Natural killer cells: fighting viruses and much more

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More than 450 immunologists recently met in Cavtat, Croatia to discuss advances in natural killer (NK) cell biology. The meeting highlighted emerging themes in NK cell responses to viruses, NK cell tolerance and potential use of NK cells in the therapy of malignancies.

NK cells provide innate defense against viruses and tumor cells. Studies presented at the NK meeting and reviewed here advance our knowledge on how NK cell recognize virally infected and malignant cells, avoiding autoreactivity. Some of these findings may open new avenues for therapeutic intervention in infectious diseases and cancer.

NK detection of cytomegalovirus

NK cells are essential for controlling cytomegalovirus infections. Mouse NK cells use multiple mechanisms to detect mouse cytomegalovirus (MCMV); the best characterized is the expression of the activating receptor Ly49H in C57BL/6 mice, which directly recognizes the MCMV-encoded cell surface molecule m157 (**Fig. 1a**). However, Ly49H is just the tip of the iceberg. Silvia Vidal (Montreal) reported that NK cells from different inbred mice strains express additional activating receptors, Ly49P, Ly49D2 and Ly49L, which recognize the MCMV-encoded molecule m04. Notably, in contrast to Ly49H, Ly49P, Ly49D2 and Ly49L detect m04 only in the presence of certain major histocompatibility complex (MHC) haplotypes (**Table 1**). Most likely, m04 associates with MHC class I molecules, generating altered MHC class I structures that engage and trigger Ly49P, Ly49D2 and Ly49L. However, Marina Babic (Rijeka, Croatia) reported that association of m04 with MHC class I of the H2d and H2k haplotypes can promote engagement of the inhibitory receptor Ly49A, thus protecting MCMV-infected cells from NK cell–mediated killing. The MHC background may also influence the ability of another receptor, Ly49G2, to detect MCMV infection (Michael Brown, Charlottesville, Virginia, USA). Collectively, these results demonstrate that the outcome of NK cell recognition of MCMV-infected cells is highly dependent on the MHC class I context.

MHC class I also affects NK cell detection of cells infected by human cytomegalovirus (HCMV). Miguel Lopez-Botet (Barcelona, Spain) showed that HCMV infection reduces cell surface expression of human leukocyte antigen HLA-E on dendritic cells. Because HLA-E is the ligand of the inhibitory receptor NKG2A, NKG2A$^+$ NK cells are released from inhibition and kill infected dendritic cells. This result is surprising given that HCMV encodes a protein, UL-40, that is digested into a peptide that forms a complex with HLA-E, promoting HLA-E expression on infected cells. UL-40s from different HCMV strains may vary in their ability to promote HLA-E expression; in addition, HLA-E alleles may differ in their affinity for UL-40 peptides.

A continuous and ongoing struggle between host defense and viral escape mechanisms was further suggested by the characterization of viral microRNAs that interfere with NK cell responses. Ofer Mandelboim (Jerusalem) presented evidence that HCMV and other viruses such as Epstein-Barr virus and Kaposi's sarcoma–associated herpesvirus encode microRNAs that inhibit expression of MICB. MICB is an alarm molecule expressed on virally infected cells that is detected by the activating receptor NKG2D. Thus, these microRNAs allow viral escape from NKG2D recognition. Lars Dölken (Munich) identified two MCMV-encoded microRNAs that promote the ability of MCMV to establish infection. The targets recognized by these microRNAs are currently under investigation using an unbiased large-scale approach based on immunoprecipitation of the RNA-induced silencing complex from infected cells followed by deep sequencing.

MCMV also escapes NK cell and T cell surveillance by encoding immunoevasins like m152, which interferes with expression of MHC class I and/or MHC class I–like molecules known as RAE-1α–RAE-1ε. These are alarm molecules that trigger mouse NKG2D, just as MICB does in humans. David Margulies (Bethesda, Maryland, USA) reported that m152 and other viral proteins, including m144 and m153 (whose precise function is unclear), are structurally related to but distinct from classical MHC class I proteins, reflecting an ongoing evolution.

