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Review

The Murine Ly49 Family: Form and Function

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Abstract. The activity of natural killer (NK) cells is regulated by surface receptors that recognize class I MHC. Murine NK cells express a large family of lectin-related receptors (Ly49s) to perform this function, while human NK cells utilize a separate group of proteins containing Ig-related domains (KIRs). Although these receptor families are not structurally related, the Ly49 family appears to be the functional equivalent of human KIRs, since it uses similar signal transduction pathways for either activation or inhibition of NK cell function. Therefore, lessons learned from the study of the murine MHC class I receptor system may be relevant to human NK function. This review summarizes the current state of knowledge of the Ly49 family.

Key words: NK cells; MHC class I; receptors; signal transduction; Ly49; gene family.

Natural killer (NK) cells constitute an important facit of the innate immune response against viruses, parasites, intracellular bacteria, and tumor cells[31]. Unlike cells of the adaptive immune system, NK cells do not express immunoglobulins or T cell receptors. Although NK cells have been studied for over 20 years, the mechanism whereby NK cells discriminate self from non-self has only recently been elucidated. Based on the observation that NK cells preferentially kill targets lacking major histocompatibility complex (MHC) class I molecules on the cell surface, LJUNGGREN and KARRE[17] proposed that NK cells recognize and eliminate cells that lack class I (the "missing self" hypothesis). As predicted by this hypothesis, human and mouse NK cells have been found to express receptors for class I MHC that deliver inhibitory signals to NK cells. The human MHC class I receptors are monomeric type I glycoproteins containing either 2 or 3 extracellular Ig-like domains. In contrast, the mouse class I MHC receptors are members of the Ly49 family of type II glycoproteins belonging to the C-type lectin superfamily. The Ly49 MHC class I receptor family is located on chromosome 6 in a region designated as the "NK gene complex"[32]. The Ly49 proteins are expressed as disulfide-linked homodimers, and analysis of Ly49 expression has demonstrated the existence of distinct subsets of NK cells that have various combinations of Ly49 molecules on their surfaces. This review will summarize the current state of knowledge regarding the Ly49 gene family and the function of the Ly49 proteins.

There are currently 14 members of the Ly49 gene family that have been identified in the C57Bl/6 (B6) mouse genome[3, 24]. Ten of these (Ly49A-J) have been shown to produce mRNAs with a complete coding region, three are likely to represent transcribed pseudo-

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-genes (Ly49K, M, and N), and the remaining potential Ly49 gene (Ly49L) has not yet been shown to produce any mRNAs in B6 mice[1, 25, 29]. However, the Ly49L gene has been shown to produce a novel activating Ly49 protein in CBA/J mice, suggesting that Ly49 family members may be silenced via germline mutations in certain mouse strains[18]. In addition, a recent study has identified two novel Ly49A-related cDNAs (Ly49O and P) from 129/J mice[19], which indicates the need to survey all available strains of mice in order to identify the full Ly49 gene repertoire.

The Ly49 genes are closely spaced with some intergenic regions less than 5kb, and they appear to be arranged in a tandem fashion with identical orientation of transcriptional units. There is no apparent clustering of Ly49 genes with respect to activating/inhibitory function or gene homology. The exon structure of the Ly49A gene has been determined[15], and current evidence indicates that other family members share the same exon organization. Ly49A is composed of 7 exons, and individual exons correspond to functional domains of the Ly49 protein. The first exon is non-coding, the second codes for the cytoplasmic region, the transmembrane domain is in the third exon, the stalk region is contained in the fourth exon, and exons 5–7 represent the carbohydrate recognition domain (CRD).

The functional roles of several family members have been studied with specific monoclonal antibodies. Ly49 A, C, I, and G have been shown to inhibit NK cell function in response to specific MHC class I ligands on target cells[13, 22, 33], while Ly49D has been shown to activate NK cell function[20]. Ly49A has been shown to recognize D^d, H-2^k, and H-2^s using functional assays, cell-cell binding assays, and class I tetramer binding studies, while Ly49C has been shown to bind K^b, K^d, D^d, and H-2^s gene products[2, 4, 11]. Of the inhibitory receptors, Ly49G appears to be the most selective, since it only appears to bind the D^d molecule[8, 22]. Surprisingly, the activating receptor Ly49D has also been shown to recognize D^d in functional studies[5, 26]. However, cell-cell binding assays have been unable to detect any binding of Ly49D to D^d-expressing cells, suggesting that the affinity is much lower or that recognition of D^d by the activating receptor requires other interactions.

