Interplay of natural killer cells and their receptors with the adaptive immune response

David H Raulet

Although natural killer (NK) cells are defined as a component of the innate immune system, they exhibit certain features generally considered characteristic of the adaptive immune system. NK cells also participate directly in adaptive immune responses, mainly by interacting with dendritic cells. Such interactions can positively or negatively regulate dendritic cell activity. Reciprocally, dendritic cells regulate NK cell function. In addition, 'NK receptors' are frequently expressed by T cells and can directly regulate the functions of these cells. In these distinct ways, NK cells and their receptors influence the adaptive immune response.

Natural killer (NK) cells, a component of the innate immune system, mediate cellular cytotoxicity and produce chemokines and inflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor (TNF), among other activities[1]. They are important in attacking pathogen-infected cells, especially during the early phases of an infection[1,2]. They are also efficient killers of tumor cells and are believed to be involved in tumor surveillance. The long-standing hypothesis that NK cells exert an immunoregulatory effect is supported by recent evidence of 'crosstalk' with dendritic cells (DCs), as will be reviewed below.

NK cells are lymphocytes and, like B and T cells, NK cells are endowed with receptors specific for target cells. Unlike B and T cells, however, NK cells use 'hard-wired' receptor systems, encoded by genes that do not undergo variable-diversity-joining recombination or sequence diversification in somatic cells[3–5]. Also unlike B and T cells, but similar to other innate immune cells[6], NK cells use a multiple receptor recognition strategy, whereby an individual NK cell can be triggered through various receptors independently or in combination, depending on the ligands presented by the target cell in a given encounter[4,7]. Most of these receptors were originally discovered in NK cells and are generally called 'NK receptors'. Many of them, however, are also expressed by other cell types, including T cells and myeloid lineage cells, where their functions are usually less well characterized.

This review will address aspects of NK cells that bear similarities to adaptive immunity and also how adaptive immune responses are influenced by NK cells, especially through the interaction of NK cells and DCs. Also to be discussed is how the adaptive immune response is shaped by NK receptors expressed directly by cells of the adaptive immune system, especially CD8+ T cells.

Department of Molecular and Cell Biology and Cancer Research Laboratory, University of California, Berkeley, California 94720-3200, USA. Correspondence should be addressed to D.H.R. (raulet@uclink.berkeley.edu).

Themes of NK cell recognition

The recognition strategies used by NK cells are diverse. These include recognition of pathogen-encoded molecules; recognition of self proteins whose expression is upregulated in transformed or infected cells ('induced-self recognition'); and inhibitory recognition of self proteins that are expressed by normal cells but downregulated by infected or transformed cells ('missing-self recognition'). What follows are examples of each.

Recognition of pathogen-encoded molecules

A form of pathogen-associated pattern recognition, as originally proposed for macrophages and DCs[8], is exemplified by the mouse Ly49H NK receptor[9]. Ly49H, which is expressed by approximately half of NK cells in certain mouse strains, is a member of the Ly49 family of receptors[10–12]. Most Ly49 receptors are specific for major histocompatibility complex (MHC) class I molecules and are inhibitory in function. This is due to the presence of immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in the cytoplasmic tail that can associate with protein tyrosine phosphatases. Ly49H, in contrast, is a stimulatory receptor by virtue of the absence of an ITIM. It is associated with the DAP12 adapter protein, which contains in its cytoplasmic tail immunoreceptor tyrosine-based activation motifs (ITAMs), which can associate with protein tyrosine kinases of the Syk−Zap70 family. Although Ly49H receptors use a signaling pathway distinct from the that of Toll-like receptors (TLRs)[10], both receptor systems stimulate immune system activation.

Ly49H is not known to bind MHC class I molecules and instead binds to a product of *m157* of mouse cytomegalovirus (MCMV)[13,14]. A dominant gene in certain mouse strains allows NK cells to control MCMV in the early stages of infection. This gene (*Klra8*) encodes Ly49H, whereas the corresponding recessive allele corresponds to a deletion of *Klra8*. The initial triggering of NK cells, which occurs within 1−2 days of infection with MCMV, does not require the action of Ly49H[15]. However, blockade of Ly49H prevents early control of MCMV infections[10] and only Ly49H+ NK cells undergo sustained

proliferation over several days after infection[15]. These results suggest that Ly49H functions as a pathogen-specific receptor that enables NK cells to limit early-stage MCMV infections and to undergo considerable proliferation. Other possible examples of NK receptors specific for pathogens are the NKp46 and NKp44 receptors, which bind the influenza virus hemagglutinin[16]. These receptors are also involved in recognition of tumor cells and will be discussed below.