Antiviral NK responses

Lewis Lanier (San Francisco) showed that mice deficient in the C-type lectin CD94 are highly susceptible to mouse poxvirus infection. CD94 can pair with NKG2A to form an inhibitory heterodimer, and with NKG2C or NKG2E to form activating heterodimers. These receptors recognize HLA-E in human and the nonclassic class I molecules Qa-1 in mouse. In cell-based assays, only the CD94-NKG2E heterodimer detected poxvirus-infected cells. However, CD94-NKG2E required the cooperation of

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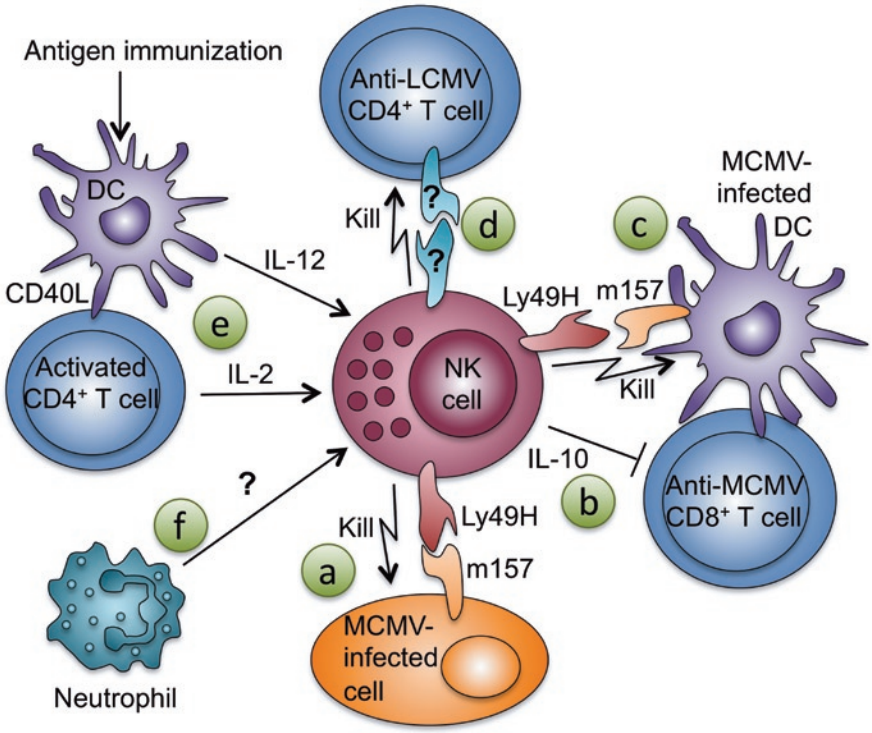

Figure 1 NK cells in regulatory networks. (**a**) NK cells recognize MCMV-infected cells via Ly49H-m157 interaction, which triggers cytotoxicity and proliferation. (**b**) When MCMV infection induces abnormal activation and proliferation, Ly49H$^+$ NK cells produce IL-10, which limits CD8$^+$ T cell–mediated tissue damage. (**c**) Ly49H$^+$ NK cells can also lyse MCMV-infected dendritic cells (DCs), thereby reducing T cell priming and viral control. (**d**) NK cells can eliminate LCMV-specific CD4$^+$ T cells, impairing viral control or preventing excessive T cell response. '?' indicates that the receptors mediating this response are not known. (**e**) CD4$^+$ T cell responses to immunogens can stimulate NK cells via IL-2 and DC-derived IL-12. (**f**) Neutrophils are required for the functional competence of NK cells via an unknown mechanism.

the activating receptor NKG2D for efficient recognition of infected cells. CD94-NKG2E may recognize a viral peptide–Qa-1 complex. Hans-Gustav Ljunggren (Stockholm) reported that a human hantavirus infection outbreak in Sweden caused the expansion of NK cells expressing CD94-NKG2C. This expansion might be the result of a hantavirus peptide presented by HLA-E, which is then particularly effective in stimulating CD94-NKG2C. Salim Khakoo (London) showed that the peptide repertoire of HLA-C can affect recognition by the killer immunoglobulin-like receptors KIR2DL2 and KIR2DL3, suggesting that viral peptide-MHC complexes may in some cases facilitate escape from NK cell recognition by engaging inhibitory receptors.

