Human neutrophil alloantigens systems

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ABSTRACT

Neutrophil alloantigens are involved in a variety of clinical conditions including immune neutropenias, transfusion-related acute lung injury (TRALI), refractoriness to granulocyte transfusions and febrile transfusion reactions. In the last decade, considerable progress has been made in the characterization of the implicated antigens. Currently, seven antigens are assigned to five human neutrophil antigen (HNA) systems. The HNA-1a, HNA-1b and HNA-1c antigens have been identified as polymorphic forms of the neutrophil Fcγ receptor IIb (CD16b), encoded by three alleles. Recently, the primary structure of the HNA-2a antigen was elucidated and the HNA-2a-bearing glycoprotein was identified as a member of the Ly-6/uPAR superfamily, which has been clustered as CD177. The HNA-3a antigen is located on a 70-95 kDa glycoprotein; however, its molecular basis is still unknown. Finally, the HNA-4a and HNA-5a antigens were found to be caused by single nucleotide mutations in the αM (CD11b) and αL (CD11a) subunits of the leucocyte adhesion molecules (β2 integrins). Molecular and biochemical characterization of neutrophil antigens has expanded our diagnostic tools by the introduction of genotyping techniques and immunoassays for antibody identification. Further studies in the field of neutrophil immunology will facilitate the prevention and management of transfusion reactions and immune diseases caused by neutrophil antibodies.

Key words: neutrophil antigens, blood transfusion, alloimmunization, transfusion reaction, neutropenia.

DEFINITION AND CLASSIFICATION

Since the beginning of the twentieth century, investigators have observed that the sera of some patients caused agglutination of leucocytes from others individuals. Granulocyte antibodies have been detected in sera of multitransfused persons, women after pregnancy, patients with neutropenia, patients with febrile transfusion reactions, and in the blood of donors that caused pulmonary transfusion reactions in the transfusion recipient. The first granulocyte-specific antigen was described in 1960 by Lalezari in a case of neonatal alloimmune neutropenia. Meanwhile, a number of granulocyte antigens has been described and characterized on the biochemical and molecular level, which allowed the development of assays for rapid antibody identification and DNA-based techniques for antigen typing (Bux et al. 1995).

Neutrophil antibodies have been shown to play a key role in the patophysiology of several clinical conditions, including neonatal alloimmune neutropenia (NAN), autoimmune neutropenia of childhood, febrile non-hemolytic transfusion reactions (FNHTR), transfusion-related acute lung injury (TRALI), immune neutropenia after bone-marrow transplantation, transfusion-related alloimmune neutropenia (TRAIN), drug-induced neutropenia, and refractoriness to granulocyte transfusion. The identification of neutrophil antigens and antibodies is relevant for the diagnosis of these disorders (Table I).
There are several clinically important human neutrophil alloantigen systems. The nomenclature used for these antigens was established in 1998 by an International Society of Blood Transfusion (ISBT) Working Party (Bux 1999) (Table II). The antigen systems are referred to as human neutrophil antigens (HNA). This nomenclature is based on the glycoprotein location of the antigens. Different polymorphisms of the same glycoprotein are designated alphabetically, in a sequential order of detection (HNA-1a, -1b, -1c) and the nomenclature of the alleles named according to the Guidelines of the International Workshop on Human Gene Mapping. Currently, the HNA systems comprise seven antigens, which are assigned to five glycoproteins (antigen systems).

**HNA-1**

**Biochemistry and Molecular Basis**

The best characterized neutrophil antigen system is HNA-1, which has three alleles, HNA-1a, -1b and -1c. HNA-1a and -1b were the first granulocyte antigens described in cases of alloimmune neonatal neutropenia (Lalezari et al. 1960). Later on, a third polymorphism was described, the SH antigen, now called HNA-1c (Bux et al. 1997b). HNA-1 alloantibodies can cause alloimmune neonatal neutropenia, TRALI and seem not to affect engraftment and neutrophil recovery in alloimmunized recipients of bone marrow transplants (Bux 2001) (Table III).

HNA-1 antibodies are located on the human neutrophil Fc gamma-receptor IIIb (FcγRIIIb) and encoded by the FCGRIIIb gene located on chromosome 1. FcγRIIIb belongs to the immunoglobulin superfamily, as it has two extracellular disulphide-bonded immunoglobulin G (IgG)-like domains. The membrane proximal domain contains residues which are critical for ligand binding (Hibbs et al. 1994), and the function of the distal domain is unknown, but it is quite polymorphic. Monoclonal antibodies reacting with FcRIIIb have been designated as CD16b. The FcγRIIIb is attached to the granulocyte membrane through a glycosyl-phosphatidylinositol (GPI) anchor that gives the receptor a high lateral mobility in the outer leaflet of the phospholipids bilayer.