Induced-self recognition

Another recognition strategy used by NK cells is exemplified by the NKG2D receptor and possibly by other stimulatory receptors such as NKp46, NKp44 and NKp30. The NKG2D receptor recognizes self proteins that are upregulated on the surface of most tumors and many infected cells. Although the tumor cell ligands for the NKp46, NKp44 and NKp30 receptors have not been identified, it is likely that these ligands are also examples of induced self molecules.

NKG2D, like Ly49H, is encoded in the NK gene complex and is a lectin-like type 2 transmembrane receptor[17]. The receptor is expressed by all NK cells and by certain T cell subsets (discussed in more detail below). The NKG2D ligands are type I transmembrane proteins encoded by the host genome: they include MHC class I chain–related A chain (MICA) or B chain (MICB) in humans[18] and a diverse family of ligands shared by human and mice called the retinoic acid early transcripts (RAET1) family, which includes Rae-1, H-60 and murine UL16-binding protein-like transcript 1 (Mult1)[19–22] in mice and the UL16-binding protein (ULBP) or human Rae-1 proteins in humans[23]. All the ligands are distant relatives of MHC class I molecules and adopt a MHC class I–like structure. However, none associate with β_2-microglobulin and, with the exception of human MICA and MICB, most of the corresponding genes are in a gene cluster separate from the MHC.

The expression patterns of different NKG2D ligands differ and are complex. In general, however, it seems that ligands are expressed poorly on the surface of normal cells but are often upregulated in tumor cells[24] or infected cells. Ligand mRNAs are upregulated in cells infected with cytomegalovirus[25]. In some cases this increase in transcription leads to upregulation of NKG2D ligands on the cell surface[26], but in other examples the virus genes encode proteins that interfere with ligand expression[25,27]. Signals associated with infections, such as TLR signaling, can lead to some upregulation of NKG2D ligands on macrophages[28]. Overall, it seems that cells use intrinsic signals associated with tumorogenesis as well as extrinsic and/or intrinsic signals associated with infections to regulate expression of NKG2D ligands.

Ligand expression by target cells provokes NK cells to attack the cells and can enhance antigen-specific CD8+ T cell responses. *In vivo*, ligand expression by subcutaneously transferred tumor cells incites immune rejection of the tumor[29,30]. Thus, the ligand upregulation that occurs normally in tumorogenesis may activate immune responses that can in some cases eliminate the tumor cells or impede their growth or spread. Although it is not yet possible to estimate the effectiveness of this response in tumor surveillance, the existence of many ligand-expressing tumor cell lines and primary tumors indicates that evasion of the response occurs frequently. In the case of certain human tumors, evasion may involve shedding of a soluble form of the MICA ligand into the circulation, where it binds to NKG2D on lymphocytes and desensitizes the cells to subsequent stimulation via NKG2D[31].

At least three other stimulatory receptors have been linked to NK-mediated lysis of tumor cells. NKp46, NKp44 and NKp30 are all members of the immunoglobulin superfamily[32–34]. NKp46 is encoded in the leukocyte receptor gene complex, which includes the genes for many other immunoglobulin-related NK receptors, whereas the NKp44 and NKp30 genes are in the human MHC[4]. All three receptors are associated in the membrane with ITAM-containing signaling adapter molecules[4]. Receptor engagement is generally sufficient to fully activate the NK cell.

Antibody blocking studies indicate that one or more of these receptors and NKG2D may participate in activating lysis, depending on the tumor cell line. Blocking all the receptors generally prevents tumor cell lysis[4]. Many NK cells coexpress these receptors: the same cell can use a different receptor or more than one receptor, depending on the ligands expressed by the target cells.

