Chapter

Neutrophil-Specific Antigens: Immunobiology, Genetics and Roles in Clinical Disorders

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Abstract

Neutrophils are the most abundant nucleated cells in blood circulation and play important roles in the innate and adaptive immune responses. Neutrophil-specific antigens, only expressed on neutrophils, are glycoproteins originally identified in studies on neonatal neutropenia due to fetal-maternal incompatibility and autoimmune neutropenia of infancy. The most investigated neutrophil–specific antigens are the NA and NB antigens that their incompatibilities also cause transfusion-induced febrile reactions and acute lung injury, a potentially fatal reaction, and in bone marrow transplantation, causing graft rejection. NA antigens are members of the immunoglobulin superfamily and are low-affinity Fc-receptors FcγRIIIb (CD16b). Fc receptors connect the F(ab), the antigen-binding fragment of the antibody molecules, to neutrophils and lead them to recognize and phagocytize the targeted antigens. The NB (CD177) antigen belongs to the urokinase-type Plasminogen Activator Receptor Superfamily (uPAR, CD59, Ly6), but its specific functions have not been fully determined. It is known, however, that NB antigen binds proteinase-3 (PR3 to the neutrophil membrane), a serine protease. In clinical studies, it was also demonstrated that NB expression is highly elevated in Polycythemia Vera and is unexpectedly expressed in some cancer tissues. Neutrophil-specific antigens are examples of antigens that have important biological and clinical activities beyond antigenicity.

Keywords: alloimmune neonatal neutropenia (ANN), autoimmune neutropenia of infancy (AINI), neutrophil-specific antigens, NA, SH, NB1, NB2, Fc gamma Receptor IIIB (FcγRIIB), HLA antigens, human neutrophil antigen (HNA) nomenclature

1. Introduction

Neutrophils are the most abundant circulating leukocytes. They are short-lived, terminally differentiated myeloid cells produced in the bone marrow. They contain intracellular granules and vesicles and a multi-lobed nucleus. The condensed nuclear chromatin is considered to indicate a limited transcriptional activity in the cells. Neutrophils are an important part of innate and adaptive immunity, and the first line of defense against invading pathogens. There are multiple types of proteins expressed on neutrophil surfaces, including Fc-receptors, adhesion molecules, integrins, multiple cytokine receptors, innate immune receptors such as Toll-like receptors, and C-type lectins. Some receptors directly recognize and bind to pathogens and play their role in the defensive functions, some other receptors, such

as Fc-receptors, bind the Fc fragment of the antibody molecules to neutrophils, which enable the neutrophils to recognize the targets of the antibodies and then perform their functions in adaptive immunity. Some other receptors recognize the inflammatory signals. The extracellular structures from neutrophil surface proteins are involved in signal transduction, cell-cell interactions, neutrophil migration, and substance releases such as cytokines, reactive oxygen species, exocytosis of intracellular granules, and neutrophil extracellular trap (NET) formation, the structure that traps and kills the invading bacteria. Some of the glycoproteins expressed on the neutrophil surface carry the Neutrophil-Specific Antigens. The protective effects of neutrophils are also contributed by their ability to receive signals through their receptors that stimulate them to rapidly generate pseudopods, move and invade their targets. They phagocytize the microorganisms and digest them by proteolytic enzymes present in their intracellular granules. Eli Metchnikoff (1845–1916) of Pasteur Institute in Paris was the first investigator who identified neutrophil immunogenicity. He reported that guinea pigs injected with rabbit blood produce antibodies against rabbit leukocytes and called the antibodies leukoagglutinins and leukolysins [1]. In 1926, Charles Doan recognized that leukocyte incompatibility causes transfusion reaction and suggested that leukocytes have their own "groups" [2]. Jean Dausset later reported that the presence of leukoagglutinins in blood could be associated with chronic autoimmune neutropenia [3]. In 1957, Tom Brittingham, of the hematology team of Washington University in St. Louis, produced leukoagglutinins experimentally [4]. He volunteered to receive weekly 100 mL blood for nine consecutive weeks from a patient who had chronic myelogenous leukemia, the last four causing chills and fever. He could prevent the reaction by minimizing the number of injected leukocytes. To study the effects of antibodies in donors' blood, he received 50 mL blood from a patient who had received numerous transfusions. This injection caused high fever, vomiting, dyspnea, cyanosis and hypotension. A chest X-ray on the following day revealed bilateral lung infiltrations, a reaction that is now recognized as transfusion-related acute lung injury (TRALI). Dausset [5] and others [6, 7] in subsequent years demonstrated inheritance of leukocyte antigens.

