA)_2$). Admittedly, one such as me is hardly in a position to challenge such a high quality laboratory, and some of the most respected scientists in the world. But it really doesn't make much sense, and this assumption, as referenced in the paper, was basically stemming from information that did not include the discovery of the antigen binding (Fab) framework portion of Ig also bound to SpA. **Finally, and most importantly, I presented a poster next to Dr. Iain B. McInnes, the head of the laboratory, at the ACR meeting in Boston, in 2014, and he also said that the assumption about the structure of the IC formed with SpA treatment commented on in this paper was possibly or even probably "bogus".**

Just considering the interactions of SpA and Ig in figure 17, one would think that such a homogenous formation of a specific, small IC, as predicted in this paper, would be highly unlikely.

VIII. The Formation of Immune Complexes and Possible Modes of action of Staph Protein A

The Prosorba Column, which had SpA covalently bound to glass beads in a plastic container, was shown to be efficacious in the treatment of RA and ITT and was approved by the FDA for these indications. Rather than having anything to do with the beads held within the container, this device probably worked because SpA leached off the column, to the tune of at least 200 ug per treatment, or possibly a great deal more SpA than that leached off the column. The Prosorba Column was approved for the treatment of ITP in late 1980 and for RA in late 1990. There has been a great deal of time to postulate on the mode of action of the Prosorba Column in RA and ITP. First and foremost, the most accepted possibility has been the theory that it worked like Intravenous immunoglobulin (IVIG) probably by interacting via inhibitory Fc receptors on macrophages. The paper quoted above from the Dr. McInnes lab would also strongly support such a mechanism for modern SpA. Such a mode of action (MOA), or mechanism of action is very attractive, because it suggests a lack of "global" immunosuppression. The nine currently FDA approved "biologics" in the USA are all clearly immunosuppressive.

If the administration of SpA is not immunosuppressive, then it could be used in combination with current biologics, and it could be used with patients with recurrent infections, etc. etc. and it would make it uniquely attractive as a new "disease modifying agent".

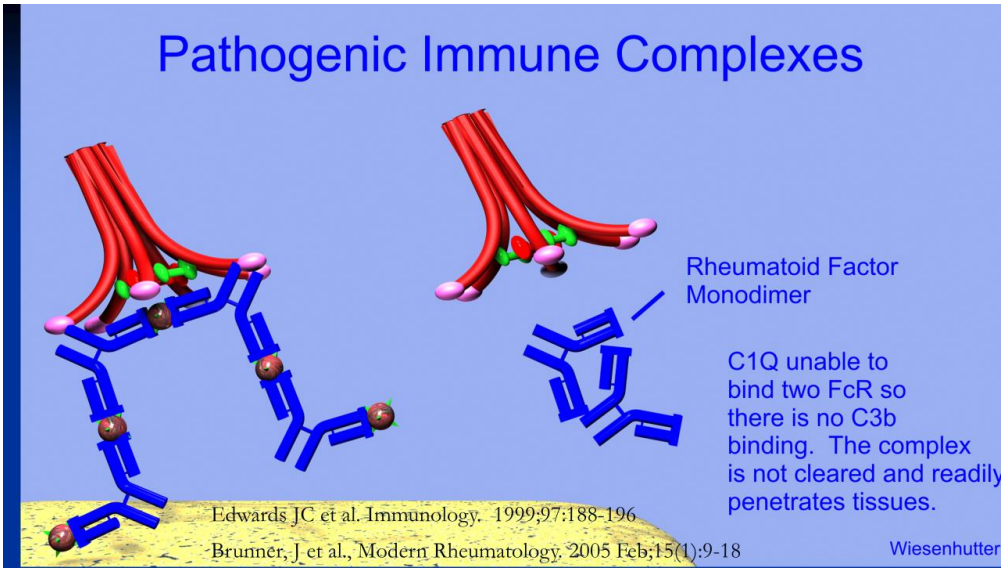


Figure 21. A pathogenic immune complex, an IgG rheumatoid factor dimer, is shown. Such a dimer will not interact with C1Q.