The NKp46, NKp44 and NKp30 ligands on tumor cells have not been identified. It is likely that the ligands are generally upregulated on transformed cells relative to normal cells[4]. Thus, recognition by these receptors probably represents another example of induced-self recognition.
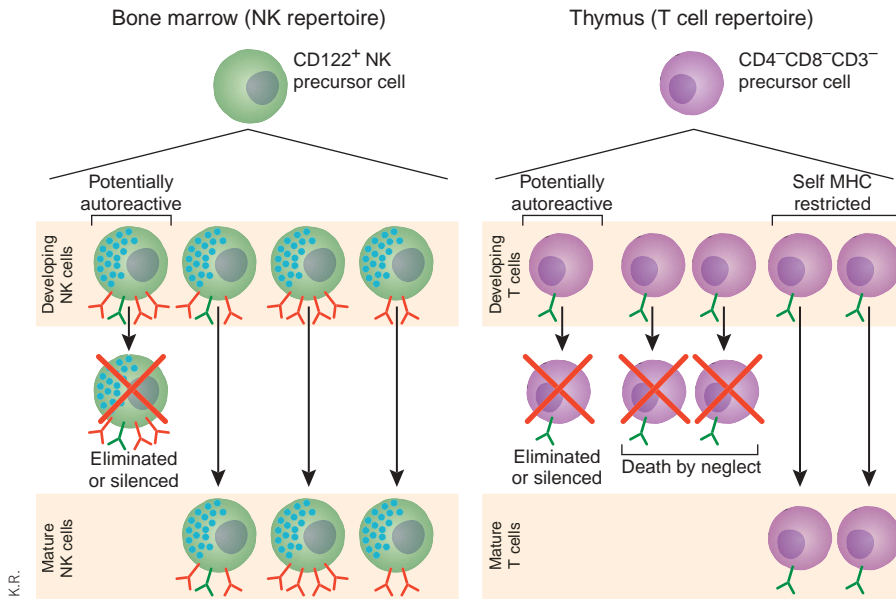
Missing-self recognition

Missing-self recognition was the first recognition strategy discovered for NK cells[35]. The principle is that the NK cell is inhibited by receptors specific for proteins expressed on the surface of normal cells. Strong inhibitory interactions can even prevent the lysis of target cells that express ligands for stimulatory NK receptors. Loss of the self protein, which can occur as a result of infection or transformation, unleashes the NK cell to attack the target cell. Missing-self recognition is also used by other immune system components such as the complement system[36]. It is likely that missing-self recognition operates only when the target cell also expresses ligands for stimulatory receptors expressed by NK cells. In this sense, missing-self recognition occurs in concert with various forms of stimulatory recognition, rather than as a completely independent form of recognition.

Missing-self recognition of MHC class I molecules was first suggested by the finding that tumor cells lacking MHC class I molecules are generally the most NK cell–sensitive target cells[37]. Even nontransformed cells that lack MHC class I molecules as a result of gene-targeting events are sensitive to destruction by NK cells[38]. Hence, NK cells preferentially attack self cells that have downregulated MHC class I molecules as a result of infection or transformation.

The mechanism of missing-self recognition was elucidated by the discovery of MHC-recognizing inhibitory receptors, including Ly49 receptors[39], the killer immunoglobulin-like receptors (KIRs)[40,41], the leukocyte immunoglobulin-like receptors (LIRs)[42,43] and the CD94-NKG2 receptors[44–46]. The Ly49 and CD94-NKG2 receptors are lectin-like type II transmembrane proteins encoded in the NK gene complex, whereas the KIR and LIR genes are immunoglobulin-like type I transmembrane proteins and are encoded in the leukocyte receptor gene complex. Ly49 receptors have a prominent function in mouse NK cells but are not functionally expressed in humans, whereas KIR and LIR are important in humans but not in mice. Genes encoding CD94-NKG2A function in both species. All of the inhibitory MHC-specific receptors contain in their cytoplasmic domains an ITIM that recruits protein tyrosine phosphatases essential for inhibitory function[47].

Each receptor family of this type also includes stimulatory isoforms that associate with DAP12 and lack ITIMs, and many of these are specific for MHC class I molecules[4,5]. The function of these stimulatory MHC-specific receptor isoforms is uncertain, but it may be to enable certain NK cells to attack target cells that express an MHC ligand, but have extinguished expression of a distinct MHC ligand for an inhibitory receptor[7].

Bone marrow (NK repertoire)

CD122⁺ NK precursor cell

Thymus (T cell repertoire)

CD4⁻CD8⁻CD3⁻ precursor cell

Figure 1 Comparison of some features of the formation of the NK cell and T cell repertoires. The T cell (and B cell) repertoires are complex because of the huge number of distinct antigen receptors represented. Although there are many fewer NK cell receptors, the repertoire is still complex because of the random coexpression of many possible combinations of NK receptors. Unlike the T cell repertoire, many of the NK cell receptors are inhibitory (red), although some are stimulatory (green). In both cell compartments, random expression of receptors can lead to the appearance of potentially autoreactive clones. In the case of NK cells, this may occur because a clone expresses a stimulatory receptor specific for self cells and/or because the clone lacks inhibitory receptors specific for self cells. Such potentially autoreactive NK clones are silenced possibly by multiple mechanisms, including by being rendered hyporesponsive. Only a minority of developing T cells express receptors that are self MHC restricted. T cell clones that are neither autoreactive nor self MHC restricted die during T cell development. A comparable positive selection step has not been demonstrated in NK cell development.