2. Alloimmune neonatal neutropenia (ANN)

Neutrophil-specific antigens were identified during the investigation on alloimmune neonatal neutropenia (ANN), proven to be due to fetal-maternal incompatibility [8]. In that study, the first child had died from neutropenia and sepsis, and the three subsequent infants also had neutropenia at birth. The diagnosis of ANN was based on the detection of a strong leukoagglutinin in the maternal serum that reacted with the paternal neutrophils but not with the maternal own cells. The blood smear from the last infant showed the absence of mature neutrophils but lymphocytes, monocytes, eosinophils, basophils and platelets were normal. Neutrophil-specificity of the antibody was established by demonstrating that the antibody could be absorbed only by isolated neutrophils and not by any other blood cells or tissue cells [9]. An anti-5b antibody [10], known to react with all blood leukocytes and many other tissue cells tested, was used as a control and showed that the ANN antibody reacted only with neutrophils. This first neutrophil-specific antigen identified was named NA1, "N" for Neutrophil, "A" for the first locus, and 1 for the first allele. Further investigation on ANN led to the identification of other antigens including NA2, NC1 [9, 11], SH [12, 13] and HNA-1d [14]. ANN could be identified because the tests were directly performed on the blood from the affected infants and their parents, and not on blood samples from multiparous female

donors who develop multiple forms of antibodies against the HLA, red blood cells and platelets. This may explain the reason for some other investigators inability to recognize ANN, and even conclude that neonatal neutropenia was the result of infection and not alloimmunization [15]. Neutrophil specificity causes ANN because the total neutrophil mass in fetus blood is small, and there are no other cell types in the body that express the same antigens and can absorb the maternal antibodies. The more common HLA antibodies in maternal blood do not cause ANN because HLA antigens are widely distributed in blood cells and other tissues, and also, as soluble antigens, can absorb the maternal-derived antibodies [16]. In one ANN example, both neutrophil-specific and HLA antibodies were detected in the maternal plasma, but only the neutrophil-specific antibody was detected in the affected newborn's blood. ANN is a self-limited disorder and lasts as long as the maternal antibodies remain in the infant's circulation, a period of a few weeks to a few months. The recovery can be predicted when the antibody can no longer be detected in the infant's plasma. In ANN, the absolute neutrophil counts are below 500 and the common symptoms are omphalitis, and infections in the skin and in other locations. It has been estimated that ANN occurs in 0.1–0.2% of pregnancies. This may be an underestimation; because now most birth deliveries are performed under sterile conditions and the newborns are not exposed to bacteria, and consequently, neutropenic infants may remain asymptomatic and undiagnosed. Also, blood counts and differentials are not routinely tested on asymptomatic newborns. ANN, in contrast to erythroblastosis fetalis, known as Rh disease, can occur in the first pregnancies. This information should warn the blood banks that blood donors with single pregnancy and asymptomatic children or undiagnosed ANN infants may be carriers of the hazardous neutrophil-specific antibodies, and transfusion of their blood may result in severe reactions and even fatalities.

2.1 Autoimmune neutropenia of infancy (AINI)

The second clinical disorder that increased interest in neutrophil-specific antigens was autoimmune neutropenia of infancy (AINI). In 1975, a strong anti-NA2 antibody was identified in the blood of a six months old infant. The antibody was not present in the mother's blood and the child was too old to have ANN [17]. Additional studies [18] demonstrated autoantibodies on 119 of 121 severely neutropenic children. The disorder, called AINI, was shown to be very common and the autoantibodies were directed against neutrophil-specific antigens found in ANN. Thus far, the antigens NA, NB, ND1 [19] NE1 [20] and 9a [18] are associated with AINI, and some of these antibodies are also found in a few cases of autoimmune neutropenia in adults [16]. Before detection of autoantibodies and establishing the autoimmune nature of this disorder, AINI was recognized as "chronic benign neutropenia," benign because it was a self-limited neutropenia disorder, lasting several months to a few years. The neutrophil count in blood of AINI patients is between zero to 500, and there is an elevation of monocytes and eosinophils, which may represent a protective defense mechanism. Pathogenesis of AINI has not yet been determined, but it has been suggested that a delay in maturation of the regulatory T-cells (T-regs) causes a lack of tolerance to neutrophil-specific antigens, and that the autoimmunity becomes corrected when T-regs become functional [21, 22].

