Arch Immunol Ther Exp, 2004, 52, 255–266
PL ISSN 0004-069X

WWW.AITE–ONLINE.ORG
Review

Received: 2004.04.26
Accepted: 2004.05.19
Published: 2004.08.15

Mouse Ly-6 proteins and their extended family: markers of cell differentiation and regulators of cell signaling

Anil Bamezai

Biology Department, Villanova University, Villanova, PA 19085, USA

Summary

The Ly-6 locus on mouse chromosome 15 encodes a family of 10–12 kDa proteins that are linked to the cell surface by a glycosylphosphatidyl-inositol anchor and have cell signaling and cell adhesion properties. Expression of Ly-6 proteins is tightly regulated during development; these proteins continue to serve as excellent differentiation surface markers on normal and abnormal cells, but their role in driving cellular differentiation is still emerging. Recent studies suggest that Ly-6 gene products participate in regulating signaling through other cell type-specific receptors, perhaps by virtue of these proteins being localized in lipid rafts that play a key role in relaying signals from the membrane to the nucleus. Ligands for some Ly-6 proteins have been reported; the consequence of their interactions with the Ly-6 receptor remains to be fully uncovered. Mouse Ly-6-like proteins have also been reported from a variety of life forms ranging from *Caenorhabditis elegans* to humans that show a limited amino acid identity and share structural features with members of mouse Ly-6. Despite these similarities, the non-murine Ly-6 proteins bind distinct ligands and appear to have different cellular functions. All members of the Ly-6 super gene family perhaps evolved from an ancestral gene by a gene duplication mechanism.

Key words: **Ly-6 • cell adhesion • cell signaling • differentiation marker • disease • lipid raft**

Full-text PDF: http://www.aite-online/pdf/vol_52/no_4/5889.pdf

Author's address: Dr. Anil Bamezai, Biology Department, Mendel Science Center, Villanova University, 800 Lancaster Avenue, Villanova, PA 19085, USA, tel.: +1 610 519 4847, fax: +1 610 519 7863, e-mail: anil.bamezai@villanova.edu

INTRODUCTION

Ly-6 gene products are a group of alloantigens first reported on mouse lymphocytes in the 1970's[77]. A body of data generated with polyclonal and monoclonal antibodies against Ly-6 proteins revealed a broad tissue expression on cells of hematopoietic origin. Additionally, the expression of Ly-6 proteins has also been observed in non-hematopoietic cells[39]. The unique expression patterns of Ly-6 proteins on a variety of cell types have enabled their use as markers of differentiation, thereby aiding in identifying and isolating different subsets of immune cells for their functional analyses *in vitro*. Although Ly-6 proteins have been recognized for their use as differentiation antigens for quite some time, their role in cellular differentiation is still emerging. The advent of hybridoma technology and its application in identifying and isolating a number of Ly-6 gene products in the mid 1980's has aided in the discovery of the Ly-6 structure and has provided clues about its function[72, 94]. Immunoprecipitation and Western blot analyses of Ly-6 proteins with anti-Ly-6 antibodies provided the first insights into the biochemical nature of Ly-6 molecules, and the amino acid sequence obtained from the immunoprecipitated Ly-6 protein provided a key to unravel the complementary and chromosomal DNA sequences encoding Ly-6 proteins[64, 84, 91]. Anti-Ly-6 antibodies were shown to initiate responses in lymphocytes, providing the first evidence of their possible role in lymphocyte signaling[72, 94]. Some recent data suggest that Ly-6 proteins regulate signaling processes initiated by the antigen receptor in lymphocytes[46, 106]. Similarly, Ly-6 proteins have also been reported to regulate signaling in non-lymphoid cells through their cell type-specific receptors[15, 16, 33, 78]. A number of mouse and human Ly-6 proteins participate in the processes of cell-cell adhesion[8, 11, 18, 41] by binding their ligands[5, 17, 30, 87]. Some Ly-6 proteins are also known to regulate cell adhesion mediated by the integrins[41]. A precise developmental or activation stage of immune cells where the Ly-6 protein might exert its function remains unknown. This review will highlight the importance of Ly-6 gene products in their usefulness as markers of differentiation. We will discuss their role in cell adhesion and cell signaling and their association with disease conditions. We will focus on the advances related to the biology of the Ly-6 gene family since 1995. For detailed early literature on this topic, the reader is directed to previous reviews[38, 71, 83, 93, 100].

EXPRESSION AND STRUCTURE OF LY-6 PROTEINS

Mouse Ly-6 proteins as differentiation antigens

Earlier studies indicated a broad tissue expression of mouse Ly-6 proteins on cells of hematopoietic origin (reviewed in [38, 83, 93, 100]) and their usefulness as differentiation markers on these cells. Recent reports have substantiated and expanded these previous findings. **Ly-6A.2** (also known as stem cell antigen-1 (Sca-1) and T cell-activating protein), one member of this gene family, is expressed on the murine bone marrow stem cells that generate all of the thymic subsets[110]. **Ly-6A/E** protein expression is tightly regulated on developing T cells in the thymus. Ly-6A/E is expressed on the first two stages (double negative I (DN I) and DN II) of the most immature, $CD4^-CD8^-CD3^-$ subset of thymocytes, these two subsets expressing CD44 and/or CD25 on their surface ($CD4^-CD8^-CD3^-CD44^+CD25^{+/-}$)[7]. Ly-6A.2 protein is downregulated in the majority of developing thymocytes that express both the CD4 and CD8 cell surface proteins ($CD4^+CD8^+$). Ly-6A.2 is then re-expressed on the mature T cell subsets in the thymus that have helper and cytolytic phenotype[17]. Its expression is maintained on approximately 70% of the peripheral $CD4^+$ T cells and about 40% of the $CD8^+$ T cells in the secondary lymphoid organs of an unmanipulated mouse[93]. **Sca-2** or thymic shared antigen-1 (TSA-1), another member of the mouse Ly-6 family, is expressed on a majority of intra-thymic precursor cells that give rise to cells of lymphoid lineage. Sca-2 protein continues to be expressed on large blast cells, whereas its surface expression is lost on both the small, non-dividing, immature and mature T cells present in the thymus and periphery[19]. Mouse **Ly-6ThB** is present on immature thymocytes and its expression is lost on naïve and activated T lymphocytes[38]. Diminished cell surface expression of Ly-6ThB is observed on a subset of $CD4^+CD8^+$ T cells undergoing positive selection in the thymus[90]. The majority of mouse bone marrow cells, a small subset of immature $CD4^-CD8^-$ thymocytes, and $CD8^+$ T cells, which mature outside the thymus, have been shown to express high levels of **Ly-6C** (reviewed in[38, 93, 100]). Allelic forms of Ly-6C (C.1 and C.2) show expression on distinct subsets of $CD4^+CD8^-$ and $CD4^-CD8^+$ spleen cells as well as some natural killer cells[98]. Ly-6C expression also marks a subset of natural killer T cells[96], memory $CD8^+$ T cells[21, 113], and B cells exposed to a T helper 1 cytokine environment[97]. More recently, a subset of naïve $CD4^+$ T cells ($CD44^{low}CD62^{high}$) with very high Ly-6C expression has been shown to promote differentiation of B cells into antibody-generating plasma cells[76]. **Ly-6G**, a granulocyte-differentiation antigen (Gr-1), has served as an excellent marker for identifying and isolating mature granulocytes[34]. Both Ly-6G and Ly-6C show high surface expression on recently described plasmacytoid dendritic cells, in a strain-dependent manner, which play a key role in regulating immunity to pathogens by generating high levels of type I

interferons (IFNs) in response to bacterial and viral gene products[6, 26]. **Ly-6I**, a new member of this family, is highly expressed on bone marrow cells. Ly-6I and Ly-6C expression is also observed on granulocytes and macrophages[86]. Low expression of Ly-6I is observed on mature B cells as well as developing T cells. Ly-6I possesses 80% sequence identity and the same exon/intron organization as Ly-6C and Ly-6E. Most of the homology of Ly-6I and other members is observed in the signal sequence and N-terminus sequence of the protein, which is required for attachment of the glycosylphosphatidyl-inositol (GPI)--anchor. Similarity between Ly-6I with other members of this family extends to their 10 cysteine residues that are conserved within the peptide backbone. Ly-6I exists in two allelic forms, Ly-6I.1 and Ly--6I.2[86]. **Ly-6M**, another novel member of the family, is also expressed on cells of hematopoietic origin, especially monocytes and myeloid precursors[49, 85]. Transcription of the Ly-6M gene was observed in a variety of tissues, including bone marrow, spleen, thymus, peritoneal macrophages, kidney, and lung[49, 85]. Other tissues, including heart, stomach, liver, small intestine, brain, and skin, show an absence of Ly-6M expression.

