CD177: A member of the Ly-6 gene superfamily involved with neutrophil proliferation and polycythemia vera

David F Stroncek*, Lorraine Caruccio and Maria Bettinotti

Address: From the Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD, USA
Email: David F Stroncek* - Dstroncek@cc.nih.gov; Lorraine Caruccio - Lcaruccio@cc.nih.gov; Maria Bettinotti - Mbettinotti@cc.nih.gov
* Corresponding author

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Abstract

Genes in the Leukocyte Antigen 6 (Ly-6) superfamily encode glycosyl-phosphatidylinositol (GPI) anchored glycoproteins (gp) with conserved domains of 70 to 100 amino acids and 8 to 10 cysteine residues. Murine Ly-6 genes encode important lymphocyte and hematopoietic stem cell antigens. Recently, a new member of the human Ly-6 gene superfamily has been described, CD177. CD177 is polymorphic and has at least two alleles, PRV-1 and NB1. CD177 was first described as PRV-1, a gene that is overexpressed in neutrophils from approximately 95% of patients with polycythemia vera and from about half of patients with essential thrombocytemia. CD177 encodes NB1 gp, a 58–64 kD GPI gp that is expressed by neutrophils and neutrophil precursors. NB1 gp carries Human Neutrophil Antigen (HNA)-2a. Investigators working to identify the gene encoding NB1 gp called the CD177 allele they described NB1. NB1 gp is unusual in that neutrophils from some healthy people lack the NB1 gp completely and in most people NB1 gp is expressed by a subpopulation of neutrophils. The function of NB1 gp and the role of CD177 in the pathogenesis and clinical course of polycythemia vera and essential thrombocytemia are not yet known. However, measuring neutrophil CD177 mRNA levels has become an important marker for diagnosing the myeloproliferative disorders polycythemia vera and essential thrombocytemia.

Introduction

CD177 is an important neutrophil gene that encodes the neutrophil membrane glycoprotein (gp) NB1. NB1 gp has been studied for more than 20 years and during that time several different names have been used to describe this gp, its antigens, and the gene that encodes this molecule. NB1 was first described by Lalezari and colleagues while investigating a case of neonatal alloimmune neutropenia [1]. Occasionally, during pregnancy, a mother produces alloantibodies to neutrophil antigens than cross the placenta, react with neutrophils in the fetus, and cause the neonate to become neutropenic. One antigen recognized by such antibodies was described as “NB1” by Lalezari in 1971 [1]. Later, this antigen was renamed as Human Neutrophil Antigen-2a (HNA-2a) and the gp carrying this antigen was called NB1 gp [2]. Monoclonal antibodies specific for NB1 gp have been produced and clustered as CD177 [3]. In 2001 Kissel and colleagues sequenced the gene encoding NB1 gp and called the gene NB1 [4]. However, this gene was highly homologous to a gene called PRV-1 that had been sequenced the year before. Temerinac and colleagues identified and sequenced PRV-1 in 2000 while searching for genes overexpressed in neutrophils from patients with polycythemia vera [5]. The
coding regions of NB1 and PRV-1 differ at only 4 nucleotides that result in amino acid changes and Caruccio, Bettinotti, and colleagues have shown that PRV-1 and NB1 are alleles of a single gene which in this review is referred to as CD177 [6-8] (Table 1).

**CD177, NB1 glycoprotein, and neutrophils from healthy subjects**

**NB1 glycoprotein**

NB1 gp has been studied for many years since it carries the neutrophil alloantigen HNA-2a. Investigations with alloantibodies and monoclonal antibodies revealed that NB1 gp has a mass of 58 to 64 kDa on analysis by SDS-PAGE and 50.5 kDa as determined by MALDI-TOF mass spectrometry [4] (Table 1). It is a glycosyl-phosphatidylinositol (GPI) anchored gp that is found on neutrophil plasma membranes and secondary granules [9,10]. NB1 gp contains N-linked carbohydrate side chains but not O-linked carbohydrates. NB1 gp is a neutrophil-specific protein in that it is expressed by neutrophils, neutrophilic metamyelocytes, and myelocytes, but not by any other blood cells [11].