There are several other possible MOA's for SpA which would require the formation of IC with treatment. Another attractive possibility stems from older theories concerning the production by a "rogue" B cell of a "pathologic small immune complex" (psIC), an IgG dimer rheumatoid factor as shown in figure 21. An IC structured like this would go undetected and be allowed to enter joints, and extra articular locations such as the lung, and lead to stimulation of B cells, and the abnormal production of more immunoglobulin, including rheumatoid factors. These small, unassuming entities, these IC, would go undetected by the usual immune system disposal mechanisms and they would survive indefinitely.

Clearly, the infusion of SpA into the vasculature, and the formation of IC as shown above, will also lead to incorporation of such pathogenic IC, which has gone previously undetected, into the newly formed immunoaggregate containing SpA, resulting in destruction of the pathogenic dimer and leading to the cessation of this profound vicious circle.

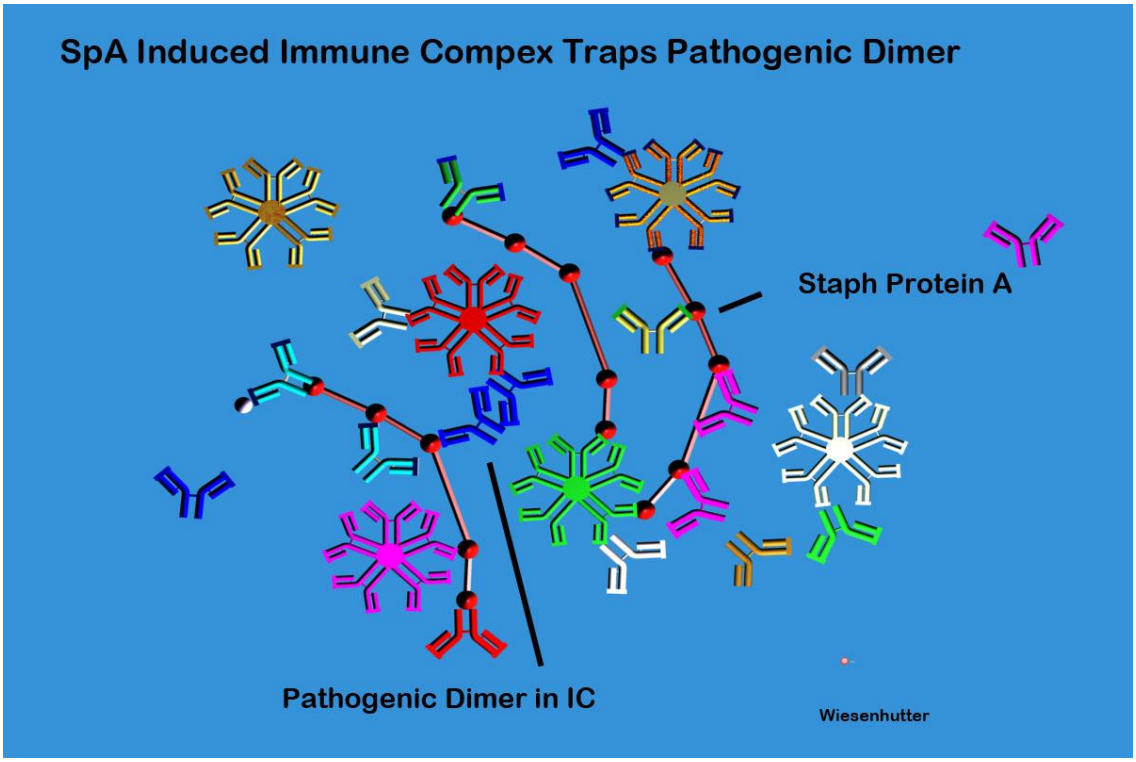


Figure 22. A pathogenic immune complex dimer is demonstrated trapped within the IC Induced by treatment with SpA

If such a MOA can be proven, then one could postulate the possibility, if patients are vigorously and aggressively given the highest exposure to drug tolerated, then a cure for RA and even other autoimmune disorders,

such as immune mediated thrombocytopenic purpura, systemic lupus erythematosus, multiple sclerosis, and inflammatory bowel disease (Crohn's disease and Ulcerative Colitis) could be possible. There are many experts who feel RA is cancer like, and should be treated as cancer, first with induction, then with a cure. SpA would fit the bill perfectly. Start with Canine B cell lymphoma, and go from there.