3. Neutrophil-specific antigens

The NA and NB antigen systems are the most investigated neutrophil-specific antigens and are discussed here, with their information summarized in

Tables 1 and **2**. The genetics and biological functions of these two systems are different and are described separately. However, their clinical impacts, such as their roles in ANN, AINI, blood transfusion, and bone marrow transplantation are similar. Also, in this report, the nomenclature used is based on the original and not the HNA that will be discussed separately.

3.1 The NA system

Four expressing alleles, NA1, NA2, NA3 (SH), and NA4 are currently known in this system (**Table 1**). NA1 was the first, described with neonatal neutropenia. The antigen SH, here referred to as NA3, was first described in one case of ANN, and subsequently in three other cases [12, 13]. The antigen referred to as NA4, was originally described in two cases of ANN [14]. In rare individuals, the NA antigens are not expressed. This abnormality, caused by gene alteration, is called NA^{null} [12, 23, 24]. The NA antigens appear at the metamyelocytic phase of neutrophil maturation and are anchored on the cell membranes by the Glycosylphosphatydylinositol (GPI) at the density of 100,000 to 300,000 copies per cell [25, 26]. Some NA molecules are also stored inside the cells and translocate to the membrane and are released during cell activation. NA antigens are the low-affinity Fc-receptors, FcγRIII [27]. Fc-receptors are glycoproteins that bind the Fc fragment of immunoglobulins and connect the humoral to the cellular components of the immune system. In this process, the effector cell, via its Fc-receptor, is connected to the F(ab)2, the antigen-binding part of the antibodies. This connection leads to phagocytosis and antibody-mediated cell cytotoxicity (ADC) [28]. The genetic polymorphisms in the coding genes for Fc-receptors influence their effectiveness. Three Fc gamma receptor (FcγR) subclasses have been identified thus far: FcγRI, FcγRII, and FcγRIII [29]. The NA epitopes are located on FcγRIIIb (CD16b), exclusively expressed on neutrophils, bind Fc fragments of IgG1 and IgG3 immunoglobulin subclasses [30, 31]. The *CD16B* gene has 5 exons and is located on chromosome 1q23.3, and it belongs to the immunoglobulin superfamily. FcγRIIIb contains two immunoglobulin G (IgG)-like domains and the proximal domain carries the IgG-binding segments. The four variations in *FCGR3B* coding region lead to the formation of the four known NA alleles (**Tables 1** and **2**). These SNPs are located on exon 3 of the *FCGR3B* gene. *FCGR3B*01* and *FCGR3B*02* are the most frequent alleles coding NA1 and NA2 respectively [32]. These two alleles are

System	Allele	Frequency	Location	Amino acids	MW (kDa)*	Chromosome	Exons	Nucleotide, base pairs
NA (CD16b)	NA1	0.560	Membrane, intracellular	233	58–65	1q23.3	5	699
	NA2	0.885		233	65–80			
	NA3 (SH)	0.05		233	65–80			
	NA4 (HNA1d)	Unknown		233	65–80			
	NA^{null}	Rare						
NB (CD177)	NB1	0.97	Membrane, secondary granules, vesicles	437	55–64	19q13.2	9; 6 in the pseudo-gene	1614

**Difference in molecular weight (MW) is due to differences in glycosylation.*

Table 1.
Neutrophil-specific antigens, the NA and NB system.