**Structure of CD177**

CD177 belongs to the Leukocyte Antigen 6 (Ly-6) supergene family and is located on chromosome 19q13.2 [4-6]. It has 9 exons and an open reading frame of 1311 bp and encodes 437 amino acids with an N-terminal signal sequence of 21 amino acids [4-6]. The predicted structure of the encoded protein is consistent with NB1 gp. The predicted protein has 3 N-glycosylation sites and a hydrophobic C terminus with a GPI attachment (o). The predicted molecular mass of the protein is 44.2 kDa [4]. The predicted protein has two highly homologous cysteine-rich domains of 188 amino acids. Each domain has 6 cysteine residues. Immediately adjacent to CD177 is a pseudogene that is highly homologous to exons 4 through 9 of CD177 in tail to head orientation [6].

**Heterogenous neutrophil expression of NB1 gp**

NB1 gp is unusual in that it is expressed on subpopulations of neutrophils. The mean size of the NB1 gp-positive subpopulation of neutrophils is 45% to 65% [12,13] and it ranges from 0% to 100%. Estrogen and possibly progesterone seem to affect the expression of NB1 gp. The expression of NB1 gp is greater on neutrophils from women than men [13]. The size of the NB1 gp-positive subpopulation of neutrophils from women is approximately 49% to 59% compared to approximately 42% to 43% for men. The expression of NB1 gp falls with age in women, but remains constant in men [13]. Neutrophil expression of NB1 gp is even greater in pregnant women than in healthy female blood donors. Approximately 67% to 70% of neutrophils from pregnant women express NB1 gp [14]. Interestingly, neutrophil counts are also greater during pregnancy. The administration of G-CSF to healthy subjects for several days increases the proportion of neutrophils expressing NB1 gp to nearly 90% by an unknown mechanism [15].

The absence of NB1 gp expression by subpopulations of neutrophils is due to the lack of CD177 mRNA transcription. A comparison of CD177 mRNA between NB1 gp expressing and non-expressing neutrophils from the same people revealed that CD177 mRNA was absent from neutrophils that did not express NB1 gp [16]. In addition, one day after the administration of 5 µg/kg of body weight of G-CSF to healthy subjects the size of the NB1 gp positive neutrophil population did not change and CD177 mRNA remained absent from NB1 gp negative neutrophils, however CD177 mRNA levels increased 1000-fold in NB1 gp expressing neutrophils.

**NB1 gp deficient neutrophils**

NB1 gp is absent from all neutrophils in some healthy people. Analysis of neutrophils from these NB1 gp deficient people with several different monoclonal and alloantibodies specific to NB1 gp have found that their membranes lack the entire NB1 gp. Approximately 3% of Caucasians, 5% of African Americans, and 1% to 11% of Japanese have NB1 gp deficient neutrophils [13,17,18].

One of the causes of NB1 gp deficient neutrophil phenotype is a CD177 mRNA splicing defect [19]. CD177 mRNA was analyzed from two NB1 gp deficient people, and although CD177 mRNA was present in both, frame shift mutations in CD177 mRNA were detected [19]. Insertions of intron sequences that created stop codons were found. The deduced protein in both people lacked transmembrane segments and GPI linkage sites. No NB1 gp or pro-

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**Table 1: Characteristics of CD177 and the NB1 glycoprotein it encodes**

| CD177 |  |
|---|---|
| Location | 19q13.2 |
| Alleles | PRV-1, NB1 |
| Expressed by | Neutrophils |
| Homologous genes | uPAR (CD87), CD59 |

| NB1 gp |  |
|---|---|
| Mass | 58 to 64 kDa |
| Number of amino acids | 416 |
| Protein type | Glycosylphosphatidylinositol anchored |
| Glycosylation | N-linked side chains |
| Neutrophil location | Plasma membranes and secondary granules |
| Epitope | HNA-2a |
tein fragments were detected on neutrophils or in their plasma [19]. People with NB1 gp deficient neutrophils are healthy, but too few have been studied to determine if the absence of this protein has a subtle effect on neutrophil counts, neutrophil function, host defense, or host response to inflammation.

