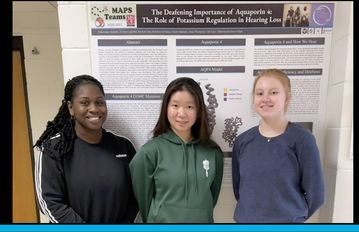
On the Flame: The Fab Fragment of Rheumatoid Arthritis



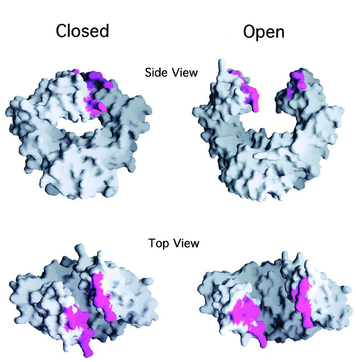
Kaelyn Chen, Katelyn Lawrence, Toluwalase Akintola | Jim Lane | Mahtomedi Senior High

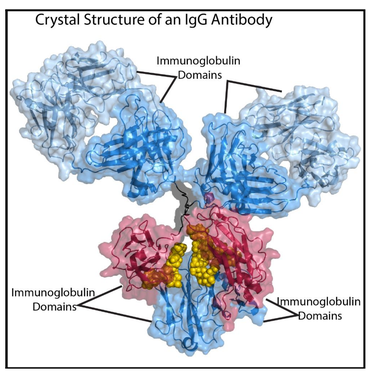
Abstract

With more than 200,000 cases per year, Rheumatoid Arthritis (RA) is one of the most common autoimmune disorders in the United States. Across the globe, up to 14 million people are affected by its symptoms of joint pain, stiffness, swelling, fatigue, and even physical deformity. Rheumatoid factors (RF) are autoantibodies that correlate with RA severity and recognize epitopes in the Fc region of its antigen immunoglobulin (Ig) G, yet it is paradoxically found circulating in the blood alongside the antigen without binding to it. According to Thomsen, et. al, a 2018 study that contested the idea of IgG aggravation as the prerequisite to RF binding, the native state of IgG is present in a closed form, where the Fab fragment shields the Fc region, only exposing the Fc effector sites when a conformational change is induced. This closed form is a result of a missing galactose between the Cγ2 domains of the heavy chains, thus causing the IgG to self associate, forming immune complexes to fix the complement system. The subsequent heightened inflammatory response exacerbates the progression of joint damage in RA and other severe symptoms. In this study, we examined IgG in relation to the complement system and RF forming immune complexes to achieve a better understanding of the immunological progression or pathogenesis of RA. The Mahtomedi MSOE Center for BioMolecular Modeling MAPS Team used 3-D modeling and printing technology to examine structure-function relationships of Fab and Fc fragments in IgG. The visual model will be a valuable tool in developing our story.

Closed Form

RF-IgG immune complexes found in the joint fluid and synovial tissues often correlate with disease activity and severity, however, in the more recent years, a paradox arose when RFs were found circulating alongside large amounts of their antigen IgG without reacting by the research of Thomsen et al. Upon examination, the well known “horseshoe” shape of the antibody Fc fragment was different, with the heavy chains folded back on themselves. This closed form soon revealed to be an important effector of inflammation progression and joint damage in RA. All because of a missing galactose. In our model of a human immunoglobulin G, model 1H3X taken from the Protein Data Bank, we highlight the specific asparagine 297 side chains where the missing sugars would be attached. In the horseshoe form of IgG-Fc, the space in the middle is filled with oligosaccharide chains, which are attached through asparagine residues 297. In a study done by Krapp et al, a terminal N- acetylglucosamine and mannose sugar residues were removed from the IgG structure. From this experiment they discovered that there were resulting conformational changes in the oligosaccharide and the polypeptide loop that contains the N-glycosylation site. These impacts in structure result in changes in IgG- Fc fragments, leading to the approach of Cγ2 domains which results in a “closed” formation, rather than the “open” formation that is dominant in fully galactosylated IgG-Gc. Without the monosaccharide sugar to mediate the restrictive motion of the oligosaccharide relative to the protein backbone, the interactions between the oligosaccharide and the peptide surface decreases and exposes the terminal N-acetyl glucosamine (GlcNAc). The IgG’s self associate to fill the hole, making a closed binding, but this self-association overfixes the complement system causing joint damage and inflammation. Which is why RF combined with IgG can circulate in high concentrations among patients with rheumatoid diseases.

