


Molecular basis of human neutrophil antigen 2 (HNA-2) expression

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HNA-2 is a neutrophil-specific antigen located on GPI-anchored glycoprotein CD177. HNA-2 is absent from the neutrophil surface of 3–5% of normal individuals, known as HNA-2_{null} individuals. The exposure of HNA-2_{null} individuals to HNA-2-positive neutrophils during pregnancy, transfusion or transplantation induces immunization against HNA-2 and production of isoantibodies. These isoantibodies are involved in the mechanism of neonatal alloimmune neutropenia (NAIN), transfusion-related acute lung injury (TRALI) and graft rejection. The presence of CD177 on a neutrophil surface of HNA-2-positive individuals follows a bimodal expression that categorizes the circulating neutrophils into HNA-2-positive and HNA-2-negative subsets. The lack of HNA-2 (in HNA-2_{null} individuals) is associated with the presence of a missense mutation, CD177*c.787A>T in exon 7 (Lysine 263 Stop) of the CD177 gene. This mutation alone or in combination with CD177*c.997delG has been introduced as the main reason for the absence of CD177 in HNA-2_{null} individuals. A pseudogene (CD177P1) is located downstream of the CD177 gene. The conversion of exon 7 of CD177P1 into CD177 is responsible for the generation of the CD177*c.787A>T missense mutation. Genotyping has identified HNA-2_{null} individuals that are heterozygous for the CD177*c.787A>T mutation without CD177*c.997delG, indicating the presence of a complementary mechanism regulating CD177 expression. In HNA-2_{null} individuals and those with atypical CD177 expression, a CD177*c.1291G>A polymorphism in combination with CD177*c.787A>T has been recently described. Altogether, these data indicate a complex compound mechanism for the regulation of CD177 expression on the neutrophil surface. This manuscript will summarize recent findings on CD177 expression and highlights the CD177 functions on neutrophil surface.

Key words: alloimmunisation, expression, CD177, genotyping, human neutrophil antigen 2 (HNA-2).

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Introduction

The human neutrophil antigen 2 (HNA-2) epitopes are located on the CD177 protein, known as NB1. CD177 is a glycosyl-phosphatidylinositol (GPI)-anchored glycoprotein

that is exclusively expressed by neutrophils. HNA-2 was first identified in 1971 in a case of alloimmune neutropenia [1]. The absence of HNA-2 on the neutrophil surface of 3–5% of individuals in the healthy population is the reason for the iso-immunization against this protein upon exposure to HNA-2-positive cells (during pregnancy, transfusion or transplantation) [1,2].

Isoantibodies directed against HNA-2 are involved in transfusion-related acute lung injury (TRALI), neonatal alloimmune neutropenia (NAIN) and graft rejection [1,2]. CD177 is detectable during terminal neutrophil differentiation and peaked in band cells [3]. The CD177 expression on the neutrophils of adult individuals follows a bimodal

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expression that generates CD177⁺ and CD177⁻ subpopulations and remains constant in time.

The function of CD177 is mainly unknown; the trans-interaction of CD177 with PECAM-1 on the endothelial surface was previously proposed [4]. This heterophilic interaction leads to endothelial junction instability and is therefore involved in neutrophil transmigration through endothelial cells [5]. In the cis-interaction, CD177 interacts with proteinase 3 (PR3), a major antigen for the antineutrophil cytoplasmic autoantibody (ANCA). The neutrophil subpopulation expressing PR3 (PR3_{pos} subpopulation) is identical to the CD177_{pos} subpopulation [6,7]. PR3 interaction with CD177 is mediated by a hydrophobic patch on the c-terminus of the PR3 protein [8]. ANCA-induced neutrophil activation is the result of CD177/PR3 interaction with the CD11b/CD18 (Mac-1) complex on the neutrophil surface [9]. Co-localization of CD177/PR3 with CD11b/CD18 on the neutrophil surface provides this complex with signalling transduction abilities, therefore allowing its participation in neutrophil activation and degranulation in response to ANCA binding.

The current review aims to summarize recent findings on CD177 expression and functions and highlights the genetic regulation of CD177 expression on the neutrophil surface.

HNA-2 expression

The HNA-2 epitope is located on glycoprotein CD177, a (GPI)-anchored glycoprotein which belongs to the LY-6 family, and contains two cysteine-rich domains. Investigation using alloantibodies and monoclonal antibodies has identified CD177 as a protein of 58–64 kDa on the surface of neutrophils and also in secondary granules [10]. CD177 appears on the surface of myelocytes, metamyelocytes and neutrophils [11].

