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Initiation and Limitation of Ly-49A NK Cell Receptor Acquisition by T Cell Factor-1¹

Vassilios Ioannidis,* Béatrice Kunz,* Dawn M. Tanamachi,† Léonardo Scarpellino,* and Werner Held²*¹

The establishment of clonally variable expression of MHC class I-specific receptors by NK cells is not well understood. The Ly-49A receptor is used by ~20% of NK cells, whereby most cells express either the maternal or paternal allele and few express simultaneously both alleles. We have previously shown that NK cells expressing Ly-49A were reduced or almost absent in mice harboring a single or no functional allele of the transcription factor T cell factor-1 (TCF-1), respectively. In this study, we show that enforced expression of TCF-1 in transgenic mice yields an expanded Ly-49A subset. Even though the frequencies of Ly-49A⁺ NK cells varied as a function of the TCF-1 dosage, the relative abundance of mono- and biallelic Ly-49A cells was maintained. Mono- and biallelic Ly-49A NK cells were also observed in mice expressing exclusively a transgenic TCF-1, i.e., expressing a fixed amount of TCF-1 in all NK cells. These findings suggest that Ly-49A acquisition is a stochastic event due to limiting TCF-1 availability, rather than the consequence of clonally variable expression of the endogenous TCF-1 locus. Efficient Ly-49A acquisition depended on the expression of a TCF-1 isoform, which included a domain known to associate with the TCF-1 coactivator β-catenin. Indeed, the proximal Ly-49A promoter was β-catenin responsive in reporter gene assays. We thus propose that Ly-49A receptor expression is induced from a single allele in occasional NK cells due to a limitation in the amount of a transcription factor complex requiring TCF-1. The Journal of Immunology, 2003, 171: 769–775.

Inhibitory receptors specific for MHC class I molecules ensure NK cell self-tolerance toward normal host tissues. The relief from MHC receptor-mediated inhibition, which occurs when target cells lose MHC class I expression, allows NK cells to kill such aberrant host cells. NK cell reactions to host cells lacking single MHC class I molecules can occur because some inhibitory MHC receptors recognize only certain MHC class I molecules and individual NK cells express various combinations, yet limited numbers of inhibitory receptors (for review, see Ref. 1). Ligand selectivity combined with clonal distribution of inhibitory NK cell receptors also explains the reactivity of NK cells to allogeneic target cells (2). This property of NK cells is of clinical relevance, because it can be exploited in graft vs leukemia reactions (3).

Clonally variable expression of inhibitory NK cell receptors thus plays an important role for NK cell biology. However, it is not well understood how this diversity is established. Although the expression of CD94/NKG2A heterodimers is regulated by cytokines such as IL-15 (4), the regulation of human killer Ig-like receptors (KIR)³ or murine Ly-49 MHC class I receptors has remained obscure. However, bone marrow stroma is required for KIR or Ly-49 acquisition in vitro (5, 6). Furthermore, Ly-49 receptor expression shows some peculiar features. Ly-49 genes are normally expressed from a single and occasionally from both alleles (7–9), which is established independently of gene-recombination events (10). The patterns of KIR gene expression are maintained via DNA methylation (11, 12). The acquisition of distinct murine MHC receptors seems to occur at sequential stages of NK cell development, yet their coexpression is not mutually exclusive nor strongly interdependent (13–15). Finally, Ly-49 receptor expression is not directed by MHC class I molecules; however, the repertoire of these receptors on NK cells is modulated by MHC class I molecules (15–18).

The prototype inhibitory MHC class I receptor in mice, the Ly-49A receptor is acquired by ~20% of NK cells (19). In contrast to other members of the Ly-49 receptor family, the acquisition of Ly-49A during NK cell development is stringently dependent on the trans-acting factor T cell factor-1 (TCF-1) (20, 21), whereas the close TCF-1 relative lymphoid enhancer factor-1 (LEF-1) is not required (22). Heterozygous TCF-1 mutant mice had 50% fewer Ly-49A NK cells than normal. This raised the possibility that TCF-1 was expressed by a subset of NK cells and that these NK cells acquired Ly-49A. Alternatively, TCF-1 may be expressed by all NK cells, but be rate limiting for Ly-49A acquisition, such that only some NK cells acquired Ly-49A.

