Commentary

Selection and Adaptation of Cells Expressing Major Histocompatibility Complex Class I–specific Receptors of the Natural Killer Complex

By Albert Bendelac

From the Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

In addition to their essential role in presenting pathogen peptides for recognition by the antigen receptor of killer CD8 T cells, MHC class I molecules can be the targets of another fundamentally different mode of recognition, also involved in fighting pathogens. Studies on NK, a component of the innate immune system that comes into action at the early phase of many intracellular infections, have uncovered an entire set of new receptors for MHC class I that are encoded in the so-called NK gene complex (NKC; 1), and have provided support for the idea that NK cells survey for the absence of self–MHC molecules (2) through these receptors (3). This function is crucial for fighting against certain viruses, such as herpes viruses (4–6). The current challenge that is addressed in this issue of *The Journal of Experimental Medicine* by Johansson et al., is to understand the rules for selection and tolerance of NK cells expressing appropriate MHC-specific receptors in the face of the polymorphism of inherited MHC genes.

MHC-specific NK Receptors and the Recognition of Missing Self. MHC-specific NKRs are encoded by genes in the NK complex. They belong to at least two different structural families, one with Ig domains and the other with a carbohydrate recognition domain, that are both represented, albeit to variable degrees, in mice and humans (7–9). Unlike TCRs, NKRs do not discriminate between most MHC–peptide complexes, despite the fact that recognition is centered around the peptide-binding groove and depends upon peptide-induced conformation of the MHC molecule. In that regard, NKR recognition of MHC is very similar to that of antibodies against the $\alpha1/\alpha2$ domains of MHC. They recognize allelic forms of MHC molecules, often cross-reacting onto several other MHC allotypes or isotypes.

A biochemical characteristic of many MHC-specific NKRs is the presence of an immunoreceptor tyrosine-based inhibitory motif in the cytoplasmic tail, which binds and activates the tyrosine phosphatase SHP-1 upon phosphorylation. Thus, normal, uninfected cells engage MHC-specific NKRs and deactivate NK cells by preventing phosphorylation-dependent cell activation transduced through activating NK receptors such as NK1.1 (10, 11). In contrast, downmodulation of MHC class I, a strategy of escape from CD8 killer cells attempted by many viruses, is countered by NK cell-mediated killing.

Coevolution of MHC and NKC Genes. Unlike TCR genes, NKR genes do not undergo somatic recombination, and the number of MHC-specific NKR genes is limited, in the range of 15–30 in mice and humans, with a degree of polymorphism that is low compared to that of MHC. Therefore, evolutionary considerations suggest that the primary pressure for diversification has driven the MHC genes, whereas NKR genes, which are encoded in a separate locus, had to coevolve in an evolutionary pursuit to match the arising new MHC alleles.

To fulfill their function of MHC surveillance at the level of the individual, NK cells must therefore tailor the use of their MHC-specific NKRs to the inherited set of MHC genes through some process of selection or adaptation. Because the MHC and the NKC are encoded on different chromosomes, and because the selection pressures for particular MHC and NKR genes may be different, exerted by different pathogens at different times or in different populations, it may not always be possible to select a perfect NKR repertoire. For example, some MHC alleles may not be recognized by existing NKRs, or on the contrary, they may be recognized with too high an affinity by some NKRs. Some individuals may therefore inherit largely incompatible sets of alleles, with NKRs that are unable to see MHC or that are insensitive to its downmodulation by viruses. Such theoretical situations could account for the existence of human patients (4) or mouse strains, such as SJL (12) or nonobese diabetic (13), that are profoundly deficient in NK function.

The Regulation of MHC-specific NKR Expression. These evolutionary considerations emphasize the complexity of matching the expression of NKR genes with the MHC genes inherited by the individual. Within the framework of the missing-self hypothesis (14), a functional NK must express one or more NKRs that bind host MHC alleles with sufficient combined avidity to prevent autoreactivity (self tolerance). This avidity should not be too high however, or the NK would be unable to detect significant decreases in MHC expression.

