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Resistance to Mousepox Virus: CD94 on a Special Mission

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NK cells play a key role in the control of ectromelia virus. In this issue of *Immunity*, Fang et al. (2011) demonstrate that the deletion of CD94 abolishes resistance to mousepox infection.

Natural killer (NK) cells are key actors in innate immunity. They protect the host from many types of viral infections by sensing proinflammatory cytokines in their environment as well as changes in the expression of major histocompatibility complex (MHC) class I and other molecules expressed on the cell surface during a viral insult. Effector functions of NK cells are regulated by integrating signals from their activating and inhibitory receptors. The engagement of inhibitory NK cell receptors by MHC class I molecules expressed by healthy cells prevents their activation. On the other hand, NK cells are activated either through the engagement of their activating receptors or a lack of engagement of their inhibitory receptors. Defusing NK cells is critical for their survival, so over time viruses have evolved a number of strategies to evade NK cell control (Lisnic et al., 2010). Virus-driven evolution of their natural hosts led to emergence of mechanisms able to oppose the viral immunoevasion. The best way to achieve this goal would be selection of activating NK receptors that specifically recognize virally infected cells. Indeed, unlike their inhibitory counterparts, many of the activating NK cell receptors bind to various molecular determinants of infection and demonstrate certain types of specificity to virally encoded molecules.

The most studied example of a virus-specific NK cell response is in C57BL/6 (B6) mice, which are constitutively resistant to murine cytomegalovirus (MCMV). These mice express Ly49H, an activating receptor on their NK cells that directly interacts with the MCMV m157 protein, leading to recognition and elimination of infected cells via cytolytic mechanisms (Arase et al., 2002). Another NK cell-dependent mechanism of MCMV resistance has been described in MA/My mice, whose activating Ly49P receptor recognizes the MHC class I allele H2-D^k bound to a viral protein encoded by the *m04* gene (Kielczewska et al., 2009). It has to be pointed out that the specificity of NK cell receptors to virally encoded molecules is not restricted to herpes viruses as shown by the fact that NKp46- and NKp44-activating NK cell receptors bind to influenza hemagglutinin (Arnon et al., 2006).

Mousepox or ectromelia virus is another virus whose pathogenesis is tightly controlled by NK cells. It belongs to *Orthopoxviruses*, a large family of DNA viruses that includes, in addition to ectromelia, the variola virus, a causative agent of smallpox, as well as vaccinia virus, cowpox virus, and monkeypox virus. Unlike several mouse strains that are highly sensitive to mousepox, B6 mice are able to successfully cope with mousepox infection without developing symptoms of the disease. The important role that NK cells play in the control of ectromelia infection is best illustrated by the fact that depletion of NK cells abolishes the resistance of B6 mice to this virus. In addition to reducing the viral burden during the early course of infection, functional NK cells are required for the generation of an optimal T cell response (Fang et al., 2008). A dramatic reduction of both $CD8^+$ and $CD4^+$ T cell responses is observed in ectromelia-infected mice depleted of NK cells, possibly as a consequence of virus-mediated eradication of dendritic cells. Although it is well established that NK cells play a key role in ectromelia virus infection in B6 mice, the molecular mechanism of the resistance and the receptors involved has remained elusive until now.

In this issue of *Immunity*, Fang et al. (2011) explain the mechanism of resistance of B6 mice to ectromelia virus through the involvement of the CD94 receptor (Figure 1). CD94-NKG2 receptors are composed of an invariant CD94 polypeptide, which forms a heterodimer with either NKG2A, NKG2C, or NKG2E

