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Neutrophil-Specific Antigen HNA-2a (NB1, CD177): Serology, Biochemistry, and Molecular Biology

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Human neutrophil antigen-2a (HNA-2a) is one of the most clinically important neutrophil alloantigens. It was previously known as NB1 (1). Monoclonal antibodies specific to HNA-2a have been clustered as CD177 (2). HNA-2a was first described in 1971 in the sera of a women alloimmunized during pregnancy by Lalezari and collegues (3). HNA-2a specific antibodies cause alloimmune neonatal neutropenia, autoimmune neutropenia, and transfusion-related acute lung injury. HNA-2a is expressed on neutrophils and neutrophilic metamyelocytes, and myelocytes from 89% to 97% of healthy individuals. It is a 56 to 64 kD glycosylphosphatidylinositol (GPI) anchored plasma membrane glycoprotein (gp) that is also expressed on secondary granules. Among HNA-2a-positive people, the antigen is expressed on subpopulations of neutrophils. Approximately 55 to 65% of neutrophils in each healthy adult express HNA-2a. Four monoclonal antibodies specific to HNA-2a have been described 1B5, 7D8, MEM-166, and TAG4 (4-7). These antibodies have been useful in identifying the structure of the 56 to 64 kD gp carring HNA-2a and in sequencing the gene encoding gp. The gene encoding HNA-2a is over expressed in people with polycythemia rubra vera, but the function of the CD177 gp is not known.

Expression of HNA-2a

HNA-2a is neutrophil-specific. It is expressed on neutrophils, neutrophilic metamyelocytes, and myelocytes (4, 8). It is not expressed by other blood cells. HNA-2a is unique in that it is expressed on subpopulations of neutrophils. The mean size of the HNA-2a-positive subpopulation of neutrophils is 56% to 64% (5-9). Neutrohils tested from the same person at different times remains constant in regards to the percent of neutrophils expressing HNA-2a (9, 10). In approximately 8% of people three subpopulations of neutrophils can be detected, one not reactive with HNA-2a-specific antibodies and two that react with HNA-2a specific antibodies though with different intensities (10). The expression of HNA-2a is greater on neutrophils from women than men (10). The size of the HNA-2a positive subpopulation of neutrophils from women is 62±20% compared to 52±20% for men. The expression of HNA-2a falls with age in women, but remains constant in men (10). Neutrophil expression of HNA-2a is greater in pregnant women than in healthy female blood donors.

The administration of G-CSF to healthy subjects for several days increases the proportion of neutrophils expressing HNA-2a to near 90% (11). The surface expression of HNA-2a is slightly upregulated by treatment with the chemotactic peptide f-met-leu-phe (9, 12). The treatment of neutrophils with f-met-leu-phe does not change the percentage of cells that express HNA-2a (9).

CD177 glycoprotein

The glycoprotein carrying HNA-2a, CD177, is located on neutrophil plasma membranes and secondary granules (9, 13) and is linked to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor (9, 12). Although some GPI proteins are shed by f-met-leu-phe treated neutrophils, CD177 is not, nor is soluble CD177 present in plasma (9).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of CD177 after immunoprecipitation or immunoblotting shows a band of Mr of 56 to 64 kD under reducing conditions and 49 to 55 kD under non-reducing conditions (9, 13). In MALDI-TOF mass spectrometry, the purified glycoprotein has a molecular mass of 50.556 kD (14, 15).

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CD177 has N-linked, but not O-linked carbohydrate side chains (9, 13-15). Removal of N-linked carbohydrates with endoglycosidase-F from immunoprecipated CD177 glycoprotein decreases the apparent molecular weight of the protein to approximately 45 kD in SDS-PAGE (9, 13) and to 43.069 kD when determined by MALDI-TOF mass spectrometry (14, 15).