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**TABLE I**

Clinical conditions associated with neutrophil antibodies.

<table>
<thead>
<tr>
<th>Alloimmune diseases</th>
<th>Autoimmune diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal alloimmune neutropenia (NAN)</td>
<td>Autoimmune neutropenia of childhood</td>
</tr>
<tr>
<td>Transfusion-related acute lung injury (TRALI)</td>
<td>Drug-induced immune neutropenia</td>
</tr>
<tr>
<td>Alloimmune neutropenia after bone marrow transplantation</td>
<td>Autoimmune neutropenia after bone marrow transplantation</td>
</tr>
<tr>
<td>Transfusion-related alloimmune neutropenia (TRAIN)</td>
<td></td>
</tr>
<tr>
<td>Refractoriness to granulocyte transfusions</td>
<td>Febrile transfusion reactions</td>
</tr>
</tbody>
</table>

**TABLE II**

ISBT Human neutrophil alloantigens (HNA) nomenclature.

<table>
<thead>
<tr>
<th>Antigen system</th>
<th>Carrier glycoproteins</th>
<th>CD</th>
<th>Antigens</th>
<th>Former names</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNA-1</td>
<td>Fcγ Receptor IIIb</td>
<td>CD16b</td>
<td>HNA-1a</td>
<td>NA1</td>
<td>FCGR3B*01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HNA-1b</td>
<td>NA2</td>
<td>FCGR3B*02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HNA-1c</td>
<td>SH</td>
<td>FCGR3B*03</td>
</tr>
<tr>
<td>HNA-2</td>
<td>NB1 glycoprotein</td>
<td>CD177</td>
<td>HNA-2</td>
<td>NB1</td>
<td>CD177*01</td>
</tr>
<tr>
<td>HNA-3</td>
<td>unknown (GP 70–95)</td>
<td>CD177</td>
<td>HNA-3a</td>
<td>5b</td>
<td>unknown</td>
</tr>
<tr>
<td>HNA-4</td>
<td>MAC-1; CR3; αMβ2-integrin</td>
<td>CD11b</td>
<td>HNA-4a</td>
<td>MART</td>
<td>ITGAM*01 (230G)</td>
</tr>
<tr>
<td>HNA-5</td>
<td>LFA-1; α1,β2-integrin</td>
<td>CD11a</td>
<td>HNA-5a</td>
<td>OND</td>
<td>ITGAL*01 (2372G)</td>
</tr>
</tbody>
</table>

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TABLE III

Clinical disorders caused by neutrophil specific antibodies.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clinical condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNA-1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alloimmune neonatal neutropenia</td>
</tr>
<tr>
<td></td>
<td>Autoimmune neutropenia</td>
</tr>
<tr>
<td></td>
<td>TRALI</td>
</tr>
<tr>
<td>HNA-2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alloimmune neonatal neutropenia</td>
</tr>
<tr>
<td></td>
<td>Autoimmune neutropenia</td>
</tr>
<tr>
<td></td>
<td>TRALI</td>
</tr>
<tr>
<td></td>
<td>Drug-induced neutropenia</td>
</tr>
<tr>
<td></td>
<td>Graft failure after bone marrow transplantation</td>
</tr>
<tr>
<td>HNA-3a</td>
<td>TRALI</td>
</tr>
<tr>
<td>HNA-4a</td>
<td>Alloimmune neonatal neutropenia</td>
</tr>
<tr>
<td></td>
<td>Autoimmune neutropenia</td>
</tr>
<tr>
<td>HNA-5a</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

of the plasma membrane (Huizinga et al. 1990a). The FcγRIIIb is a heavily glycosylated protein with different relative molecular weights of 50-65 kDa and 65-80 kDa for the HNA-1a and -1b isoforms, respectively (Ory et al. 1989).

FcγRIIIb is the clinically most important immunogenic glycoprotein of the neutrophil membrane. In addition, 30% of granulocyte autoantibodies recognize epitopes on the FcγRIIIb with a preferential binding to the HNA-1a polymorphic form of this receptor (Bux et al. 1997a). The glycoprotein is constitutively only expressed on neutrophils in mean copy numbers of 190,000 (range 120,000-400,000) (Huizinga et al. 1989).

