Ly6 family proteins in neutrophil biology

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ABSTRACT

The murine Ly6 complex was identified 35 years ago using antisera to lymphocytes. With advances in mAb development, molecular cloning, and genome sequencing, >20 structurally related genes have been identified within this complex on chromosome 15. All members of the Ly6 family and their human homologues share the highly conserved LU domain and most also possess a GPI anchor. Interestingly, many Ly6 proteins are expressed in a lineage-specific fashion, and their expression often correlates with stages of differentiation. As a result, Ly6 proteins are frequently used as surface markers for leukocyte subset identification and targets for antibody-mediated depletion. Murine neutrophils display prominent surface expression of several Ly6 proteins, including Ly6B, Ly6C, and Ly6G. Although the physiology of most Ly6 proteins is not well understood, a role in neutrophil functions, such as migration, is recognized increasingly. In this review, we will provide an overview of the Ly6 complex and discuss, in detail, the specific Ly6 proteins implicated in neutrophil biology.


Introduction

The Ly6 complex in mice was first described >30 years ago [1, 2]. To date, >20 structurally related genes have been identified within the murine Ly6 complex on chromosome 15. Ly6 proteins share a conserved motif known as the LU domain, and most are membrane-bound molecules attached to the cell surface via a C-terminal GPI anchor [3]. Although the physiologic function of most Ly6 proteins remains enigmatic, the relatively lineage-specific expression of many of these proteins has rendered several Ly6 family members useful markers for cellular differentiation and subset identification, as well as targets for antibody-mediated depletion in vivo.

In this review, we will provide an updated overview of the murine Ly6 complex. As the historical perspectives on the discovery of the Ly6 family and involvement of Ly6 proteins in lymphocyte differentiation and signaling has been reviewed in detail [4–6], we will focus our discussion on the increasingly recognized role of Ly6 proteins in neutrophil biology. As a result of the complexity and redundancy of the past nomenclature, we will refer to Ly6 proteins using standardized nomenclature from the Mouse Genomic Informatics Database throughout this review [7].

ARRANGEMENT OF THE MURINE LY6 COMPLEX

The Ly6 complex was discovered initially using polyclonal sera against murine lymphocytes to identify markers to distinguish lymphocyte subsets. Two distinct haplotypes, referred to as Ly-6.1 and Ly-6.2, were described, based on the reactivity to these antisera [8]. The Ly-6.1 strains, including A, BALB/c, CBA, C3H/J, DBA/1, and NZB, typically showed lower antisera reactivity in peripheral lymphocytes compared with their Ly-6.2 counterparts, which included 129, AKR, C57 (including C57BL/6), DBA/2, SJL, and SWR. A wave of subsequent studies using mAb found that the Ly6 complex encoded several structurally similar genes with different patterns of tissue expression [3, 8–12]. Genomic studies illustrated the existence of two alleles for many of the Ly6 genes; alleles of the Ly-6.1 haplotype are generally expressed at lower levels compared with those of the Ly-6.2 haplotype, providing an explanation to the early observations of haplotype difference in antisera reactivity [5]. The proximity of Ly6 genes within the complex was supported by their stable haplotype transmission and later confirmed by genetic mapping of the entire Ly6 complex on chromosome 15 [13]. A minor exception for the haplotype

Abbreviations: Gr-1, granulocyte antigen-1, ImmGen=Immunological Genome Project Database, LL=lymphocyte antigen 6/urokinase-type plasminogen activator receptor, Ly6=lymphocyte antigen 6, P1-PLC=phosphatidylinositol-specific PLC, PR3=proteinase 3, Sca=stem cell antigen, SLURP=secreted lymphocyte antigen 6/urokinase-type plasminogen activator receptor-related protein-1, TAP=T cell-activating protein, TSA=thymic-shared antigen, uPAR=urokinase-type plasminogen activator receptor

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transmission was found in NZB mice, which possesses all Ly-6.1 specificities except for the Ly-6B.2 allele [12].