Lastly, and the facts could even be more complexed than this, B cells that are VHIII positive could be incorporated into these newly formed complexes. Such an occurrence could lead to at least two major new MOA possibilities. One of which is B cell depletion, which has been repeatedly shown to occur. More importantly for SpA, this depletion of B cells seems to mainly occur in Marginal Zone B cells and B1 B cells, which is ideal, since these population of B cells have been most implicated in autoimmunity.

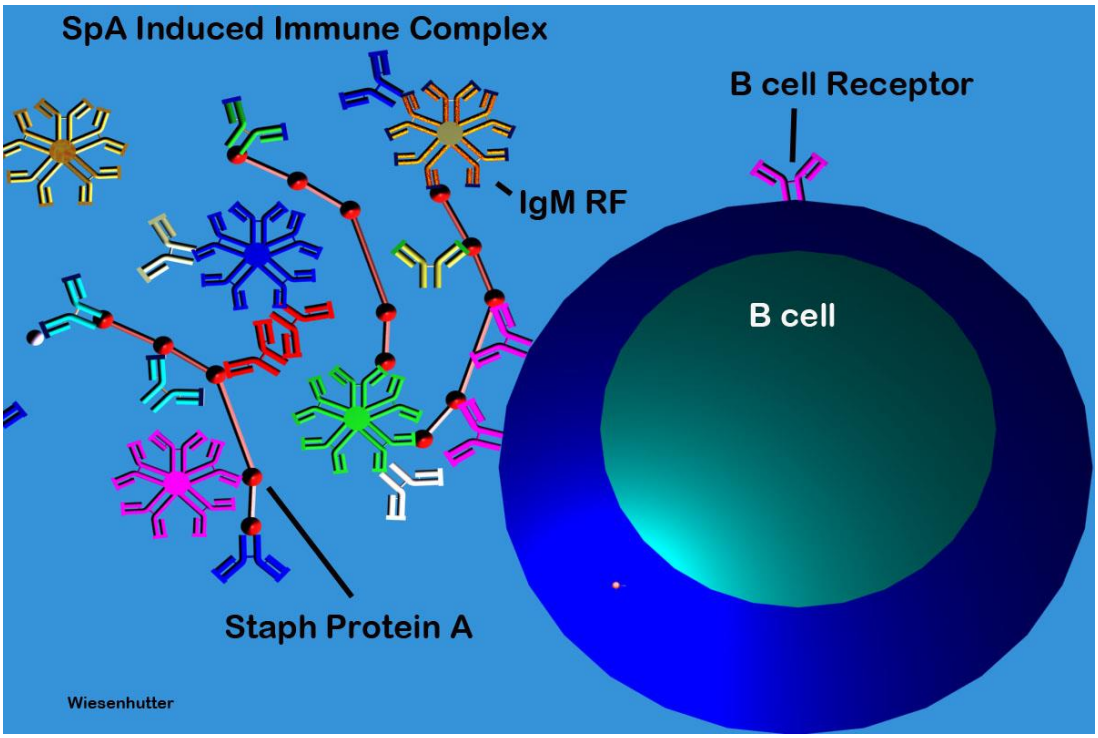


Figure 23. The immune complex induced by SpA treatment binds to a B cell.

Finally, the SpA binding to B cells with SpA infusions could lead to alteration of migration of B cells which would be therapeutic, possibly preventing the B cells from entering the joint, and thereby preventing the production of rheumatoid factors, and the formation of immune

aggregates that will never leave the joint. It has been shown that the formation of these aggregates stimulate the "pathogenic" B cells to produce more Ig. This never ending pathologic circle could be distrupted.

The anti TNF and anti IL6 and anti CD 28 drugs; the incredibly successful biologics that make billions and billions per year, only sop up the interleukins produced by what's happening with this process. All of the currently approved biologics work immunologically very downstream.

SpA most likely works far upstream, at the beginning of the pathoimmunoloic process, preventing the start of this never ending vicious circle.

I would like to mention one last possibility. Complement is consumed when SpA enters the vasculature. Complement fragments are formed, such as C3a, C5a, and a C4 fragment as well. I suggest that these fragments could enter the joint space, where they bind to resident mast cells, and to the immune aggregates there, resulting in the solubilization of these aggregates, allowing for their removal from the joint space via egress by the lymphatic system. Once entering into the vasculature, by way of the thoracic duct, they are identified and destroyed.

Craig Wiesenhutter, M.D.
January 2016