Cross-talk with NK cells

Mouse Ly49H$^+$ NK cells typically proliferate and release lytic granules and IFN-γ in response to MCMV infection. Christine Biron (Providence, Rhode Island, USA) showed that this is not the case in immunocompetent mice infected with high MCMV loads or perforin-deficient mice infected with standard MCMV loads. In these settings, Ly49H$^+$ NK cells proliferate but produce IL-10. This response was essential for surviving MCMV infection as IL-10 limited excessive CD8 T cell–mediated tissue damage (**Fig. 1b**). Mariapia Degli-Esposti (Perth, Australia) showed that in C57BL/6 mice, Ly49H$^+$ NK cells kill MCMV-infected dendritic cells during the early phase of infection, impairing ongoing T cell priming and facilitating persistent infection (**Fig. 1c**). In contrast, in mouse strains lacking Ly49H, infected dendritic cells are spared by NK cells, facilitating priming of specific T cells and faster control of infection. Thus, NK cells can control antiviral T cell responses by manipulating antigen-presenting cells. Notably, Irene Slavuljica (Rijeka, Croatia) demonstrated that a recombinant MCMV encoding a RAE-1 ligand for NKG2D promotes rather than impairs T cell priming. Thus, the outcome of interaction between NK and dendritic cells may depend on the type of receptor-ligand pairs involved.

Stephen Waggoner (Worcester, Massachusetts, USA) addressed the impact of NK cells on T cell responses after infection with lymphocytic choriomeningitis virus (LCMV). In this model, NK cells do not have a direct role in controlling viral replication. However, NK cells kill activated LCMV-specific CD4$^+$ T cells (**Fig. 1d**). At low viral loads, NK cell control of T cells is irrelevant, as T cells effectively clear the virus without apparent immunopathology. At intermediate viral loads, NK cell control of T cells is detrimental because it impairs effective viral control, leading to T cell–mediated immunopathology. At high viral loads, which are normally associated with T cell exhaustion, NK cells are beneficial because they prevent overzealous antiviral T cell responses. Thus, NK cells can control viral clearance and immunopathology even in infections caused by viruses resistant to NK cells. Eleanor Riley (London) and Veronique Braud (Nice, France) reported that specific CD4$^+$ T cells induced by vaccination with inactivated rabies virus in humans and immunization with *Leishmania major* in mouse promote NK cell activation. Mechanistically, CD4$^+$ T cells act directly, by releasing IL-2, and indirectly, by stimulating dendritic cells to release IL-12 via interaction of CD40, a maturation marker of dendritic cells, with CD40L, the ligand for CD40 (**Fig. 1e**). Going beyond interactions between NK and dendritic cells, Eric Vivier (Marseille, France) reported that NK cells also require neutrophils for activation. Accordingly, impaired NK cell maturation and acquisition of effector functions were observed in neutrophil-deficient mice as well as patients suffering from severe congenital neutropenia (**Fig. 1f**).

Memory NK cells

NK cells are generally considered innate cells that respond to virally infected and neoplastic cells without previous immunization. This view has been challenged by studies showing that NK cells can develop memory for chemical haptens. Moreover, MCMV infection can induce the expansion of Ly49H$^+$ 'memory' NK cells. Can NK cells develop memory for just a limited repertoire of antigens or for many antigens as T cells do? Silke Paust (Boston) showed that hepatic NK cells can mediate memory responses to influenza virus and vesicular stomatitis virus, as well as influenza-encoded matrix protein 1 or HIV-1–encoded group antigen and/or envelope proteins. What receptors are implicated in recognition of these antigens, and how is specificity achieved? Although these questions remain unanswered, Paust pointed out that memory function in hepatic NK cells depends on expression of the chemokine receptor CXCR6; CXCL16, the ligand for CXCR6, is constitutively expressed in the liver. This may help explain why CXCR6$^+$ adaptive NK cells are not found in other organs, such as the spleen.