The presence of allelic variations, together with the proximity and sequence homology of Ly6 family members, has created considerable confusion in the interpretation of early studies. The field is complicated further by the overlapping specificities of the several mAb. Ly-6A.2 and Ly-6E.1, for example, were among the early genes identified from the complex, but cloning studies later showed that they represent two alleles of the same gene, now designated as Ly6A [10]. Ly6A has also been referred to as Ly6A/E, TAP, and Sca-1 by different groups [14, 15]. Some genes in this family remain incompletely annotated. For example, Ly6B (discussed further below) does not appear under this name in many database searches but is designated as I830127L07Rik, based on its record from genomic DNA sequencing.

A second cluster of Ly6-related genes is located in the MHC class III region of murine chromosome 17 [16]. The nomenclature of these genes (for example, Ly6g5c, Ly6g6e) can suggest a close relationship with genes in the chromosome 15 locus, but sequence alignment suggests only a distant relationship (see Supplemental Fig. 1). Whereas many of these genes have human orthologs (Supplemental Fig. 1), gene expression profiling using the ImmGen Database fails to disclose significance (see Supplemental Fig. 1). Whereas many of these genes possess a LU domain (for example, Ly6g5c, Ly6g6e) can suggest a close relationship with genes in the chromosome 15 subcluster of murine genes for which no syntenic region is present in humans or rats. This observation suggests that the region may have arisen through gene duplication after evolution.

### Table 1. Genes of the Ly6 Complex on Mouse Chromosome 15

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Full name</th>
<th>Orientation</th>
<th>MGI ID#</th>
<th>Location (Mb)</th>
<th>Mapping (cM)</th>
<th>Other alias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psca</td>
<td>Prostate Sca</td>
<td>+</td>
<td>1919623</td>
<td>74.71</td>
<td>34.26</td>
<td></td>
</tr>
<tr>
<td>Slurp1</td>
<td>Secreted Ly6/Plaur domain-containing 1</td>
<td>–</td>
<td>19090923</td>
<td>74.72</td>
<td>34.26</td>
<td>ARS; ArSB</td>
</tr>
<tr>
<td>Lypd2</td>
<td>Ly6/Plaur domain-containing 2</td>
<td>–</td>
<td>1915561</td>
<td>74.73</td>
<td>34.27</td>
<td>VLL; LypdC2</td>
</tr>
<tr>
<td>Lynx1</td>
<td>Ly6/neurotoxin</td>
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<td>1345180</td>
<td>74.74</td>
<td>34.27</td>
<td>SLURP2</td>
</tr>
<tr>
<td>Ly6d</td>
<td>Ly6 complex locus D</td>
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<td>96881</td>
<td>74.76</td>
<td>34.27</td>
<td>Thb; Ly6d</td>
</tr>
<tr>
<td>Ly6k</td>
<td>Ly6 complex locus K</td>
<td>–</td>
<td>1923736</td>
<td>74.79</td>
<td>34.28</td>
<td></td>
</tr>
<tr>
<td>Gnl</td>
<td>GPI-anchored molecule-like protein</td>
<td>–</td>
<td>3644767</td>
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<td>34.28</td>
<td>HemT-3</td>
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<tr>
<td>2010109I03Rik</td>
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<td>–</td>
<td>1914288</td>
<td>74.87</td>
<td>34.29</td>
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<td>106651</td>
<td>75.95</td>
<td>34.29</td>
<td></td>
</tr>
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<td>Ly6I</td>
<td>Ly6 complex locus I</td>
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<td>–</td>
<td>107527</td>
<td>74.99</td>
<td>34.29</td>
<td>TAP; Sca-1; Ly-6A; Ly-6A/E; Ly-6E.1</td>
</tr>
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<td>75.04</td>
<td>34.29</td>
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</tr>
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<td>Ly6 complex locus C2</td>
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<td>3712069</td>
<td>75.10</td>
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<td>Ly6C.2</td>
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<td>Ly6B</td>
<td>Ly6 complex locus B</td>
<td>–</td>
<td>107526</td>
<td>75.13</td>
<td>34.29</td>
<td>7/4; GM-2.2; I830127L07Rik</td>
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<tr>
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<td>Ly6 complex locus G</td>
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<td>75.16</td>
<td>34.29</td>
<td>Gr-1</td>
</tr>
<tr>
<td>BC025446</td>
<td>BC025446</td>
<td>+</td>
<td>2385015</td>
<td>75.22</td>
<td>34.29</td>
<td></td>
</tr>
<tr>
<td>Ly6F</td>
<td>Ly6 complex locus F</td>
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<td>109441</td>
<td>75.27</td>
<td>34.29</td>
<td></td>
</tr>
<tr>
<td>9030619P08Rik</td>
<td>9030619P08Rik</td>
<td>–</td>
<td>3612405</td>
<td>75.42</td>
<td>34.35</td>
<td></td>
</tr>
<tr>
<td>Ly6H</td>
<td>Ly6 complex locus H</td>
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<td>1346030</td>
<td>75.56</td>
<td>34.52</td>
<td>NMLY6</td>
</tr>
<tr>
<td>GPIHBP1</td>
<td>GPI-anchored HDL-binding protein 1</td>
<td>+</td>
<td>1915703</td>
<td>75.59</td>
<td>34.59</td>
<td></td>
</tr>
</tbody>
</table>