TCF-1 and other members of the TCF/LEF family (i.e., TCF-1, TCF-3, TCF-4, and LEF-1) bind DNA in a sequence-specific manner, yet possess no intrinsic ability to modulate transcription (23). Nevertheless, TCF/LEF factors contribute to transcriptional responses in reporter gene assays in various ways. For instance, LEF-1 was shown to play an architectural role, which depended on DNA bending. This facilitated the formation of a transcriptionally competent higher order nucleoprotein complex at the minimal TCR α enhancer (24–26). All TCF/LEF factors can serve as dock-}

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¹ Abbreviations used in this paper: KIR, killer Ig-like receptor; LEF, lymphoid enhancer factor; NKC, NK gene complex; TCF-1, T cell factor-1; Tg, transgene/transgenic; TLE, transducin-like enhancer of split.

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² Address correspondence and reprint requests to Dr. Werner Held, Ludwig Institute for Cancer Research, Lausanne Branch, University of Lausanne, Epalinges, Switzerland, and Department of Molecular and Cell Biology, University of California, Berkeley, CA 94729.
split (TLE) proteins leads to promoter repression (27), while β-catenin is an interaction partner, which activates transcription (28, 29). The availability of β-catenin as an interaction partner for TCF/LEF is regulated by extrinsic factors such as some members of the Wnt family of signaling molecules. TCF/LEF thus act as nuclear effectors of the canonical Wnt signaling pathway, which plays important roles in embryogenesis, the establishment and/or maintenance of self-renewing tissues, and tumorigenesis (28-31).

The interaction of TCF/LEF with the coactivator β-catenin or Groucho/TLE corepressors is mediated via distinct, nonoverlapping domains (27). This property has allowed us to begin to dissect the dependence of Ly-49A acquisition on a particular function of TCF-1. We find that a β-catenin-responsive TCF-1 isoform is required for Ly-49A acquisition by NK cells and that this was a rare event due to a limiting TCF-1 dosage.

Materials and Methods

Mice

C57BL/6 (B6) and BALB.B mice were purchased from Harlan Olac (Horst, The Netherlands). Tcf-1 (exon VII)-deficient mice have been described before (32). Tcf-1 transgenic (Tg) mice were generated by inserting human p45 TCF-1 (25) or the murine p33 TCF-1 (M2a) (33) cDNA clones into the H-2K b promoter-Ig μ enhancer cassette (34), as described (35). Tg mice were generated by standard methods using fertilized (B6 × DTA/2F1) F1 oocytes. Tg lines were established by backcrossing to B6 and TCF-1-deficient mice. The C57BL/6 (B6) and BALB.B mice were purchased from Harlan Olac (San Jose, CA).

Flow cytometry and cell sorting

For flow cytometry with B6 allele-specific fluorescence, cells were resuspended in PBS/3% FCS/0.5% saponin and incubated for 1 hr with a Cy3-conjugated goat anti-mouse IgG1 Ab (Jackson ImmunoResearch, West Grove, PA). After one wash in PBS/3% FCS/0.5% saponin, cells were resuspended in PBS/3% FCS and analyzed, as above.

Western blot

IL-2-expanded, plastic-adherent cells (1 × 10^6, >90% NK1.1^+ CD3^- cells) were washed once in PBS and lysed in sample buffer (62.4 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-ME, and 0.02% bromphenol blue). Samples were boiled for 5 min before SDS-PAGE and blotted onto Hybond ECL nitrocellulose membranes (Amersham, Little Chalfont, U.K.). Blots were blocked with 5% dry milk in PBS containing 0.005% Tween (PBS-T) and incubated overnight at 4°C with the primary Ab. The following Abs were used: rabbit anti-TCF-1 (37) (kindly provided by Y. Katsura, Kyoto, Japan) and mouse anti-α-tubulin (B-5-1-2; Sigma-Aldrich). After washing, HRP-conjugated secondary Abs to mouse or rabbit IgG (Sigma-Aldrich) were added for 1 hr at room temperature. Western blots were revealed using an ECL detection kit (Pierce, Rockford, IL). Blots were stripped for 30 min at 50°C in stripping buffer (2% w/v SDS, 62.5 mM Tris-HCl, pH 6.8, 100 mM 2-ME) before reprobing.

