Paroxysmal Nocturnal Hemoglobinuria Diagnosed after Influenza Vaccine: Coincidence or Consequence?

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For Editorial see page 113

Paroxysmal nocturnal hemoglobinuria is a rare disease caused by the absence or reduced expression of glycosylphosphatidylinositol – anchor proteins on the membrane of red blood cells [1]. Two of these proteins, CD55 (decay-accelerating factor) and CD59 (membrane attack complex inhibitory factor), play a role in neutralizing the complement system. Deficiency or reduced expression of these proteins leads to increased sensitivity of the affected cells to the action of complement. The consequences are the manifestations of PNH, which include hemolytic anemia, venous thrombosis, abdominal pain and dysphagia.

Vaccination against influenza virus is the most effective method to prevent the disease. Intramuscular influenza vaccines are inactivated preparations of either whole virus or subvirion components. The protection is based on induction of virus-neutralizing antibodies, mainly against the viral hemagglutinin [2].

The efficacy of most vaccines currently used depends on the presence of an adjuvant in conjunction with the infectious antigen. An adjuvant increases the exposure of the immune system to the antigen by giving it physical protection in the regional lymph node. Its effect is accomplished via a mechanism that involves both the innate (conferred by the complement system) and adaptive immune system. However, by triggering the immune system an adjuvant can also induce an autoimmune response, recently defined as "ASIA" (autoimmune/autoinflammatory syndrome induced by adjuvants) [3]. We present a patient diagnosed with PNH 2 days after receiving an influenza vaccine. We discuss the possible association between these two conditions.

PNH = paroxysmal nocturnal hemogobinuria

PATIENT DESCRIPTION

A 29 year old woman was admitted to our department due to intermittent headaches and fatigue over the previous 2 weeks; there were no other complaints. Her past medical history was unremarkable apart from iron deficiency anemia diagnosed 6 months before her admission. She was taking iron and folic acid supplements. Her headaches appeared to be of migrainotic type, with a preceding visual aura that responded to paracetamol and rest. She had received an influenza vaccine 2 days prior to the onset of symptoms. She was vaccinated against influenza in the previous year and there were no adverse events.

On admission she did not appear to be in distress. Vital signs were normal. She was jaundiced; the rest of her examination was unexceptional. Laboratory tests showed a macrocytic anemia with a hemoglobin level of 6.9 g/dl and mean corpuscular volume 101 fl, platelet count in the lower limit of normal, mild elevation of serum aspartate aminotransferase, indirect hyperblirubinemia up to 2.5 mg/dl, and lactate dehydrogenase level of approximately 6000 U/L. Coagulation tests were normal. Anemia workup revealed reticulocytosis with an absolute reticulocyte count of 300,000/µl, undetectable haptoglobin level, and negative Coombs test. Ferritin level was 22 ng/ml; folic acid and B12 were within normal range. These findings were consistent with non-immune hemolytic anemia. No schistocytes or helmet cells were detected on peripheral blood smear. Urine was positive for hemosiderin. Abdominal ultrasound showed no hepatomegaly or splenomegaly.

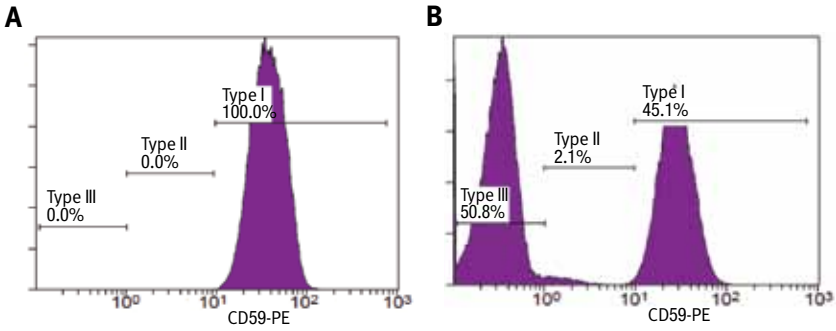
A designated flow cytometry blood test revealed the patient's granulocytes and monocytes to be deficient in CD59 and deficient in CD24/FLAER (GPI by fluorescent aerolysin) with granulocyte clone of 90% [Figure]. A bone marrow biopsy showed hypercellularity with no evidence of aplastic anemia or myelodysplastic syndrome. The patient was administered one packed red blood cell unit, resulting in elevation of hemoglobin to 9.5 mg/dl. She was prescribed 1 mg/kg of prednisone and folic acid was continued. A week later her hemoglobin level was stable.