*Gene location based on annotation from sequencing of chromosome 15 (measured in megabases). Plaur, Plasminogen activator, urokinase receptor.
tionary divergence from these other species and/or was lost in other species but preserved in mice (Fig. 1B).

The organization of Ly6 genes is highly conserved, beginning with a short signal peptide, encoded by the first of three exons that will undergo translation. In some cases, an additional exon, containing a portion of the 5' untranslated region, is present upstream of the start codon. The mature protein is typically encoded by the last two exons and features cysteine residues in conserved positions that generate the LU domain structure (discussed below) [5]. The hydrophobic GPI anchor is located in the C-terminus followed by the 3' untranslated region.

**THE LU DOMAIN**

A core structural element of the Ly6 protein family is an ~90-aa LU domain, named for its presence in the Ly6 proteins and in the related uPAR. Ly6 proteins on murine chromosome 15 each contain at least one such motif; uPAR contains three; and murine CD177 contains four. The LU domain assumes a “three finger-fold” structure through disulfide bonds, formed by eight to 10 conserved cysteines at the base of the protein; variability in the length of the fingers and in the sequence of the tips of each loop renders this motif a flexible platform for intermolecular interactions [19]. Figure 2 depicts a theoretical structure for Ly6G, modeled on a LU domain from another family member.

Most LU proteins, including all of those within the murine chromosome 15 Ly6 locus, except for SLURP1, are tethered to the cell surface via a GPI linker attached near the C terminus. Enzymatic cleavage of this linkage or in other residues connecting the protein to the cell membrane offers a mechanism to regulate the surface expression of these proteins and could potentially enable the released protein to conduct independent biological activity [21]. LU family members lacking a GPI attachment site can be secreted immediately. The known and potential functions of LU proteins are correspondingly diverse. Whereas none is known to have direct enzymatic activity, LU
proteins may tether enzymes and thereby, focus their activity; examples include binding of the proteases uPA by uPAR and PR3 by human CD177 [19, 22]. Unable to signal directly without a cytoplasmic tail, LU family members may interact with transmembrane proteins to initiate signaling cascades, a capacity that is likely potentiated by the propensity of GPI-linked proteins to associate with lipid rafts [23, 24]. Additional potential functions of this protein family, exemplified by uPAR, include direct binding to ECM (vitronectin) [25], modulation of β integrin affinity via effects on integrin head group conformation [26], and regulation of the uptake of apoptotic cells by macrophages [27, 28]. Enzymatic cleavage of uPAR attenuates its surface functions but renders soluble uPAR a chemotactic ligand for the fMLPR [21, 29, 30]. Thus, proteins containing the LU domain may engage in a wide variety of biological processes, and individual proteins may have multiple, distinct activities.