In summary, the mouse Ly-6 gene products continue to be used as differentiation markers for subsets of immune cells. Even though each member of the Ly-6 family is not expressed in a lineage-specific manner, and in many instances their expression overlaps, nonetheless all members of this gene family, in conjunction with other stage-specific differentiation antigens, have become important markers for identifying and isolating discrete lineages of cells.

Expression of mouse Ly-6 genes in non-lymphoid tissues

In addition to Ly-6 expression on cells of hemopoietic origin, the mouse Ly-6 proteins are also expressed on a variety of non-lymphoid tissues. Expression of some members of this family has been reported in the brain, bone, and kidney. Strain-dependent expression of Ly-6A/E has been observed in the mouse brain on vascular elements throughout the brain in BALB/c mice. In contrast, the C57BL/6 strain of mice shows expression within the white matter that is limited to the hippocampal and midbrain regions[20]. Mouse brain also expresses another member of this family, Ly-6C, one report suggests its expression on microglia in the fetal and adult brain[107]. Another report describes Ly-6C expression on endothelial cells[3]. Expression of Ly-6 proteins on mouse kidney has been long recognized; early studies established the specificity of Ly-6 expression on kidney tissues by ruling out the expression of this protein on the passenger leukocytes present in the kidney[39]. Ly-6A/E is extensively expressed on vascular endothelium and tubular epithelium in the kidney. Expression of this protein is upregulated upon the advent of murine lupus nephritis[9]. More recently, the gene expression profiles of mouse kidney proximal tubules exposed to proteinuria[82] or γ radiation[63] show upregulated expression of TSA-1, thereby providing a useful marker for detecting kidney pathology. Ly-6A and Ly-6C have also been used as markers for identifying mouse osteoblasts that arise from mesenchymal stem cells[50].

Ly-6 supergene family: an extended mouse Ly-6 gene family

The mouse Ly-6 proteins show a limited similarity both at the nucleotide and amino acid levels and share some key structural features with Ly-6-like proteins from other species, ranging from *Caenorhabditis elegans* to humans. However, orthologues of mouse Ly-6 proteins in these organisms have been difficult to establish. The *C. elegans* odr-2 gene encodes a novel Ly-6-related protein that is required for olfaction[16]. In addition to the limited identity in the amino acid sequence of Ly-6 proteins across various species, an interesting feature of the Ly-6-like proteins is their cysteine-rich nature. The position of these cysteine residues within the primary amino acid sequence of various Ly-6 proteins is conserved. All the 8–10 cysteine residues in the primary amino acid sequence among Ly-6-like proteins can be aligned. Well--spaced cysteines in the amino acid backbone of Ly-6 molecules facilitate their folding and contribute to their tertiary structure. Modeling of mouse Ly-6 proteins based on the crystal structure of cobra toxin, which shows some homology with the mouse Ly-6 proteins, suggests the presence of 2 or 3 protruding fingers coinciding with the number of either 8 or 10 cysteine residues present (reviewed in [108]). Based on this limited amino acid identity and similar structural features among all Ly-6-like proteins, these proteins have been categorized into the Ly-6 super gene family (Ly-6SF). Despite sharing similarities in 3-dimensional structure, different members of the Ly-6SF perform diverse cellular functions. This diversity is consistent with their ability to bind distinct, non-overlapping ligands that are not shared among the Ly-6 proteins. It is likely that the members of the Ly-6 gene family have evolved from an ancestral gene by gene duplication.

To be able to identify Ly-6 genes in other species, mouse Ly-6A and Ly-6C cDNA probes were used and Ly-6-related transcripts were detected in rat kid-

ney. These four cDNA sequences isolated from the rat kidney cDNA library contain a remarkably conserved leader sequence as well as the 33 amino acid sequence at the C-terminus. Southern blot analysis of rat genomic DNA with a labeled rat Ly-6 cDNA probe showed multiple bands, thereby suggesting the multigenic nature of the rat Ly-6 gene family[36], similar to what has been reported for the mouse.

Previous efforts to isolate human Ly-6 genes by screening human cDNA libraries with labeled mouse Ly-6 cDNA probes using southern blotting were unsuccessful. Recent efforts have identified a few human gene products, which show a limited amino acid identity with mouse Ly-6 proteins. These human Ly-6 genes are localized to a part of the human chromosome 8, which is syntenic to the mouse chromosome 15, where the Ly-6 locus resides, and therefore might be considered as true homologues. In some cases, the human Ly-6 proteins do not share the tissue expression of their mouse counterparts. E48 is a human Ly-6 protein expressed on keratinocytes and its expression is absent in lymphocytes. In contrast, the mouse counterpart of E48, Ly-6ThB, shows expression on keratinocytes as well as lymphocytes[11]. The exclusive expression of human E48 on keratinocytes and the loss of its expression on lymphocytes appears to be due to functional elimination of ancestral Ly-6 – exon 1 out of the total of four exons constituting the mouse gene[12]. Messenger RNA for human retinoic acid-induced gene E (RIG-E), a retinoic acid-inducible differentiation marker, on acute promyelocytic leukemia cells is detected on a number of lymphoid and non-lymphoid tissues, including liver, spleen, kidney, and uterus. RIG-E expression is not observed in stomach, colon, and placenta[73]. Human TSA-1/Sca-2 is expressed on lymphocytes and has been mapped to human chromosome 8q24.3[14]. The highest expression of human TSA--1/Sca-2 was observed in spleen, ovary, and pineal gland. In addition, the expression was observed in brain, breast tissue, and fetal liver[14]. Northern blot analysis demonstrated the presence of 9804 mRNA, a homologue of mouse TSA-1, in a variety of human fetal and adult tissues[99]. These observations suggest the presence of TSA-1/Sca-2-related gene sequences in human tissues. Monoclonal antibodies against 9804 protein, which is currently unavailable, will provide insights into the biochemical nature of these human Ly-6 gene products. Expression of secreted Ly-6/uPAR-related protein 2 (SLURP-2), another homologue of mouse Ly-6, was reported on epithelial cells of human skin[109]. In contrast, SLURP-2 mRNA was found to be absent in bone marrow and spleen, the two organs where cells of developing and mature cells of hematopoietic origin reside, respectively. In contrast, the expression of this protein is observed in the thymus[109]. Whether this later expression is specific for the thymic stroma or lymphoid population is unknown.

Although most Ly-6 proteins have 10 cysteine residues, the SLURP-1 protein, a secreted mammalian member of the Ly-6 gene family that lacks the GPI-anchoring signal sequence, has 8 cysteine residues[1]. A human SLURP-1-like Ly-6-related protein in the xenopus (XLURP-1) is expressed on progenitors of myeloid cells, first detected at the anteroventral site in the early tail-bud stage of xenopus embryos[103]. It has been proposed that transient presence of XLURP-1-expressing cells, bisecting the cardiac mesoderm prior to ear tube formation, has a role in cardiac morphogenesis[103]. Ly-6-related protein, SP-10, has been identified in sperm acrosome, but its role in fertilization has not yet been investigated[83].

In summary, these reports show the existence of a number of human, rat, and xenopus genes that have a potential homology with the mouse Ly-6 proteins. Both sequence similarity and syntenic relationship between mouse and human Ly-6 sequences will be important in determining their homologous nature. The tissue expression of Ly-6 proteins across the species, the nature of their ligands, and the consequences of their interaction with ligand(s) will provide more insights into the immunobiology of the Ly-6 supergene family.

REGULATION OF LY-6 PROTEIN EXPRESSION

Cytokine/signaling-regulated expression of Ly-6 proteins

The expressions of Ly-6 proteins are upregulated on a variety of mouse tissues in the body during immune responses targeted against either a foreign or self antigen (reviewed in[38, 71, 83, 93, 100]). Exposure of immune cells to type I and II IFNs upregulates the expression of Ly-6 gene products under *in vitro* and *in vivo* experimental settings (reviewed in[38, 71, 83, 93, 100]). Inclusion of antibodies against IFN-γ in assays that measure T lymphocyte activation resulted in significantly diminished upregulation of Ly-6 molecules[28]. Similar IFN-γ inducibility is observed for osteoclast--inhibitory protein-1/hSca, a Ly-6-like gene product that inhibits osteoclast formation, a process central to bone development[61]. These results suggest a direct role of IFN-γ in upregulating Ly-6 protein expression. The role of protein kinase C (PKC) in the induction of Ly-6 protein expression has been previously recognized[27]. Pharmacological agents that activate PKC upregulate the expression of Ly-6 proteins in γδ-positive dendritic epithelial cells present in the mouse

epidermis[48]. Ly-6D expression is upregulated in mouse liver by perioxisome proliferators-activated receptor α[22]. While a number of cytokines upregulate the expression of Ly-6 proteins, expression of Ly-6 is downregulated by tumor necrosis factor α (TNF-α) and anti-Fas antibodies, which induce apoptosis in tumor cell lines[95]. These reports demonstrate that cytokines secreted during both normal and abnormal immune responses alter surface expression of Ly-6 proteins.