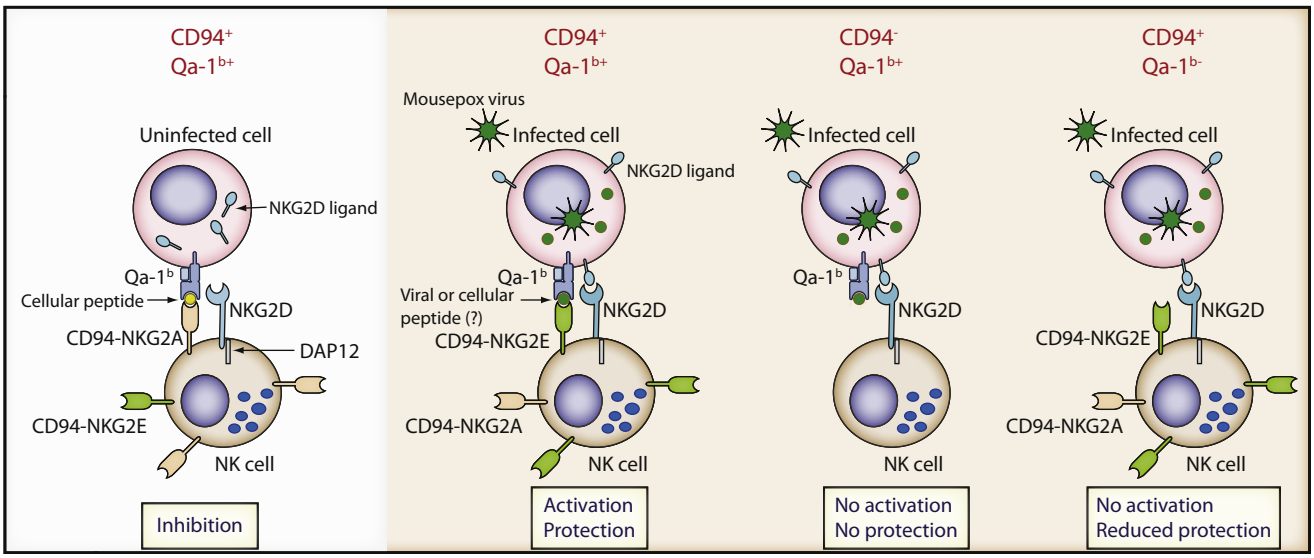

Figure 1. CD94-NKG2E and NKG2D Synergize in Response to Mousepox Virus
Under uninfected conditions, cells express Qa-1^b molecules loaded with peptides derived from the signal sequence of MHC class Ia, which engages the CD94-NKG2A inhibitory receptor. Because CD94-NKG2A dominates over activating CD94-NKG2 receptors, this interaction prevents reactivity of NK cells against healthy cells. Infection with ectromelia virus induces the expression of activating CD94-NKG2 receptors, which recognize infected cells via Qa-1^b possibly with higher affinity because of expression of viral peptides. NKG2D expression synergizes with CD94-NKG2E, resulting in NK cell activation and protection against poxvirus. CD94 deficiency prevents the expression of CD94-NKG2E, leading to abolished NK cell activation and subsequent CD8$^+$ T cell response. This results in loss of resistance to poxvirus. Likewise, Qa-1^b deficiency prevents ligation of CD94-NKG2E, which leads to insufficient activation of NK cells and reduced protection.

chains, all of them using the MHC class Ib HLA-E molecule in humans and the Qa-1^b molecule in mice as their ligands. These molecules present peptides derived from the signal peptide of MHC class Ia molecules that can be substituted with a viral peptide as part of a viral immune evasion strategy. Contrary to NKG2A, which is an inhibitory molecule possessing an immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytoplasmic domain, NKG2C and NKG2E possess a charged amino acid in the transmembrane domain that allows them to dock to signaling adaptor protein DAP12, thus eliciting an activating signal. Because CD94 is required for transport of NKG2A, NKG2C, and NKG2E to the cell membrane, the deletion of CD94 completely prevents the expression of CD94-NKG2 receptors (Orr et al., 2010). Interestingly, *Klrd1*$^{-/-}$ mice lacking the gene encoding CD94 do not differ from control mice with respect to the number of NK cells, their maturation, cytolytic capacity, or the expression of various markers and recruitment to draining lymph nodes. Moreover, the control of various pathogens such as MCMV, lymphocytic choriomeningitis virus (LCMV), vaccinia virus, or *Listeria monocytogenes* is not altered in *Klrd1*$^{-/-}$ mice (Orr et al., 2011; Fang et al., 2011). On the contrary, the deletion of CD94 dramatically abolished the resistance of mice to ectromelia virus (Fang et al., 2011). This phenomenon was confirmed by blocking CD94-NKG2 receptors with antibodies and by the reversion of resistance to ectromelia after the CD94 gene had been introduced into a *Klrd1*$^{-/-}$ mouse. Furthermore, B6 mice carrying the natural killer gene complex (NKC) haplotypes from the mousepox-susceptible DBA/2J strain with a spontaneous mutation in the *Klrd1* gene are also susceptible to ectromelia. As one would expect, mice lacking Qa-1^b, a natural ligand for CD94-NKG2 (Vance et al., 1998, 1999), also show a markedly reduced ability to cope with mousepox infection.