**Expression and Function of Ly6 Proteins**

Early studies using polyclonal sera provided initial support for the use of the Ly6 complex as a marker of lymphocyte differentiation, given the variable reactivity among T cell subsets [1, 2]. The development of mAb and cloning of the individual Ly6 members improved our understanding of the expression profile of Ly6 family members. Several Ly6 proteins have become essential markers for the identification of leukocyte subsets. For example, Ly6A (more commonly termed Sca-1) is commonly used to identify hematopoietic stem cells from the bone marrow, whereas Ly6G is a specific marker that separates neutrophils from other leukocytes. With the use of the ImmGen Database [17], we summarize the mRNA expression profile of major murine chromosome 15 Ly6 family members in Table 2.

Expression of Ly6 proteins is not limited to hematopoietic cells. In the kidney, the vascular endothelium and tubular epithelium display surface expression of Ly6A at baseline, which is enhanced further in the setting of experimental nephritis [31]. Ly6 proteins have also been described on bone marrow and thymic stromal cells, endometrial cells in the uterus, and intestinal epithelial cells [32–34].

The function of Ly6 proteins, however, is less well understood. A supportive role for proliferation was first proposed, based on T cell proliferation in response to mAb against Ly6A.

### Table 2. mRNA Expression of Ly6 Genes in Leukocyte Subsets from the ImmGen Database [17]

<table>
<thead>
<tr>
<th>Gene</th>
<th>B cells</th>
<th>T cells</th>
<th>NK cells</th>
<th>Monocytes</th>
<th>Neutrophils</th>
<th>DCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ly6A</td>
<td>+ + + a</td>
<td>+ + a</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ + + a</td>
</tr>
<tr>
<td>Ly6B</td>
<td>–</td>
<td>–</td>
<td>+ a</td>
<td>+ + a</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Ly6C1</td>
<td>–</td>
<td>++ a</td>
<td>+</td>
<td>++ a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ly6C2</td>
<td>–</td>
<td>++ a</td>
<td>+</td>
<td>++ a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ly6D</td>
<td>+ a</td>
<td>+ a</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ + + a</td>
</tr>
<tr>
<td>Ly6E</td>
<td>+ + a</td>
<td>+ + a</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ + a</td>
</tr>
<tr>
<td>Ly6F</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ly6G</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ +</td>
</tr>
<tr>
<td>Ly6H</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ly6I</td>
<td>–</td>
<td>–</td>
<td>+ + a</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ly6K</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*a Variable expression among cell subsets.
[35]. Certain Ly6 proteins are in close proximity with essential signaling components of the BCR and TCR complex, and several LU family members have been observed to associate with β integrins [36–39]. Ly6 proteins have been implicated in a number of cellular functions, including leukocyte differentiation, cell adhesion, cell migration, and cytokine production. Many of these findings are based on in vitro studies, whereas the in vivo function of most Ly6 proteins remains largely unclear. Recognizing that exploration of the function of murine Ly6 proteins remains at a very early stage, we will summarize here the current understanding of Ly6 proteins expressed on the surface of neutrophils.

LY6B

Whereas most Ly6 proteins were noted to be expressed predominantly by lymphocytes, mAb, produced by two independent groups, identified a new member of the family, known as Ly6B, on the surface of bone marrow and peripheral neutrophils [12, 40]. Ly6B is a heavily N-glycosylated protein of 25 kD with greater expression in Ly-6.2 strains, with the exception of NZB mice, as discussed earlier [12]. Around the same time, a neutrophil differentiation antigen was discovered using a rat mAb, called 7/4 [41]. The 7/4 antigen was present on maturing neutrophils in the bone marrow but not on progenitor cells [42]. Expression of the 7/4 antigen was polymorphic, and the pattern was compatible with Ly-6 haplotype differences. Indeed, a recent study confirmed that Ly6B is synonymous with the 7/4 antigen [43].

Ly6B is also found on the surface of immature monocytes that express Ly6C, the chemokine receptor CCR2, and the selectin CD62 ligand. Ly6B⁺ monocytes possess greater proliferative capacity compared with their Ly6B⁻ counterparts. Moreover, Ly6B expression has been found in activated tissue macrophages in the setting of inflammation and infection [43–45]. Together, with other myeloid markers, such as F4/80, CCR2, and CD11b, Ly6B is a useful marker to delineate neutrophils, as well as monocyte subsets.

