FACTORS AFFECTING HEMOLYSIS

The rate of hemolysis in hemolytic anemia is related to several factors:

- Class of immunoglobulin
- Amount of antibody coating the cell
- Ability of the antibody to activate complement
- Thermal amplitude of the antibody
- Macrophage activity

1. The class of immunoglobulin (Ig) coating the erythrocyte is important in determining if, and at what rate, hemolysis will occur. Macrophages have three different Fc receptors (FcR) that have varying affinities for different subclasses of IgG and for monomeric IgG.  
   - FcR I (CD64)
   - FcR II (CD32)
   - FcR III (CD16)

   These receptors are able to bind to a specific domain on the Fc region of an immunoglobulin. All three receptors bind IgG1 and IgG3 avidly (IgG3 > IgG1) but have little or no affinity for IgG2 and IgG4. The FcR I receptor has a high affinity for monomeric forms of IgG1 and IgG3. FcR II and FcR III bind only IgG dimers and are responsible for initially binding the erythrocyte coated with IgG. The differential affinity of Fc receptors for IgG subclasses explains the different rates of erythrocyte survival based on the subclass of IgG bound to the cell. Thus, IgG2 and IgG4 antibodies have little affect on erythrocyte survival. Despite the fact that IgG1 has a high affinity for corresponding erythrocyte antigen, up to 35% of individuals with IgG1 sensitized erythrocytes have no signs of decreased cell survival.  

2. The amount of antibody bound and its avidity for the erythrocyte antigen is important in determining the rate of hemolysis. It has been shown that a certain number of IgG molecules per erythrocyte are needed for hemolysis to occur. If the antigen density (number of antigenic sites) on the erythrocyte membrane is high, more antibodies can be bound. Thus, autoantibodies specific for high-density antigens are more likely to cause hemolysis than autoantibodies to low-density antigens.

3. Antibodies that are able to activate complement may increase the rate of hemolysis. Complement activation opsonizes the sensitized cell and leads to an increase in the rate of removal by macrophages via complement receptors. Both IgM and IgG have the ability to activate complement; however, the pentameric structure of IgM makes it more efficient at activation. Subclasses of IgG differ in their potential for complement activation (IgG1 > IgG3 > IgG2 > IgG4). Regardless of the IgG subclass, however, the two molecules of IgG must be located within 30-40 nm of each other on the erythrocyte membrane in order to activate complement.
4. The thermal amplitude of the antibody (maximum temperature at which the antibody will exhibit activity) affects hemolysis. Most warm reacting IgG antibodies will cause hemolysis and related clinical symptoms because optimal reactivity of the antibody is 37°C (body temperature). Cold-reacting antibodies vary in their ability to cause hemolysis depending upon their thermal range of reactivity. Naturally occurring cold-reacting antibodies optimally bind to the antigens at 4°C and their usual thermal amplitude is 20 to 25°C. Thus, they cause no in vivo destruction. However, pathologic cold agglutinins have reactivity up to 32°C and can cause hemolysis and subsequent clinical symptoms. The antibody attaches to the cell and activates complement when the peripheral circulation cools to 28°–32°C. As the blood warms to 37°C, the antibody dissociates but the complement remains fixed to the cell membrane.

5. Macrophage activity is also involved with the rate of hemolysis. The ability of the individual’s macrophages to sequester and remove sensitized cells is probably an important determinant in the rate at which cells are destroyed. In most patients receiving glucocorticoids, hemolysis is dramatically slowed. This response is most likely mediated by suppression of Fc site receptors in macrophages.

References