The FCGR3B gene is located on the long arm of the chromosome 1 and consists of five exons with 699 bp encoding 233 amino acids, including a signal peptide of 17 amino acids. cDNA analysis of the gene revealed five nucleotide substitutions (nucleotides 141, 147, 227, 277 and 349) associated with the HNA-1a/b polymorphisms. All substitution sites are located in the third exon that codes for the membrane distal domain. The five nucleotide differences result in four amino acid substitutions (positions 36, 65, 82 and 106) with two additional N-linked glycosilation sites, so that the HNA-1b polymorphic form of the FCGR3B has six potential N-linked glycosilation sites, compared to four of the HNA-1a isoform. This explains the observed differences in their relative molecular weights (Ravetch and Perussia 1989).

An additional polymorphism of the FCGR3B is the HNA-1c antigen that has been associated with a later single nucleotide substitution in the allele encoding HNA-1b (Bux et al. 1997a). HNA-1c genetics is complicated by the fact that some of the HNA-1c-positive individuals, mainly Europeans, possess three FCGR3B genes in their genome with a HNA-1a and -1c allele combination on the chromosome (Koene et al. 1998). An unequal crossing-over event during meiosis may be the reason for FCGR3B gene duplication in which the allele encoding HNA-1a became associated with the HNA-1c allele (Steffensen et al. 1999).

In contrast to individuals with a hyperexpression of the FcγRIIIb, few individuals do not express this receptor on their neutrophils due to FCGR3B gene deficiency, showing the HNA-1 null phenotype (de Haas et al. 1995). Most of the FcγRIIIb deficient individuals do not suffer from repeated infections, autoimmune or immune complex diseases, but pregnant women can form FcγRIIIb-specific alloantibodies causing alloimmune neutropenia in the neonate (Huizinga et al. 1990b).

FUNCTION

The FcγRIIIb is a low affinity receptor for IgG1 and IgG3. It binds with its membrane proximal domain to the Fe parts of polymeric IgG antibodies. Resting neutrophils primarily engage FcγRIIIb for the binding of immune complexes and clearing them from the circulation. The receptor contributes also to the phagocytosis of opsonized micro-organisms (Bux 2008).

FREQUENCY

The HNA-1 frequencies vary widely among different populations (Kuwano et al. 2000, Kissel et al. 2000, Lin et al. 1994, Ohno and Matsuo 1989, Han and Um 1997). Using flow cytometry technique to phenotype randomly healthy Brazilian blood donors, we found a frequency of 65% and 83% for HNA-1a and HNA-1b, respectively (Norcia et al. 2006) (Table IV).

HNA-2

BIOCHEMISTRY AND MOLECULAR BASIS

The HNA-2a antigen was described as neutrophil-specific antigen ‘NB1’ by Lalezari and co-workers in 1971 (Lalezari et al. 1971). HNA-2a is exclusively expressed...
TABLE IV
Human neutrophil alloantigens (HNA) frequencies (%).

<table>
<thead>
<tr>
<th>Population</th>
<th>HNA-1a</th>
<th>HNA-1b</th>
<th>HNA-1c</th>
<th>HNA-1 null</th>
<th>HNA-2a</th>
<th>HNA-3a</th>
<th>HNA-4a</th>
<th>HNA-5a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africans</td>
<td>46–66</td>
<td>78–84</td>
<td>23–31</td>
<td>4</td>
<td>98</td>
<td>NT</td>
<td>NT</td>
<td>88</td>
</tr>
<tr>
<td>Chinese</td>
<td>90</td>
<td>52</td>
<td>0</td>
<td>0–0.2</td>
<td>99</td>
<td>NT</td>
<td>NT</td>
<td>65</td>
</tr>
<tr>
<td>Asian Indians</td>
<td>44</td>
<td>83</td>
<td>16</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Japanese</td>
<td>88</td>
<td>51–61</td>
<td>0</td>
<td>&lt; 0.4</td>
<td>89–99</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Koreans</td>
<td>78</td>
<td>75</td>
<td>&lt; 1</td>
<td>NT</td>
<td>86</td>
<td>NT</td>
<td>99</td>
<td>96</td>
</tr>
<tr>
<td>Europeans</td>
<td>54–52</td>
<td>87–89</td>
<td>5–7</td>
<td>0.2–0.8</td>
<td>87–97</td>
<td>89–99</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>North Americans</td>
<td>56–62</td>
<td>89</td>
<td>5</td>
<td>NT</td>
<td>97</td>
<td>NT</td>
<td>NT</td>
<td>96</td>
</tr>
<tr>
<td>Brazilians</td>
<td>100</td>
<td>83</td>
<td>11</td>
<td>NT</td>
<td>97</td>
<td>86–95</td>
<td>96</td>
<td>91</td>
</tr>
<tr>
<td>Brazilian Indians</td>
<td>83</td>
<td>36</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>100</td>
<td>96</td>
</tr>
</tbody>
</table>