COMMENT

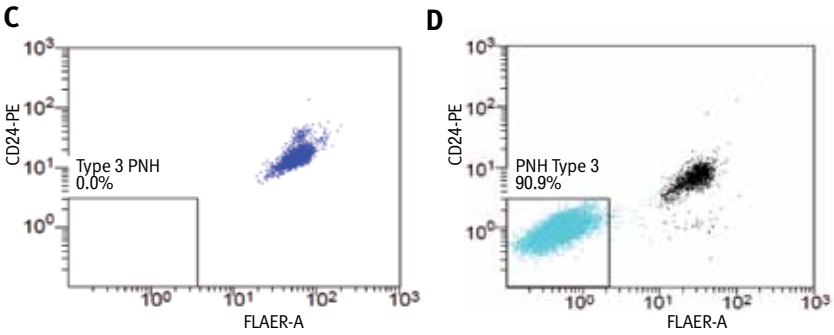
PNH is a rare condition characterized by the partial or complete absence of GPI-anchor due to an acquired defect in the *PIG-A* gene [1]. Its diagnosis is based on the combination of both clinical characteristics and specific immunophenotypic markers by flow cytometry. Decay-accelerating factor (DAF, CD55) disables C3 and C5 con-

GPI = glycosylphosphatidylinositol

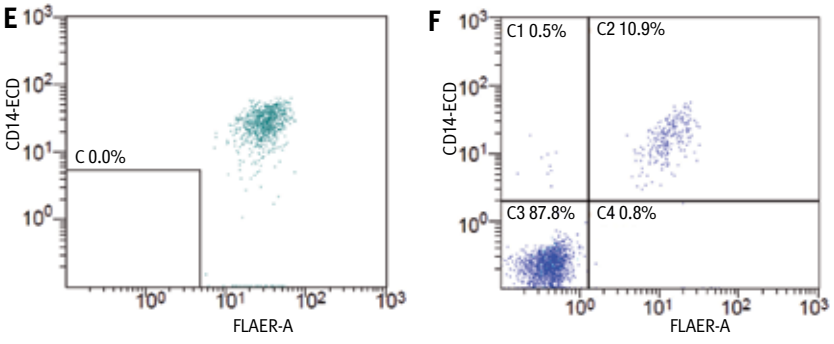
CD59 deficiency expression of gated population of red blood cells.
[A] Normal control with a single high intensity type I peak. **[B]** PNH showing low high intensity type I peak and high type III peak (50.8%) with very low type II peak (2.1%)



CD24 deficiency and aerolysin (FLAER) expression on gated population of granulocytes.
[C] Normal control showing a single positive CD24/FLAER population. **[D]** PNH showing a low positive CD24/FLAER population and a high (90.9%) negative type III CD24/FLAER population



CD14 deficiency and aerolysin (FLAER) expression on gated population of monocytes.
[E] Normal control showing a single positive CD14/FLAER population. **[F]** PNH showing a low positive CD14/FLAER population and a high (87.8%) negative type III CD14/FLAER population.



vertase, thereby regulating the early part of the complement cascade. CD59 inhibits the terminal complement complex formation, known as the membrane attack complex, by blocking incorporation of C9 onto C5b-8, causing lytic pore formation and lysis of red blood cells. PNH red blood cells, deficient in or lacking these proteins, are prone to complement-induced hemolysis [1].

Vaccines that contain infectious antigens, either attenuated or recombinant, may rarely induce autoimmunity via similar 'infectious' mechanisms. We hypothesize that the influenza vaccine given to our patient triggered complement activation in one of two possible pathways: a response to the presentation of the viral antigen itself or to the adjuvant via the mechanism of the syndrome ASIA.

An essential first step in antibody response to influenza vaccination is for B cells to come in contact with the antigen. B cells and dendritic cells co-express complement receptors of C3 complement component, namely CR2 (CD21) and CR1 (CD35). These receptors act as a link between innate immunity (conferred by the complement system) and adaptive (humoral) immunity. They bind activated C3b and C4b and the immune complexes of C3d-Ag. Complement C3 and CD21/CD35 are important components in humoral immunity to influenza [4]. Complement system activation in response to influenza occurs via the classical, alternative, or lectin pathway [4]. Knockout mice deficient in complement C3 or CD21/CD35 display a reduced ability to neutralize influenza virus [4].