The physiological function of Ly6B is unknown, and few studies have been performed to address this issue. In vivo administration of mAb against Ly6B leads to depletion of neutrophils and monocytes [43]. Unlike Ly6G (discussed below), ligand of Ly6B does not affect the migration of neutrophils [39]. Interestingly, removal of the GPI anchor, using PI-PLC, releases Ly6B from the surface of monocytes but not neutrophils [43]. A potential explanation is the presence of Ly6B-binding partners on the surface of neutrophils that prevent PI-PLC from reaching the inositol ring [46]. Further studies are needed to identify the function and potential binding partners of Ly6B.

LY6C

Ly6C is a 14-kD protein, first identified as an antigen shared by ~50% of bone marrow cells and a small population of T lymphocytes in the periphery [12]. The nomenclature of Ly-6C.1 and Ly-6C.2 was commonly used in early studies, based on the hypothesis that these proteins represented allelic products of the same gene. However, subsequent studies showed that Ly-6C.1 and Ly-6C.2 actually represent two separate genes (now referred to as Ly6C1 and Ly6C2) [6, 47].

Ly6C1 and Ly6C2 are located adjacent to each other in the mid-section of the Ly6 complex. They share >95% similarity in their genomic and protein sequences. With a nearly identical structure, expression profile, and reactivity to antibodies, they are collectively known as Ly6C. No antibodies currently available distinguish the two molecules.

Expression of Ly6C is found on neutrophils, monocytes, DCs, and subsets of CD4⁺ T cells and CD8⁺ T cells (Table 2). Prominent expression is seen on myeloid cells in the bone marrow as they terminally differentiate into neutrophils and monocytes [48]. In addition to the profile of intrinsic expression, Ly6C expression is inducible by type I and type II IFNs, by virtue of IFN-response elements present in the promoter region [49]. Ly6A and Ly6E are also IFN-inducible [49, 50], whereas Ly6G is not (unpublished data). The role of these Ly6 proteins in the immune response downstream of IFN stimulation is not clear.

Whereas Ly6C is expressed uniformly by neutrophils in the bone marrow and in the circulation, the level of Ly6C expression is used to distinguish monocyte subsets. Ly6Chi monocytes (also called immature or inflammatory monocytes) express the chemokine receptor CCR2 and migrate from the bone marrow to the site of infection or inflammation [51, 52]. This subset of monocytes gives rise to a number of cell lineages, including DCs in the periphery, Langerhans cells of the skin, and microglial cells in the CNS [53, 54]. By contrast, Ly6Clo monocytes (also called mature or residential monocytes) express the chemokine receptor CX3CR1 and act as the precursor for resident macrophages in different tissues [52]. Details of Ly6Chi and Ly6Clo monocyte subsets are discussed extensively in previous reviews [55, 56].

The discovery of a granulocyte-differentiation antigen, known as Gr-1, using the mAb RB6-8C5, has fostered some confusion in the literature on Ly6C. Surface expression of Gr-1 is abundant on immature myeloid cells and neutrophils in the bone marrow but rare on lymphocytes, macrophages, or uncommitted precursor cells [57]. Gr-1 expression increases with cell maturation in neutrophils but appears to be transient on monocytes. However, RB6-8C5 reacts with Ly6C and Ly6G [58]. Interpretation of Gr-1 expression, therefore, is confounded by the dual specificity of the RB6-8C5 antibody. Although neutrophils and most monocytes in the bone marrow are Gr-1-positive, monocytes express Ly6C but not Ly6G, whereas neutrophils display abundant expression of both proteins [51]. Nevertheless, descriptors, such as Gr-1hi and Gr-1lo monocytes (corresponding to Ly6C1hi and Ly6C2lo monocytes), are still commonly used in the literature.

Early investigation on the function of Ly6C focused on T lymphocytes. The addition of mAb targeting Ly6C induced proliferation and activation of cultured cytotoxic T cells in a TCR- and IL-2-independent manner [59]. However, this effect was not observed consistently using other clones of anti-Ly6C mAb [60]. Subsequent studies demonstrated a novel role of Ly6C on CD8⁺ T cell homing and migration by inducing the
activation and clustering of the integrin LFA-1 (CD11a/CD18) [61], although the physiological ligand of Ly6C remains an enigma.