NT: not tested.

on neutrophils and can be found on the plasma membrane and membranes of secondary granules and secretory vesicles (Goldschmeding et al. 1992). An unique characteristic of HNA-2a is its heterogenous expression, i.e. a single individual has a neutrophil subpopulation which expresses HNA-2a and another that does not. The mean proportion of neutrophils that express HNA-2a ranges from 0-100% and is slightly greater in females (63%) than in males (53%) (Matsuo et al. 2000, Moritz et al. 2007). The HNA-2a expression has been reported to drop in older women but not in men, suggesting that estrogen may influence the antigen expression. This is in accordance with the finding that the HNA-2a expression increases in pregnancy (Caruccio et al. 2003). HNA-2a-negative individuals and the negative neutrophil subset of HNA-2a-positive individuals show in fact a null phenotype since their neutrophils are deficient in the carrier glycoprotein (Kissel et al. 2002). The alloantibodies formed by HNA-2a-negative individuals are important in neonatal alloimmune neutropenia, TRALI, autoimmune drug-induced neutropenia, and graft failure after bone marrow transplantation.

HNA-2a has been characterized as a 56-64 kDa glycoprotein, which is like the FcyRIIIb linked to the cell membrane via a GPI anchor (Stroncek et al. 1990). Monoclonal antibodies directed against HNA-2a have been clustered as CD177. The glycoprotein has two cysteine-rich domains and three N-linked glycosylation sites. Homology of the cysteine-rich domains suggests that CD177 belongs to the Ly-6/uPAR/snake-toxin family of proteins (Kissel et al. 2001).

Kissel et al. (2001) sequenced the gene encoding HNA-2a and found it to be located on chromosome 19q13.2. In addition, there is a pseudogene homologous to exons 4 through 9 that is located adjacent to the HNA-2a gene, but oriented in the opposite direction. The HNA-2a cDNA consist of 1311 bp coding for 437 amino acids, including a signal peptide of 21 amino acids. The HNA-2a null phenotype was found to be the result of incorrect splicing, leading to mRNA strands containing intron sequences with stop codons.

FUNCTION
CD177 is involved in the adhesion of neutrophils to endothelial cells and their transendothelial migration by cationic-dependent interaction with the heterophilic domains of PECAM-1 (CD31) (Sachs et al. 2007). Recently, it has been demonstrated that the subset of neutrophils that express CD177 on their plasma membrane also displays the neutrophil serine protease proteinase 3 (mPR3), which is usually located intracellularly. The function of this co-expression of CD177 and mPR3 on the plasma membrane of a neutrophil subset is unknown (Bauer et al. 2007). A highly significant HNA-2a up-regulation was observed in patients with bacterial infections and polycythemia vera, as well as in stem cells donors stimulated with granulocyte colony-stimulating factor (Göhring et al. 2004).

FREQUENCY
HNA-2a is a high frequency antigen in North Americans, Europeans (97%) and Japanese (89-99.5%). Phenotyping studies performed in our laboratory employing flow cytometry have shown that the frequency of HNA-2a in Brazilians is similar to that described by North Ameri-
HUMAN NEUTROPHIL ALLOANTIGENS SYSTEMS

563


HNA-3

BIOCHEMISTRY AND MOLECULAR BASIS

HNA-3a antigen has been introduced by van Leeuwen et al. in 1964. It has been suggested that the antigen is located on a 70- to 95 kDa protein, which is not linked to the plasma membrane via a GPI anchor as the HNA-1 and -2 antigens (de Haas et al. 2000). Cyto-genetics studies suggested that the HNA-3a antigen is the product of a gene located on the chromosome 4. However, the primary structure of HNA-3 remains to be elucidated.