System	Gene	Protein	Antigen name	Nucleotide	Amino acids
FcγRIIIb (CD16)	*FCGR3B*	CD16	NA1	108G, 114C, 194A, 233C, 244G, 316G	36R, 38 L, 65 N, 78A, 82D, 106 V
			NA2	108C, 114 T, 194G, 233A/C, 244A, 316A	36S, 38 L, 65S, 78D/A, 82 N, 106I
			NA3 (SH)	108C, 114 T, 194G, 233A, 244A, 316A	36S, 38 L, 65S, 78D, 82 N, 106I
			NA4 (HNA1-d)	108C,114 T,194G, 233A, 244A, 316A	36S, 38 L, 655S, 78A, 82 N,106I
			NA^{null}	—	—
CD177	*CD177*	CD177	NB1	787A	Full length
CD177 negative	*CD177* altered	NB2(?)*	NB2(?)* NB1-neg	787 T	264 (Truncated length)
CD177	*CD177* modified	NB1 soluble in plasma	NB1	1291G > A	Truncated at the GPI-binding site

**NB2 is suggested to be a truncated protein, but needs to be confirmed.*

Table 2.
Neutrophil-specific antigens alleles; nucleotide and amino acid differences.

determined by five nucleotides in positions 108, 114, 194, 244, and 316, and except for a silent position in position 114, the other SNPs result in the replacement in positions 36, 65, 82, and 106 of IgG-like domains distal to the cell membrane [33]. The presence of asparagine in position 65 is determinant for the formation of the NA1 epitope. For the formation of the NA2 epitope, the presence of amino acid Ser in position 36 and Asn in position 82 is necessary. Therefore, to predict the NA phenotype, it is necessary to determine the FCGRIIIB genotype in positions 108 and 244. Since no evidence exists regarding the involvement of amino acid 106 in the NA epitope formation, it does not necessary to determine the FCGRIIIB genotype in position 316 [33]. A polymorphism in nucleotide c.233 of *FCGR3B*02* is responsible for the generation of the third allele, *FCGR3B*03* known as NA3 (SH). This SNP changes the amino acid alanine in position 78 to Asp [13]. The glycoprotein encoded by the *FCGRB*03* allele bears both epitopes for NA2 and NA3. The fourth *FCGR3B* allele, *FCGR3B*04* encoded by *FCGR3B*01* is formed by an SNP c.316G > A, changing amino acids in positions p.106Val > Ile. NA4 epitope is coded by *FCGR3B*02*. Alloantibodies against NA4 were produced by individuals typed *FCGR3B*01/FCGR3B*03* (NA1/NA3) against NA2 coded by *FCGR3B*02*. Therefore, immunization against the NA4 is only formed in NA1/NA3 individuals against NA2 individuals [14]. NA1 and NA2 are different in molecular weight caused by disparate N-glycosylation sites: 4 glycosylation sites for NA1 and 6 glycosylation sites for NA2 [34]. In some individuals in the European population (about 0.15% of the normal population), the *FCGR3B* gene, is missing with no detectable FcγRIIIb on the neutrophil surface [35]. Accordingly, some individuals carry three copies of *FCGR3B* genes [36]. FcγRIIIb contributes to phagocytosis, elimination of immune complexes, and antibody-mediated cell cytotoxicity [37]. The co-localization of FcγRIIIb with CD11b/CD18 on lipid rafts participates in signal transduction involved in neutrophil activation and production of reactive oxygen species (ROS) [31]. A clinical study on individuals with NA^{null} phenotypes has found only four out of 21 subjects to develop frequent infections [38]. Although, NA^{null} individuals are mostly healthy without symptoms, their exposure

to NA positive cells during pregnancy, after transfusion or transplantation induces immunization and consequently the production of iso-antibodies against NA antigen (s). The clinical relevance of NA antigens other than ANN, AINI and autoimmunity include TRALI, febrile transfusion reactions and bone marrow transplantation incompatibility issues. NA2 in some populations has been documented as a susceptibility to the development of systemic lupus erythematosus (SLE) [39]. Also, NA antigens are known to be lost in Paroxysmal Nocturnal hemoglobinurea (PNH) [40], because of loss of GPI in PNH. A separate gene, *CD16A* discovered by Retch [32], produces another Fc-receptor that has medium affinity, and also binds IgG1 and IgG3 immunoglobulins. These two proteins (FcγRIIIa and FcγRIIIb) are highly homologous; however, *FCGR3A* codes a protein with 21 transmembrane extra amino acid and is longer than *FCGR3B* and is expressed on natural killer cells, a subset of monocytes, macrophages, and T-cells, but not on neutrophils [41]. The amino acid differences in the four NA expressing alleles are NA1 and NA2 differ in three amino acids, and NA3 and NA4 are variations of NA2. NC1, as previously described [11], has been reported to be related to, or identical to NA2 [42]. Neutrophil stimulation induces a proteolytic cleavage and releases membrane FcγRIIIb molecules to plasma [43].