The mechanisms that underlie the up-regulation and down-regulation of Ly-6 proteins by IFNs and other cytokines remain unclear. Bothwell and colleagues have identified an 80-bp region, within the Ly-6E promoter containing a signature IFN-γ activation site (GAS), that binds a 91-kDa phosphorylated protein induced during exposure to IFN-γ[58]. This GAS site is part of the distal regulatory region upstream, –1760 to –1220, of the Ly-6E promoter in fibroblasts. Several possible regulatory sequences have been found in the putative promoter region that lies 5' to the first exon of the Ly-6C.1 gene. These regulatory sequences of the Ly-6C.1 gene include a previously reported 28-base sequence closely resembling the consensus IFN-responsive sequence found in other IFN-responsive genes[10]. Other studies suggest two distinct regions required for IFN responsiveness; one region lies upstream of –1760 of the promoter and the second is the G region corresponding to –1245 to –1214 bp of the Ly-6E promoter[59]. The G region contains a GAS sequence known to bind STAT1, in which binding is critical for induction of Ly-6E expression by both IFN-γ and IFN-α/β, in T cell lines. This study also demonstrates the importance of a number of other transcriptional factors, e.g. Oct-1 and Oct-2, as well as chromatin-associated high mobitity glycoprotein proteins, in the IFN-dependent expression of Ly-6 proteins[59].

The above findings highlight the similarity between the mechanisms underlying the induction of Ly-6 proteins by IFN-γ and IFN-α/β, but how the two mechanisms are distinct remains unclear[60]. The mechanism underlying the enhanced expression of other members of the Ly-6 gene family by type I and type II IFNs in lymphoid and non-lymphoid cells is unknown. Moreover, the mechanisms that result in the synergistic action of IFN-γ with other cytokines in a cell-specific manner are unknown[70]. Future experiments need to address these issues to provide insights into the possible physiologic significance of Ly-6 upregulation under specific immune responses. We and others have hypothesized that the upregulated expression of Ly-6 proteins during cell activation and by cytokines generated during immune response play a role in regulating cell growth and differentiation[38, 46, 83, 93, 100]. However, the significance of Ly-6 protein induction by cytokines in innate and adaptive immunity is unclear.

Significance of regulated expression of Ly-6 proteins in development

Exogenous factors (e.g. cytokines) influence the expression of Ly-6 proteins. Some members of the Ly-6 family are known to show alterations in their expression during development, perhaps by endogenous factors that are also developmentally regulated. Sca-1 was identified as a marker for bone marrow stem cells, the precursors to all the T cells developing in the thymus[104]. Biochemical characterization and molecular cloning of cDNA encoding Sca-1 revealed it to be indistinguishable from the Ly-6A.2 molecule[111]. Some recent findings in which regulatory DNA sequences of Ly-6A.2 (Sca-1) were fused to reporter genes to generate transgenic mice have reconfirmed the expression of Ly-6A.2 in stem cells of T cell lineage[69]. These findings have provided an impetus to study other Ly-6A.2 (Sca-1) protein expression during early developmental stages. Ly-6 proteins are expressed in a cell line derived from the aorta-gonads-mesonephros (AGM)[117]. The earliest expression of Ly-6A.2 is observed during embryogenesis in the AGM region of mice embryos[24], the site of the first hematopoietic stem cells (HSCs), which give rise to all blood lineages in the adult organism. Transgenic mice expressing the green fluorescence protein (GFP) marker under the control of the Sca-1 transcriptional regulatory elements show a clear localization of GFP-expressing cells to the endothelial cell layer of the dorsal aorta in the AGM region[42]. Collectively, these observations demonstrate the expression of a Ly-6 protein during the early stages of embryogenesis; subsequent alterations of its expression during late embryogenesis have not been investigated.

The role of Ly-6A.2 (Sca-1) protein in the development of immune and non-immune cells has been a recent focus of study. Stanford and his colleagues have generated Sca-1-deficient mice to study this issue[53]. These studies have revealed that Sca-1 participates and contributes to regulating HSC renewal and the development of committed progenitor cells, megakaryocytes, and platelets[53]. Ly-6A.2 continues to be expressed on the most immature, $CD4^-CD8^-CD3^-$, T cell subset in the thymus, where expression is lost during the transition from the $CD44^+CD25^+CD3^-CD4^-CD8^-$ (stage II) to the $CD44^-CD25^+CD3^-CD4^-CD8^-$ (stage III) stage[7]. Although $CD4^+CD8^+$ (double positive – DN) T cells do not express Ly-6A.2 protein, it is re-expressed on mature $CD4^+CD8^-$ and

$CD4^-CD8^+$ (single positive) T cells[38, 93, 100]. The underlying mechanism related to the regulated expression of Ly-6 proteins during development has only been the focus of investigation in recent years[7, 44]. In mice, dysregulation of Ly-6A.2 protein during T cell development in the thymus by ectopic expression on thymic subsets shows a block in development at the $CD4^-CD8^-CD3^-$ subset stage, where normal expression of Ly-6A.2 is lost[7]. Reduced amounts of linker of activated T cells (an adaptor protein critical for the progression of DN T cells to the DP stage) in immature T cells from the Ly-6A.2-dysregulated mice may explain the block observed in these mice[44]. Another molecule, $p56^{lck}$, which plays an important role in the transition from the DN to the DP stage, is not altered. The cells that mature in the Ly-6A.2-dysregulated mice show broad usage of the V_β T cell repertoire, suggesting that rearrangement of T cell receptor (TCR) genes was not affected. Alterations in the V_β TCR repertoire was observed, perhaps due to the enhanced cell death by apoptosis observed in the thymus of these mice[44]. Abnormal development of $CD4^+$ T cells in mice, where Ly-6A.2 is ectopically expressed on the $CD4^+CD8^+$ T cells, even in the absence of the MHC class I and II underscores the importance of regulated Ly-6A.2 expression on the developing T cells in the thymus[45]. These findings are surprising, since the MHC proteins, complexed with self peptides presented in the thymus, are central to the developmental processes. Collectively, these published reports suggest that dysregulating Ly-6A.2 expression on developing thymocytes inhibits their growth during the $CD4^-CD8^-$ stage of their development; cells that manage to develop in the Ly-6A.2-dysregulated mice have an altered V_β T cell repertoire, thereby suggesting that the loss of Ly-6A.2 expression within the $CD4^-CD8^-$ subset is essential for normal T cell development and constitutes an important checkpoint in this process.

Ly-6ThB, another member of the Ly-6 gene family, appears to have a reciprocal pattern of expression on developing T cells to that of Ly-6A.2. The majority of $CD4^+CD8^+$ thymocytes express Ly-6ThB, and about 20–40% of $CD4^+CD8^+$ T cells downregulate Ly-6ThB expression during thymic selection processes[90]. The importance of developmentally regulated expression of ThB and other Ly-6 molecules remains to be addressed.

FUNCTION OF LY-6 PROTEINS

Role of Ly-6 in cell signaling

Our insights into the expression, biochemistry, and structure of mouse Ly-6 proteins are far more advanced than our understanding of the physiological role of this gene family. Attempts to resolve the function of the Ly-6 gene family have posed many challenges. The first set of observations that suggested the role of Ly-6 proteins in T cell responses were made in Ken Rock and Ethen Shevach's laboratories in the mid 1980's. These investigators generated monoclonal antibodies against Ly-6 proteins. Cross-linking of Ly-6 proteins with anti-Ly-6 antibodies caused transcription, translation, and synthesis of interleukin 2 (IL-2), a key growth factor for T lymphocytes[72, 94]. These observations set up the first paradigm to address the function of this gene family. To examine the importance of Ly-6 expression to lymphocyte activation, Yeh et al.[118] subjected a T-T hybridoma to mutagenesis and screened for variants lacking the expression of Ly-6A.2 protein. These extensive analyses suggested that the lack of Ly-6 expression on T-T hybridoma cells results in reduced responses initiated by the antigen receptor[118]. Consistent with these data are the observations that silencing of the Ly-6A expression on $CD4^+$ lymph node T cells, as well as a T cell line, with an antisense oligonucleotide complimentary to the 5' of the Ly-6A gene[35] or by expression (moderate) of antisense Ly-6 RNA stably transfected in a T cell line resulted in the inhibition of responses through the antigen receptor[66]. These early studies suggest that Ly-6 protein expression plays an important role in T cell activation.