Despite the prominent expression of Ly6C on myeloid cells, few studies have been performed to address the function of this molecule on monocytes and neutrophils. A potential role of Ly6C in signal transduction is suggested by its interaction with Fgr, a member of the Src family of tyrosine kinases, with established roles in neutrophil migration and activation [62–64]. Supporting a proinflammatory role of Ly6C, Butovsky and colleagues [65] found that the Ly6C blockade decreased inflammatory cytokine production, monocyte infiltration, neutrophil loss, and death in a murine model of amyotrophic lateral sclerosis. As is the case for Ly6B, the binding partner(s) and function of Ly6C may be cell type-specific. Significant differences in the kinetics of Ly6C up-regulation in response to cytokines and in hydrolysis by PI-PLC have been described between neutrophils and monocytes [66].

An inherent problem of functional studies using mAb is whether the downstream effects of antibody-mediated ligation reflect the physiological function of the targeted protein. For example, whereas a stimulatory role of Ly6A on T lymphocytes was demonstrated initially by studies using mAb [35], an inhibitory role of the molecule was shown in T cells from Ly6A-deficient animals [67]. Ly6C-deficient mice are not yet available, and given the high degree of homology between Ly6C1 and Ly6C2, deletion of both may be necessary to generate an informative strain.

**LY6G**

Ly6G was identified as a novel member of the Ly6 family in 1993 [58, 68]. Ly6G is a small protein of 25 kD that is tethered to the cell membrane via a GPI linker [58]. Unlike the Gr-1 antigen, which encompasses epitopes from Ly6G and Ly6C, expression of Ly6G is markedly restricted. Use of 1A8, a mAb considered specific for Ly6G, shows that expression of Ly6G in circulating blood, spleen, and pulmonary exudates is limited to neutrophils, whereas monocytes, macrophages, lymphocytes, and plasmacytoid DCs are negative, a conclusion supported by gene expression data (Table 2) [58, 69–71]. Circulating FcεRI+CD49+ basophils also lack surface Ly6G (unpublished data). Eosinophils are reported variably as Ly6G-negative, low, or intermediate [71–75]. Ly6G is expressed by granulocytic myeloid-derived suppressor cells and also by a recently described population of cells from viral-infected skin that has anti-inflammatory activity yet mononuclear morphology [76, 77]. In bone marrow, Ly6G (assessed using RB6-8C5) appears to distinguish the granulocyte lineage, with highest expression in the most mature cells without colony-forming potential [42, 57]. Ly6G expression is typically higher in circulating than in bone marrow neutrophils and higher in cells recruited to inflamed sites (arthritic joints, thioglycollate-stimulated peritoneum) than in paired blood (unpublished data). Whether all murine neutrophils express Ly6G is unknown. In marrow and peripheral blood, CD45<sup>hi</sup> cells with the scatter properties of neutrophils are uniformly Ly6G-positive. Gr-1-negative cells with ring-shaped nuclei have been observed in Borrelia-infected arthritic joints in animals depleted of circulating neutrophils with RB6-8C5, although the identity of these cells is uncertain, as not all cells with ringshaped nuclei are neutrophils [78, 79].

Antibodies targeting Ly6G (RB6-8C5 or 1A8) are commonly used in studies aimed at identifying the role of neutrophils in murine disease models. These antibodies are highly efficient, depleting circulating neutrophils within minutes of administration. RB6-8C5 also depletes Ly6C<sup>hi</sup> (inflammatory) monocytes and other Ly6G-expressing cells, and effects observed after administration of this antibody may diverge from those observed after 1A8 [69, 80]. The mechanisms underlying neutrophil depletion are not understood completely, but opsonization by complement likely plays a key role, as C3<sup>−/−</sup> mice, but not mice receiving a C5 inhibitor, fail to deplete, whereas normal depletion is observed in mice lacking CD18, CD11b, or the FcyR chain or treated with heparin or antagonists of P- and E-selectin [80–83]. Studies with radiolabeled RB6-8C5 have been interpreted to suggest that neutrophils are cleared in the liver and spleen, although these data are complicated by expression of Ly6G by splenic macrophages, and studies seeking depleted neutrophils by histology or MPO assays have not consistently found missing neutrophils in these organs [71, 80, 81].