Inhibitory recognition of classical MHC class I molecules (class Ia) is mediated mainly by KIR in humans and Ly49 receptors in mice[48]. Despite the differences in structure and genetic origin, KIR and Ly49 receptors are very similar in their pattern of expression and function. Each family consists of relatively few genes in a given individual (approximately ten), which bind different subsets of MHC class I molecules[7]. In both cases, these receptors are expressed on subsets of NK cells in an overlapping way so that each NK cell randomly expresses an average of three or four receptor genes from the available set. Each NK cell can therefore discriminate between cells expressing different MHC alleles, selectively attacking cells whose MHC molecules fail to engage the inhibitory receptors that are expressed by the NK cell.

NK cells also carry out a second type of missing-self recognition mediated by inhibitory receptors. The NKR-P1B and NKR-P1D receptors are inhibitory members of the NK gene complex–encoded NKR-P1 family of lectin-like type 2 transmembrane receptors[5]. The ligand for NKR-P1B and NKR-P1D was recently identified as Ocil, also known as Clr-b[49,50]. Ocil, like the NKR-P1 receptors that recognize it, is a member of a family of lectin-like type 2 transmembrane proteins, and the Ocil-Clr gene family members are interspersed with NKR-P1 genes in the NK gene complex[5]. The Ocil–NKR-P1 family interactions represent the only known cases where the NK receptor and ligand are of the same structural type and are encoded by closely linked genes. Ocil, like MHC class I molecules, is broadly expressed by normal cell types and is often downregulated in tumor cells[50]. Thus, the Ocil–NKR-P1 interaction may serve a function similar to that of MHC-Ly49 or MHC-KIR interactions in enabling NK cells to discriminate transformed cells from normal cells.

NK cells have a complex repertoire

The inhibitory MHC-specific receptors such as Ly49, KIR and CD94-NKG2A are considered receptors of the innate immune system, but some features of this recognition system are reminiscent of adaptive immune receptor systems. Most salient is that each NK cell expresses a different set of MHC-specific receptors, generating a repertoire of NK clones with distinct specificities[7] (**Fig. 1**). The process that the cell uses to activate expression of only a subset of the genes encoding the KIR or Ly49 receptors in each cluster seems to be initially random and usually results in expression of only one of the two alleles at each locus of an expressed gene encoding Ly49, KIR or NKG2A, a phenomenon called 'monoallelic expression'[51]. The frequency of cells showing monoallelic expression of the genes encoding Ly49 is regulated to some extent by a feedback mechanism dependent on engagement of MHC ligands by inhibitory receptors expressed during the process of NK cell development[52]. These features are at least superficially similar to features of the processes that select immunoglobulin or T cell receptor (TCR) variable-region genes for expression in developing B or T cells, which also involves a largely random process of receptor variable gene selection and results in allelic exclusion of receptor genes through a feedback mechanism. There are, of course, considerable differences in these processes, including the absence of gene rearrangement in the NK receptor loci, the much smaller repertoire of distinct NK receptors, the common expression of multiple receptors by NK cells and the fact that monoallelic expression of NK receptor genes is not stringent[7].