What could be the protective principle in B6 mice infected with ectromelia? The infection is accompanied by decreased expression of NKG2A receptors and an increase in expression of NKG2C and NKG2E receptors. Yet, contrary to a dramatic loss of virus control after the deletion of CD94, mousepox-infected cells failed to activate reporter cells expressing CD94 and different isoforms of NKG2. Based on their previous data indicating the role of NKG2D in early control of ectromelia virus, the authors proposed that CD94-NKG2 might work in concert with this activating receptor, which is otherwise structurally and functionally different from other NKG2 members and does not pair with CD94. In order to prove this hypothesis, Fang et al. (2011) constructed a series of CD94 reporter cell lines expressing NKG2C, NKG2E, and a short form of the NKG2D receptor in all possible combinations. The results revealed that only CD94-expressing reporter cells possessing a combination of NKG2E and NKG2D could be activated by ectromelia-infected fibroblasts. Although the molecular mechanism for the synergy between the two receptor systems remains unsolved, the authors speculate that stimulation through both receptors is required in order to overcome the inhibition through NKG2A or other receptors. Moreover, the engagement of both receptors might favor a stronger activating signal through the recruitment of DAP12.

Altogether, the work by Fang et al. (2011) establishes a role for CD94 in the control of mousepox virus and illustrates how a limited number of NK cell receptors can be used in complex ways. This

work has important implications for our understanding of natural resistance to viral disease and as such may lead to the development of novel approaches for coping with viral infections. However, as in the case of all important new findings, the results from this study set the stage for many new questions that need to be addressed. As suggested by the authors, it is likely that the infection results in the presentation of novel viral or cellular peptide(s) by Qa-1^b because reporter cells expressing CD94-NKG2E receptors were not stimulated unless target cells were infected. Could it be that Qa-1^b-peptide complexes on ectromelia-infected cells exhibit a higher affinity for CD94-NKG2E? The effect of Qa-1^b deficiency on the mortality rate is much lower than in *Klrd1*$^{-/-}$ mice, suggesting the existence of another molecule involved in CD94-NKG2 signaling. It is known that CD94 is also expressed on NKT, γδT cells, and some activated $CD8^+$ T cells, so it is important to assess the contribution of these individual cell subsets in the described phenotype. Late kinetics of mortality in mice lacking CD94 suggests that, in addition to NK cells, a compromised $CD8^+$ T cell response might be an immediate cause of death of mice.

The most puzzling question is the mode of synergism between NKG2D and CD94-NKG2E receptors. Is the engagement of NKG2D by its natural ligands required to synergize with CD94-NKG2E or does NKG2D act by stabilizing the CD94-NKG2E complex by docking next to it? Alternatively, does CD94-NKG2E act to stabilize sustained NKG2D expression? The expansion of CD94-NKG2C has also been established during HCMV infection, suggesting the possibility that the involvement of these activating receptors is not necessarily specific for a single virus (Guma et al., 2006). Having in mind that poxviruses share great homology and the fact that CD94-NKG2 receptors are well conserved between rodents and primates, one could speculate that CD94 function might be driven by poxvirus evolution. Results obtained with the mousepox virus model indicate that there is constant pressure for a novel innate immune response mechanism to resist viral immunoevasion and virus-induced pathology.

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At 17, In-10's Passion Need Not Inflame

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In this issue of *Immunity*, Chaudhry et al. (2011) and Huber et al. (2011) report that control of Th17 cell responses during colonic inflammation requires direct signaling by IL-10 in regulatory T cells and Th17 cells.

Inflammatory responses due to commensal microorganisms or pathological stimuli are held in check by a variety of processes involving suppressive cytokines, other soluble mediators, and suppressive populations of inflammatory cells. The cytokine interleukin-10 (IL-10) and regulatory T (Treg) cells, such as $FoxP3^+$ Treg and $FoxP3^-$ Tr1 cells, play both unique and overlapping roles in this control. IL-10 has regulatory functions on both innate and adaptive responses that are mediated in part by its effects on antigen-presenting cells, in which it reduces secretion of proinflammatory cytokines such as TNF and IL-12 and lowers expression of costimulatory molecules (Ma and Trinchieri, 2001; Moore et al., 2001). IL-10 is produced by several cell types including Treg cells. Treg cells, on the other hand, regulate immune responses through multiple mechanisms including the production of suppressive cytokines such as IL-10, TGF-β, and IL-35, but also depletion of energy and growth factors (e.g., ATP and IL-2), cytolysis of antigen-presenting cells, and inhibition of antigen-presenting cell maturation (Vignali et al., 2008). In vitro assays of Treg cell activity are poor at modeling their requirements for IL-10. However, a clear requirement for Treg cell IL-10 is seen in vivo where it plays a key role in regulation of inflammation at environmental interfaces such as the intestine and the lung. The studies by