HNA-3a is expressed on neutrophils and lymphocytes, whereas the reported expression on platelets is controversial. Alloantibodies to HNA-3a were found in occasional cases of febrile transfusion reactions (Lalezari and Bernard 1965) and neonatal immune neutropenia (de Haas et al. 2000). HNA-3a alloantibodies were increasingly reported in conjunction with TRALI, especially with severe cases, in which patients required artificial ventilation, or with fatal reactions (Davoren et al. 2003). This is possibly the result of the neutrophil priming capacity for reactive oxygen species production, and probably the marked capability of the HNA-3a antibodies to agglutinate neutrophils.

FUNCTION

Nothing is known about the function of HNA-3a.

FREQUENCY

HNA-3a is a high frequency antigen with reported phenotype frequencies ranging from 89-99% in Europeans (van Leeuwen et al. 1964, de Haas et al. 2000, Lalezari and Bernard 1965). Using flow cytometry technique, we found a HNA-3a frequency of 95% in randomly investigated healthy Brazilian blood donors (Norcia et al. 2006) (Table IV).

HNA-4

BIOCHEMISTRY AND MOLECULAR BASIS

The HNA-4a antigen and the identification of HNA-4a-negative individuals were first reported in 1986 by Kline et al. The HNA-4 system is located on the β2-integrin, a member of the Leu-CAM family and integrin superfamily, which shares a common β subunit (β2 or CD18) noncovalently associated with four different α subunits. The HNA-4a antigen is a polymorphic variant of αM (CR3; CD11b) subunit as the result of a single nucleotide change G302A, leading to an arginine instead of a histidine at position 61 (Simsek et al. 1996). HNA-4a alloantibodies can cause neonatal immune neutropenia (Fung et al. 2003). Two types of HNA-4a antisera have been identified, with different effects in cell interactions, suggesting that the humoral response to this antigen is heterogeneous and differs from one immunized person to another (Sachs et al. 2004). The CD11b/CD18 is also the target of autoantibodies reported not only to cause neutropenia, but also to be able to impair neutrophil adhesion (Hartman and Wright 1991).

FUNCTION

CD11b/CD18, also known as Mac-1, CR3 or αMβ2-integrin, is expressed on neutrophils, monocytes and natural killer cells, and plays an important role in the leukocyte adhesion to endothelial cells and platelets, as well as in phagocytosis. It is not known whether the function of CD11b/CD18 is influenced by the HNA-4a polymorphism.

FREQUENCY

HNA-4a genotypes were found in > 90% of whites, Asians and Brazilians (Kline et al. 1986, Han and Han 2006, Cardone et al. 2006) (Table IV).

HNA-5

BIOCHEMISTRY AND MOLECULAR BASIS

The existence of a leukocyte non-HLA, now termed HNA-5a, defined by antibodies in a serum named ‘OND’, was reported by Decary et al. in 1979. HNA-5a has been located on the αL (CD11a; LFA-1) chain of the leukocyte β2-integrin family, and was found to be due to a G2466C substitution in the coding sequence predicting the amino acid change Arg776Thr (Simsek et al. 1996). HNA-5a alloantibodies have not yet been involved in neutropenia.

An Acad Bras Cienc (2009) 81 (3)
ELYSE MORITZ et al.

FUNCTION

The CD11a/CD18 complex, also known as LFA-1 or \( \alpha_M\beta_2 \) -integrin, is expressed on all leucocytes and function as a leucocyte adhesion molecule. It is unknown whether the integrin function is influenced by the HNA-5a polymorphism.

FREQUENCY

Genotyping resulted in HNA-5a frequencies between 79% and 88% in different populations (Sachs et al. 2005). We have found a higher frequency of HNA-5a positive genotype in Brazilian blood donors (91%), and Brazilian Amazon Indians (96%) (Cardone et al. 2006) (Table IV).

LABORATORIAL ASSAYS FOR DETECTING NEUTROPHIL ANTIGENS AND ANTIBODIES

PHENOTYPING AND GENOTYPING OF NEUTROPHIL ANTIGENS

Traditionally, neutrophil antigen phenotyping has been performed using human alloantibodies in the granulocyte agglutination test (GAT), or the granulocyte immunofluorescence test (GIFT). However, alloantisera specific to neutrophil antigens are not always available. Alloantibodies to HNA-1a, -1b, -2a, and -3a are available, but antibodies to HNA-1c, -4a, and -5a are difficult to obtain.