Table 1 Activating Ly49 receptors of different mouse strains specific for MCMV-infected cells in restricted MHC contexts

Ly49 receptor	Mouse strain	MCMV protein	MHC context	Immunoevasin
Ly49H	C57BL/6	m157	None	?
Ly49P	MA/My	m04	H2k, H2d	Yes[a]
Ly49L	BALB/c	m04	H2k, H2f, H2d	Yes[a]
Ly49P1	NOD/Ltj	m04	H2k	Yes[a]
Ly49D2	PWK/Pas	m04	H2k	Yes[a]

[a]m04 can function as an immunoevasin by stabilizing surface expression of MHC class I molecules and engaging inhibitory Ly49A receptor during MCMV infection. ?, not known.

NK cell adaptation to self-MHC

NK cell tolerance to self is controlled by inhibitory receptors for MHC class I. In mice lacking MHC class I, NK cells should be autoreactive because their inhibitory receptors are no longer engaged, but instead are hyporesponsive and self-tolerant. To explain this phenomenon, Werner Held (Lausanne, Switzerland) discussed 'licensing' and 'disarming' models as well as alterations of the NK cell repertoire that could be caused by NK cell adaptation. Although the debate continues, there was a general agreement on three points. First, receptors specific for self-MHC class I molecules are involved in NK cell education. Second, NK cell adaptation is not an all-or-none phenomenon. In fact, NK cells behave like rheostats that quantitatively calibrate both their repertoire and responsiveness on the basis of the number of inhibitory signals they receive from the microenvironment, as proposed by Petter Höglund (Stockholm). Additionally, two studies by Julie Elliott (St. Louis, Missouri, USA) and Nathalie Joncker (Berkeley, California, USA) demonstrated that NK cell responsiveness is not necessarily a set feature acquired during development but can be dynamic and change in response to the MHC class I microenvironment. Donor NK cells adoptively transferred to recipient mice with different levels of MHC class I readjusted their responsiveness to the recipient MHC class I levels in days. Understanding the mechanisms that tune NK cell responsiveness remains a difficult task. However, Andrew Makrigiannis' (Ottawa) generation of a transgenic mouse that has markedly reduced expression of the entire Ly49 gene complex (and NKG2 family) may minimize the complexities inherent in analyzing expression of multiple receptors on individual NK cells.

NKG2D in cancer

NK cells recognize tumor cells expressing NKG2D ligands, including MICA, MICB and ULBP in humans and RAE-1α–RAE-1ε and H-60 in mice. Expression of NKG2D ligands is induced in tumor cells as part of the DNA damage response. Stephan Gasser (Singapore) demonstrated that intracellular DNA sensors and the downstream signaling effectors TBK1 and IRF3 link the DNA damage response with induction of NKG2D ligands. Thomas Spies (Seattle) reported the notable observation that some human tumors have adopted the NKG2D-DAP10 receptor complex for their own benefit. Tumor cells express NKG2D-DAP10, which engages tumor NKG2D ligands, generating an autocrine loop that promotes transformation and tumor survival.

Signals for cytotoxicity

NK cell–mediated lysis of target cells requires the formation of a cytolytic immune synapse between NK cells and target cells and subsequent release of lytic granules containing perforin and granzymes. Actin polymerization is an essential event in this process. Jordan Orange (Philadelphia) showed that polymerization of actin occurs not only via the *Wiskott-Aldrich* syndrome protein (WASp) but also through an alternative pathway mediated by WAVE2, which is induced by IL-2. This new observation provided the basis for a clinical trial to treat the cytolytic defect in patients with Wiskott-Aldrich syndrome by administering IL-2. Visualization of the immune synapse between NK cells and dendritic cells (Rosa Barreira da Silva, Zurich) extended the importance of actin polymerization to NK cell activation by dendritic cells.

