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Review

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

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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 thrombocythemia. *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 thrombocythemia are not yet known. However, measuring neutrophil *CD177* mRNA levels has become an important marker for diagnosing the myeloproliferative disorders polycythemia vera and essential thrombocythemia.

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 kD 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 containes 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].

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	Gycosylinositol anchored
Glycosylation	N-linked side chains
Neutrophil location	Plasma membranes and secondary granules
Epitope	HNA-2a

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 (ω). 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-

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 receptor (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 thromobpoiesis. 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

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 thromobocythemia	21%, 100%, 17%, 67%, 33%	[5,26,29-31]
Idiopathic myelofibrosis	67%	[26]
Chronic myelogenous leukemia	0%	[5]

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. Erthyroid colonies from patients with polycythemia vera are hypersentive 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 hydridization 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 myleoproliferative 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

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 eigth 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 hematopietic 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 (*SLUPR-1*), *SLUPR-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, monocytes 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

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
PSCA	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 Mal de Meleda syndrome	8q24.3
SLURP-2		1	Secreted	Increased expression in skin keratinocytes in psoriasis vulgaris	8q24.3
E-48 (LY6D)	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
LY6H	Brain and acute lymphoblastic leukemia	1	GPI-anchored		8q24.3
LYGK	Keratinocytes	1	GPI-anchored	Overexpressed in head-and-neck squamous cell carcinoma	
GML		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	Spermatozoan	1	GPI-anchored		19q13.33
CD177	Neutrophil	2	GPI-anchored	Increased expression in polycythemia vera	19q13.2
uPAR	Neutrophils and monocytes	3	GPI-anchored	A high affinity receptor for urokinase type plasminogen activator	19q13.3

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

of plasmin convert pro-uPA to uPA. The membrane associated uPA bound to uPAR then converts large quantities of plaminogen to plasmin, a proteolytic enzyme with degrades fibrin.

uPAR effects cell function in several ways. It plays a role in cell-cell and cell-extracelluar 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 maxtrix 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.

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 vulgarsis. *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 histocompatiblity 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 myelopoesis 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 if 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 pathogenisis or clinical course of polycythemia vera.

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