What controls the expression of NKR genes? A key observation is that subsets of NK can be defined by the expression of different MHC-specific NKRs (15). The frequency of NK expressing a particular MHC-specific NKR does not vary much according to the MHC haplotype. For example, B6 ($H2^b$) NK cells express the D^d-specific NKR Ly49A, a member of the Ly49 family of NKR genes, with the same frequency (15–20%) as NK cells in B10.D2 ($H2^d$) mice. Thus, NK cells seem to express useful, as well as pre-

J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/08/349/03 $2.00
Volume 186, Number 3, August 4, 1997 349–351
http://www.jem.org

sumably useless, MHC-specific NKRs. A second important observation is that the frequency of NK cells expressing two different NKRs of the Ly49 family is close to what would be expected in a purely stochastic model of Ly49 gene activation, i.e., the product of the frequency of single expressors, suggesting that different genes and alleles are randomly activated at a low frequency. These results strongly support the idea that stochastic activation of Ly49 genes contributes the primary NKR repertoire (16, 17).

Since little bias is observed in the frequency of Ly49 isotype expression by NK cells of different MHC genotypes, the possibility exists that NK cells expressing inappropriate MHC-specific NKRs may not be deleted. It could be argued that because not all NKR specificities are known yet, it is not possible to rule out deletion-based selection as the main process for forging an appropriate NKR repertoire. Indeed, a definite, though modest, increase in the frequency of expression of additional MHC-specific NKRs has been observed in NK cells expressing a useless receptor, suggesting that at least some of the NK cells that fail to express an appropriate NKR might be deleted.

Clonal Adaptation. Other observations, however, suggest that cellular adaptation rather than deletion may be prominent in shaping the NKR repertoire. For example, it has been observed that the surface concentration of Ly49A, an NKR for D^d, is decreased twofold in D^d-expressing mice (18). Conversely, many Ly49 isotypes have been shown to be upregulated in β2m-deficient mice, possibly to make up for the decreased concentration of MHC molecules (19). It is not clear yet whether these modulations merely result from receptor engagement, or whether they might reflect a process of receptor calibration (14). In such a calibration model, NK cells would each increase or decrease their level of receptors so as to be unresponsive to cells expressing the baseline concentration of existing MHC class I alleles while being maximally sensitive to MHC downmodulation. It is noteworthy in the context of this hypothesis that several potentially autoreactive T cell subsets, some of which also express NK receptors, have been reported to express decreased levels of TCR or CD8 coreceptor (20–23).

Another form of cellular adaptation is clonal inactivation. In this issue of *The Journal of Experimental Medicine*, Johansson et al. examined NK cell tolerance in transgenic mice with a mosaic expression of the D^d. Mosaic expression of a transgene is thought to occur as a result of stochastic gene inactivation linked to particular sites of integration, and results in a proportion of cells that do not express the transgene. In this case, the cells do not express an MHC allele expressed by other cells in the mouse. A somewhat similar situation may be achieved in hemopoietic radiation chimeras reconstituted with a mixture of β2m-sufficient and -deficient fetal liver cells (24). In both cases, NK cells appeared to be tolerant of the cells that do not express MHC class I. However, when Johansson et al. isolated D^d-positive NK cells from the D^d-negative NK cells and cultured them for 24 h, the cells completely recovered the ability to kill D^d-negative targets.

This striking result argues that NK cells capable of reactivity to the D^d-negative targets had not been deleted in the mosaic mouse. Several possibilities may account for their extremely rapid functional recovery. One possibility is that the autoreactive NK cells were only tolerant in appearance; when the mosaic populations were tested against MHC-negative targets, their killing ability was hidden because of the competition by the MHC-negative cells in the mosaic. Thus, the killing activity was revealed after removal of these MHC-negative cells. This seems rather unlikely, since the mosaic mice appeared to have stable proportions of MHC-positive and -negative cells. Moreover, in the case of mixed fetal liver chimeras, the proportion of β2m-positive and -negative hemopoietic cells roughly corresponded to that used for reconstitution, arguing against a continuous attack of the latter by NK cells. Another possibility is that the NK cells were 'anergic' or desensitized to the absence of D^d, and could very promptly recover in the absence of the tolerogen. Finally, existing functional NK cells may have adapted to their new environment by recalibrating their various NK receptors to match the new MHC levels. As discussed above, the latter possibility might be difficult to test at present because subtle adjustments of the expression of several MHC-specific receptors might be involved.