In contrast to the early studies, some recent findings with Ly-6A knock-out and Ly-6A.2 transgenic mice suggest an inhibitory role of these proteins. The $CD4^+$ cells from Ly-6 knock-out mice, which lack Ly-6A protein expression, showed modest hyper-proliferative responses to anti-CD3 antibodies. These experiments also revealed that the signaling through the TCR/CD3 complex in $CD4^+$ T cells from the Ly-6A knock-out mice lasted longer than the responses generated by $CD4^+$ T cells from normal mice. Experimental support to this observation is lent by analyses of $CD4^+$ cells from $Ly\text{-}6Tg^+ \times$ DO11 TCR double transgenic mice. $CD4^+$ T cells from these mice, which overexpressed Ly-6A.2 protein, showed a reduced growth in response to a peptide antigen, chicken ovalbumin peptide (cOVA323-339)[46]. These recent studies suggest an inhibitory role of Ly-6 expression, recapitulating what has been observed with cell lines where both Ly-6 and TCR molecules are engaged concurrently, with their respective antibodies[71]. Co-engagement of the antigen receptor along with other accessory proteins that are anchored to the plasma membrane by a GPI-anchor (including Ly-6 proteins) also shows repressed responses, thereby suggesting that the inhibitory mechanisms may be

common to all proteins that are anchored to the membrane by a GPI-anchor (reviewed in[66, 74]). The mechanism underlying the inhibitory and activating responses through Ly-6 protein are not completely known and current understanding of this issue is discussed below.

Models that would consistently explain the apparently contradictory literature related to the signaling mediated by Ly-6 proteins are lacking. Signaling through Ly-6 proteins has been studied in two different contexts: first, anti-Ly-6 antibodies have been used to engage the Ly-6 molecules singularly, in the absence of concurrent signaling through the antigen receptor. Second, both the Ly-6 and the antigen receptor have been concurrently engaged. In the first context, the outcome of cross-linking of Ly-6 proteins with their antibodies has resulted in the production of the cytokines such as IL-2 and IFN-γ by T cell lines as well as normal T cells. The cytokine responses by normal T cells require additional factor(s) whose action is mimicked by the pharmacological agent phorbol myristate acetate, which is known to activate PKC[93]. It has remained enigmatic how Ly-6 and other proteins, tethered to the outer leaflet of the membrane by a GPI-anchor, are able to signal inside the cell despite the absence of their transmembrane and cytoplasmic tail. Expression of the TCR and its signaling components are critical for the activation of T cells by a number of GPI-anchored proteins, including Ly-6. Engagement of Ly-6 proteins with anti-Ly-6 antibodies causes phosphorylation of the TCR-ζ chain[115]. T cell hybridomas that express TCR-ζ with truncated cytoplasmic domains, which contain the three signaling immunoreceptor tyrosine-based activation motifs (ITAM), fail to generate cytokines[114]. In some instances the contribution of components of the TCR/CD3 complex other than TCR-ζ appears to be sufficient to signal into the cell interior through Ly-6 and another GPI-anchored protein Thy-1[47]. These studies might suggest that Ly-6 and other GPI-anchored proteins may co-opt TCR and its associated signal-transducing components (CD3/TCR-ζ) for signaling into the cell interior. It is possible that Ly-6 proteins may achieve this function by physically associating with TCR/CD3/ζ complex on the plasma membrane to transduce signals[62].

Recent data suggest that the presence of specialized clusters of saturated sphingolipids, and cholesterol in the lipid bilayer, jointly create "lipid rafts"[13, 56, 101]. These structures are thermodynamically more stable and have higher melting temperatures, as opposed to the more fluid, liquid-disordered phase of the membrane, composed of phospholipids with unsaturated fatty acyl chains. Lipid rafts play an important role in cell signaling. Inhibitors that de-stabilize the lipid raft, either by sequestering, depleting, or suppressing synthesis of cholesterol, inhibit the signaling through a number of cell surface receptors in a variety of cell types[81, 116]. These findings suggest the importance of intact lipid microdomains as relay stations on the plasma membrane, from where signaling is transmitted to the cell interior. Engagement of the TCR with an appropriate MHC-peptide complex or by antibodies directed against the antigen receptor and its signaling components results in their aggregation and the trafficking of the TCR and its components into the lipid rafts, along with other signaling molecules[4, 55, 81, 116]. It is also generally believed that cross-linking of the receptor also initiates the coalescing of the lipid rafts that are small and unstable[81]. GPI-anchored proteins, including Ly-6 molecules tethered on the outer leaflet of the membrane with a lipid anchor, are housed in the sphingolipid-cholesterol rafts[43, 102]. The location of Ly-6 proteins within the lipid rafts may place them in the vicinity of some key signaling proteins[52, 120] that are either housed in the lipid rafts or accumulate in it during cell signaling events. Perhaps this may prove to be a way for Ly-6 molecules to co-opt their signaling properties. Future experiments are necessary to address these issues.

Signaling through Ly-6 proteins has also been studied in the context of signaling through the antigen receptor. Concurrent engagement of the Ly-6 protein and the antigen receptor by the agonists that bind them results in a more inhibitory response than the response observed when either antigen receptor or Ly-6 is engaged singularly. It has been proposed that interaction of Ly-6 with its ligand initiates inhibitory signals. $CD4^+$ T cells from the lymph nodes of Ly-6 knockout mice, which lack Ly-6A expression and are therefore unable to engage its ligand, show a modestly higher proliferation than control cells[106]. The $CD4^+$ T cells from Ly-6A.2 transgenic mice, where Ly-6A.2 protein is overexpressed and therefore likely to engage its ligand more avidly, show substantial growth inhibition in the presence of a model peptide antigen[46]. These observations suggest that Ly-6A.2 expression regulates signaling initiated through the antigen receptor. The biochemical events associated with the inhibitory signals delivered by Ly-6 protein have not been fully investigated. Marmor and Julius[75] have suggested that inhibitory signals through GPI-anchored proteins (including Ly-6) prevent the IL-2R-α chain's association with the IL-2R-β and -γ, which is necessary to activate the Janus kinases and other downstream membrane signaling events. Another possible mechanism for Ly-6 expression-dependent inhibitory signals may directly implicate the components of the TCR/CD3 complex. Since signaling initiated by Ly-6, as well as the antigen recep-

tor, requires expression of TCR-ζ, it is likely that the signaling pathways initiated by concurrent engagement of these two receptors compete for the same set of membrane proximal signaling components, thereby inhibiting signals initiated by cross-linking Ly-6 molecule. Future investigations need to address this issue.

Recent advances in T cell signaling have utilized powerful imaging devices to gain insight into the topological and spatial constraints of membrane-proximal signaling events[51]. An activated T cell forms an immunological synapse (IS) with an appropriate antigen-presenting cell (APC)[29, 79]. Signaling proteins in T cells cluster at the contact site of the T cell – APC conjugates[51, 75, 79]. TCR and PKC-θ are localized to the center of the IS and lymphocyte function antigen-1 and talin at the periphery of the synapse[29, 80]. Certain proteins known to inhibit T cell activation (CD45 and CD43) are excluded from the center of the synapse[2, 25, 105]. Some recent data suggest that the IS may be a site where T cell responses are regulated[65]. The localization of Ly-6 proteins within IS is unknown. It is possible that Ly-6A.2 expression might influence the organization of signaling proteins within or outside these structures and therefore alter signaling in T cells. The physiological significance of signals emanating from Ly-6 is unknown. Collectively, these results suggest that Ly-6A.2 participates as an accessory cell protein in T cell responses. These responses are primarily initiated by the antigen receptor (signal 1) and the co-stimulatory molecule CD28 (signal 2) by binding to foreign antigen-MHC complexes and B7 proteins, respectively. Antigen-stimulated $CD4^+$ T cells double every 4.5 h and therefore have a potential to generate 1×10^{12} cells in a week's time[67]. This profound proliferation, compounded by limited space in the lymphoid compartment, may have toxic effects and autoimmune consequences. Therefore, these processes appear to be regulated by Ly-6 and a number of other proteins (CTLA-4, IL-2, and members of TNF gene family). This idea is consistent with the expression pattern of Ly-6A.2, which shows minimal expression on naïve T cells and its profound upregulation upon T cell activation.