Transfections and reporter gene assays

The 5’ region (~1066 (EcoRI) to +44 (EcoRV)) of the Ly-49A gene (38) and the corresponding fragment, in which the two TCF/LEF sites were mutated, were inserted into the luciferase (luc) reporter plasmid pGL3 (Promega, Madison, WI). This construct was sequenced to ensure that only the intended mutations were introduced. The expression constructs for β-catenin and β-catenin-TCF/LEF reporter plasmids (pTOPFLASH and control pOPFLASH) were described before (39). Human 293 cells were transfected using Ca phosphate, and the cells were collected and assayed for luciferase activity after 48 hr. CMV-enhanced green fluorescence protein or CMV-renailla luciferase expression constructs were cotransfected to normalize for transfection efficiency. Transfections were done using 1 μg of green fluorescence protein or 50 ng of renilla control plasmid with 4 μg of luciferase reporter and 5 μg of expression β-catenin expression plasmid. Where needed, appropriate empty expression vectors were added to keep the amount of DNA constant.

Results

Ly-49A acquisition in TCF-1 transgenic mice

We have previously shown that the acquisition of the Ly-49A NK cell receptor was dependent on the trans-acting factor TCF-1. TCF-1 seemed to limit Ly-49A acquisition, based on a 2-fold smaller Ly-49A NK cell subset in mice with a single as compared with two functional TCF-1 alleles (20). This was not due to differential expression of TCF-1 in Ly-49A^- NK cells, because comparable levels of TCF-1 protein were detected in the two NK cell subsets (Fig. 1a).

To functionally address the basis for the limited Ly-49A acquisition, we analyzed mice expressing a p45 TCF-1 isoform (Fig. 1b) under the control of the H-2K b promoter/Igμ enhancer expression cassette (35). In Tg mice, CD3^- DX5^ splenic NK cells expressed the human TCF-1 protein at appreciable uniformity, as judged by intracellular flow cytometry specific for the uniform levels of TCF-1. Based on the mean fluorescence intensity of staining, NK cells from Tg line 2 expressed somewhat higher levels of the Tg TCF-1 as compared with line 11 (Fig. 2a). Moreover, p45 Tg levels in IL-2-expanded NK cells were comparable, yet below those of endogenous TCF-1 based on Western blot analyses (Fig. 2b).

The impact of Tg expression on Ly-49A NK cell receptor usage was investigated using B6 backcross two to four mice. All mice were lacking the strong Ly-49A ligand H-2D b to avoid modulation of Ly-49A usage based on Ly-49-MHC class I interactions. Moreover, they all harbored two B6-derived copies of the NK gene complex, which contains the polymorphic Ly-49 gene cluster. As shown in Fig. 3a, the Ly-49A NK cell subset in spleens of Tg lines 2 and 11 was significantly expanded (36.4 ± 5.5% and 30.6 ± 1.6% of NK cells, respectively) as compared with non-Tg littermate

mAb (7H3) (36) diluted in PBS/3% FCS/0.5% saponin (Sigma-Aldrich, Buchs, Switzerland). Cells were then washed once in PBS/3% FCS/0.5% saponin and incubated for 1 hr with a Cy3-conjugated goat anti-mouse IgG1 Ab (Jackson ImmunoResearch, West Grove, PA). After one wash in PBS/3% FCS/0.5% saponin, cells were resuspended in PBS/3% FCS and analyzed, as above.
Groucho family, and a NH2-terminal domain, a domain that mediates the interactions with corepressors of the Ponceau red staining.

TCF-1 expression and TCF-1 transgenic mice. a, IL-2-expanded NK cells (CD3− NK1.1+) were sorted into Ly-49A+ and Ly-49A− subsets. Nuclear extracts prepared from 10^6 sorted cells were subjected to immunoblot analysis with a polyclonal antiserum to TCF-1. Arrows indicate TCF-1 p45 and p33 isoforms. Equal protein loading was ensured by Ponceau red staining. b, The TCF-1 p45 isoform includes a DNA binding domain, a domain that mediates the interactions with corepressors of the Groucho family, and a NH2-terminal β-catenin binding domain. The latter domain is not present in the naturally occurring p33 isoform.

Compared with non-Tg mice, the usage of the B6 allele (A1+) NK cells) decreased 2- and 15-fold in TCF-1+/− and TCF-1−/− mice, respectively, whereas that of the BALB allele (TNTA− NK cells) decreased 1.5- and 4-fold (Fig. 4). Decreasing TCF-1 gene dosages reduced the acquisition of either Ly-49A allele, whereby the BALB allele was less affected. The TCF-1 gene dosage thus regulates the acquisition of individual Ly-49A alleles in F1 NK cells.