We have found that, when used in flow cytometry, the neutrophil alloantibodies allow a higher sensitivity rate do detect HNAs when compared to the GIFT (A.M. Norcia et al., unpublished data). The GAT and GIFT assays require training, as the results are evaluated with a microscope rather than by the flow cytometer.

Monoclonal antibodies specific to HNA-1a, -1b and -2a have been described, are commercially available, and have been used to phenotype neutrophils using flow cytometry. This method is faster and easier, since the assay can be done with the whole blood instead of isolated neutrophils.

Genotyping assays to HNA-1a, -1b, -1c, -4a and -5a have been developed. The characterization of the genes encoding the HNA-1 antigens has allowed for the development of genotyping assays for these antigens (Bux et al. 1995, Kissel et al. 2000). HNA-1a, HNA-1b and HNA-1c are encoded by FCGR3B*1, FCGR3B*2 and FCGR3B*3, respectively. FCGR3B*1 and FCGR3B*2 differ at 5 nucleotides, one of which is silent. One nucleotide differs between FCGR3B*2 and FCGR3B*3. Although distinguishing single nucleotide polymorphisms is usually simple, genotyping for FCGR3B alleles is complicated by the high degree of homology between FCGR3B and the gene that encodes F\( \gamma \)RIIIa, FCGR3A. Among the 5 nucleotides that differ between FCGR3B*1 and FCGR3B*2, FCGR3A is the same as FCGR3B*1 at 3 nucleotides and the same as FCGR3B*2 at 2 nucleotides (Stroncek 2004). As a result most laboratories, including ours, have employed PCR-SSP to distinguish FCGR3B alleles (Fig. 1).

![Fig. 1](image-url)
polymorphism. HNA-4a is due to a single nucleotide substitution in the α_M subunit of the β_2 integrin, G302A, which predicts an Arg61His amino acid polymorphism. HNA-5a is the result of a single nucleotide substitution in the α_L subunit of the β_2 integrin, G2466C, which predicts an Arg766Thr amino acid polymorphism. A variety of methods can be used to type these alleles. A PCR-SSP method is being used to type HNA-4a (Clague et al. 2003). HNA-5a can be typed using a PCR-SSP method or, alternatively, by a PCR-RFLP method described by our group (Cardone et al. 2006) (Fig. 2).

![Fig. 2](image)

Although the molecular basis of HNA-2a has already been described, HNA-2a genotyping methods are not available, yet. The HNA-2a-negative phenotype is due to CD177 mRNA splicing defects (Kissel et al. 2002). The CD177 mRNA from people with HNA-2a-negative neutrophils contains additional sequences of varying length that are homologous to CD177 intron sequences. However, no mutations have been detected in the CD177 introns or exons from people with a negative phenotype. It may be possible to distinguish the HNA-2a-positive from HNA-2a-negative phenotypes by analyzing neutrophil CD177 mRNA for accessory sequences, but working with mRNA is much more difficult than working with DNA, and no laboratory is currently analyzing granulocyte mRNA to assess HNA-2a antigen expression.

Because the gene encoding HNA-3a has not been identified, no genotyping assays is available for this antigen.

**Neutrophil Antibodies**

Screening for neutrophil antibodies remains technically challenging. No single technique has thus far been shown to consistently detect all clinically relevant granulocyte antibodies.

The assays used to detect neutrophil antibodies are GAT, GIFT or flow cytometry, and monoclonal antibody immobilization of granulocyte antigens (MAIGA). In the GAT, antibodies cause neutrophils to actively agglutinate (McCullough et al. 1988). It is a very reliable assay, but less sensitive than other methods. The GAT can detect antibodies to HNA-1, -2, -3, -4, and -5 antigens, and it is the assay that can best identify antibodies specific for HNA-3a.

In the microscopic GIFT, antigen-antibody reactions are detected using fluorescence-conjugated secondary antibodies and a fluorescent microscope (McCullough et al. 1988). Strong reactions are readily distinguished, but considerable training is required to distinguish weak reactions from background staining. Testing for neutrophil antibodies with flow cytometry is the same as the GIFT, except that neutrophils are evaluated with a flow cytometer rather than a fluorescence microscope.