3.2 NB antigen

NB1 [44, 45, 46], also known as CD177, is a cysteine-rich glycoprotein that expresses at the promyelocytic phase of neutrophil maturation [47]. This early expression on immature neutrophils causes the bone marrow of the newborns affected with NB1 alloimmune neonatal neutropenia, to show a "maturation arrest" profile, and even be misdiagnosed as acute leukemia. NB1 is anchored on the neutrophil membrane, and also mediates the expression of proteinase 3 (PR3), a serine protease enzyme, on the neutrophil membrane [48]. NB1 is also present in neutrophil secondary granules and intracellular vesicles. Stroncek and Skubitz determined that NB1 is a 58–65 KDa, GPI anchored glycoprotein [49, 50]. Not all circulating neutrophils in NB1-positive individuals carry the NB1 antigen on their surfaces [19, 51, 52]. This bimodal expression divides the neutrophils into NB1-positive and NB1-negative subpopulations in NB1-positive blood. Although, the percentage of NB1-positive neutrophils remains stable, infection, pregnancy, treatment with granulocyte-colony stimulating factor (G-CSF), and Polycythemia Rubra Vera (PRV) upregulate, CD177 expression [49, 53, 54]. CD177 is not detectable on neutrophils of 3–5% of normal populations. These individuals are defined as CD177-null. Kissel [55] sequenced the gene and determined it to be composed of 1614 base pair nucleotides, belonging to the urokinase-type Plasminogen Activator Receptor Superfamily (uPAR, CD59, Ly6), located on chromosome 19q13.3 [53]. The gene has 9 exons and a pseudogene composed of 6 exons derived from the original gene and is reversely positioned [52, 56–58]. A missense mutation in exon 7 of CD177 gene, c.787A > T replaces amino acid 263 with a stop codon and induces production of a truncated protein and consequently loss of CD177 expression on the neutrophil surface (**Table 2**) [57]. The stop codon responsible for the absence of CD177 protein on the neutrophil surface arises when exon 7 in the CD177 coding region is provided by the CD177 pseudogene, called CD177P1 [52]. The heritable ratio between CD177/CD177P1 determines CD177 high and low expression; individuals homozygous for the CD177 gene have higher CD177 expression whereas the existence of CD177P1 sequence in the CD177 gene leads to the presence of CD177 negative subpopulation [52]. However, in only 40% of CD177-null individuals, the presence of c.787 T homozygote is responsible for the absence of CD177 protein from the neutrophil surface [58, 59]. Later studies have added c.1291G > A SNP that affects the GPI anchor region of CD177 molecule and converts membrane-bound

to the soluble form of CD177. This polymorphism has been introduced as a genetic regulator for atypical/low expression that participates in the mechanism of CD177 deficiency. The combination of SNPs, c.787 T and c.1291A is responsible to regulate the presence of CD177 on the neutrophil surface [60]. A study on the epigenetic component that regulates CD177 expression, has documented a non-classical random monoallelic expression (MAE) on neutrophil subsets of CD177 positive individuals [61]. The complete absence of gene transcription (neither complete, nor truncated) in the CD177 negative neutrophil subpopulation has been introduced as a mechanism regulating the absence of CD177 protein on the CD177 negative subpopulation. However, later study analyzing mRNA content in sorted CD177 positive and negative subpopulations has shown the presence of CD177 mRNA in both neutrophil subsets and doubted the previous observation [59]. Additional interpretation on the potential role of c.787 T and c.1291 on NB system is presented in the NB2–dilemma subsection, separately.