A fraction of Ly-49A+ NK cells in normal mice expresses simultaneously both alleles. To investigate whether the development of such NK cells was also regulated by TCF-1, we first used an artificial mixture of B6 and BALB.B NK cells to ensure that the flow cytometric analysis did not detect artifactual A1+TNTA− double-positive NK cells (Fig. 4a). Among TCF-1+/− (B6 × BALB.B)F1 mice, 2.0 ± 0.5% of total NK cells simultaneously expressed both Ly-49A alleles (Fig. 4d). The frequency of biallelic Ly-49A NK cells increased to 8.9 ± 0.8% in Tg-TCF-1+/- mice and decreased to 0.2 ± 0.1% in TCF-1−/− mice. The TCF-1 gene dosage thus also regulates the development of cells that express simultaneously both Ly-49A alleles. Abundant biallelic expression of Ly-49A cells may be the result of an increased expression of the endogenous TCF-1 locus in some immature NK cells. To address this possibility, we analyzed TCF-1 Tg (line 2) TCF-1−/− mice, respectively, whereas that of the BALB allele (TNTA− NK cells) decreased 1.5- and 4-fold (Fig. 4). Decreasing TCF-1 gene dosages reduced the acquisition of either Ly-49A allele, whereby the BALB allele was less affected. The TCF-1 gene dosage thus regulates the acquisition of individual Ly-49A alleles in F1 NK cells.

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Ly-49A acquisition is dependent on α/β-catenin-responsive TCF-1 isoform

The mechanism by which TCF-1 mediates Ly-49A acquisition may contribute to its role as a rate-limiting factor. The function of TCF/LEF is dependent on interactions with cofactors, which are mediated by distinct domains in TCF/LEF proteins. This property has allowed us to begin to dissect the dependence of Ly-49A acquisition on a particular function of TCF-1. In addition to p45, we have analyzed transgenic mice, which express a p33 TCF-1 Tg (35). This naturally occurring TCF-1 isoform includes the DNA as well as the Groucho/TLE binding domains, yet lacks the β-catenin interaction domain present in p45 (Fig. 1b). Both types of transgenic mice were backcrossed to mice lacking endogenous TCF-1 (35).

The expression of the p45 transgene (in the absence of endogenous TCF-1) restored Ly-49A usage by NK cells to levels observed in TCF-1−/− mice (Fig. 5A). Consistent with the somewhat higher TCF-1 Tg expression (Fig. 2a), the Ly-49A NK cell subset in line 2 was larger (22.8 ± 3.8% of NK cells) as compared with line 11 (13.9 ± 3.2%). In contrast, the p33 Tg, which was expressed at levels similar to p45, resulted only in a minor effect on Ly-49A usage (Fig. 5, a and b). The p33 Tg did not act in a dominant-negative fashion based on the observation that the Ly-49A NK cell subset was slightly larger (rather than reduced) in

FIGURE 3. Ly-49A acquisition is influenced by the TCF-1 gene dosage. a, Dot plots show nylon-wool-nonadherent spleen cells stained with mAbs to CD1 and NK1.1 in the indicated types of mice. Numbers indicate the mean percentage (±SD) of NK1.1+ CD3− NK cells of three or more mice. Histograms show Ly-49A usage among gated NK1.1+ CD3− NK cells. Numbers indicate the mean percentage (±SD) of Ly-49A+ NK cells of the above mice. b, Bar graphs show the percentage of Ly-49A+ NK cells among splenic NK cells of the indicated strains of mice. Data indicate mean percentage (±SD) of seven or more independent determinations. c, Bar graphs show the absolute numbers of NK cells in spleens of the indicated types of mice. Data are derived from nylon-wool-nonadherent cells and are shown as mean absolute number (±SD) of seven or more independent determinations.