The expression of NB1 gp is also absent from neutrophils from people with paroxysmal nocturnal hemoglobinuria (PNH) and in many people with CML [10,11]. NB1 gp is absent on neutrophils from people with PNH since it is a GPI-anchored protein and GPI anchored proteins are absent from blood cells of people with PNH. It is not known why some patients with CML do not express NB1 gp. It is not known if the lack of expression of NB1 gp on neutrophils from patients with PNH or CML has any clinical significance.

**CD177 polymorphisms**

Several polymorphisms of CD177 have been described. The most common allele of CD177 is the allele that Temerniac et al described as PRV-1 [5]. Kissel and colleagues described a second allele, NB1 [4]. The PRV-1 and NB1 alleles differ at only 4 bp that result in amino acid changes [4]. These single nucleotide polymorphisms are a G to C change of bp 42, a C to T change at bp 390, a G to A change at bp 1003, and a T to C change at 1171. Initially it was not appreciated that PRV-1 and NB1 were alleles of the same gene. Bettinotti and colleagues used Human Genomic Project databases to characterize the structure of the PRV-1 and NB1 genes [6]. They described the intron and exon structure of PRV-1, but they found only one gene homologous to both PRV-1 and NB1 suggesting that they are alleles of the same gene that is now called CD177. In addition, they found a pseudogene homologous to exons 4 through 9 of CD177 [6].

The most common polymorphism in CD177 is a single nucleotide G to C change at bp 42 that results in an amino acid substitution in the protein signal sequence. This polymorphism is present in approximately 40% of healthy subjects [7]. It appears that this polymorphism is associated with an increase in size of the neutrophil population that expresses NB1 gp. Caruccio and colleagues found that the size of the NB1 gp expressing neutrophil population in 42 G homozygous individuals was 41% compared to 66% in 42 C homozygous individuals [7]. They also identified additional single nucleotide polymorphisms (SNPs) predicted to result in amino acid substitutions including A to T at bp 123, G to A at bp 145, G to A at bp 1077 and A to T at bp 1099 [7]. However, since these SNPs were present in only about 10% of subjects it could not be determined if they affected NB1 gp expression. Wolff and colleagues also found that G42C was associated with the size of the neutrophil population expressing NB1 gp [16].

In addition, they found that SNPs at 786 and 1077 were associated with changes in the size of the neutrophil population expressing NB1 gp. Wolff and colleagues sequenced the CD177 promoter region from the beginning of exon 1 and prior to 162 bp upstream, but no polymorphisms were found [16]. While SNPs likely contribute to the variable expression of NB1 gp, other mechanisms that have not yet been identified are also likely involved with the heterogenous expression of NB1 gp.

**Increased neutrophil CD177 mRNA levels in infection and inflammation**

Increases in neutrophil CD177 mRNA levels are seen in clinical conditions associated with increased neutrophil production such as people with severe infections or burns and in healthy subjects given G-CSF [16,20]. In patients given 10 µg/kg of G-CSF twice daily over 4 days CD177 mRNA levels increased markedly [5]. Neutrophil CD177 mRNA levels are not increased in patients with chronic myelogenous leukemia or acute myelogenous leukemia [5].

**Function of CD177**

People with a NB1 gp null phenotype are healthy and the function of their neutrophils is normal. These results suggest the function of NB1 gp may be duplicated by another protein. One study suggests that NB1 gp has a role in the adhesion of neutrophils to endothelial cells [21]. The neutrophil protein that is most similar in structure to NB1 gp is urokinase type plasminogen activator (uPAR or CD87). uPAR has a number of roles in cell function. Interestingly, it is involved in leukocyte adhesion and adhesion to marrow stroma [22]. It is not known if NB1 gp affects adhesion to marrow stroma.