Although more refined genetic approaches are on their way to further probe the rules of selection and adaptation in NK cells, the results reported by Johansson et al. already suggest that NK cells may have solved the problem of tolerance in a unique fashion. Future studies in this field may also illuminate the intriguing observation that MHC-specific NKRs are also expressed by other cell lineages, including some T cell subsets (21, 25, 26) and mast cells (27).

Address correspondence to Dr. Albert Bendelac, Department of Molecular Biology, Princeton University, Washington Road, Princeton, NJ 08544. Phone: 609-258-5454; FAX: 609-258-2205; E-mail: abendelac@molbiol.princeton.edu

Received for publication 19 June 1997.

References

1. Yokoyama, W.M., J.C. Ryan, J.J. Hunter, H.R.C. Smith, M. Stark, and W.E. Seaman. 1991. cDNA cloning of mouse NKR-P1 and genetic linkage with Ly-49. Identification of a natural killer cell gene complex on mouse chromosome 6. *J. Immunol.* 147:3229–3236.
2. Karre, K., H.G. Ljunggren, G. Piontek, and R. Kiessling. 1986. Selective rejection of H-2 deficient lymphoma variants suggests alternative immune defence strategy. *Nature (Lond.).*

319:675–678.
3. Karlhofer, F.M., R.K. Ribaudo, and W.M. Yokoyama. 1992. MHC class I alloantigen specificity of Ly49+ IL-2–activated natural killer cells. *Nature (Lond.).* 358:66–70.
4. Biron, C.A., K.S. Byron, and J.L. Sullivan. 1989. Severe herpes virus infections in an adolescent without natural killer cells. *N. Engl. J. Med.* 320:1731–1735.
5. Farrell, H.E., H. Vally, D.M. Lynch, P. Fleming, G.R. Shellam, A.A. Scalzo, and N.J. Davis-Poynter. 1997. Inhibition of natural killer cells by a cytomegalovirus MHC class I homologue in vivo. *Nature (Lond.).* 386:510–514.
6. Reyburn, H.T., O. Mandelboim, M. Vales-Gomez, D.M. Davis, L. Pazmany, and J.L. Strominger. 1997. The class I MHC homologue of human cytomegalovirus inhibits attack by natural killer cells. *Nature (Lond.).* 386:514–517.
7. Yokoyama, W.M., B.F. Daniels, W.E. Seaman, R. Hunziker, D.H. Margulies, and H.R. Smith. 1995. A family of murine NK cell receptors specific for target cell MHC class I molecules. *Semin. Immunol.* 7:89–101.
8. Lanier, L.L. 1997. Natural killer cells: from no receptors to too many. *Immunity.* 6:371–378.
9. Raulet, D.H. 1996. Recognition events that inhibit and activate natural killer cells. *Curr. Opin. Immunol.* 8:372–377.
10. Burshtyn, D.N., A.M. Scharenberg, N. Wagtmann, S. Rajagopalan, K. Berrada, T. Yi, J.P. Kinet, and E.O. Long. 1996. Recruitment of tyrosine phosphatase HCP by the killer cell inhibitor receptor. *Immunity.* 4:77–85.
11. Nakamura, M.C., E.C. Niemi, M.J. Fisher, L.D. Shultz, W.E. Seaman, and J.C. Ryan. 1997. Mouse Ly-49A interrupts early signaling events in natural killer cell cytotoxicity and functionally associates with the SHP-1 tyrosine phosphatase. *J. Exp. Med.* 185:673–684.
12. Kaminsky, S.G., I. Nakamura, and G. Cudkowicz. 1985. Genetic control of the natural killer cell activity in SJL and other strains of mice. *J. Immunol.* 135:665–671.