The second possibility is that it was the adjuvant that triggered the immune response [3]. In order to recognize and react against foreign antigens, the antigen needs to be presented to T and later B lymphocytes (adaptive system) by specialized antigen-presenting cells such as dendritic cells and macrophages (the innate system). The role of adjuvants is to enhance the innate response by prolonging the exposure to the antigen [3,5]. As mentioned earlier, however, in rare cases the adjuvant itself induces an autoimmune response, termed ASIA [3]. Influenza vaccines of the last pandemics contain MF59 or AS03 oil-in-water adjuvants. MF59 was found to be a strong inducer of cytokines, cytokine receptors and antigen-presenting genes [5]. The immune response to this adjuvant is very rapid, triggering both innate and adaptive systems. Although the criteria for ASIA comprise mainly typical clinical manifestations known to be associated with an immune response such as fever and arthritis, we believe that the current case may be attributed to the same syndrome [3,5].

CR = complement receptor

The temporal relation between the emergence of our patient's symptoms and the influenza vaccination she received less than 48 hours earlier may be more than merely a coincidence. Since complement activation facilitates hemolysis and other clinical features of PNH, we hypothesize that the desired and normal immune response to the vaccine began a vicious cycle of PNH-cell destruction by activated C3 components.

To the best of our knowledge, this is the first report suggesting a possible association between the influenza vaccine and a hemolytic episode of PNH. Given that all PNH patients are prone to the damage of complement activation, the question is whether influenza vaccine should be administered to these patients? On the other hand, influenza virus can cause a severe and even fatal disease, especially since the appearance of the 2009 pandemic H1N1. A registry of vaccination and post-vaccination complaints and findings in PNH patients might resolve this question.

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Capsule

CNVs conferring risk of autism or schizophrenia affect cognition in controls

In a small fraction of patients with schizophrenia or autism, alleles of copy-number variants (CNVs) in their genomes are probably the strongest factors contributing to the pathogenesis of the disease. These CNVs may provide an entry point for investigations into the mechanisms of brain function and dysfunction alike. They are not fully penetrant and offer an opportunity to study their effects separate from that of manifest disease. Stefansson et al. show in an Icelandic sample that a few of the CNVs clearly alter fecundity (measured as the number of children by age 45). Furthermore, they use various tests of cognitive function to demonstrate that control subjects carrying the CNVs perform at a level that is between that of schizophrenia patients and population controls. The CNVs do not all affect the same cognitive domains, hence the cognitive deficits that drive or accompany the pathogenesis vary from one CNV to another. Controls carrying the chromosome 15q11.2 deletion between breakpoints 1 and 2 (15q11.2(BP1-BP2) deletion) have a history of dyslexia and dyscalculia, even after adjusting for IQ in the analysis, and the CNV only confers modest effects on other cognitive traits. The 15q11.2(BP1-BP2) deletion affects brain structure in a pattern consistent with both that observed during first-episode psychosis in schizophrenia and that of structural correlates in dyslexia.

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Capsule

Human-pathogen co-evolution

Helicobacter pylori is the principal cause of gastric cancer, the second leading cause of cancer mortality worldwide. However, *H. pylori* prevalence generally does not predict cancer incidence. To determine whether co-evolution between host and pathogen influences disease risk, Kodaman et al. examined the association between the severity of gastric lesions and patterns of genomic variation in matched human and *H. pylori* samples. Patients were recruited from two geographically distinct Colombian populations with significantly different incidences of gastric cancer, but virtually identical prevalence of *H. pylori* infection. All *H. pylori* isolates contained the genetic signatures of multiple ancestries, with an ancestral African cluster predominating in a low risk coastal population and a European cluster in a high risk mountain population. The human ancestry of the biopsied individuals also varied with geography: mostly African ancestry in the coastal region (58%) and mostly Amerindian ancestry in the mountain region (67%). The interaction between the host and pathogen ancestries completely accounted for the difference in the severity of gastric lesions in the two regions of Colombia. In particular, African *H. pylori* ancestry was relatively benign in humans of African ancestry but was deleterious in individuals with substantial Amerindian ancestry. Thus, co-evolution likely modulated disease risk, and the disruption of co-evolved human and *H. pylori* genomes can explain the high incidence of gastric disease in the mountain population.

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