**CD177 and polycythemia vera**

**Polycythemia vera**

Polycythemia vera is a myeloproliferative disorder. In addition to polycythemia vera, traditional classifications of myeloproliferative disorders include essential thrombocythemia, chronic myelogenous leukemia (CML), and idiopathic myelofibrosis. All four disorders are clonal hematopoietic progenitor disorders involving erythropoiesis, myelopoiesis, and thrombopoiesis. The predominant characteristic of polycythemia vera is the overproduction of red blood cells. Essential thrombocythemia is characterized best by the overproduction of platelets. In CML granulocytes are overproduced. In idiopathic myelofibrosis marked fibrosis of the bone marrow is present. The molecular abnormalities in CML are well characterized. CML is characterized by the Philadelphia chromosome which is due to a reciprocal translocation between chromosomes 9 and 22, t(9;22)(q34;q11.2). In
contrast, until recently little was known of the molecular basis of the other myeloproliferative disorders [23-25].

The incidence of polycythemia vera in North America is approximately 2 per 100,000. Polycythemia vera is most common in patients greater than 40 years of age and the median age at diagnosis is 60 years. Polycythemia vera is slightly more common in men than women [25]. The predominant clinical feature of polycythemia vera is the overproduction of red blood cells and increased red blood cell mass. Other clinical features of polycythemia vera include increased platelet counts, increased neutrophil counts and splenomegaly. Neutrophil counts are not, however, increased to the same degree as the red cell mass and platelet counts.

The growth of hematopoietic progenitors from patients with polycythemia vera is abnormal. In healthy subjects the growth of erythroid colonies from the blood or bone marrow requires the addition of growth factors including erythropoietin. Erythroid colonies from patients with polycythemia vera are hypersensitive to erythropoietin and a number of other hematopoietic growth factors. Erythroid colonies from patients with polycythemia vera form in culture in the absence of the addition of exogenous erythropoietin. These seemingly growth factor independent colonies are known as erythropoietin independent erythroid colonies.

**Polycythemia vera and neutrophil overexpression of CD177**

Several chromosomal abnormalities have been described in patients with polycythemia vera, but most are found in 30% or less of patients. No one chromosomal abnormality has been found in all patients, but recently a molecular abnormality has been found in nearly all patients with polycythemia vera. Several studies have found that 95% to 100% of patients with polycythemia vera have markedly elevated levels of neutrophil CD177 mRNA [5,26-30]. Temerinac and colleagues compared genes expressed by neutrophils from patients with polycythemia vera with those expressed by neutrophils from healthy subjects [5]. They used subtractive hybridization to clone complementary DNA (cDNA) of genes that were overexpressed or underexpressed by neutrophils from patients with polycythemia vera in comparison to healthy individuals. They found that only one gene was strongly overexpressed in the polycythemia neutrophils, CD177. They tested 19 patients with polycythemia vera and found high numbers of neutrophil CD177 mRNA in all 19, but neutrophil CD177 mRNA was nearly absent from neutrophils from all 21 healthy subjects tested. Temerinac also studied neutrophils from a small number of patients with essential thrombocythemia and idiopathic myelofibrosis and some were also found to have increased neutrophil CD177 mRNA levels [5].

Several other studies have confirmed the overexpression of neutrophil CD177 mRNA in polycythemia vera, and two other myeloproliferative disorders, essential thrombocythemia and idiopathic myelofibrosis [5,26-31] (Table 2). The number of patients expressing increased quantities of CD177 mRNA varies among the disease type and among studies. The proportion of patients with elevated CD177 mRNA levels is greatest in those with polycythemia vera and least in those with idiopathic myelofibrosis. Elevated CD177 mRNA levels have been found in 90% to 100% of patients with polycythemia vera, 30% to 50% of patients with essential thrombocythemia, and 10% to 30% of patients with idiopathic myelofibrosis. Patients with essential thrombocythemia and increased levels of neutrophil CD177 mRNA are at increased risk of thromboembolic and major bleeding complications compared to essential thrombocythemia patients with normal CD177 mRNA levels [32]. It is not known if increased expression of CD177 mRNA levels are directly responsible for the increased risk of vascular complications or if increased CD177 mRNA levels are simply a marker of a subset of patients with more severe disease.