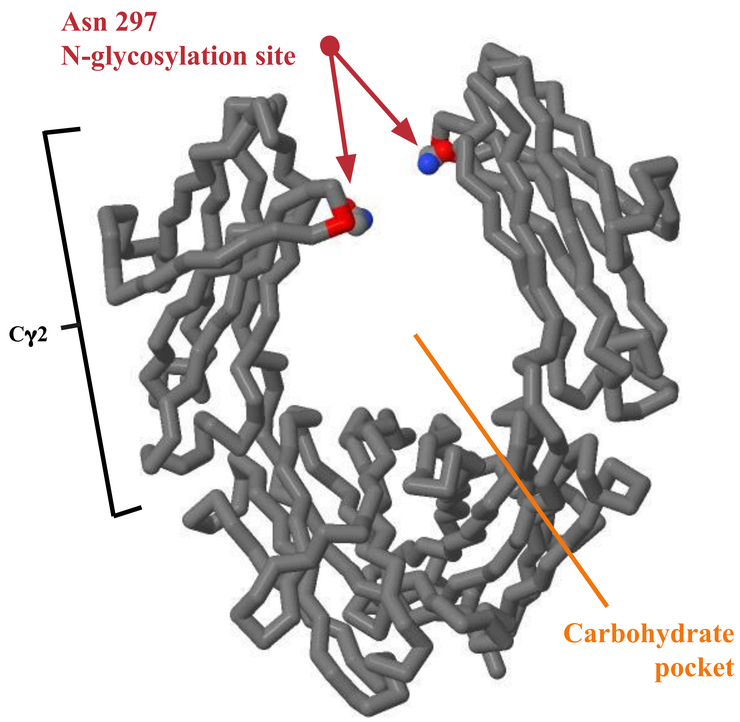




Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune disease that affects the joints – specifically the tissue lining. This occurs when the body's immune system mistakenly attacks the body instead of protecting it. Common symptoms range from pain and stiffness in the joints, tenderness and swelling of the joints, fatigue, and weakness. The joints are damaged when there is an excess amount of synovial fluid, as a result from synovial swelling. While the synovial fluid is an important cushion surrounding most joints, too much of it causes pain and stiffness as bone erosion and wearing down of the cartilage occurs. The large accumulation of fluid stretches out synovium, making the joint less stable and resulting in damage. Overtime, the inflammation associated with the disease can cause serious bone erosion and joint deformity.

IgG-Fc Model



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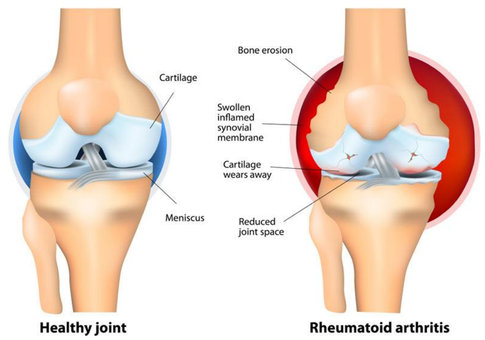
IgG-Fc and RF-Fab

The first autoantibody found in RA was the Rheumatoid factor (RF). Although it is now known not to be unique to the disease and can be a sign of other autoimmune diseases, infections, or certain cancers, the elevated presence of RF in the bloodstream is commonly used to help diagnose RA and is associated with a higher severity. Along with several other autoantibodies, they activate the tissue-resident macrophages and mast cells, leading to the secretion of inflammatory cytokines and chemokines. These pro-inflammatory mediators further induce the recruitment of neutrophils and monocytes from the circulation, leading to excessive joint inflammation. Specifically, RF is an antibody against the Fc portion of IgG and can join to form immune complexes that contribute to the disease process. The Fab fragment of the RF binds to epitopes within the Fc of IgG, forming RF-IgG immune complexes in the joint fluid and synovial tissues of patients with RA and activating the classical (antibody binding) pathway of the complement system and induce further inflammation.

Complement System Activation

The innate complement system plays a major role when it comes to RA pathogenesis. The complement system is composed of distinct plasma proteins, and they react and mark pathogens so that phagocytes can destroy them, leading to an inflammatory response. In terms of rheumatoid arthritis, the complement system can be activated in two ways; via antibody binding or via mannose binding protein (MBP). In normal IgG, the classical pathway of the complement system is activated by the binding between the Fab arms of the rheumatoid factor and the Fc receptor binding site on IgG. In the agalactosyl IgG, this activation is closed off due to the closed form structure. Instead, the now exposed terminal GlcNAc residues can now interact with the C-type carbohydrate-recognition domains (CRDs) of MPB, which recognizes pathogens containing high concentrations of mannose or GlcNAc residues on their surface, which is the case with the agalactosyl IgG. MBP-mediated complement activation is only available to IgG antibodies with the closed Fc structure, and the occurrence of both in the synovial fluid suggests that the complement activation via MBP is contributing to the chronic inflammation of the affected joints, which provides an answer to the paradoxical discovery of disease severity linked to deglycosylated IgG even despite the lack of its binding to RF.



Conclusion

Rheumatoid arthritis is a common and painful autoimmune disease with complex origins. Looking at the rheumatoid factor Fab fragment and the structure of immunoglobulin G we found other effectors for joint inflammation than just an overproduction of synovial fluid. Experiments done by several studies proved that the missing galactose at the N-glycosylation site of the Asn 297 sidechain resulted in a closed formation of IgG. This, along with the collapsation of the Fc fragment led to its self-association and involvement with the complement system via MBP. From these new understandings of complement system activation in RA, we can discover new ways of treatment for RA patients and change the future progression of the disease.