Genomic Sequence

The cDNA encoding the HNA-2a protein was found to consist of 1311 bp coding for a protein of 416 and a signal peptide of 21 amino acids (14, 15). The predicted protein has two cysteine-rich domains, three potential N-linked glycosylation sites, and a potential w-site for attachment of the GPI anchor (14, 15). This gene belongs to the Ly-6 snake toxin superfamily. Other genes in this family include urokinase type plasminogen activator receptor (uPAR) (CD87), decay acceralerating factor (CD59), and polycythemia rubra vera-1 (*PRV-1*) gene. *PRV-1* is over expressed in neutrophils from people with polycythemia rubra vera (16). The gene encoding HNA-2a and *PRV-1* differ at only 4 bp (15). Bettinotti and colleagues used Human Genomic Project databases to characterize the structure of the *PRV-1* and NB1 genes (17). They described the intron and exon structure of NB1 but they found only one gene homologous to both *PRV-1* and NB1 suggesting that they are alleles of the same gene. In addition, they found a pseudo gene homologous to exons 4 through 9 of *PRV-1/NB1*.

CD177 Polymorphisms

HNA-2a is expressed on neutrophils by approximately 97% of Caucasians, 95% of African Americans, and 89% to 99% of Japanese (6, 10, 18, 19, 20). There is some variation in the expression of HNA-2a among antigen-positive people. When the expression of two HNA-2a-specific CD177 monoclonal antibodies, 1B5 and 7D8, was compared the mean size of the subpopulation of neutrophils that reacted with these two antibodies was the same (53.0±23.3% and 55.3±22.0%, respectively), but the reactions of the two antibodies differed significantly in a few donors (10). In 10% of the donors the percentage of neutrophils that reacted with 1B5 and 7D8 differed by more than 12% (10). These differences in the 1B5- and 7D8-subpopulation size occurred in 18% of Caucasian donors, but only in 4% of the African American donors (10). Two other HNA-2a specific CD177 antibodies have been described, MEM-166 and TAG4. The two antibodies also recognized two subpopulations of neutrophils in most healthy adults and the size of the subpopulation of neutrophils recognized by MEM-166 and TAG4 is the same as that recognized by 7D8 (7).

HNA-2a has been reported to have an allele, NB2, but the product of this gene can not be reliably identified with alloantisera and no monoclonal antibody specific for NB2 has been identified (21-23). Rabbit polyclonal antibodies that react with CD177 glycoprotein do not react with any glycoprotein on HNA-2a-negative neutrophils in flow cytometry, immunoblotting, or immunoprecipation assays (22).

HNA-2a genes from two women with HNA-2a-negative neutrophils who produced HNA-2a-specific alloantibodies have been studied and PRV-1/NB1 cDNA sequences were present in both women (24). Their neutrophil mRNA was isolated, converted to cDNA and analyzed. PCR products of variable lengths were detected and sequencing detected both exons and accessory sequences that were considered to be introns. Some cDNAs containing the entire *PRV-1/NB1* coding sequence were identified, but all cDNA had some accessory sequences.

Disease Relevance

The expression of HNA-2a is reduced on neutrophils from people with paroxysmal nocturnal hemoglobinuria (PNH) and chronic myelogenous leukemia (CML) (8, 9). It is not known if the lack of expression of HNA-2a on neutrophils from patients with PNH or CML has any clinical significance. PRV-1/NB1 mRNA is over expressed by neutrophils from patients with polycythemia rubra vera. The significance of this is not certain.

The 3% of people whose neutrophils lack HNA-2a are not at increased risk of infection or autoimmune disorders. However, HNA-2a-negative mothers with HNA-2a-positive neonates can produce HNA-2a-specific alloantibodies which can cause the neonate to experience neutropenia lasting several weeks (25-27). Transfusion of blood components containing alloantibodies specific for HNA-2a can cause severe transfusion-related acute lung injury (TRALI) in the transfused patients (25, 28). Antibodies specific for HNA-2a have also been produced by some patients with quinine-induced neutropenia (29).

Alloantibodies to HNA-2a can also be produced by patients transfused with granulocyte concentrates (30). When patients with HNA-2a specific alloantibodies are transfused with HNA-2a-positive granulocytes they can experience febrile transfusion reactions and shorten survival of the transfused granulocytes can occur.

Role of CD177 in Cellular Function

The role of CD177 in neutrophil function is unknown. In some studies, HNA-2a-positive neutrophils were significantly less adherent to human umbilical vein endothelial cells than HNA-2a-negative neutrophils (31). Upregulation of HNA-2a after f-met-leu-phe stimulation, HNA-2a internalization upon antibody cross-linking, and activation of the respiratory burst after binding of HNA-2a antibodies to HNA-2a-bearing neutrophils suggest a possible role as receptor molecule (32).

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