Samuel Chiang (Stockholm) showed that calcium mobilization is an essential signal for NK cell function; he reported a marked defect in target cell–induced NK cell degranulation and cytokine production in patients with a genetically inherited defect in the calcium-responsive channel ORAI1. Two-photon imaging of interactions between NK and tumor cells (Jacques Deguine, Paris) captured only brief interactions that led to transient calcium mobilization. In contrast, more prolonged interactions of cytotoxic T lymphocytes with tumor targets correlated with sustained calcium mobilization. This difference is unexpected and deserves more in-depth study, as does the observation that NK cells can kill target cells remotely by delivering cytolytic granules through nanotubes (Daniel Davis, London). Eric Long (Rockville, Maryland, USA) demonstrated that integrin LFA-1 promotes polarization of lytic granules and focuses degranulation to a central stable region within the immune synapse. Signals from NK cell receptor CD16 cooperate with LFA-1 to stimulate directed degranulation. Both CD16 and LFA-1 activate the kinase Syk, but their signaling pathways bifurcate further downstream, as LFA-1–mediated granule polarization depends on phosphorylation of the cytoskeletal-associated protein paxillin, whereas CD16-mediated degranulation depends on phosphorylation of the adaptor molecule LAT. Carsten Watzl (Heidelberg, Germany) used mathematical modeling to investigate the cross-talk between activating and inhibitory signals in NK cells. These opposing stimuli intersect at the signaling molecule Vav-1. This leads to a switch-like regulation of Vav-1 phosphorylation, in which dephosphorylation of Vav-1 by inhibitory receptors is dominant over Vav-1 phosphorylation induced by activating receptors. Kerry Campbell (Philadelphia) identified a mechanism to terminate signaling of the activating receptor KIR2DL4, which involves the recruitment of E3 ubiquitin ligase Triad3A. Alessandro Moretta (Genova, Italy) showed that KIR3DL2 can mediate an unconventional signaling pathway; by binding and internalizing CpG oligonucleotides it triggers intracellular TLR9 and its downstream effectors.

NK cells express multiple receptors of the signaling lymphocytic-activation molecule (SLAM) family that transmit activating signals through three related intracellular adaptors known as SAP, EAT2 and ERT (the latter is only expressed in mouse). Because SLAM-related receptors interact with self or with other family members and are expressed on cells of the hematopoietic system, these receptors and their adaptors are probably essential for NK cell recognition and killing of hematopoietic cells. By studying knockout mice lacking all three SLAM signaling adaptors (TKO), André Veillette (Montreal) found that, indeed, TKO NK cells could not kill the predicted targets. Unexpectedly, he also found that TKO NK cells were more efficient in killing nonhematopoietic cells, suggesting that SLAM-related receptors are involved in adjusting the threshold of NK cell activation.

Exploiting KIR-MHC mismatches

Human inhibitory KIR receptors primarily recognize four polymorphic epitopes carried by HLA class I molecules. Analysis of KIR-MHC interactions in nonhuman primates (Peter Parham, Stanford, California, USA)

showed how KIR have evolved along with the MHC class I repertoire of each species. Analysis of KIR haplotypes in human populations showed substantial variability in the numbers of some KIR loci as well as copy number variation (John Trowsdale, Cambridge, UK). This may be facilitated by nonallelic homologous recombination, which leads to duplications, deletions, formation of hybrid genes and restoration of pseudogenes. Array-based comparative genomic hybridization analysis of different mouse strains showed substantial variation in gene content of the *Nkrp1* and *Ly49* genes within the NK gene complex encoding mouse NK cell receptors (Wayne Yokoyama, St. Louis, Missouri, USA).

Given that the MHC and KIR gene complexes are polygenic and polymorphic and segregate independently, NK cells could express KIRs that are not engaged by endogenous MHC, for instance after hematopoietic stem cell transplantation (HSCT) between unrelated donors. KIR-MHC mismatches may be exploited in cell therapy of malignancies to elicit a graft-versus-leukemia effect. In unrelated donor HSCT, Jeffrey Miller (Minneapolis, Minnesota, USA) found that donors with a homozygous KIR haplotype B motif (Cen-B/B) protect against acute myeloid leukemia (AML) relapse and lead to superior disease-free survival. In a nontransplant setting, encouraging results were also obtained with adoptive transfer of donor NK cells that share only one MHC haplotype with the recipient. Conditioning by total-body irradiation and administration of IL-15 are being tested to promote expansion, long-term survival and function of adoptively transferred NK cells.