The role of CD177 on neutrophil function is complicated and poorly understood. Physical association of CD177 with CD11b/CD18, concentrated on lipid rafts, support transduction of signals initiated after binding of anti-PR3 antibodies (such as Anti-Neutrophilic Cytoplasmic Autoantibody (ANCA)) on CD177/PR3 complex toward neutrophil cytoplasm and consequently leads to neutrophil activation, degranulation, and superoxide production [62]. The association of CD177 in ANCA-mediated neutrophil degranulation may explain the selective activation of CD177 positive neutrophil subsets involved in the mechanism of ANCA-associated vasculitis (AAV). Analysis has introduced an epitope formed by CD177/PR3 complex as a relevant epitope for CD177 autoantibodies but not isoantibodies. Interestingly this epitope is a signaling relevant epitope that upon binding of relevant antibodies induces neutrophil activation [63]. A previous study indicated CD177 as a heterophilic binding partner for platelet endothelial cell adhesion molecule 1 (PECAM-1). Inhibitory analysis has introduced PECAM-1 membrane-proximal domain 6 to mediate the binding of CD177. The later study however introduced PR3 as a binding partner for PECAM-1 but not CD177 alone [63]. The CD177/PECAM-1 interaction is considered to induce a signal in endothelial cells, destabilize VE-cadherin from the endothelial junction, and lead to the preferential trans-endothelial migration ability of CD177-positive neutrophils [64, 65]. The binding of monoclonal antibodies to CD177 (such as MEM166) enhances the expression of β2 integrins, activates CD177 positive neutrophils, raises neutrophil adhesion, and interrupts neutrophil's migratory ability [66]. This effect rather than direct CD177/PECAM-1 binding, explains a neutrophil adhesion via a CD177-driven pathway [67]. In CD177 deficient mice, besides a slight decrease in neutrophil counts, no defect in neutrophil function, chemotaxis, and clearance of bacterial infection was observed [68]. Although, no distinct difference between CD177 positive and negative neutrophil subsets have been described and both CD177 positive and null individuals are healthy, in multiple diseases the CD177 expression/upregulation has been introduced as a risk factor. In comparison to healthy donors, a higher proportion of CD177 positive neutrophils have been detected in patients with ANCA vasculitis [69]. The study documented a differential gene expression between CD177 negative and positive neutrophils; CD177 positive produces a lower level of pro-inflammatory cytokines and exhibits increased bactericidal activities such as ROS production and neutrophil extracellular trap (NET) [70]. A decreased percentage of CD177 positive neutrophils in Myelodysplastic Syndrome has been documented [71]. In contrast, in Kawasaki disease, an epigenetic hypomethylation and consequently increased CD177 gene transcription has been reported [72]. Beyond neutrophils, the presence of CD177 on epithelial cells, in cervical cancer, prostate cancer and ovarian cancer has been documented. Analysis of CD177 in mammary epithelial cells

revealed a strong CD177 expression on epithelial cells that was significantly reduced in paired cancer specimens [73]. These data suggest CD177 molecule as a cancer cell-intrinsic tumor suppressor and introduced this molecule as a potential good prognostic factor in different cancer types [73]. A recent study documented TRALI induction in a CD177 pre-immunized recipient after transfusion with packed red blood cells (PRBC) from CD177 positive donors, but not CD177 negative donors. In vitro analysis has documented the presence of soluble CD177/PR3 complex in plasma from CD177 donors that was significantly increased after PRBC filtration. The molecular mechanism regulating the secretion of CD177 in plasma is yet to be resolved. PECAM-1 on pre-activated endothelial cells absorbs soluble CD177/PR3 complex from plasma. Binding of CD177 isoantibodies to the absorbed CD177/PR3 complex on endothelial cells, induced endothelial barrier dysfunction implicated in the mechanism of anti-CD177 mediated reverse TRALI [74]. In severe cases of COVID-19 infections, progressive respiratory failure results from immunothrombosis that is driven by activated neutrophils and platelets. As an activation marker, CD177 molecules were upregulated in severe COVID-19 cases [75]. Further studies have documented a correlation between CD177 upregulation in the serum and disease severity and mortality in COVID-19 [76].

3.3 NB2 dilemma

The presence of a stop codon on the CD177 gene is shown to prevent NB1 biosynthesis [77]. Based on this observation, the NB-negative individuals are called NB^{null}. This interpretation also suggests that there is no allele to NB1. In contrast to this conclusion, a NB1 positive mother was reported to have a child with ANN and have a neutrophil-specific antibody that reacted only with NB1-negative neutrophils. This antibody could be absorbed only by NB1-negative neutrophils and was called anti-NB2, an allele to NB1 [78]. In this case, the maternal neutrophils were also found to be 9a-negative, which suggested a possible relationship between NB2 and 9a, an antigen previously described by van Rood. Also, examination of data from testing neutrophils from 76 members of 11 families, tested with anti-9a and anti-NB2 showed identical results in 72 of 76 donors (these results were obtained during 1967 HL-A workshop experiments in Torino, Italy). Anti-NB2 antibodies were also reported to cause febrile transfusion reaction [79] and TRALI [80]. Future studies are needed to confirm that NB2, an allele to NB1, exists or if its identification has been the result of serological errors. If confirmed, NB2 would be a truncated NB1 molecule in NB-negative individuals who carry the c.787 stop codon, which causes the absence of amino acids responsible for CD177 expression (**Table 2**). Therefore, NB2 will be CD177-negative but not NB^{null}. It should also be recognized that individuals with SNP c.1291 G > A expression have soluble NB1 antigens present in their plasma and not on their neutrophils, and thus will be classified as NB1 negative, however, this individual would not necessarily be NB2 positive.