Some of the differences in the proportion of polycythemia vera patients with elevated CD177 mRNA levels may be due to variations in treatment. The treatment of polycythemia vera by phlebotomy or hydroxyurea has no effect on neutrophil CD177 mRNA levels, however, CD177 mRNA levels are reduced by interferon-α treatment. Fruehauf and colleagues monitored four patients with polycythemia vera who had elevated CD177 mRNA.

### Table 2: Proportion of patients with myeloproliferative disorders who have elevated neutrophil CD177 mRNA levels

| Disorder                      | Increased with CD177 levels (%) | References          |
|-------------------------------|---------------------------------|---------------------|
| Polycythemia vera             | 95%, 69%, 91%, 100%             | [5,26,29,31]        |
| Essential thrombocythemia     | 21%, 100%, 17%, 67%, 33%        | [5,26,29-31]        |
| Idiopathic myelofibrosis      | 67%                             | [26]                |
| Chronic myelogenous leukemia  | 0%                              | [5]                 |
levels during interon-α treatment and found that CD177 mRNA levels fell to normal within six months in all four patients [28].

Patients with secondary erythrocytosis have increased hemoglobin levels but they do not have hematopoietic stem cell abnormalities. Neutrophils from patients with secondary erythrocytosis do not have increased levels of neutrophil CD177 mRNA [5,27]. As a result the measurement of CD177 mRNA levels has become a useful diagnostic tool for distinguishing polycythemia vera from secondary erythrocytosis.

While CD177 is almost universally overexpressed in polycythemia vera, the mechanisms of overexpression are not known. No abnormalities in CD177 have been found in polycythemia vera. Analysis of the structure of CD177 in patients with polycythemia vera by Southern blotting and fluorescence in situ hybridization (FISH) did not reveal any abnormalities in the CD177 gene. FISH analysis of CD177 from bone marrow cells from 26 patients with polycythemia vera did not reveal any deletions, translocations, or insertions of CD177 [33]. Southern blotting analysis of neutrophil DNA from eight patients with polycythemia vera did not reveal any gross abnormalities [34]. It is not known if single nucleotide polymorphisms (SNPs) in CD177 are more common in patients with polycythemia vera.

**NB1 glycoprotein expression in polycythemia vera**

NB1 gp is the neutrophil protein encoded by CD177. Although the expression of CD177 mRNA is markedly elevated in neutrophils from patients with polycythemia vera, the quality of NB1 gp expressed by neutrophils from polycythemia vera patients is similar to healthy subjects [34].

**Ly6/uPAR gene family**

The Ly-6 gene superfamily is also known as the uPAR or snake toxin family. This superfamily is characterized by conserved cysteine rich domains. Typically these domains contain 70 to 100 amino acids including eight to ten cysteine residues spaced at conserved distances. The Ly-6 superfamily includes two subfamilies. One subfamily encodes GPI-anchored glycoproteins and the other subfamily encodes secretory proteins without a GPI anchor. In general, the GPI-anchored Ly-6 proteins have domains with ten cysteines and the secretory proteins have eight. The protein encoded by CD177, NB1 gp is an exception in that it is GPI anchored, but it has only six cysteine residues in its cysteine rich domains. Most Ly-6 proteins have one cysteine rich domain. Two exceptions are uPAR which has three cysteine rich domains and NB1 gp which has two [22]. Members of this family tend to have little homology. At most, 20% to 30% of amino acids are conserved among members. The functions of these proteins are diverse, but not well understood.

