The intensity of agglutination

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Antibodies are proteins produced during bodies defence against foreign antigens and they are driven from plasma cells. In the event of an immune response B lymphocytes initiate the production of IgM antibody. In comparison to other immunoglobulin's IgM is the largest and earliest antibody available in response to an antigen (Bailey & Johnson, 2006). The large structure of this antibody is because it consists of an additional domain in its constant area (Overfield et al, 2007). This antibody has a polymeric structure & it consists of heavy and light chains. The binding between two heavy chains or between heavy and light chains is facilitated via the disulphide bond. IgM antibody has a pentameric structure consisting of five subunits. These subunits are joined together via a disulphide bond which occurs between the Fc region and the intersubunit, interasubunit- J chain. Two fab antigen binding sites are available on each IgM monomer and since IgM has a pentameric structure ten Fab antigen binding sites are available that can potentially interact with ten antigens (Overfield et al, 2007) (Khurana, 2006). The initial aim of this practical was to discover if red blood cell antigens can interact with IgM anti-D (Rh) antibody and weather as a consequence of this interaction agglutination occurs. The second aim was to discover weather dithiothreitol (DTT) reducing agents is capable of altering the structure of IgM antibody at different concentration hence affecting the level of agglutination and finally to discover if indirect anti-IgM antibody is capable of facilitating agglutination. The large and pentameric structure of IgM antibody can potentiate the possibility of its interaction with red blood cell antigens resulting in formation of agglutination.

Material & Method

For instructions on how to conduct the experiment with the relevant materials used please refer to the practical schedule. The concentrations of DTT added to the nine tubes where as following (0. 001, 0. 002, 0. 003, 0. 004, 0. 005, 0. 006, 0. 007, 0. 008, 0. 009 & 0. 01).

Results

Table 1: The above table illustrates the number of tubes labelled from 1-10 and the concentrations of DTT in (Mol/L). As illustrated in the above table the control tube which is tube 1 lacked DTT while tubes which were numbered as (2, 3, 4, 5, 6 &7) consisted of different concentrations of DTT as shown here (0. 001, 0. 002, 0. 003, 0. 004, 0. 005, 0. 006 & 0. 007). According to the first observation results tubes numbered 1-7 expressed signs of agglutination as indicated by a positive sign (+). Instead tubes numbered (8, 9 &10) which had the following DTT concentrations (0. 007, 0. 008 & 0. 009) expressed no indications of agglutination hence they were marked as negative (-). Due to time limitations results for the second antibody labelling could not be obtained.

Discussion

The intensity of agglutination in these tubes depended on the concentration of DTT. The control tube which is tube 1 is DTT deficient which is accompanied with agglutination. Tubes labelled 2-7 express different concentrations of DTT starting from the lowest hence escalating slowly. In these tubes agglutination is still observed since the effect of DTT is still not strong enough to break the bonds expressed in IgM antibody while as the concentration of DTT escalates further in tubes 8-10 agglutination is not

evidenced. DTT is a reducing agent capable of mediating intersubunit and interasubunit-J chain cleavage hence facilitating IgM subunit (µ2Î» 2) synthesis (Kownatzki & Drescher, 1973). As the concentration of DTT escalates its capability to break these bonds with greater intensity increases as seen in tubes 8-10 leading to greater IgM subunit formation (µ2Î» 2) and lessens the possibility of antigen antibody interaction hence lack of agglutination. In addition DTT affects the structure of IgM heavy and light chains by preventing them from unfolding and causes this chain too separate accordingly leading to agglutination deficiency. A continuous raise in DTT concentration as evidenced in tubes 8-10 causes a decline the probability of disulphide bonds from resuming their function in IgM antibody (Valetti & Sitia, 1994). According to the study conducted by (Marrodan et al, 2001 & Morris et al, 1974) DTT reducing agent restrains agglutination from occurring by facilitating the disulphide bond located in the IgM antibody to break. In addition the 19 S IgM antibody is cleaved by DTT into a 7S subunit. The 7S antibody subunits are rendered incapable of maintaining IgM antibody’s function and therefore won’t be able to interact with red blood cell antigens leading to lack of agglutination (Knight, 1978).

Due to time limitation for the experiment results for the second antibody labelling could not be obtained. According to (Overfield et al, 2007) the lacking agglutination as a consequence of DTT effect can be reversed by adding anti-IgM antibody hence signs of agglutination will appear but the extent of agglutination will depend on whether the IgM antibody subunits have maintained their ability to bind to red blood cells antigen or due to high level of DTT concentration they have been completely deformed.

According to the study conducted by (Emmerich et al, 2006) IgM antibody can be used in the diagnosis of Lassa virus infection which is highly predominant in Western African patients. This diagnosis is achieved via using reverse enzyme immunoassay (ELISA) technique to identify anti-Lassa IgM antibody. The result of this study implemented that via using reverse ELISA in 20 patients with sign of fever high level of anti-Lassa IgM antibody was diagnosed indicating the presence of the Lassa virus. In a study conducted by (Varsano et al, 1995) the presence of IgM antibody against respiratory syncytial virus antigen (RSV) was examined in 145 patients via using the ELISA technique. According to the result of this study ELISA-IgM antibody detection is a highly efficient method in the diagnosis of RSV at early stage of the disease. In another study by (Tsuda et al, 2001) polymerase chain reaction (PCR) technique was used to detect for the presence of IgM antibody against TT virus (TTV) in the diagnosis of human circovirus. The result of this experiment suggests that healthy volunteers were defective of anti-TTV IgM antibody whereas infected individuals showed signs of its presence suggesting that this method is beneficial for diagnosis purposes of human circovirus.

Immunoglobulin cleavage can be triggered via the action of different enzymes or chemicals. Papain is an enzyme that cleaves IgG antibody into three segments of FC, heavy and light chains. Furthermore IgM antibody can be cleaved by pepsin enzyme either into an antibody that weights less accompanied with FC fragments (Rudmann, 2005)(Svehag et al, 1969). Protease enzyme is driven from Neisseria gonorrhoeae bacteria capable of cleaving IgA antibody (Pouedras et al, 1992). According to (Akesson et al,

2006) streptococcus pyogenes bacteria is responsible in mediating diseases such as gotonsillitis, septicaemia and it intervenes its action by causing IgG antibody cleavage via using an enzyme called Ides. The action of this virus is to insure that the antibody is unavailable to destroy the bacteria. Furthermore trypsin is another enzyme capable of cleaving IgM antibody at temperature above 50 C leading to different FC fragment synthesis (Andrew et al, 1970).

Conclusion

Normally red blood cell antigens are capable of interacting with IgM antibody resulting in agglutination while in the presence of DTT reducing agent this binding is inhibited leading to lack of agglutination. The extent of this inhibition will depend on the concentration of DTT and the extend of IgM J chain, interchain & intrachain cleavage via DTT. The greater the concentration of DTT the stronger its effect is on this chain which lessens the likelihood of this chain regaining their binding capacity hence their ability to regain antigen binding activity. The concept of antigen antibody binding can be used for the diagnosis purpose of many diseases.