13. Schultz, L.D., P.A. Schweitzer, S.W. Christianson, B. Gott, I.B. Schweitzer, B. Tennent, S. McKenna, L. Mobraaten, T.V. Rajan, D.L. Greiner, and E.H. Leiter. 1995. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J. Immunol.* 154:180–191.
14. Hoglund, P., J. Sundback, M.Y. Olsson-Alheim, M. Johansson, M. Salcedo, C. Ohlen, H.G. Ljunggren, C.L. Sentman, and K. Karre. 1997. Host MHC class I gene control of NK-cell specificity in the mouse. *Immunol. Rev.* 155:11–28.
15. Ciccone, E., D. Pende, O. Viale, C. Di Donato, G. Tripodi, A.M. Orengo, J. Guardiola, A. Moretta, and L. Moretta. 1992. Evidence of a natural killer (NK) cell repertoire for (allo) antigen recognition: definition of five distinct NK-determined allospecificities in humans. *J. Exp. Med.* 175:709–718.
16. Held, W., J. Roland, and D.H. Raulet. 1995. Allelic exclusion of Ly49-family genes encoding class I MHC-specific receptors on NK cells. *Nature (Lond.).* 376:355–358.
17. Raulet, D.H., W. Held, I. Correa, J.R. Dorfman, M.F. Wu, and L. Corral. 1997. Specificity, tolerance and developmental regulation of natural killer cells defined by expression of class I–specific Ly49 receptors. *Immunol. Rev.* 155:41–52.
18. Olsson, M.Y., K. Karre, and C.L. Sentman. 1995. Altered phenotype and function of natural killer cells expressing the major histocompatibility complex receptor Ly49 in mice transgenic for its ligand. *Proc. Natl. Acad. Sci. USA.* 92:1649–1653.
19. Held, W., J.R. Dorfman, M.F. Wu, and D.H. Raulet. 1996. Major histocompatibility complex class I dependent skewing of the natural killer cell Ly49 repertoire. *Eur. J. Immunol.* 1996:2286–2292.
20. vonBoehmer, H., J. Kirberg, and B. Rocha. 1991. An unusual lineage of αβ T cells that contains autoreactive cells. *J. Exp. Med.* 174:1001–1008.
21. Bendelac, A., M.N. Rivera, S.-H. Park, and J.H. Roark. 1997. Mouse CD1-specific NK1 T cells. Development, specificity, and function. *Annu. Rev. Immunol.* 15:535–562.
22. Schultz, R.J., A. Parkes, E. Mizoguchi, A.K. Bhan, and S. Koyasu. 1996. Development of CD4-CD8- αβTCR+ NK1.1+ T lymphocytes. Thymic selection by self antigen. *J. Immunol.* 157:4379–4389.
23. Curnow, S.J., C. Boyer, M. Buferne, and A.M. Schmitt-Verhulst. 1995. TCR-associated ζ-FcεRIγ heterodimers on $CD4^-CD8^-$ NK1.1+ T cells selected by specific class I MHC antigen. *Immunity.* 3:427–438.
24. Wu, M.F., and D.H. Raulet. 1997. Class I–deficient hemopoietic cells and nonhemopoietic cells dominantly induce unresponsiveness of natural killer cells to class I-deficient bone marrow cell grafts. *J. Immunol.* 158:1628–1633.
25. Lanier, L.L., and J.H. Phillips. 1996. Inhibitory MHC class I receptors on NK cells and T cells. *Immunol. Today.* 17:86–91.
26. Guy-Grand, D., B. Cuenod-Jabri, M. Malassis-Seris, F. Selz, and P. Vassalli. 1996. Complexity of the mouse gut T cell immune system: identification of two distinct natural killer T cell intraepithelial lineages. *Eur. J. Immunol.* 26:2248–2256.
27. Rojo, S., D.N. Burshtyn, E.O. Long, and N. Wagtmann. 1997. Type I transmembrane receptor with inhibitory function in mouse mast cells and NK cells. *J. Immunol.* 158:9–12.