Ly-6 genes were first described in mice. They were found to be expressed by subpopulations of murine lymphoid and myeloid cells and are now widely used as markers of murine T cell differentiation and hematopoietic stem cells [35-38]. Ly-6A/E (Stem cell antigen 1 or Sca-1) is used as a marker of hematopoietic precursor cells. All murine hematopoietic stem cells express Sca-1. In addition, Sca-1 may be important in murine T cell activation. Ly-6B is a marker of immature thymocytes and myeloid cells and may play a role in T cell costimulation. Ly-6C is a marker of peripheral blood T cell activation. Although the exact functions of these molecules are not known, they likely play roles in signal transduction and cell adhesion.

Several Ly-6 superfamily genes in addition to CD177 have been found in humans (Table 3). Human Ly-6 genes that encode GPI-anchored proteins with a single cysteine rich domain include CD59, Sperm Acrosomal Membrane-Associated Protein 14 (SAMP14), prostate stem cell antigen (PSCA), RIG-E, GML, LYGH, E-48, Ly-6K, Secreted Ly-6/uPAR-Related Protein 1 (SLURP-1), SLURP-2 and SP-10. Two human Ly6 genes are secreted proteins, SLURP-1 and SLURP-2. CD177 is most similar to uPAR, but these two proteins are only 23% homologous. Most Ly-6 genes are found in one of three regions of the human genome: 19q13.3, 8q24, and 6p21.3.

Among the LY-6 genes found in humans, CD59 and uPAR are best described. CD59 or membrane inhibitor of reactive lysis is an important red blood cell membrane molecule that inhibits complement mediated hemolysis. It inhibits the terminal step of complement activation cascade by preventing the binding of C9 to C5b-8. As a result CD59 prevents the formation of the polymeric membrane attack complex (MAC) and protects cells from MAC induced lysis. CD59 is expressed by erythrocytes and leukocytes. CD59 is an 18-kDa GPI-anchored cell membrane glycoprotein [39]. CD59 gene has been localized to chromosome 11p13 [40].

uPAR is a high affinity receptor for urokinase-type plasminogen activator (uPA). uPAR is located on chromosome 19q13.3 near CD177. The uPAR gene encodes 335 amino acids including a signal sequence of 33 amino acids [22]. It is an approximately 55 kDa GPI-anchored membrane glycoprotein and its protein backbone is approximately 35 kDa. uPAR is expressed by neutrophils, macrophages and their precursors. Antibodies specific for uPAR cluster as CD87.

uPAR is an important activator of the proteolytic enzyme plasmin [22]. When uPAR binds pro-uPA trace amounts
of plasmin convert pro-uPA to uPA. The membrane associated uPA bound to uPAR then converts large quantities of plasminogen to plasmin, a proteolytic enzyme with degrades fibrin.

uPAR effects cell function in several ways. It plays a role in cell-cell and cell-extracellular matrix adhesion [22]. uPAR affects cell adhesion by binding the extracellular matrix molecule vitronectin or cell membrane adhesion molecules β1 and β2 integrins. uPAR plays a role in cell migration. In migrating monocytes and neutrophils, membrane bound uPAR is redistributed to the cell's leading edge. Antibodies to uPAR inhibit monocyte and neutrophil chemotaxis. uPAR may play an indirect role in myelopoiesis by activating uPA and plasmin which may release or activate cytokines or cytokine precursors sequestered in the extracellular matrix or bound to cell membranes [22].

SAMP14 is a Ly-6 gene that is expressed only in testis [41]. It has been localized to the outer and inner acrosomal membranes and acrosome matrix of sperm. SAMP14 may have a role in sperm-egg interactions. The gene is located on chromosome 19q13.33 and the protein is predicted to have a cysteine rich domain, be GPI-anchored and about 14 kDa.