FIGURE 4. The TCF-1 gene dosage influences mono- and biallelic Ly-49A expression. Dot plots depict an expression analysis of Ly-49A alleles among gated DX5− CD3− NK cells. a, Control staining using B6 (H-2b) and BALB.B (H-2b) NK cells was used to ensure the specificity of the mAbs. The Ly-49A B6 allele was detected using mAb A1, while mAb TNTA is specific for Ly-49A BALB. An artificial mixture of B6 and BALB.B cells ensured that no artifactual A1/TNTA cells were detected. Numbers indicate the percentage of cells in the respective quadrants of a representative experiment. b, NK cells from mice with the indicated TCF-1 genotypes and with a heterozygous B6/BALB.B NK gene complex (NKC) were analyzed for Ly-49A B6 (using mAb A1) and Ly-49A BALB (mAb TNTA) usage. Numbers indicate the percentage of cells in the respective quadrants of a representative experiment. c, Ly-49A B6 (mAb A1) and Ly-49A BALB (mAb TNTA) usage among NKC B6/BALB heterozygous mice of the indicated TCF-1 genotypes. Data are depicted as mean values (±SD) and are derived from three or more mice of each type. d, Experimental values (open bars) indicate the percentage of NK cells, which simultaneously express the Ly-49A B6 and Ly-49A BALB alleles (A1+ TNTA+ double-positive NK cells). Filled bars indicate the predicted value for independent acquisition of the two alleles. This value was calculated for each mouse by multiplying the percentage of A1+ NK cells with that of TNTA+ NK cells divided by 100.
The Ly-49a promoter is β-catenin responsive

To test whether the Ly-49a promoter was β-catenin responsive, we chose 293 cells, which express all the TCF/LEF family factors as interaction partners for β-catenin (44). Indeed, the transfection with β-catenin trans-activated the Ly-49a promoter 2- to 3-fold in reporter gene assays (Fig. 6). That was of comparable magnitude to the trans-activation obtained for other TCF/LEF target genes such as myc or cyclin D1 (45, 46). Similar to some other TCF/LEF target genes, no further promoter induction was obtained by cotransfecting p300, which represents a β-catenin coactivator (47) (data not shown). The Ly-49a promoter contains two consensus TCF/LEF binding sites (CTTTGA/TA/T) close to the transcriptional start site (Fig. 6a) (20). Point mutations in the two binding sites increased the basal promoter activity in 293 cells, suggesting that in the absence of β-catenin the wild-type Ly-49a promoter was repressed by TCF/LEF. That is in agreement with findings for other TCF/LEF target genes (48). Importantly, the mutant Ly-49a promoter was no longer β-catenin responsive, suggesting that the induction by β-catenin is mediated by TCF/LEFs (Fig. 6b). These findings suggest that the Ly-49a promoter is a direct target of TCF/LEF-β-catenin complexes.

Discussion

The Ly-49A receptor is acquired by some, but not all NK cells during development. The data presented in this work are compatible with the view that the stable induction of Ly-49A gene expression is regulated by the formation of a nucleoprotein complex at the Ly-49A promoter, which requires TCF-1. Indeed, Ly-49A induction is dependent on a β-catenin-receptive TCF-1 isoform, and the Ly-49a promoter is β-catenin responsive in reporter gene assays. These findings suggest that the requirement of a complex, which includes TCF-1 as well as its coactivator β-catenin. TCF-1 seems to represent a rate-limiting factor, such that the assembly of a functional complex at the Ly-49A promoter occurs only in occasional NK cells. The TCF-1 dosage may thus set a probability of all-or-non-Ly-49A acquisition events.

The question as to whether cellular signaling results in binary (on/off) or graded transcriptional responses in individual cells has recently received renewed attention. Indeed, the competition between activators and repressors of transcription produced a binary response in distinct model systems. In both cases, the concentration of the exogenous stimulating agent influenced the proportion of cells expressing a reporter gene rather than the rate of transcription in each cell (49, 50). Similarly, for the developmentally regulated acquisition of Ly-49A, the TCF-1 dosage would influence the probability to stably switch on Ly-49A expression.

We have previously shown high (50-fold) reporter gene activation by the Ly-49a promoter in EL-4 cells (20). This activity was
further increased by transfecting p33 (1.3-fold) (20) or p45 (1.9-fold over the constitutive activity) (not shown). These effects were observed in the absence of significant activation of the TCF optimal promoter reporter gene, which depends on the presence of TCF/β-catenin complexes. These data suggested that β-catenin was not required for Ly-49A promoter activity in a cell line with constitutive expression of the endogenous Ly-49A locus. The behavior of the Ly-49A promoter was thus similar to that of the minimal TCF/β enhancer, which was induced by either p33 or p45 in a B cell line (25). Reporter gene activity in these situations may thus reflect an architectural role of TCF-1, which may play a role in maintaining constitutive gene expression.