Interestingly, direct videomicroscopy of circulating neutrophils after administration of anti-Ly6G shows very different effects based on the state of the animal. In healthy mice, RB6-8C5 induces detachment of most rolling/adherent cells, whereas residual neutrophils assume a “hairy” appearance associated with accumulation of platelets on the neutrophil surface [82, 84]. This effect is not unique to RB6-8C5 but also observed with anti-CD44 and P-selectin Fc [84]. However, in mice pretreated with TNF, RB6-8C5 or 1A8 induces arrest of rolling cells, vascular leak, shock, and death, which can be prevented by combined treatment with heparin and antagonism of P- and E-selectin [82]. A similar shock-like response occurs in mice pretreated with LPS and is mediated by platelet-activating factor released by neutrophils [85].

The function of Ly6G remains to be fully defined. No Ly6G-deficient animal or neutrophil cell line has been developed, such that studies to date extrapolate from the effects of antibody ligation. Ribechini et al. [86] found that RB6-8C5 induced up-regulation of CD115, F4/80 and asialo GM1 on CD11b+ populations in bone marrow, accompanied by morphological changes, suggesting myeloid expansion. Murine marrow, cultured ex vivo in the presence of RB6-8C5, showed modest up-regulation of phospho-STAT-1, -3, and -5 staining in surviving Ly6G<sup>−</sup> cells, potentially reflecting a signaling effect akin to GM-CSF, although unlike GM-CSF, antibody treatment provided no survival advantage [86]. In thioglycollate peritonitis, treatment with i.p. RB6-8C5, 4 h after initiation of peritonitis, was associated with the appearance of pyknotic nuclei at harvest, 16 h later, potentially reflecting induction of apoptosis; absence of contemporaneous controls renders this finding difficult to interpret, and other authors have found no effect of 1A8 on apoptosis [39, 86].

Recent data have begun to implicate Ly6G in neutrophil migration. Wang et al. [39] observed that administration of doses of RB6-8C5 and 1A8, low enough to have only modest,
transient effects on the number of circulating neutrophils, could nevertheless abrogate K/BxN serum transfer arthritis, a neutrophil-dependent model of immune complex joint inflammation. Joint tissues from 1A8-treated mice remained free of neutrophils. Further study showed that 1A8, but not control antibodies, markedly attenuated neutrophil migration toward multiple chemoattractants and that this effect was mediated through impaired surface expression and function of β2 integrins, as residual migration in CD18−/− neutrophils was not affected. Ly6G was found to associate with LFA-1 (CD11a/CD18) and macrophage 1 antigen (CD11b/CD18), suggesting a direct effect of Ly6G antibody ligation on β2 integrin function [39]. The nature and mechanism of this effect and the extent to which it reflects the biology of endogenous Ly6G remain to be determined.

Interestingly, RB6-8C5 and (to a lesser extent) 1A8 are commonly used as labeling reagents for in vivo microscopy, including in studies of neutrophil migration. Whereas some authors have identified marked changes in neutrophil rolling and adhesion after in vivo antibody administration, others have not [80, 84, 87–90]. The basis for these divergent findings remains to be determined. Neutrophil migration exhibits differential dependence on integrins depending on the tissue and the stimulus, such that the explanation may lie in the specific experimental system used [91]. Alternately, it may be that conjugation alters the functional properties of the antibody.

OTHER LY6 FAMILY MEMBERS ON NEUTROPHILS

Ly6E, also known as TSA-1 or Sca-2, is a protein whose expression is better studied in lymphocytes than in neutrophils, although consistent with gene expression data (Table 2), a population of Ly6E+Gr-1+ cells has been observed in bone marrow [92]. A small, LU-containing protein of 136 aa, Ly6E was identified as a thymic differentiation antigen with selective expression in a population of maturing T lymphocytes and epithelial cells within the thymus [93]. Subsequent work showed that it is also expressed on B cells and may interact with FcyRIIB [94]. Ly6E expression extends beyond hematopoietic lineages, and deletion is embryonic-lethal, as a result of cardiac and adrenal abnormalities, although lymphoid development remains grossly intact [95].