Another important shared feature of NK cells and B or T cells concerns self-nonself discrimination (**Fig. 1**). As in the adaptive immune system, the clonally diverse repertoire of NK cells necessitates mechanisms to prevent autoimmunity. Random expression of receptor genes can theoretically lead to the appearance of NK clones lacking inhibitory receptors specific for self MHC or clones expressing stimulatory receptors for self MHC. Either type of NK clone could potentially mediate autoimmunity, but a variety of studies have demonstrated that self-tolerance is imposed somatically[7]. The underlying processes are not well understood, but include a mechanism that renders potentially autoreactive NK cell clones hyporesponsive, similar in some respects to the state of 'anergy' or hyporesponsiveness observed in potentially autoreactive B and T cells[7,53,54].
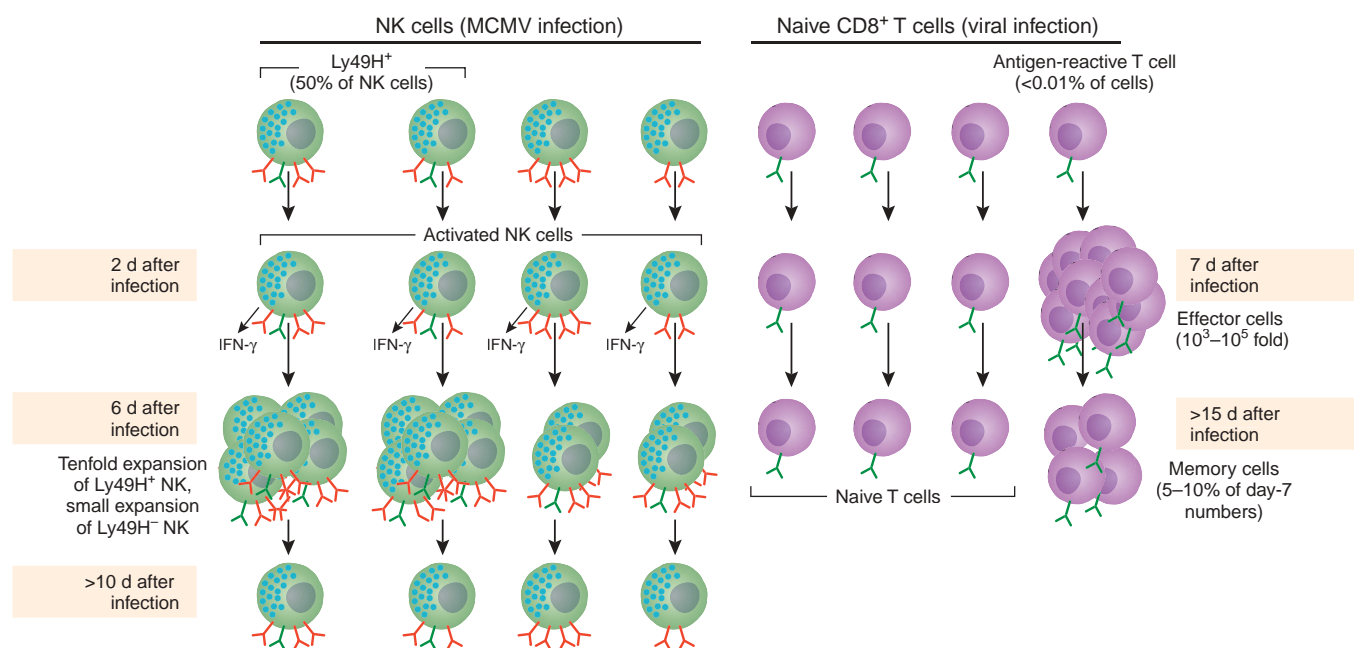
Figure 2 Comparison of NK cell and T cell activation and 'clonal expansion'. Naive CD8^{+} T cells and 'resting' NK cells are relatively noncytotoxic and functionally nonresponsive; functional activities are induced during immune responses. Both types of cells undergo clonal expansion as well. Clonal expansion of CD8^{+} T cells is considerable. The frequency of naive T cells specific for a viral antigen is usually less than 1 in 10,000, but such clones expand more than 1,000-fold during the first week after infection to form the effector cell pool. After considerable cell death, memory cells persist, representing approximately 5–10% of the number of cells in the effector cell population. NK cells undergo a more modest clonal expansion in response to MCMV. Within a day or so after infection, many NK cells of diverse specificity are activated to produce IFN-γ. Subsequently, the 50% or so of NK cells that express Ly49H undergo a selective expansion of about 10- to 15-fold, presumably as a result of engagement of Ly49H by the viral *m157* product, expressed in infected cells. Ly49H^{−} NK cell populations undergo a much smaller expansion. After several more days, the expanded Ly49H^{+} population is believed to contract to preinfection levels, consistent with the absence of substantial long-term memory in the NK response.

Similarities between NK and T cell activation

Although NK cells are usually thought of as constitutively active cells, they actually exist in various states of activation. Unstimulated, or 'resting', NK cells typically have relatively low functional activities *ex vivo*. Strong functional activity is induced *in vivo* by a brief exposure to NK-sensitive tumor cells[29,55], pathogens[2] or pathogen-associated molecular patterns such as double-stranded RNA or CpG oligonucleotides[1]. This process may normally involve interactions between NK cells and DCs (discussed later). The resulting activated pool of NK cells persists for several days, providing heightened NK cell–mediated protection during this period. In some cases, especially strong activation is restricted to an NK cell subset, such as the Ly49H+ subset in mice infected with MCMV. Shortly after the initiation of infection, Ly49H+ NK cells undergo 'clonal expansion' and accumulate over a period of a few days[15] (**Fig. 2**). These features of activation and clonal expansion bear some similarities to the priming and clonal expansion of naive T and B cells (**Fig. 2**). There are, however, substantial differences. The frequency of antigen-responsive cells is much lower in the case of T cells and the magnitude of the expansion is much greater[56]. Most importantly, although the expanded Ly49H+ NK cell populations may help clear the virus, they do not provide long-lasting NK cell–mediated memory.