The inhibitory Ly49s contain an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domains that becomes phosphorylated in response to receptor ligation, leading to recruitment of SHP-1 phosphatase and attenuation of intracellular signaling[21, 27, 28]. Ly49D requires the association of a signaling molecule (DAP-12) containing an immunoreceptor tyrosine-based activation motif (ITAM) in order to transmit activating signals[23, 30]. A transmembrane arginine residue in Ly49D is required for DAP-12 association, and this charged residue is also present in the Ly49H protein. Ly49H has been shown to associate with DAP-12, suggesting that it will also function as an activating molecule in NK cells[7, 30]. Three of the potential new genes found in the B6 genome are similar to either Ly49D or H, suggesting that they may also represent activating Ly49s. Ly49P is a DAP-12-associated activating receptor identified in 129/J mice[18], however, a gene corresponding to Ly49P has not yet been localized in the B6 genome.

The activation of Ly49 genes during murine NK cell development presents an interesting system for the study of selective gene expression. Ly49A and C have been shown to be subject to allelic exclusion[10], however, this exclusion is not complete, suggesting that the activation of Ly49 gene expression is controlled by a probabilistic mechanism, and thus the activation of two identical family members in the same NK cell is a rare event. It has been proposed that the activation of Ly49 gene transcription is controlled by a stochastic process, since the proportion of NK cells that express two Ly49 proteins is roughly equivalent to the product of the proportion of NK cells expressing the individual Ly49 proteins[9]. Single cell RT-PCR analysis of Ly49 expression[16] has shown that the majority of NK cells express from one to four different Ly49s per cell and NK cells with 5 or more Ly49s are extremely rare, supporting the theory that the Ly49 genes are activated in a random fashion by a limiting factor present in bone marrow. No increase in Ly49 gene transcription has been shown to occur in response to cytokine. The level of Ly49 protein expression on NK cells has been related to the expression of the corresponding MHC class I ligand in a given mouse strain[12]. For example, Ly49C expression levels are lower in congenic strains that express the H-2^b or H-2^k MHC haplotypes recognized by Ly49C when compared with mice expressing the H-2^d haplotype[6]. In addition, adoptive transfer experiments have shown that down-regulation of Ly49A occurs as a rapid adaptation process in mature NK cells after interaction with the H-$2D^d$ ligand *in vivo*[14]. This suggests that Ly49 levels are not fixed, but can be changed in mature NK cells if they are exposed to a different MHC class I environment. Whether or not there is any transcriptional component to this type of Ly49 regulation is unknown.

An additional mechanism controlling the level of Ly49 expression has been revealed in studies using PCR to amplify cDNAs of specific family members. In a study reported by McQueen et al.[25], the majority of

Ly49J transcripts were found to lack exon 3, which encodes the transmembrane domain. Exon 3 was skipped preferentially in Ly49J, since loss of exon 3 in the related Ly49C and I mRNAs was shown to occur only at low frequency. The loss of exon 3 does not disrupt the open reading frame of the Ly49s, and McQUEEN et al. suggest that there may be an intracellular function for these transmembrane-lacking variants of the Ly49s. The characterization of Ly49 cDNAs from 129/J mice in our laboratory resulted in the isolation of several variants lacking either the transmembrane exon, the stalk exon, or both. The loss of either of these exons does not disrupt the open reading frame, raising the possibility that alternate protein products could be produced. In order to address this possibility, clones were expressed in 293 cells and Ly49 proteins were detected by Western blotting with a polyclonal anti-Ly49 antiserum. Only complete Ly49 cDNAs containing all 7 exons were capable of expressing Ly49 protein, suggesting that exon skipping represents a mechanism whereby the level of Ly49 protein expression can be limited in a particular strain. The molecular basis of gene-specific exon skipping probably relates to differences in exon definition between different Ly49 genes. Single nucleotide changes in splice donor, acceptor, branchpoint, or enhancer sequences could result in less efficient splicing of a particular exon.

NK cells are often thought of as "precursors" of T cells in an evolutionary sense. The mechanism used to generate a diverse receptor repertoire in NK cells seems to reflect their more primitive nature. The expression of many related receptor genes by NK cells is a simple alternative to the genomic recombination mechanism used to generate T cell receptor diversity. The evolutionary divergence between rodents, which use Ly49 to recognize class I MHC, and primates, which use KIR molecules to recognize class I MHC, is puzzling. Both types of NK receptors play similar roles, yet are not related. This example of convergent evolution of function may have occurred due to differences in intracellular pathogens found in mice versus humans. However, this would imply a role for the peptide during class I MHC recognition by Ly49/KIR. Interestingly, Ly49I has been shown to preferentially bind certain MHC tetramers depending on the type of peptide presented[8]. Further insight into the role of Ly49 molecules in NK function may be gained from gene-deletion technology. However, since different Ly49s are known to bind the same types of MHC, such redundancy may result in no detectable phenotype. The deletion of groups of Ly49 genes may be more informative in this regard.

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