On the neutrophil surface of 3–5% of normal individuals, HNA-2 is not detectable; these are known as HNA-2_{null} individuals. The HNA-2_{null} individuals are healthy, with normal neutrophil function, suggesting the possible presence of an alternative protein that duplicates the function of HNA-2 on neutrophils [12]. In HNA-2-positive individuals, the bimodal expression of HNA-2 divides neutrophils to two HNA-2_{pos} and HNA-2_{neg} subpopulations. The percentage of CD177_{pos} neutrophils remains stable within an individual in physiological conditions. In women, the size of HNA-2_{pos} neutrophils is higher than in men [13]. Also, pregnancy and the administration of G-CSF enhance the proportion of HNA-2_{pos} neutrophils [11].

The CD177 gene

CD177 gene located on chromosome 19 contains 9 exons and two UTRs and coding for a protein of 437 amino

acids that carries the HNA-2 antigen. HNA-2 was originally identified in a case of neonatal neutropenia by Lalezari *et al.* [1]. The coding sequence of CD177 was later isolated from granulocytes of polycythemia patients and named PRV1 [14]. Shortly thereafter, in an independent work, Kissel and colleagues identified neutrophil-specific mRNA, named NB1 or HNA-2, which is highly homologous to PRV1 and is only different in sequences at positions 3, 119, 323 and 379; this suggested PRV1 as the allele for NB1 [15]. The CD177 gene is located on chromosome 19q13.31, where a pseudogene harbouring exons 4–9 was also identified in the telomeric region on the anti-sense strand [16].

Frame-shift mutations in CD177 and the insertion of intronic sequences that creates a premature stop codon in CD177 coding region have been considered as responsible mechanism for the absence of the protein on the surface of neutrophils in two women who have children with neonatal alloimmune neutropenia [17].

While several studies have analysed the molecular mechanism of CD177 regulation, the precise mechanism of CD177 expression is still unclear. However, several SNPs have been attributed to the regulation of CD177 expression on the neutrophil surface in different individuals [2,16,18]. Recently, the c.787A>T polymorphism (rs879198465) in CD177, which results in a premature stop codon, has been shown either in combination with c.997delG (compound heterozygote) or alone (homozygote) to be responsible for the absence of CD177 in HNA-2_{null} individuals [19]. The c.787A>T polymorphism resulted from a gene conversion of exon 7 from the CD177 pseudogene into the CD177 gene [20]. However, multiple studies have shown the c.787A>T variant in the heterozygous state in HNA-2_{null} individuals. Since the wild-type allele in c.787A>T heterozygous individuals is expected to be translated into the full protein, the absence of the CD177 protein in such individuals provides evidence for the presence of a complementary mechanism in addition to the c.787A>T SNP contributing to the regulation of CD177 expression [21].

Another polymorphism, c.1291G>A in exon 9 of the CD177 gene, has been associated with the absence of CD177 protein in c.787A>T heterozygous individuals, as well as in those with the atypical expression of the HNA-2 antigen on the neutrophil surface (three peaks: one negative and two positive peaks). In this study, the 787A-1291A (AA) haplotype could lead to low levels and/or the absence of HNA-2 expression in human subjects [22]. CD177 contains a short stretch of hydrophobic amino acids that forms the GPI signal. The polymorphism c.1291G>A (Gly431Arg) is located within the carboxy-terminal hydrophobic region of CD177. The amino acid change from glycine to arginine may affect the

hydrophobicity of CD177 GPI signal, leading to the destabilization of the CD177 protein from the neutrophil surface [22].

DNA methylation has been considered as an epigenetic regulatory mechanism for CD177 expression in physiological and pathological conditions [23]. Polymorphism in the promoter region or in the transcription factor binding sites has also been considered the reason for the absence of CD177 on the CD177_{neg} neutrophil subpopulation [18]. A recent study using the HeLa cell model showed an association of the CD177_{neg} fraction with CpG methylation in the *CD177* regulatory promoter that causes silencing of the *CD177* allele and leads to the monoallelic expression of CD177 [24].

In contrast, our recent analysis using sorted CD177_{neg} and CD177_{pos} neutrophils from a CD177-positive phenotyped donor revealed the presence of CD177 mRNA in both neutrophil subpopulations, indicating the presence of an active *CD177* gene in both neutrophil subpopulations, regardless of the CD177 phenotype. The sequencing of *CD177* mRNA showed identical sequences in both subpopulations, rejecting gene silencing as the mechanism.