Table 3: Human genes belonging to Leukocyte Antigen 6 (Ly-6) gene super family

| Gene      | Cellular expression | # Cysteine domains | Type of protein   | Comments                                                                 | Location     |
|-----------|---------------------|--------------------|-------------------|--------------------------------------------------------------------------|--------------|
| CD59      | Erythrocytes        | 1                  | GPI-anchored      | Protects cells from membrane attack complex induced lysis                | 11p13        |
| PSACA     | Prostate            | 1                  | GPI-anchored      | Marker of prostate cancer and increase prostate cell division            | 8q24.2       |
| SLURP-1 (ARS B) |                | 1                  | Secreted          | Mutations are associated with Male de Meleda syndrome                    | 8q24.3       |
| SLURP-2   | Keratinocytes       | 1                  | Secreted          | Increased expression in skin keratinocytes in psoriasis vulgaris          | 8q24.3       |
| E-48 (L6D) | Keratinocytes       | 1                  | GPI-anchored      | Overexpressed in head-and-neck squamous cell carcinoma                    | 8q24.3       |
| RIG-E     | Acute promyelocytic leukemia cells | 1 | GPI-anchored | Expression is induced by all-trans-retinoic acid                          | 8q24.3       |
| LYH       | Brain and acute lymphoblastic leukemia | 1 | GPI-anchored |                                                                      | 8q24.3       |
| LYGK      | Keratinocytes       | 1                  | GPI-anchored      | Overexpressed in head-and-neck squamous cell carcinoma                    | 8q24.3       |
| GML       | Erythrocytes        | 1                  | GPI-anchored      | Expression is induced by suppressor gene p53                             | 8q24.3       |
| SP-10     | Sperm and testis    | 1                  | GPI-anchored      | Abnormal RNA splicing is common                                            | 11q23-q24    |
| SAMP14    | Spermatozoa         | 1                  | GPI-anchored      |                                                                      | 19q13.33     |
| CD177     | Neutrophil          | 2                  | GPI-anchored      | Increased expression in polycythemia vera                                 | 19q13.32     |
| uPAR      | Neutrophils and monocytes | 3 | GPI-anchored | A high affinity receptor for urokinase type plasminogen activator          | 19q13.33     |

PSCA = Prostate Stem Cell Antigen SLURP-1 = Secreted Ly-6/uPAR Related Protein 1 SLURP-2 = Secreted Ly-6/uPAR Related Protein 2 SAMP14 = Sperm Acrosomal Membrane-Associated Protein 14

PSCA is expressed by normal prostate tissue and its expression is localized to basal cell epithelium in an area that contains the stem cell compartment of the prostate [42,43]. PSCA is upregulated in prostate cancer. It encodes a GPI-anchored cell surface protein of 123 amino acids and has four N-glycosylation sites [43]. PSCA is located on chromosome 8q24.2.

RIG-E was first described as a gene expressed by all-trans-retinoic acid differentiated acute promyelocytic leukemia and HLA-60 cell lines [44]. It is also highly expressed by ovary and malignant thymocytes in T-acute lymphoblastic leukemia and at lower levels by liver, spleen, lung, uterus, fetal brain, and fetal thymus. RIG-E is located on chromosome 8q24 and encodes a protein with 131 amino acids of which 20 amino acids are a signal peptide sequence. RIG-E is GPI anchored [44].

GML expression is induced by tumor suppressor gene p53 [45]. GML is located on chromosome 8q24.3 and it encodes a GPI-anchored protein.

LYGH is expressed by brain and acute lymphoblastic leukemia cells [46]. It also has a single cysteine rich domain and a GPI-anchor and is located on chromosome 8q24.3.
E48 or Ly-6D and Ly-6K are expressed by normal squamous epithelial cells and are overexpressed in head-and-neck squamous cell carcinoma [47,48]. Ly-6D is located on chromosome 8q24.3 and encodes a 15 to 20 kDa GPI-anchored protein with one cysteine rich domain [47].

The ARS B gene encodes SLURP-1, a 9 kDa protein [49,50]. It contains no GPI-anchor, is not glycosylated, and is secreted. ARS B is located on chromosome 8q24.3 and mutations in ARS B are associated with Mal de Meleda syndrome which is also know keratosis palmoplantaris transgrediens of Seimens. Mal de Meleda syndrome is a rare autosomal recessive hyperkeratotic skin disorder involving the palms of the hand and soles of the feet.