4. The HLA and HNA nomenclature

In 1964, Bernard Amos of Duke University was appointed by the National Research Council to organize an international research team to identify the antigens involved in organ transplantation. Over a few years, different antigens were identified and were named 'HL-A' [81, 82], in which 'H' and 'L' were the terms used by different investigators for the antigens they had discovered, and 'A' was for the first locus identified. It was later recognized that the system was too complicated, and HL-A was changed to HLA to make place for other antigens and loci. Therefore, the

HLA is not "Human Leukocytes Antigens" nor is "Human lymphocyte Antigens," as used by the current literature. The HLA antigens are widely distributed in various tissues and not leukocyte-specific. It should also be noted that these antigens have their biological functions. and are not designed for organ transplantation that is a medical procedure. The term "HNA," proposed to be used for neutrophil-specific antigens, is a misinterpreted copy of HLA nomenclature and creates confusing issues: In the HNA nomenclature, the letter 'H' for human is wrong because these antigens are not specific for humans, and are present in various other animals, 'N' for neutrophil is wrong, because none of the antigens, HNA3, HNA4 nor HNA5, described in NHA systems are neutrophil-specific or cause ANN, AINI or neutropenia.

5. Basis of leukoagglutinin methodology

Many techniques are used for investigating neutrophils antigens [83, 84]. However, leukoagglutination is one of the most essential laboratory tests for neutrophil antibody detection. Unfortunately, some facilities avoid neutrophil agglutination testing because of the lack of reproducibility due to the use of inappropriate technology. The mechanism of neutrophil agglutination was investigated by time-lapse cinematography [85] and showed that after the addition of antibodies to neutrophil suspensions, a period of 5–10 minutes incubation at 37°C was required to activate the neutrophils. After this silent period, neutrophils develop many pseudopods and begin to move toward each other (agglutinate). This suggests that neutrophil agglutination is a chemotactic response and requires a biological environment. Damage to neutrophils during isolation, storage, presence of contamination, and centrifugation cause non-specific clumping. Another technical difficulty is the mixed agglutination that occurs when there are red cells (or red cell ghosts) in the cell preparation and red cell antibodies present. This red cell incompatibility, in the presence of complement, causes neutrophil stimulation to phagocytize the red cells, and or red cell ghosts, and form massive neutrophil aggregates. EDTA In the medium can prevent this process. Maintaining a highly viable environment for neutrophils is essential for the neutrophil agglutination test.

6. Conclusion

Neutrophil-Specific antigens are biological structures on blood neutrophils, the most frequent nucleated cells in blood circulation. Fetal-maternal incompatibility on these antigens causes neonatal neutropenia, and their autoimmunity results in neutropenia in children and adults. Also, the presence of antibodies against these antigens causes serious complications in blood transfusions, and incompatibility in bone marrow transplantation can cause graft rejection. Beyond antigenicity and immunological effects, these molecules have major roles on neutrophil functions: NA antigens connect neutrophils to antibodies to perform their phagocytic and other defense functions. The NB antigen interacts with Proteinase 3, Platelet-Endothelial Cell Adhesive Molecule 1 and other molecules to perform various neutrophil functions, including protein digestion and penetration across endothelial cells. This may be part of the mechanism of the development of serious lung injuries associated with COVID-19 infection and transfusion-related acute lung injury. It is also recognized that NB antigen expression is increased in Polycythemia Vera and in several cancer tissues. More investigation is needed to understand the significance of the appearance of NB on neoplastic tissues. This information would contribute to

the understanding of the development of malignancies, their progression, and lead to the development of new approaches for their treatment.

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Conflict of interest

The authors declare no conflict of interest.

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