Given that mouse NK cells adjust rapidly to changes in the MHC class I environment, could similar adaptation abrogate the therapeutic potential of NK cell alloreactivity induced by HSCT or NK cell adoptive transfer between haploidentical donor-recipient pairs? Katharine Hsu (New York) studied the functional competence of human NK cells developing in the first 3 months after HLA-matched HSCT. Even without HLA mismatch, a variable number of developing NK cells that express KIRs are not engaged by recipient MHC. In contrast to expectations, these 'unlicensed' or 'unarmed' NK cells are functionally competent and may contribute to overall and disease-free survival of AML patients.

IL-22–producing NK-like cells

A population of innate lymphoid cells (ILC) that expresses NK cell markers and the transcription factors RORγt and aryl hydrocarbon receptor (AHR) and that also secretes IL-22 was recently discovered in the gut lamina propria and gut-associated lymphoid tissues. These cells may have an important function in the protection and repair of the epithelial barrier during infections. The origin of these cells and their relationship with lymphoid tissue inducer (LTi) cells was extensively debated at the meeting. Michael Caligiuri (Columbus, Ohio, USA) analyzed the *in vitro* differentiation of human NK cell precursors isolated from tonsils and showed that IL-22–producing NK cells might represent an intermediate step during development of conventional IFN-γ–producing NK cells, and that IL-1 provides an important stimulus for their expansion. Andreas Diefenbach (Freiburg, Germany) adoptively transferred mouse RORγt$^+$ LTi-like cells and NK cells into alymphoid mice and showed that RORγt$^+$ LTi but not NK cells can generate IL-22–producing NK-like cells. In some organs (for example, colon) these cells lose RORγt expression and become IFN-γ–producing cells. Although the functional program of RORγt$^-$ LTi-derived cells resembles that of conventional NK cells, genetic lineage tracing data demonstrate that they are distinct innate lymphocyte lineages. Collectively, these data suggest intrinsic plasticity in the development and maintenance of IL-22–producing LTi-like cells. Jim Di Santo (Paris) showed that the development of gut IL-22–producing NK-like cells requires the transcription factors RORγt and Id2 and the cytokine IL-7. Moreover, he tracked the fate of cells that express RORγt in the gut by a RORγt fate map, and found that IL-22$^+$ NK-like cells and conventional NK cells have distinct lineages. Evidence was also provided that IL-22–producing ILC and LTi cells have separate lineages.

AHR is a transcription factor that senses xenobiotics and endogenous metabolites such as those produced by the gut microbiota. AHR is expressed at high levels in T_h17 T cells and IL-22–producing NK-like cells. Marco Colonna (St. Louis, Missouri, USA) showed that AHR-deficient mice lack IL-22–producing NK-like cells in the lamina propria and Peyer's patches. In addition, human IL-22–producing NK-like cells were shown to have functional plasticity and to produce the B cell–activating factor BAFF. Thus, NK-like cells in the gut may be involved in stimulating mucosal B cell responses.

Important advances were also reported in the development of conventional NK cells. Hugh Brady (London) showed that E4bp4 is a novel transcription factor required for NK cell development. Selective deletion of the transcription factor STAT5 in NK cells led to a marked reduction of NK cell numbers (Eva Eckelhart, Vienna). Francesco Colucci (Cambridge, UK), found that the transcription factor Bcl11b promotes T cell over NK cell development such that deletion of Bcl11b redirects T cell progenitors to differentiate into NK cells.

Concluding remarks

Although this report can give only a brief overview of some of the highlights of this conference, NK cell biology is clearly flourishing. The molecular mechanisms of antigen recognition by NK cells and NK cell memory and education are some of the exciting aspects that wait to be revealed at the next Meeting of the Society for Natural Immunity (NK2012) in Asilomar, California.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.