Identified by two independent groups in 2001, Ly6I (also known as Ly6M) is among the newest members in the Ly6 family. Ly6I is comprised of 134 aa, and its protein sequence closely resembles Ly6A and Ly6C, with >70% homology [96, 97]. The expression profile for Ly6I, however, is more closely related to Ly6C with a myeloid predominance. The highest expression is found on Ly6C+ monocytes, whereas granulocytes display moderate surface expression. Subsets of immature B cells and peripheral T cells also express this protein. Unlike Ly6A and Ly6C, Ly6I is only weakly inducible by IFNs [96].

Little is known regarding the function of Ly6I, and only one published study has examined this issue [98]. By creating a chimeric protein comprised of Ly6I and IgM, Pluthig et al. [98] showed binding of Ly6I on the surface of B lymphocytes. Similar results were seen with Ly6A and Ly6C chimeric proteins using this approach. Transfection studies identified CD22 as a potential ligand on B cells that interact with the chimeric Ly6 proteins. However, a direct interaction between the native Ly6I protein and CD22 has not been demonstrated. The effect of antibody ligation has not been addressed for Ly6I as a result of the absence of a suitable mAb.

More distant family members containing multiple LU domains include uPAR itself (discussed above) and by gene expression, CD177. Comprised of 17 exons, the murine CD177 gene is a substantially larger ortholog of human CD177, which contains only nine exons. While human CD177 has been the subject of detailed study by several groups, no data are available regarding the surface expression or function of CD177 on murine neutrophils.

HUMAN RELEVANCE OF THE MURINE LY6 GENE FAMILY

As evident from Fig. 1B and Supplemental Fig. 1, many murine LU family members have potential human orthologs, but this is not the case for the common neutrophil Ly6 proteins, Ly6B, Ly6C, and Ly6G. Their abundance and lineage-selective expression suggest that they are likely to have important roles in murine neutrophil biology. Absence in humans (and rats) may indicate that these roles are not as important in other species. More likely, perhaps, is that their biological roles are assumed by other proteins. If this is the case, human neutrophil LU proteins could represent functional homologs. Similarities among proteins in this family suggest some degree of functional overlap. For example, like Ly6G, uPAR and CD177 associate with β2 integrins and are implicated in the regulation of migration [38, 99, 100]. CD177 is perhaps particularly interesting in this respect, as its expression appears largely limited to neutrophils [101]. Human and murine CD177 diverge substantially in sequence (human CD177 has two LU domains, whereas murine CD177 has four), and human CD177 serves as a docking site for PR3, whereas sequence analysis of murine CD177 suggests that it is unlikely to do so [22, 102]. Whether CD177 or other proteins have adapted to accept certain functions of the missing murine neutrophil Ly6 proteins remains to be determined. Given that the functional tasks of murine and human neutrophils substantially overlap, better understanding the function of murine Ly6 family proteins is likely to provide important insights into human neutrophil biology.

CONCLUDING REMARKS

Starting from the initial recognition of the Ly6 as a group of alloantigens on lymphocytes, tremendous progress has been made in understanding the arrangement of this gene complex during the past three decades. More than 20 Ly6 or Ly6-like genes have been identified within the Ly6 complex on chromosome 15, and data on their nucleic acid sequence, structural motifs, and expression profile are now readily available. Knowledge about the physiological function of Ly6 proteins, on the other hand, lags behind. Whereas abundant expression of several Ly6 proteins on neutrophils has been known for
years, we are only beginning to uncover the role of these molecules in neutrophil biology. Further effort is needed to define the function and potential binding partners for members of the Ly6 complex. A number of Ly6-like genes located elsewhere in the genome also await further investigation. Results from these studies will shed light on the function of human orthologs and functional homologs of the murine Ly6 proteins.

AUTHORSHIP

P.Y.L., J-X.W., and P.A.N. wrote the manuscript. E.P. calculated the theoretical structure of Ly6G presented in Fig. 2. C.C.D. performed sequence analysis of the LU protein family.

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