Role of Ly-6 proteins in regulating signaling in non-lymphoid cells

In mice, the expression of Ly-6 proteins appears to regulate TCR-dependent growth and cytokine production. Several members of the Ly-6 supergene family, including Ly-6 homologous proteins in other species, appear to participate in regulating important functions in non-lymphoid tissues by yet unidentified mechanisms. While searching for novel protein factors with the potential to inhibit osteoclast formation, Reddy and colleagues isolated a candidate gene belonging to the Ly-6 gene family from the human osteoclast-like multinucleated cell (MNC) cDNA library by expression cloning[15]. The product of this gene significantly reduced the osteoclast-like MNC formation induced by 1.25-dihydroxyvitamin D3 in both mouse and human bone marrow cultures. These inhibitory effects were cell specific; this member of the human Ly-6 protein family did not inhibit growth in a variety of non--osteoclast cells. Mutations in the odr-2 gene, which encodes a Ly-6 related protein, causes a defect in the ability to chemotax two odorants that are recognized by the two AWC olfactory neurons in *C. elegans*[16]. A mouse lynx1, which is expressed on hippocampus, cortex, and cerebellum cells, modulates nicotinic acetylcholine receptors in the mammalian brain[78]. Moreover, a mutation in SLURP-1, a human Ly-6 protein, causes a rare autosomal recessive skin disorder, Mal de Meleda, characterized by transgressive palmoplanter keratoderma, keratotic skin lesions, and perioral erythema[33]. The regulatory function of the Ly-6 protein in T cells and other cells is likely to stem from two major properties reported for some members of this gene family: cell signaling[72, 94] and cell adhesion[8, 11, 18, 41]. Cell-cell adhesion properties of mouse (Ly-6A.2, TSA-1)[8, 18] and human (E48, a human homologue of mouse Ly-6ThB)[11] Ly-6 proteins have been reported. These observations provided the impetus to identify the Ly-6 ligands. We recently raised monoclonal and polyclonal antibodies against a Ly-6A.2 ligand. Our data indicate that the ligand is a 66-kDa protein expressed in the thymus and the spleen as well as on the majority of B cells and macrophages[30]. CD22 expressed on B cells appears to be another promiscuous ligand that binds multiple members of the Ly-6 family[87]. A ligand for another member of the Ly-6 gene family, Ly-6d, was recently identified and cloned[5]. Ly-6d-ligand is a 9-kDa cell surface protein with broad tissue expression[5]. Interestingly, the Ly-6d ligand has homology with mouse notch proteins, which are evolutionarily conserved and play an important role in development /lineage commitment in a number of cell types, including lymphocytes (reviewed in[89]). The Ly-6d-ligand is transcribed and translated from the same gene as GPY-7, an 80-kDa GTPase activator protein, but in reverse orientation[17]. Our results suggest that one way in which Ly-6 proteins modulate signaling and mediate their function is by interacting with their ligand(s). The precise functional consequences and physiological significance of Ly-6 – ligand interactions are currently unknown.

Ly-6 and disease conditions

Expression of Ly-6 proteins is upregulated on a variety of tissues during malignancy and immune disor-

ders[23, 57]. Lymphocytes from MRL-lpr/lpr autoimmune mice show upregulated expression of Ly-6 proteins[23]. More recently, in an effort to identify disease-specific genes using microarray assays, a gene sequence identical to secreted Ly-6/uPAR-related protein 2, SlURP-2, a novel member of Ly-6SF, was identified in psoriasis vulgaris[109]. Ly-6 proteins are also upregulated in cells that have undergone malignant transformation. A number of proteins belonging to the Ly-6SF, including uPAR[37], Ly-6A.2[57], and prostate stem cell antigen (PSCA)[92], are known to be highly expressed in malignantly transformed cells. Some reports have shown correlation of high mouse Ly-6A/E, human E48, and PSCA expression with high metastatic phenotype in mice and humans (reviewed in[40,112]). The role of Ly-6 proteins, especially involved in adhesion and cell signaling, in the onset and progression of cancer has not been fully addressed. It also remains to be seen if the upregulation of Ly-6 proteins during cancer development is simply the consequence of the pathophysiology associated with the disease condition. Ly-6 genes are known to carry IFN-regulatory sequences and, therefore, it is highly likely that type I and II IFNs generated during the disease process cause upregulation of Ly-6 expression[68]. Some recent data suggest that human E48 upregulates the expression of the FX enzyme, a key component in the biosynthesis pathway of selectin ligand, sialyl Lewis'a. Using anti-sense E48 it was demonstrated that E48 expression is involved in upregulation of selectin ligands as well as adhesion to activated HUVECs[32]. Regardless of the mechanism of tumorigenesis and the role of Ly-6 proteins in this process, the higher expression of Ly-6 members on malignant tissues allows them to be an attractive target for diagnosis of malignancy as well as immunotherapy. Progress in this area has been forthcoming, and the reader is directed to a recent review on this subject[31, 54]. In summary, Ly-6 gene products provide additional cancer-associated molecules that have the potential to be used for the diagnosis and therapy of a variety of cancers arising from different tissues.

ACKNOWLEDGMENT

The author would like to acknowledge Professor Russell Gardner and members of his laboratory, including Jennifer Reed, Matthew D. Nelson and Joe Comber, for helpful suggestions.

REFERENCES

1. Adermann K., Wattler F., Wattler S., Heine G., Meyer M., Forssmann W. G. and Nehls M. (1999): Structural and phylogenetic characterization of human SLURP-1, the first secreted mammalian member of the Ly-6/uPAR protein superfamily. Protein Sci., 8, 810–819.

2. Allenspach E. J., Cullinan P., Tong J., Tang Q., Tesciuba A. G., Cannon J. L., Takahashi S. M., Morgan R., Burkhardt J. K. and Sperling A. I. (2001): ERM-dependent movement of CD43 defines a novel protein complex distal to the immunological synapse. Immunity, 15, 739–750.

3. Alliot F., Rutin J. and Pessac B. (1998): Ly-6C is expressed in brain vessels endothelial cells but not in microglia of the mouse. Neurosci. Lett., 251, 37–40.

4. Alonso M. A. and Millan J. (2001): The role of lipid rafts in signalling and membrane trafficking in T lymphocytes. J. Cell Sci., 114, 3957–3965.

5. Apostolopoulos J., McKenzie I. F. and Sandrin M. S. (2000): Ly6d-L, a cell surface ligand for mouse Ly6d. Immunity, 12, 223–232.

6. Asselin-Paturel C., Boonstra A., Dalod M., Durand I., Yessaad N., Dezutter-Dambuyant C., Vicari A., O'Garra A., Biron C., Briere F. and Trinchieri G. (2001): Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat. Immunol., 2, 1144–1150.

7. Bamezai A., Palliser D., Berezovskaya A., McGrew J., Higgins K., Lacy E. and Rock K. L. (1995): Regulated expression of Ly-6A.2 is important for T cell development. J. Immunol., 154, 4233–4239.

8. Bamezai A. and Rock K. L. (1995): Overexpressed Ly-6A.2 mediates cell-cell adhesion by binding a ligand expressed on lymphoid cells. Proc. Natl. Acad. Sci. USA, 92, 4294–4298.

9. Blake P. G, Madrenas J. and Halloran P. F. (1993): Ly-6 in kidney is widely expressed on tubular epithelium and vascular endothelium and is up-regulated by interferon γ. J. Am. Soc. Nephrol., 4, 1140–1150.

10. Bothwell A., Pace P. E. and LeClair K. P. (1988): Isolation and expression of an IFN-responsive Ly-6C chromosomal gene. J. Immunol., 140, 2815–2820.

11. Brakenhoff R. H., Gerretsen M., Knippels E. M., van Dijk M., van Essen H., Weghuis D. O., Sinke R. J., Snow G. B. and van Dongen G. A. (1995): The human E48 antigen, highly homologous to the murine Ly-6 antigen ThB, is a GPI-anchored molecule apparently involved in keratinocyte cell-cell adhesion. J. Cell Biol., 129,1677–1689.

12. Brakenhoff R. H., van Dijk M., Rood-Knippels E. M. and Snow G. B. (1997): A gain of novel tissue specificity in the human Ly-6 gene E48. J. Immunol., 159, 4879–4886.

13. Brown D. A. and London E. (1998): Functions of lipid rafts in biological membranes. Annu. Rev. Cell Dev. Biol., 14, 111–136.

14. Capone M. C., Gorman D. M., Ching E. P. and Zlotnik A. (1996): Identification through bioinformatics of cDNAs encoding human thymic shared Ag-1/stem cell Ag-2. A new member of the human Ly-6 family. J. Immunol., 157, 969–973.

15. Choi S. J., Devlin R. D., Menaa C., Chung H., Roodman G. D. and Reddy S. V. (1998): Cloning and identification of human Sca as a novel inhibitor of osteoclast formation and bone resorption. J. Clin. Invest., 102, 1360–1368.