All together, these data suggest the unrevealed multi-genetic factor mechanism for the regulation of CD177 expression on the surface of CD177_{pos} and CD177_{neg} neutrophils.

HNA-2 antibodies

Autoimmune neutropenia is known as the most frequent neutropenia in children. Autoantibodies against HNAs have been demonstrated in young children with chronic neutropenia [25]. CD177 autoantibodies are also involved in immune neutropenia cases [26]. The exposure of CD177_{null} individuals to CD177-positive cells during pregnancy, transfusion or transplantation induces immunization and consequently the production of anti-CD177 isoantibodies. The passive transfer of anti-CD177 isoantibodies from the mother through the placenta and sensitization of fetal neutrophils can cause the destruction of fetal neutrophils, resulting in neonatal alloimmune neutropenia (NAIN) [26]. Isoantibodies against CD177 have been identified in cases of TRALI [27,28]. In an *ex vivo* rat lung model, isoantibodies against CD177 were able to induce neutrophil activation and the production of reactive oxygen species (ROS) dependent on the density of the cognate antigen on the neutrophil surface [4]. Activated neutrophils have been considered to be responsible for endothelial barrier damage and consequently lung oedema [4]. However, multiple observations questioned the role of neutrophils as a sole inducer of lung injury implicated in TRALI [29–32]. How the CD177

isoantibodies affect the function of cells other than neutrophils and participate in the mechanism of TRALI remains unsolved.

HNA-2 interactions

The function of CD177 on neutrophils is mainly unknown. PR3 docking on the surface of neutrophils is largely mediated by CD177 [2,31]. Anti-PR3 in patients with antineutrophil cytoplasmic antibodies (ANCA) selectively binds CD177-positive neutrophils and induces neutrophil activation and degranulation (Fig. 1) [9]. Residue substitution of PR3 revealed a non-conserved hydrophobic patch (amino acids: 166, 218, 219 and 223) on PR3 as the docking site of this molecule on CD177. Conformational distortion of these hydrophobic patches triggers the rapid solubilization of PR3 and removal from the surface of CD177-transfected cells [8].

The analysis of lipid rafts and immunoprecipitation demonstrated the co-localization of CD177/PR3 with $\beta 2$ integrins, principally Mac-1 (CD11b/CD18), which mediates the transduction of signals initiated by anti-PR3 antibodies in the neutrophil cytoplasm [9]. The binding of antibodies against CD11b and CD18 inhibits neutrophil activation triggered by anti-PR3 antibodies [9] (Fig. 1).

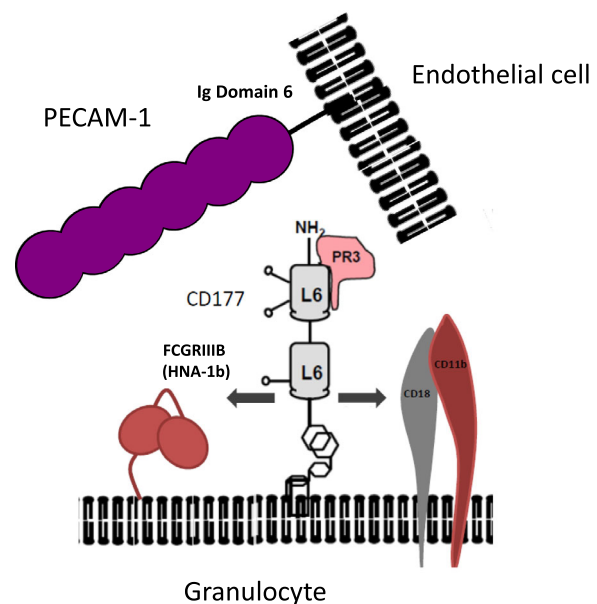


Fig. 1 In cis interaction, CD177 mediates membrane expression of PR3 and interacts with CD11b/CD18 and Fc γ RIIIB (HNA-1b) on the neutrophil surface. The interaction of CD177 with CD11b/CD18 supports transduction of signals triggered with antibodies that bind CD177/PR3 complex. In trans-interaction, CD177/PR3 complex interact with PECAM-1 immune globulin domain 6 on the endothelial surface.