SLURP-2 is expressed by a number of tissues, but it is expressed most prominently in skin and keratinocytes [51]. It is also expressed on the cervix, esophagus, brain, lung, stomach, small intestine, colon, rectum, uterus and thymus. SLURP-2 is overexpressed in skin keratinocytes in people with psoriasis vulgaris. SLURP-2 encodes a protein predicted to have 97 amino acids and one domain with eight conserved cysteine residues. SLURP-2 lacks a GPI-anchor and a transmembrane domain and as a result is secreted. It is located on chromosome 8q24.3.

Some Ly-6 genes are associated with abnormal RNA splicing. A cluster of Ly-6 genes is also located in the Class III region of the major histocompatibilty complex (MHC) on chromosome 6p21.3. Five genes are located in this area: LY6G6C, LY6G6D, LY6G6E, LY6G5B, and LY6G5C. The function of these genes is not known, but they are characterized by frequent transcription misssplicing [52]. Abnormal splicing of another Ly-6, gene SP-10, is also common. Sperm-specific antigen SP-10 is expressed in the testis. It is located on chromosome 11q23-q24. This protein differs from other Ly-6 members in that it is not GPI-anchored. It encodes a glycosylated polypeptide of between 18 and 34 kDa due to post-translational proteolytic events and alternative splicing of RNA transcripts [53,54].

**Role of CD177 in myeloproliferation**

Neutrophil CD177 mRNA levels are elevated in several conditions associated with increased neutrophil counts. Neutrophil CD177 mRNA levels are elevated in patients with severe sepsis or burns, in healthy subjects given G-CSF, and in patients with myeloproliferative disorders [5,16,21,27,30-32]. Elevated levels of neutrophil CD177 mRNA are clearly associated with increased neutrophil production and quantitating neutrophil CD177 mRNA has become a useful diagnostic tool for polycythemia vera. However, there are several other neutrophil protein markers of increased myelopoiesis that have been used to diagnosis polycythemia vera including vitamin B12 serum levels, leukocyte alkaline phosphatase, and lactoferrin plasma levels [55]. It is not yet certain if CD177 mRNA levels are more useful for diagnosing polycythemia vera than B12, leukocyte alkaline phosphatase or lactoferrin.

The more important question concerning CD177 is what role any do elevated CD177 mRNA levels play in the pathogenesis or complications of polycythemia vera? While this question is still being investigated it is likely that CD177 plays an important role in the pathogenesis and clinical course of polycythemia vera. Another neutrophil granule protein has been found to play an important role in myelopoiesis. The molecular defect in most patients with severe congenital neutropenia and in all patients with cyclic neutropenia involves a well-described neutrophil protein that previously was not thought to be involved with myelopoiesis, neutrophil elastase [56-58]. Neutrophil elastase is a serine protease found in neutrophil primary or azurophilic granules. Recently, mutations in the gene encoding neutrophil elastase, ELA-2, have been found to be associated with cyclic neutropenia and congenital neutropenia. This suggests that CD177, an abundant neutrophil membrane secondary granule protein could also be important in hematopoiesis.

The fact that several mouse Ly-6 genes play an important role in proliferation, differentiation, and homing of hematopoietic cells and lymphocytes and that several human Ly-6 genes are overexpressed in rapidly proliferating and/or malignant cells suggests that CD177 is not just a marker of increased neutrophil production, but it may play an important role in the overproduction of neutrophils in polycythemia vera. Polycythemia vera is a clonal disorder that may involve several molecular abnormalities. The abnormal expression of CD177 may not be the initiating or the most important event, but its overexpression likely contributes to the pathogenesis of the disorder.

**Conclusions**

Neutrophil CD177 is upregulated when myeloproliferation is increased. An elevation in neutrophil CD177 mRNA levels has become an important marker of myeloproliferative disorders. Further studies are needed to determine if elevated neutrophil CD177 mRNA levels are simply a marker of increased production of neutrophils or if it plays a role in the pathogenesis or clinical course of polycythemia vera.

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