Crosstalk between NK cells and DCs

NK cells influence the adaptive immune response in several ways and by interacting with several cell types. These interactions also influence the activity of NK cells. Crosstalk between NK cells and DCs is believed to be especially important and may profoundly influence both NK and T cell responses (**Fig. 3**).

Activation of NK cells *in vivo* may be in large part due to interactions with DCs, as first demonstrated in studies with mouse NK cells[57] and later generalized to human NK cells[58–60]. Freshly isolated 'resting' NK cells proliferate and acquire cytotoxic activity and the capacity to produce IFN-γ when cultured for a few days with DCs, but not when cultured with susceptible tumor target cells[57–59] (**Fig. 3**). In most studies, NK cell activation has required direct contact with DCs[58,59]. Thus, receptor-ligand interactions occurring between the cells are important, although DC-derived cytokines are also involved. The receptor-ligand interactions may differ for different DCs. DCs prestimulated with IFN-α upregulate the MICA and MICB NKG2D ligands, which contribute to activating NK cells in cocultures[15]. In mice infected with MCMV, CD8α+ DCs are necessary for the expansion of Ly49H+ NK cell populations and blocking Ly49H prevents NK population expansion[61]. In other cases, receptor-ligand interactions leading to NK cell activation by DCs have not been defined. The importance of the DC−NK cell interaction in tumor rejection was suggested by the finding that injected DCs or substances that activate DCs substantially augment NK cell–mediated protection from transferred tumor cells[57].

Communication between NK cells and DCs is not unidirectional. In fact, the maturation of DCs stimulated by NK cells may represent a key mechanism to bridge the NK response to the stimulation of T cell responses (**Fig. 3**). In coculture with NK cells, immature DCs undergo maturation, produce TNF and interleukin 12 (refs. 59,60) and upregulate costimulatory ligands such as CD86. Efficient DC activation in cell culture requires contact with NK cells, with the NKp30 receptor being important in the interaction[62]. TNF produced in the cocultures also contributes to DC activation[59,60]. In the case of
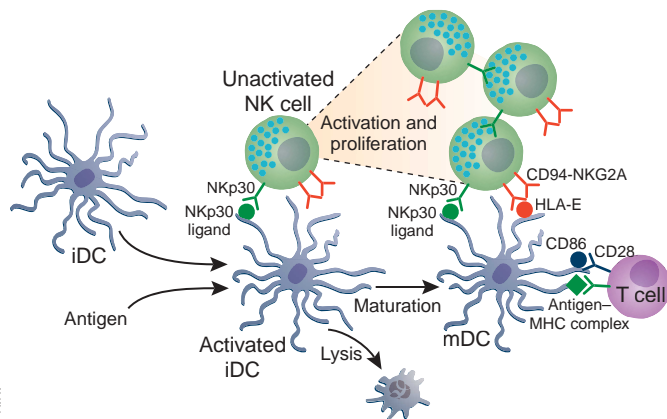
Figure 3 Reciprocal interactions of NK cells and DCs may influence the adaptive immune response. Unactivated NK cells show relatively little capacity to rapidly produce IFN-γ or kill target cells. In cell cultures, NK cell activation and modest proliferation is efficiently induced by interactions with DCs, dependent on engagement of the NKp30 NK cell receptor by its undefined ligand. Reciprocally, the interaction of NK cells with immature DCs (iDC) can lead to two outcomes, which presumably have opposite effects on the T cell response: maturation of the DC (mDC) or lysis of the DC. DC maturation results in upregulation of MHC complexes and costimulatory molecules on the DC, enabling productive stimulation of antigen-specific T cells. Upregulation of MHC molecules, especially HLA-E, also protects the DC from NK cell attack by engaging inhibitory NK receptors such as CD94-NKG2A. Presumably, the cues that determine whether NK cells kill or activate immature DCs are central in this immunoregulatory circuit but remain to be defined.

mice infected with MCMV, virus-responsive Ly49H+ NK cells are necessary for maintenance of CD8α+ DCs in the spleen[61]. In a different *in vivo* system, NK cells activated by encounters with MHC class I^{lo} tumor cells stimulate DCs to produce interleukin 12 and enhance the induction of CD8+ T cell responses[63]. The cell culture studies support the conclusion that mature DCs resulting from NK interactions are more effective at inducing T cell responses and NK responses than are immature DCs[59]. T cell responses are further influenced by IFN-γ produced by NK cells, which promotes antigen processing and presentation to T cells and T helper type 1 cell polarization.