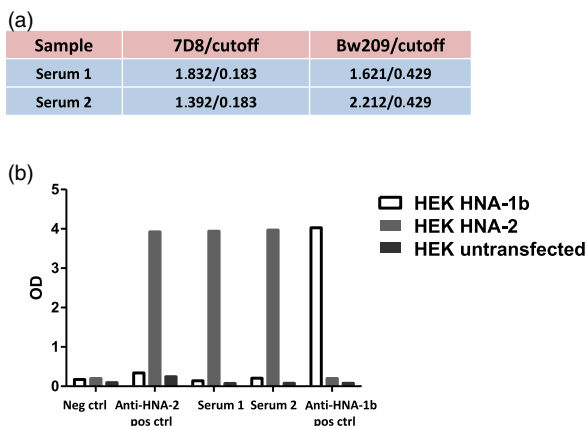


Fig. 2 Analysis of two serums contain anti-HNA-2 isoantibodies in monoclonal antibody-immobilized granulocyte/transfected cells antigen assay. On neutrophils surface CD177 interacts with Fc γ RIIIb (HNA-1b). This interaction leads to false-positive detection of some anti-HNA-2 isoantibodies in MAIGA (a). Analysis of two anti-HNA-2 positive sera, positive and negative controls (ab) in antigen-capture assay using HEK-transfected cells expressing HNA-2 or HNA-1b showed specific reaction of these serums with HNA-2 expressing cells. No reactivity of anti-HNA-2-positive serums with HNA-1b transfected cells was observed.

CD177 has been known as a heterophilic ligand for platelet cell adhesion molecule 1 (PECAM-1), a member of the immune globulin superfamily [4,33]. PECAM-1 immune globulin domain 6 mediates the interaction of CD177 with PECAM-1 on the endothelial surface. The *in vitro* interaction of CD177-positive neutrophils induces PECAM-1 dephosphorylation and endothelial junction instability that supports neutrophil migration [5]. Accordingly, CD177 ligation with the anti-CD177 antibody (MEM166) efficiently blocks the migration of CD177-positive neutrophils through the endothelial monolayer in a two-compartment chamber [4]. In contrast to the *in vitro* results, no migratory prevalence of CD177-positive over CD177-negative neutrophils was observed *in vivo*; CD177-positive and CD177-negative cells migrate equally to inflamed joints, the peritoneum and the oral cavity [34]. These controversial results introduce a complex role for CD177 in different pathways orchestrating the interactions of neutrophils with other cells *in vivo*.

This effect has been recently correlated with the dynamic co-operation between CD177 and Mac-1, which mediates β 2-dependent signalling and orchestrates a set of mechanisms and impairs neutrophil migration [34], therefore enhancing neutrophil adhesion to endothelial cells [35].

In serology analysis of anti-HNA-2 sera, the cross-reactivity of anti-HNA-2 isoantibodies with the immobilized HNA-1b (Fc γ RIIIb) antigen in an antigen-capture

assay has been observed (personal communications). Our study, using stable transfected cell expressing HNA-2 or HNA-1b in MAIGA, revealed the presence of only anti-HNA-2 in these sera, suggesting the possibility that CD177 undergoes cis-interaction with Fc γ RIIIb on the neutrophil surface as a mechanism responsible for false-positive reactivity of the serum containing anti-HNA-2 isoantibodies in HNA-1b MAIGA (Fig. 2). Such interactions on the neutrophil surface may lead to false-positive detection of anti-HNA-1b alloantibodies in antigen-capture assays such as the MAIGA.

Conclusion

The regulation of CD177 protein expression on the neutrophil surface follows a complex mechanism. At the gene level, nucleotide polymorphisms in the coding region of the *CD177* gene lead to the production of a truncated protein or alterations of the GPI-anchor sequence. In HNA-2_{null} individuals, these polymorphisms alone or in combination lead to the absence of the protein from the neutrophil surface and consequently HNA-2_{null} phenotype.

CD177 interacts with the partner proteins in two ways, *cis* and *trans*. In the *cis*-interaction with CD177, PR3 is presented on the neutrophil surface. The *cis*-interaction of CD177 with CD11b/CD18 conducts the signals initiated by the binding of antibodies to PR3 or the CD177/PR3 complex, towards the neutrophil cytoplasm, and therefore induce neutrophil activation and degranulation. The *trans*-interaction of CD177 with PECAM-1 mediates cross-talk between neutrophils and endothelial cells implicated during neutrophil transmigration.

The transcription products of the *CD177* gene on the neutrophil surface may serve as target in neutrophil specific therapeutic strategies. Moreover, CD177 interactions may be of relevance to suppress neutrophil activation in pathological conditions such as vasculitis.

Conflict of interests

The author declares no conflict of interests.

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