16. Chou J. H., Bargmann C. I. and Sengupta P. (2001): The *Caenorhabditis elegans* odr-2 gene encodes a novel Ly-6-related protein required for olfaction. Genetics, 157, 211–224.

17. Classon B. J. (2001): Ly-6 ligands remain elusive. Trends Immunol., 22, 126–127.

18. Classon B. J. and Boyd R. L. (1998): Thymic-shared antigen-1 (TSA--1). A lymphostromal cell membrane Ly-6 superfamily molecule with a putative role in cellularadhesion. Dev. Immunol., 6, 149–156.

19. Classon B. J. and Coverdale L. (1994): Mouse stem cell antigen Sca-2 is a member of the Ly-6 family of cell surface proteins. Proc. Natl. Acad. Sci. USA, 91, 5296–5300.

20. Cray C., Keane R. W., Malek T. R. and Levy R. B. (1990): Regulation and selective expression of Ly-6A/E, a lymphocyte activation molecule, in the central nervous system. Brain Res. Mol. Brain Res., 8, 9–15.

21. Curtsinger J. M., Lins D. C. and Mescher M. F. (1998): $CD8^+$ memory T cells ($CD44^{high}$, Ly-$6C^+$) are more sensitive than naive cells to ($CD44^{low}$, Ly-$6C^-$) to TCR/CD8 signaling in response to antigen. J. Immunol., 160, 3236–3243.

22. Dadras S. S., Cook W. S., Yeldandi A. V., Cao W. Q., Rao M. S., Wang Z. and Reddy J. K. (2001): Peroxisome proliferator-activated receptor alpha-dependent induction of cell surface antigen Ly--6D gene in the mouse liver. Gene Expr., 9, 173–181.

23. Davidson W. F., Dumont F. J., Bedigian H. G., Fowlkes B. J. and Morse H. C. 3rd. (1986): Phenotypic, functional, and molecular genetic comparisons of the abnormal lymphoid cells of C3H--lpr/lpr and C3H-gld/gld mice. J. Immunol., 136, 4075–4084.

24. De Bruijn M. F., Ma X., Robin C., Ottersbach K., Sanchez M. J. and Dzierzak E. (2002): Hematopoietic stem cells localize to the endothelial cell layer in the midgestation mouse aorta. Immunity, 16, 673–683.

25. Delon J., Kaibuchi K. and Germain R. N. (2001): Exclusion of CD43 from the immunological synapse is mediated by phosphorylation-regulated relocation of the cytoskeletal adaptor moesin. Immunity, 15, 691–701.

26. Diebold S. S, Kaisho T., Hemmi H., Akira S., Reis e Sousa C. (2004): Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science, 303, 1529–1531.

27. Dumont F. J., Altmeyer A., Staruch M. J., Dijkmans R., Palfree R. G. and Fischer P. A. (1990): Role of protein kinase C in IFN--mediated Ly-6E antigen induction. J. Immunol., 144, 3795–3803.

28. Dumont F. J., Dijkmans R., Palfree R. G., Boltz R. D. and Coker L. (1987): Selective up-regulation by interferon-γ of surface molecules of the Ly-6 complex in resting T cells: the Ly-6A/E and TAP antigens are preferentially enhanced. Eur. J. Immunol., 17, 1183–1191.

29. Dustin M. L. and Shaw A. S. (1999): Costimulation: building an immunological synapse. Science, 283, 649–650.

30. English A., Kosoy R., Pawlinski R. and Bamezai A. (2000): A monoclonal antibody against the 66-kDa protein expressed in mouse spleen and thymus inhibits Ly-6A.2-dependent cell-cell adhesion. J. Immunol., 165, 3763–3771.

31. Eshel R., Neumark E., Sagi-Assif O. and Witz I. P. (2002): Receptors involved in microenvironment-driven molecular evolution of cancer cells. Semin. Cancer Biol., 12, 139–147.

32. Eshel R., Zanin A., Sagi-Assif O., Meshel T., Smorodinsky N. I., Dwir O., Alon R., Brakenhoff R., van Dongen G. and Witz I. P. (2000): The GPI-linked Ly-6 antigen E48 regulates expression levels of the FX enzyme and of E-selectin ligands on head and neck squamous carcinoma cells. J. Biol. Chem., 275,12833–12840.

33. Fischer J., Bouadjar B., Heilig R., Huber M., Lefevre C., Jobard F., Macari F., Bakija-Konsuo A., Ait-Belkacem F., Weissenbach J., Lathrop M., Hohl D. and Prud'homme J. F. (2001): Mutations in the gene encoding SLURP-1 in Mal de Meleda. Hum. Mol. Genet., 10, 875–880.

34. Fleming T. J., Fleming M. L. and Malek T. R. (1993): Selective expression of Ly-6G on myeloid lineage cells in mouse bone marrow. RB6-8C5 mAb to granulocyte-differentiation antigen (Gr-1) detects members of the Ly-6 family. J. Immunol., 151, 2399–2408.

35. Flood P. M., Dougherty J. P. and Ron Y. (1990): Inhibition of Ly--6A antigen expression prevents T cell activation. J. Exp. Med., 172, 115–120.

36. Friedman S., Palfree R. G., Sirlin S. and Hammerling U. (1990): Analysis of three distinct Ly6-A-related cDNA sequences isolated from rat kidney. Immunogenetics, 31, 104–111.

37. Gondi C. S., Lakka S. S., Yanamandra N., Siddique K., Dinh D. H., Olivero W. C., Gujrati M. and Rao J. S. (2003): Expression of antisense uPAR and antisense uPA from a bicistronic adenoviral construct inhibits glioma cell invasion, tumor growth, and angiogenesis. Oncogene, 22, 5967–5975.

38. Gumley T. P., McKenzie I. F. and Sandrin M. S. (1995): Tissue expression, structure and function of the murine Ly-6 family of molecules. Immunol. Cell Biol., 73, 277–296.

39. Halloran P. F., Richards R. R., Stubbs M. and Gidon M. (1981): Evidence for expression of Ly-6.2 on non-bone marrow-derived cells in kidney, skin and connective tissues. J. Immunogenet., 8, 323–334.

40. Han K. R., Seligson D. B., Liu X., Horvath S., Shintaku P. I., Thomas G. V., Said J. W. and Reiter R. E. (2004): Prostate stem cell antigen expression is associated with gleason score, seminal vesicle invasion and capsular invasion in prostate cancer. J. Urol., 171, 1117–1121.

41. Hanninen A., Jaakkola I., Salmi M., Simell O. and Jalkanen S. (1997): Ly-6C regulates endothelial adhesion and homing of CD8(+) T cells by activating integrin-dependent adhesion pathways. Proc. Natl. Acad. Sci. USA, 94, 6898–6903.

42. Hanson P., Mathews V., Marrus S. H. and Graubert T. A. (2003): Enhanced green fluorescent protein targeted to the Sca-1 (Ly-6A) locus in transgenic mice results in efficient marking of hematopoietic stem cells *in vivo*. Exp. Hematol., 31, 159–167.

43. Harder T. and Simons K. (1999): Clusters of glycolipid and glycosylphosphatidylinositol-anchored proteins in lymphoid cells: accumulation of actin regulated by local phosphorylation. Eur. J. Immunol., 29, 556–562.

44. Henderson S. C. and Bamezai A. (2003): Loss of Ly-6A.2 expression on immature developing T cells in the thymus is necessary for their normal growth and generation of the V_β T-cell repertoire. Tissue Antigens, 62, 117–132.

45. Henderson S. C., Berezovskaya A., English A., Palliser D., Rock K. L. and Bamezai A. (1998): $CD4^+$ T cells mature in the absence of MHC class I and class II expression in Ly-6A.2 transgenic mice. J. Immunol., 161, 175–182.

46. Henderson S. C., Kamdar M. M. and Bamezai A. (2002): Ly-6A.2 expression regulates antigen-specific $CD4^+$ T cell proliferation and cytokine production. J. Immunol., 168, 118–126.

47. Hermans M. H. and Malissen B. (1993): The cytoplasmic tail of the T cell receptor ζ chain is dispensable for antigen-mediated T cell activation. Eur. J. Immunol., 23, 2257–2262.

48. HogenEsch H., de Geus B., Tielen F. and Rozing J. (1993): Constitutive expression of Ly-6.A2 on murine keratinocytes and inducible expression on TCR $\gamma\delta^+$ dendritic epidermal T cells. J. Dermatol. Sci., 5, 114–1121.