Paradoxically, although NK cells promote the activation of immature DCs, they also kill them[64,65] (**Fig. 3**). Lysis of DCs is mediated mainly by recognition through the NKp30 receptor and to a lesser extent the NKp46 receptor[58,66]. Whether this occurs in DCs from mice, which apparently lack the NKp30 receptor, remains to be determined. Immature DCs but not mature DCs are lysed by NK cells, apparently because the mature DCs have high expression of HLA-E molecules that can inhibit NK cell cytolysis, whereas the immature DCs do not[67]. In cell culture experiments, DC lysis is favored when NK cells are present in excess of immature DCs, whereas DC activation is favored when DCs are in excess[60].

The rules that govern whether immature DCs are lysed or activated *in vivo* remain to be fully determined. Lysis of immature DCs might be seen as one of several checkpoints for preventing T cell responses in the absence of the microbial or viral signals necessary to induce DC maturation. In the absence of these signals, lysis of immature DCs with the potential to present self antigens could help prevent autoimmune reactions.

Adaptive immune system cells express 'NK receptors'
The receptors that regulate NK cells are thought of as components of the innate immune system, but many of them are also expressed by T

cells and are probably important in the regulation of T cell responses. Both inhibitory and certain stimulatory NK receptors are expressed by subsets of T cells, including NKG2D and NKR-P1C stimulatory receptors and the KIR, Ly49, CD94-NKG2A and LIR inhibitory receptors[68]. The discussion here will be limited to NKG2D, KIR, Ly49 and CD94-NKG2A.

The best characterized 'NK stimulatory receptor' expressed by T cells is NKG2D. The receptor is expressed by CD8+ T cells, some γδ T cells and some NK1.1+ T cells[17]. In mouse CD8+ T cells, NKG2D is only expressed after the cells are activated through TCR signaling, but in humans even unactivated CD8+ T cells express some NKG2D. Although resting or recently activated CD4+ T cells do not express NKG2D, CD4+NKG2D+ T cells have been found in the joints of humans with rheumatoid arthritis[69].

Expression of NKG2D ligands by target cells can enhance virus-specific CD8+ T cell responses *in vitro*, especially production of cytokines[26,29]. *In vivo*, ligand expression by subcutaneously transferred tumor cells can enhance T cell responses specific for the tumor cells[29,70]. The extent to which the enhancement of tumor-specific T cell responses is due to the expression of NKG2D by CD8+ T cells rather than being an indirect effect of NK cells or antigen-presenting cells has not been fully elucidated.

In NK cells, NKG2D engagement is typically a sufficient signal to trigger activation[71], but in CD8+ T cells receptor engagement usually serves to enhance, or costimulate, responses to specific antigens[26]. In this manner, the 'hardwired' NKG2D recognition system is exploited for somewhat distinct tasks in the innate versus adaptive immune systems. The key shared feature is that receptor engagement can occur only when ligand upregulation has occurred as a result of infection or transformation. In the case of NK cells at some states of activation, NKG2D engagement amplifies, or costimulates, a response stimulated through distinct ITAM-associated receptors[72].

Costimulatory function in T cells is accomplished by the association of NKG2D with the DAP10 signaling adapter molecule, which is believed to be comparable in function to the CD28, inducible costimulator and CD19 costimulatory receptors, and signals via phosphatidylinositol-3 kinase activation[73,74]. DAP10-associated NKG2D may also amplify activation of NK cells stimulated through distinct ITAM-associated receptors. The direct activating function of NKG2D in NK cells seems to have multiple explanations. In mice, NKG2D exists in two isoforms: one that associates only with DAP10 and another that associates with DAP10 as well as the aforementioned ITAM-containing signaling molecule DAP12 (ref. 75). DAP12, which is expressed in NK cells but not T cells, is necessary for NKG2D-induced cytokine production; it is also necessary for maximal cytotoxic activity by recently activated mouse NK cells[75]. Highly activated NK cells, however, apparently become less dependent on DAP12 for cytotoxicity[76]. The situation is different in humans, in which a human NKG2D isoform that associates with DAP12 has not been detected and NK cytotoxicity induced by NKG2D engagement is believed to be mediated solely through DAP10 (refs. 77,78). It remains to be explained how DAP10 signaling suffices to induce cytotoxicity in NK cells but is not sufficient to trigger cytolysis by most DAP10-expressing CD8+ T cells.