The MAIGA assay allows the detection of antibodies to specific neutrophil membrane glycoproteins (Bux et al. 1993). It can be used to detect antibodies specific to HNA-1a, -1b, and -1c antigens on FcγRIIIb (CD16), An Acad Bras Cienc (2009) 81(3)
HNA-2a on NB1gp (CD177), HNA-4a on complement component C3bi receptor (CR3 or CD11b), and HNA-5a on leukocyte function antigen-1 (LFA-1 or CD11a). The use of neutrophils from panels of donors with known HNA-1 phenotypes allows the identification of antibodies specific to HNA-1a, -1b, and -1c. In addition, antibodies that are directed to FcγRIIIb, but are not specific to HNA-1 antigens, are sometimes detected. The MAIGA assay permits the recognition of antibodies to specific neutrophil glycoproteins even when antibodies to HLA antigens are present.

Because the gene encoding HNA-3a has not been identified, recombinant technology cannot be used to produce HNA-3a antigen. As a result, most laboratories are using intact neutrophils for antibody screening assays. Unfortunately, neutrophils have a short life span, so fresh neutrophils must be prepared from fresh whole blood using density gradient separation. Patient sera are tested against panels of neutrophils prepared from donors with known phenotypes to distinguish antibodies with different specificities. The presence of HLA antibodies can make the detection of neutrophil antibodies difficult. HLA-specific antibodies can be separated from neutrophil-specific antibodies by absorbing serum with platelets (Sachs et al. 2005). Alternatively, monoclonal antibody capture assays can be used to test for antibodies specific to neutrophil membrane glycoproteins. Mammalian cell expression systems have been used to express HNA-1a, HNA-1b and HNA-2a, and these can be used to assess antibodies in flow cytometry, but these cells are not commercially available.

**CONCLUSIONS**

During the last years, considerable progress has been made in the characterization of granulocyte antigens. Glycoprotein location of the antigens allowed the development of the antigen-specific MAIGA. Elucidation of their molecular basis now makes genotyping by PCR-SSP possible. However, our understanding of the biochemical and molecular nature of neutrophil antigens is still incomplete and many questions remain: the clinical significance of HNA-5 is not known, characterization of the HNA-1 group is not yet complete, detailed description of the HNA-2 antigen is ongoing, and characterization of the HNA-3a antigen is just beginning.

There are many barriers to neutrophil antibody testing, including limited availability of reagent, lack of commercially available test kits, and the need to use fresh neutrophils. However, neutrophil antibodies remain clinically important and there are only few laboratories specialized in granulocyte immunology; therefore, not all cases of alloimmune neutropenia are probably investigated. Further studies in the field of neutrophil immunology will improve our diagnostic tools, and will consequently facilitate the prevention and management of transfusion reactions and immune diseases caused by neutrophil antibodies.

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**RESUMO**

Os aloantígenos de neutrófilos estão associados a várias condições clínicas como neutropenias imunes, insuficiência pulmonar relacionada à transfusão (TRALI), refratariedade à transfusão de granulócitos, e reações transfusionais fébris. Na última década, foi observado considerável progresso na caracterização dos aloantígenos envolvidos nestas condições clínicas. Atualmente sete antígenos estão incluídos em cinco sistemas de antígenos de neutrófilo humano (HNA). Os antígenos HNA-1a, HNA-1b e HNA-1c foram identificados como formas polimórficas do receptor FcγRIIIb (CD16b), codificados por três alelos. Recentemente, a estrutura primária do antígeno HNA-2a foi elucidada e a glicoproteína carreadora do antígeno foi identificada como um membro da superfamília Ly-6/uPAR e designada como CD177. O antígeno HNA-3a está localizado em uma glicoproteína de 70-90 kDa, entretanto sua base molecular ainda é desconhecida. Finalmente, os antígenos HNA-4a e HNA-5a são resultantes de mutações de um único nucleotídeo nas subunidades αM (CD11b) and αL (CD11a) das moléculas de adesão de leucócitos (β2 integrinas). A caracterização molecular e bioquímica dos antígenos neutrofílicos permitiu a expansão das ferramentas diagnósticas pela introdução de técnicas de genotipagem e imunoensaios para a identificação de anticorpos. Novos estudos envolvendo a imunologia de granulócitos serão de grande valor para a prevenção e tratamento de reações transfusionais e doenças imunes causadas por aloantígenos de neutrófilos.

**Palavras-chave:** antígenos de neutrófilos, transfusão sanguínea, aloimunização, reação transfusional, neutropenia.
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An Acad Bras Cienc (2009) 81 (3)