49. Horie M., Okutomi K., Taniguchi Y., Ohbuchi Y., Suzuki M. and Takahashi E. (1998): Isolation and characterization of a new member of the human Ly6 gene family (LY6H). Genomics, 53, 365–368.

50. Horowitz M. C., Fields A., DeMeo D., Qian H. Y., Bothwell A. L. and Trepman E. (1994): Expression and regulation of Ly-6 differentiation antigens by murine osteoblasts. Endocrinology, 135, 1032–1043.

51. Huppa J. B. and Davis M. M. (2003): T-cell-antigen recognition and the immunological synapse. Nat. Rev. Immunol., 3, 973–983.

52. Ilangumaran S., Arni S., van Echten-Deckert G., Borisch B. and Hoessli D. C. (1999): Microdomain-dependent regulation of Lck and Fyn protein-tyrosine kinases in T lymphocyte plasma membranes. Mol. Biol. Cell, 10, 891–905.

53. Ito C. Y., Li C. Y., Bernstein A., Dick J. E. and Stanford W. L. (2003): Hematopoietic stem cell and progenitor defects in Sca--1/Ly-6A-null mice. Blood, 101, 517–523.

54. Jalkut M. W. and Reiter R. E. (2002): Role of prostate stem cell antigen in prostate cancer research. Curr. Opin. Urol., 12, 401–406.

55. Janes P. W., Ley S. C. and Magee A. I. (1999): Aggregation of lipid rafts accompanies signaling via the T cell antigen receptor. J. Cell Biol., 147, 447–461.

56. Karnovsky M. J., Kleinfeld A. M., Hoover R. L. and Klausner R. D. (1982): The concept of lipid domains in membranes. J. Cell Biol., 94, 1–6.

57. Katz B. Z., Eshel R., Sagi-Assif O. and Witz I. P. (1994): An association between high Ly-6A/E expression on tumor cells and a highly malignant phenotype. Int. J. Cancer, 59, 684–691.

58. Khan K. D., Shuai K., Lindwall G., Maher S. E., Darnell J. E. Jr. and Bothwell A. L. (1993): Induction of the Ly-6A/E gene by

interferon α/β and gamma requires a DNA element to which a tyrosine-phosphorylated 91-kDa protein binds. Proc. Natl. Acad. Sci. USA, 90, 6806–6810.

59. Khodadoust M. M., Khan K. D. and Bothwell A. L. (1999): Complex regulation of Ly-6E gene transcription in T cells by IFNs. J. Immunol., 163, 811–819.

60. Khodadoust M. M., Khan K. D., Park E. H. and Bothwell A. L. (1998): Distinct regulatory mechanisms for interferon-α/β (IFN--α/β)- and IFN-γmediated induction of Ly-6E gene in B cells. Blood, 92, 2399–2409.

61. Koide M., Maeda H., Roccisana J. L., Kawanabe N. and Reddy S. V. (2003): Cytokine: regulation and the signaling mechanism of osteoclast inhibitory peptide-1 (OIP-1/hSca) to inhibit osteoclast formation. J. Bone Miner. Res., 18, 458–465.

62. Kosugi A., Saitoh S., Noda S., Miyake K., Yamashita Y., Kimoto M., Ogata M. and Hamaoka T. (1998): Physical and functional association between thymic shared antigen-1/stem cell antigen-2 and the T cell receptor complex. J. Biol. Chem., 273, 12301–12306.

63. Kruse J. J., te Poele J. A., Velds A., Kerkhoven R. M., Boersma L. J., Russell N. S. and Stewart F. A. (2004): Identification of differentially expressed genes in mouse kidney after irradiation using microarray analysis. Radiat. Res., 161, 28–38.

64. LeClair K. P., Palfree R. G., Flood P. M., Hammerling U. and Bothwell A. (1986): Isolation of a murine Ly-6 cDNA reveals a new multigene family. EMBO J., 5, 3227–3234.

65. Lee K. H., Dinner A. R., Tu C., Campi G., Raychaudhuri S., Varma R., Sims T. N., Burack W. R., Wu H., Wang J., Kanagawa O., Markiewicz M., Allen P. M., Dustin M. L., Chakraborty A. K. and Shaw A. S. (2003): The immunological synapse balances T cell receptor signaling and degradation. Science, 302, 1218–1222.

66. Lee S. K., Su B., Maher S. E. and Bothwell A. L. (1994): Ly-6A is required for T cell receptor expression and protein tyrosine kinase fyn activity. EMBO J., 13, 2167–2176.

67. Lenardo M., Chan K. M., Hornung F., McFarland H., Siegel R., Wang J. and Zheng L. (1999): Mature T lymphocyte apoptosis – immune regulation in a dynamic and unpredictable antigenic environment. Annu. Rev. Immunol., 17, 221–253.

68. Lollini P. L., Landuzzi L., Nicoletti G., de Giovanni C., Giovarelli M., Lalli E., Facchini A. and Nanni P. (1992): Ly-6A/E gene is widely expressed among transformed nonhematopoietic cells. Autocrine modulation by interferon. Anticancer Res.,12, 2245–2252.

69. Ma X., de Bruijn M., Robin C., Peeters M., Kong-A-San J., de Wit T., Snoijs C. and Dzierzak E. (2002): Expression of the Ly-6A (Sca-1) lacZ transgene in mouse haematopoietic stem cells and embryos. Br. J. Haematol., 116, 401–408.

70. Malek T. R., Danis K. M. and Codias E. K. (1989): Tumor necrosis factor synergistically acts with IFN-γ to regulate Ly-6A/E expression in T lymphocytes, thymocytes and bone marrow cells. J. Immunol., 142, 1929–1936.

71. Malek T. R., Fleming T. J. and Codias E. K. (1994): Regulation of T lymphocyte function by glycosylphosphatidylinositol (GPI)--anchored proteins. Semin. Immunol., 6, 105–113.

72. Malek, T. R., Ortega G., Chan C., Kroczek R. A. and Shevach E. M. (1986): Role of Ly-6 in lymphocyte activation. II. Induction of T cell activation by monoclonal anti-Ly-6 antibodies. J. Exp. Med., 164, 709–722.

73. Mao M., Yu M., Tong J. H., Ye J., Zhu J., Huang Q. H., Fu G., Yu L., Zhao S. Y., Waxman S., Lanotte M., Wang Z. Y., Tan J. Z., Chan S. J. and Chen Z. (1996): RIG-E, a human homolog of the murine Ly-6 family, is induced by retinoic acid during the differentiation of acute promyelocytic leukemia cell. Proc. Natl. Acad. Sci. USA, 93, 5910–5914.

74. Marmor M. D. and Julius M. (2000): The function of GPI--anchored proteins in T cell development, activation and regulation of homeostasis. J. Biol. Regul. Homeost. Agents, 14, 99–115.

75. Marmor M. D. and Julius M. (2001): Role for lipid rafts in regulating interleukin-2 receptor signaling. Blood, 98, 1489–1497.

76. McHeyzer-Williams L. J. and McHeyzer-Williams M. G. (2004): Developmentally distinct Th cells control plasma cell production *in vivo*. Immunity, 20, 231–242.

77. McKenzie I. F., Gardiner J., Cherry M. and Snell G. D. (1977): Lymphocyte antigens: Ly-4, Ly-6, and Ly-7. Transplant. Proc., 9, 667–669.

78. Miwa J. M., Ibanez-Tallon I., Crabtree G. W., Sanchez R., Sali A., Role L. W. and Heintz N. (1999): lynx1, an endogenous toxin-like modulator of nicotinic acetylcholine receptors in the mammalian CNS. Neuron, 23,105–114.

79. Monks C. R., Freiberg B. A., Kupfer H., Sciaky N. and Kupfer A. (1998): Three-dimensional segregation of supramolecular activation clusters in T cells. Nature, 395, 82–86.

80. Monks C. R., Kupfer H., Tamir I., Barlow A. and Kupfer A. (1997): Selective modulation of protein kinase C-τ during T-cell activation. Nature, 385, 83–86.

81. Montixi C., Langlet C., Bernard A. M., Thimonier J., Dubois C., Wurbel M. A., Chauvin J. P., Pierres M. and He H. T. (1998): Engagement of T cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. EMBO J., 17, 5334–5348.

82. Nakajima H., Takenaka M., Kaimori J. Y., Nagasawa Y., Kosugi A., Kawamoto S., Imai E., Hori M. and Okubo K. (2002): Gene expression profile of renal proximal tubules regulated by proteinuria. Kidney Int., 61, 1577–1587.

83. Palfree R. G. (1996): Ly-6-domain proteins – new insights and new members: a C-terminal Ly-6 domain in sperm acrosomal protein SP-10. Tissue Antigens, 48, 71–79.

