B-CELL ORIGIN OF COLD AGGLUTININS

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INTRODUCTION

Cold agglutinins (CA) are RBC specific autoantibodies that agglutinate RBCs optimally at 4 - 22°C in vitro and may be of widely divergent clinical significance. Virtually all sera from healthy individuals contain low titered CA which cause no apparent immune hemolysis and may be defined as natural or benign RBC autoantibodies. In contrast, the pathologic (e.g. hemolytic) counterparts of these autoantibodies are generally derived from clonal B cell expansions that may progress to frank lymphoma (Table 1).

The occurrence of normal and pathogenic serum autoantibodies suggests that under certain conditions, autoreactive B cell clones escape from tolerance mechanisms. For example, in certain transgenic mice, it has been known that functionally inactivated (e.g. anergic) B-cells can be rescued and induced to produce self reactive antibody by polyclonal activation with bacterial polysaccharide antigens. These experiments may therefore explain why autoimmune hemolytic syndromes, especially in children, are often associated with viral and bacterial infections. Conceivably, the causative autoantibodies result from the dysregulation of certain autoreactive B-cell clones, and in some instances, these dysregulated B-cell clones may undergo neoplastic transformation (e.g. cold agglutinin disease).

<table>
<thead>
<tr>
<th>Monoclonal *</th>
<th>Polyclonal</th>
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<tbody>
<tr>
<td>idiopathic/chronic</td>
<td>benign/natural post infectious</td>
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<tr>
<td>B-cell lymphoma</td>
<td>(e.g. Mycoplasma pneumonia,</td>
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<tr>
<td></td>
<td>Epstein-Barr virus (EBV); Human</td>
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<td></td>
<td>immunodeficiency virus (HIV);</td>
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<td>collagen vascular disorders.)</td>
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* Monoclonal cold agglutinins are derived from a spectrum of clonal B-cell expansion ranging from pre neoplastic (e.g. no evidence of malignancy) to frank lymphoma 1-3.
The majority of benign and pathologic CA are IgM autoantibodies. While the benign CA are considered to be polyclonal, the pathologic CA are invariably monoclonal and detectable as paraproteins in the serum. The pathologic CA usually occur spontaneously, during the course of a lymphoproliferative disorder, or rarely as a post-infectious complication of mycoplasma pneumonia or infectious mononucleosis \(^4, 5\). Patients with cold agglutinin disease (i.e. CA induced autoimmune hemolytic anemia) may have CA titers in the thousands or even millions compared to normal individuals who may have low titer (<32) IgM cold agglutinins. Because of their low thermal binding properties, these IgM autoantibodies appear to bind to red cells and fix complement in the peripheral circulation where temperatures fall below \(-32^\circ\text{C}\). As the cells return to warmer parts of the circulation, the IgM dissociates leaving the cells coated with only complement. Complement activation may then lead to intravascular hemolysis.

Paroxysmal cold hemoglobinuria (PCH), like cold agglutinin disease, is caused by cold-reactive autoantibodies that react with red cells in cooler parts of the body, cause complement to irreversibly bind to cells, and then elute off of the erythrocyte surface when warmed. However, in PCH, the autoantibodies are IgG molecules usually directed at P blood group antigens \(^6\) and are present in relatively low titers (<64). Because the presence in serum of these biphasic IgG antibodies may be difficult to detect by standard serological methods, specialized tests which use in vitro hemolysis as an indicator of IgG-induced complement sensitization can be employed (Donath-Landsteiner test, Donath and Landsteiner 1904). PCH may be idiopathic or secondary to syphilis or viral infection.

Although PCH is the rarest form of autoimmune hemolytic anemia (AIHA), historically it was the first type recognized probably because of its graphic clinical presentation, i.e. the sudden onset of shaking chills, back and leg pain, abdominal cramps, high fever, and the passage of black urine. Reports in the medical literature began to appear in the mid-1800's that described attacks of hemoglobinuria after exposure to cold \(^7, 8\). In 1879, Rosenbach \(^9\) reported how he had induced hemoglobinuria by immersing his patient's feet in ice water, and two years later Paul Erlich \(^10\) reported his observation of hemolysis and erythrophagocytosis in the blood obtained from the chilled finger of a patient with the disease.

For the next fifty years, laboratory investigation into the biology of AIHA was limited to the study of rare, lytic antibodies, or to cold-reactive IgM autoantibodies which would directly agglutinate red cells in vitro due to their multivalent, red cell-bridging properties.

**TARGETS FOR COLD-REACTIVE AUTOANTIBODIES**

**I/i-blood Group Specificity**

Cold-reactive anti-red cell autoantibodies typically bind to carbohydrate structures on membrane glycolipids and/or glycoproteins. The majority of cold agglutinins are IgM immunoglobulins that are directed at I/i blood group antigens \(^11, 12\). This antigen system comprises oligosaccharide chains composed of repeating N-acetyllactosamine (Gal[β1→4]GlcNAc[β1→3]) units (Feizi 1980) linked to ceramide or the membrane glycoproteins band 3 and band 4.5 \(^13, 14\) (Figure 1). The best available evidence indicates that the difference between I and i antigens relates to branching of the oligosaccharide chain; anti-i antibodies recognize a linear N-acetyllactosamine oligosaccharide while anti-I antibodies recognize a similar chain that is also branched \(^15-17\). There appears to be a developmentally-regulated transition in expression of the I/i antigens; fetal and newborn red cells express mostly i antigen while adult red cells demonstrate the opposite pattern \(^18\) (Figure 1). This transition appears to involve the acquisition of a "branching enzyme" (a [β1→6]-N-acetylglucaminyl transferase) \(^17\). It is of interest to note that the conversion of the