In contrast to EL-4, Ly-49A promoter activity in 293 cells is low (3-fold over a promoterless construct) (20). This activity was augmented 2.5-fold by the addition of β-catenin (Fig. 6). In this situation, endogenous TCF/LEFs are used to mediate reporter gene activation. Indeed, 293 cells express all four TCF/LEF family members (44). This is indicated by the fact that reporter gene activation was only observed with the wild-type Ly-49A promoter, which contains two TCF binding sites, but not with the control promoter, which contains mutant sites. Although we show that β-catenin/TCF functionally interact with the Ly-49A promoter, the physiological consequence of this binding in chromatin may be the modification of local chromatin structure via associated p300/CBP or Brg (47, 51, 52). That would explain its apparent role as a developmental switch.

A role of TCF-1 as an on switch incorporates a particular feature of Ly-49A gene expression: most Ly-49A+ NK cells express a single receptor allele. Complex formation in an individual NK cell seems to suffice only to initiate Ly-49A expression only from a single Ly-49A allele. The observation that individual NK cells can express either the maternal or the paternal allele is consistent with this possibility, as the formation of the factor complex may occur randomly on either chromosome. In addition, some Ly-49A NK cells express simultaneously both alleles. Such cells are also observed when NK cells express a fixed amount of TCF-1 (in TCF-1 Tg TCF-1−/− mice), suggesting that they develop independently of clonal variations in TCF-1 levels. Rather, the fraction of NK cells expressing both Ly-49A alleles increased in parallel to the increased usage of the individual alleles, suggesting that biallelic Ly-49A gene expression is a function of the chance that the expression of the two alleles coincides. In fact, the percentage of NK cells expressing simultaneously both alleles (A1 +ANTTA + NK cells) indicates whether the two alleles are expressed independently. Purely stochastic expression of the two alleles is predicted when the frequency of double expressors matches the product of the frequency to express either Ly-49A allele (also termed product rule (53)). In our analyses, the experimental value was always within a factor of two of the predicted one (Fig. 4d). The slight overrepresentation of biallelic Ly-49A cells occurred thus independent of the TCF-1 gene dosage and was not related to the expression pattern of the endogenous TCF-1 locus. These findings suggest that factors other than TCF-1 introduce this bias.

The comparison of (B6 × BALB.B)F1 mice, expressing variable amounts of TCF-1, revealed another unexpected feature. Changes in the TCF-1 dosage resulted in the biased usage of the B6 vs the BALB Ly-49A alleles (Fig. 4e). One possibility is that TCF-1 binds preferentially to the B6 allele of Ly-49A. Indeed, there exist two nucleotide differences between the proximal Ly-49A promoter of BALB and B6 origin (B. Kunz and W. Held, unpublished observation). Although these polymorphisms do not locate directly to the two consensus TCF binding sites, they are in their vicinity and may thus influence the binding of additional trans-acting factors involved in Ly-49A acquisition. Alternatively, an allelic bias could in principle result from a differential MHC class I-dependent effect on NK cells, which have acquired the B6 as compared with the BALB allele of Ly-49A. However, we have used H-2b mice for our analyses, in which the two Ly-49A alleles do not encounter a class I ligand that is sufficient to inhibit NK cells (54). Nevertheless, it cannot be excluded that the two alleles display some differential affinity for class I (55), which may be sufficient to influence the Ly-49 receptor repertoire.

Our findings indicate that Ly-49A acquisition is limited via the TCF-1 dosage and occurs in a β-catenin-dependent fashion. It is thus possible that, similar to TCF-1, the levels of β-catenin in NK cells influence Ly-49A acquisition. The availability of intracellular free β-catenin is dependent on extrinsic factors such as wnt family proteins (30). In this context, it is known that bone marrow stromal cells as well as hematopoietic cells express wnts (56) (our unpublished observation). Wnt5a is expressed in primary bone marrow stroma cells, while wnt10b and wnt3a are expressed by hematopoietic cells (56). Indeed, stroma cells allow the acquisition of Ly-49A in vitro NK cell differentiation assays (3, 13, 14). Thus, our analysis raises the possibility that wnts represent one of the eliciting signals to initiate Ly-49 expression by developing NK cells.

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