84. Palfree R. G., LeClair K. P., Bothwell A. and Hammerling U. (1987): cDNA characterization of an Ly-6.2 gene expressed in BW5147 tumor cells. Immunogenetics, 26, 389–391.

85. Patterson J. M., Johnson M. H., Zimonjic D. B. and Graubert T. A. (2000): Characterization of Ly-6M, a novel member of the Ly-6 family of hematopoietic proteins. Blood, 95, 3125–3132.

86. Pflugh D. L., Maher S. E. and Bothwell A. L. (2000): Ly-6I, a new member of the murine Ly-6 superfamily with a distinct pattern of expression. J. Immunol., 165, 313–321.

87. Pflugh D. L., Maher S. E. and Bothwell A. L. (2002): Ly-6 superfamily members Ly-6A/E, Ly-6C, and Ly-6I recognize two potential ligands expressed by B lymphocytes. J. Immunol., 169, 5130–5136.

88. Pike L. J. (2003): Lipid rafts: bringing order to chaos. J. Lipid Res., 44, 655–667.

89. Radtke F., Wilson A., Mancini S. J. and MacDonald H. R. (2004): Notch regulation of lymphocyte development and function. Nat. Immunol., 5, 247–253.

90. Reese J. T., Mehta H., Chappell C. H. and Bamezai A. (2001): Downregulated expression of Ly-6-ThB on developing T cells marks $CD4^+CD8^+$ subset undergoing selection in the thymus. Dev. Immunol., 8, 107–121.

91. Reiser H., Coligan J., Benacerraf B. and Rock K. L. (1987): Biosynthesis, glycosylation, and partial N-terminal amino acid sequence of the T-cell-activating protein TAP. Proc. Natl. Acad. Sci. USA, 84, 3370–3374.

92. Reiter R. E., Gu Z., Watabe T., Thomas G., Szigeti K., Davis E., Wahl M., Nisitani S., Yamashiro J., Le Beau M. M., Loda M. and Witte O. N. (1998): Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. Proc. Natl. Acad. Sci. USA, 95, 1735–1740.

93. Rock K. L., Reiser H., Bamezai A., McGrew J. and Benacerraf B. (1989): The LY-6 locus: a multigene family encoding phosphatidylinositol-anchored membrane proteins concerned with T--cell activation. Immunol. Rev., 111, 195–224.

94. Rock K. L., Yeh E. T., Gramm C. F., Haber S. I., Reiser H. and Benacerraf B. (1986): TAP, a novel T cell-activating protein involved in the stimulation of MHC-restricted T lymphocytes. J. Exp. Med.,163, 315–333.

95. Sagi-Assif O., Traister A., Katz B. Z., Anavi R., Eskenasy M. and Witz I. P. (1996): TNFα and anti-Fas antibodies regulate Ly-6E.1 expression by tumor cells: a possible link between angiogenesis and Ly-6E.1. Immunol. Lett., 54, 207–213.

96. Sato N., Yahata T., Santa K., Ohta A., Ohmi Y., Habu S. and Nishimura T. (1996): Functional characterization of $NK1.1^+$ Ly--$6C^+$ cells. Immunol. Lett., 54, 5–9.

97. Schlueter A. J., Krieg A. M., De Vries P. and Li X. (2002): B cells express Ly-6C in a Th1 but not Th2 cytokine environment. J. Interferon Cytokine Res., 22, 799–806.

98. Schlueter A. J., Malek T. R., Hostetler C. N., Smith P. A., deVries P. and Waldschmidt T. J. (1997): Distribution of Ly-6C on lymphocyte subsets. I. Influence of allotype on T lymphocyte expression. J. Immunol., 158, 4211–4222.

99. Shan X., Bourdeau A., Rhoton A., Wells D. E., Cohen E. H., Landgraf B. E. and Palfree R. G. (1998): Characterization and mapping to human chromosome 8q24.3 of Ly-6-related gene 9804 encoding an apparent homologue of mouse TSA-1. J. Immunol., 160, 197–208.

100. Shevach E. M. and Korty P. E. (1989): Ly-6: a multigene family in search of a function. Immunol. Today, 10, 195–200.

101. Simons K. and Ikonen E. (1997): Functional rafts in cell membranes. Nature, 387, 569–572.

102. Simons K. and Toomre D. (2000): Lipid rafts and signal transduction. Nat. Rev. Mol. Cell Biol., 1, 31–39.

103. Smith S. J., Kotecha S., Towers N., Latinkic B. V. and Mohun T. J. (2002): XPOX2-peroxidase expression and the XLURP-1 promoter reveal the site of embryonic myeloid cell development in Xenopus. Mech. Dev., 117, 173–186.

104. Spangrude G. J., Klein J., Heimfeld S., Aihara Y. and Weissman I. L. (1989): Two monoclonal antibodies identify thymic-repopulating cells in mouse bone marrow. J. Immunol., 142, 425–430.

105. Sperling A. I., Sedy J. R,. Manjunath N., Kupfer A., Ardman B. and Burkhardt J. K. (1998): TCR signaling induces selective exclusion of CD43 from the T cell-antigen-presenting cell contact site. J. Immunol., 161, 6459–6462.

106. Stanford W. L., Haque S., Alexander R., Liu X., Latour A. M., Snodgrass H. R., Koller B. H. and Flood P. M. (1997): Altered proliferative response by T lymphocytes of Ly-6A (Sca-1) null mice. J. Exp. Med., 186, 705–717.

107. Tanaka M., Marunouchi T. and Sawada M. (1997): Expression of Ly-6C on microglia in the developing and adult mouse brain. Neurosci. Lett., 239, 17–20.

108. Tsetlin V. (1999): Snake venom α-neurotoxins and other "three--finger" proteins. Eur. J. Biochem., 264, 281–286.

109. Tsuji H., Okamoto K., Matsuzaka Y., Iizuka H., Tamiya G. and Inoko H. (2003): SLURP-2, a novel member of the human Ly-6 superfamily that is up-regulated in psoriasis vulgaris. Genomics, 81, 26–33.

110. Uchida N., Aguila H. L., Fleming W. H., Jerabek L. and Weissman I. L. (1994): Rapid and sustained hematopoietic recovery in lethally irradiated mice transplanted with purified Thy-1.1lo Lin-Sca-1$^+$ hematopoietic stem cells. Blood, 83, 3758–3779.

111. Van de Rijn M., Heimfeld S., Spangrude G. J. and Weissman I. L. (1989): Mouse hematopoietic stem-cell antigen Sca-1 is a member of the Ly-6 antigen family. Proc. Natl. Acad. Sci. USA, 86, 4634–4638.

112. Van Houten V. M., van den Brekel M. W., Denkers F., Colnot D. R., Westerga J., van Diest P. J., Snow G. B. and Brakenhoff R. H. (2000): Molecular diagnosis of head and neck cancer. Recent Results Cancer Res., 157, 90–106.

113. Walunas T. L., Bruce D. S., Dustin L., Loh D. Y. and Bluestone J. A. (1995): Ly-6C is a marker of memory CD8$^+$ T cells. J. Immunol., 155, 1873–1883.

114. Wegener A. M., Letourneur F., Hoeveler A., Brocker T., Luton F. and Malissen B. (1992): The T cell receptor/CD3 complex is composed of at least two autonomous transduction modules. Cell, 68, 83–95.

115. Weissman A. M., Ross P., Luong E. T., Garcia-Morales P., Jelachich M. L., Biddison W. E., Klausner R. D. and Samelson L. E. (1988): Tyrosine phosphorylation of the human T cell antigen receptor ζ-chain: activation via CD3 but not CD2. J. Immunol., 141, 3532–3536.

116. Xavier R., Brennan T., Li Q., McCormack C. and Seed B. (1998): Membrane compartmentation is required for efficient T cell activation. Immunity, 8, 723–732.

117. Xu M. J., Tsuji K., Ueda T., Mukouyama Y. S., Hara T., Yang F. C., Ebihara Y., Matsuoka S., Manabe A., Kikuchi A., Ito M., Miyajima A. and Nakahata T. (1998): Stimulation of mouse and human primitive hematopoiesis by murine embryonic aorta-gonad-mesonephros-derived stromal cell lines. Blood, 92, 2032–2040.

118. Yeh E. T., Reiser H., Bamezai A. and Rock K. L. (1988): TAP transcription and phosphatidylinositol linkage mutants are defective in activation through the T cell receptor. Cell, 52, 665–674.

119. Zhang W., Trible R. P. and Samelson L. E. (1998): LAT palmitoylation: its essential role in membrane microdomain targeting and tyrosine phosphorylation during T cell activation. Immunity, 9, 239–246.