Inhibitory NK receptors expressed by T cells
Inhibitory MHC-specific receptors of the KIR, Ly49, CD94-NKG2A and LIR families are in some cases expressed by T cells, especially CD8+ T cells. Two patterns of receptor expression are seen, depending on the receptors studied. Some receptors, such as CD94-NKG2A, are expressed rapidly after activation by most or all activated cells,

whereas others, such as KIR and Ly49 receptors, are expressed sporadically in conditions of activation that remain poorly defined[7,68]. Indeed, in infected mice, most antigen-responsive CD8+ T cells do not upregulate Ly49 receptors even in the case of infection with a virus that causes chronic infections[68,79]. One report demonstrates that Ly49 receptor upregulation occurs when CD8+ T cells are stimulated by autoantigens *in vivo*, raising the possibility that it occurs as a result of chronic stimulation in the absence of inflammatory signals[80]. Consistent with this hypothesis, a substantial population of CD8+ T cells, which accumulates with age and bears markers of memory cells, expresses Ly49 (mouse) or KIR (human).

For unknown reasons, the capacity of inhibitory receptor engagement by its ligand to inhibit T cell functions varies considerably in different studies[68]. For example, engagement of the CD94-NKG2A receptor failed to inhibit CD8+ T cells specific for lymphocytic choriomeningitis virus (LCMV), *Listeria monocytogenes* or ovalbumin[79], but inhibition was observed for CD8+ T cells specific for polyoma virus[81]. The variable inhibition of T cell functions has led to the hypothesis that inhibitory MHC-specific receptors may generally have a distinct function in T cell biology. A possibility is that inhibitory receptor engagement inhibits activation-induced cell death and consequently enhances the accumulation of memory CD8+ T cells[82].

In addition to being expressed by CD8+ T cells, inhibitory NK receptors are expressed by many NK1.1+ T cells. Although the normal function of the receptors in NK1.1+ T cells is not established, one study showed that chronic exposure of mice to the synthetic glycolipid antigen for NK1.1+ T cells, α-galactosylceramide, results in a large increase in the incidence of inhibitory Ly49 expression by NK1.1+ T cells[83]. The inhibitory receptors prevent a functional response to α-galactosylceramide, suggesting that the inhibitory NK receptors can impose tolerance of NK1.1+ T cells. It remains to be determined whether this also applies to other types of T cells.

Perspective

Although well accepted as a component of the innate immune system, NK cells have features characteristic of the adaptive immune system, such as a complex repertoire and modest clonal expansion of a virus-specific subset during MCMV infection. Although the clonal expansion may not lead to potent NK cell memory, it may provide some short-term protection from pathogens. From an evolutionary perspective, these commonalities of NK cells and B and T cells suggest that clonal variation in specificity and clonal selection of specific cells are valuable mechanisms in both the innate and adaptive immune compartments, which have apparently evolved independently in these distinct cell and receptor lineages.

It is likely that the importance of crosstalk between NK cells and DCs will be increasingly appreciated as studies on this subject accrue. DCs activated by microbial stimuli may represent a common stimulus for the activation of NK cell responses *in vivo*. Conversely, by participating in the maturation of DCs, NK cells may amplify T cell activation, serving as one of several 'alarm systems' that effectively inform the adaptive immune system of potential dangers. In other conditions, the elimination of immature DCs by NK cells may repress T cell responses. A principal unresolved issue is how the choice between these opposing outcomes is regulated in physiological conditions.

Finally, an important challenge will be to clarify the physiological significance of NK receptors expressed by T cells. In the longer term, the bigger challenge will be to develop new approaches to understand how these complex cellular and receptor interactions between NK cells and the adaptive immune system are integrated to yield beneficial immune responses.

1. Trinchieri, G. Biology of natural killer cells. *Adv. Immunol.* **47**, 187–376 (1989).
2. Biron, C.A., Nguyen, K.B., Pien, G.C., Cousens, L.P. & Salazar-Mather, T.P. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu. Rev. Immunol.* **17**, 189–220 (1999).
3. Lanier, L.L. NK cell receptors. *Annu. Rev. Immunol.* **16**, 359–393 (1998).
4. Moretta, A. *et al.* Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis. *Annu. Rev. Immunol.* **19**, 197–223 (2001).
5. Yokoyama, W.M. & Plougastel, B.F. Immune functions encoded by the natural killer gene complex. *Nat. Rev. Immunol.* **3**, 304–316 (2003).
6. Medzhitov, R. & Janeway, C.A. Jr. Innate immunity: the virtues of a nonclonal system of recognition. *Cell* **91**, 295–298 (1997).
7. Raulet, D.H., Vance, R.E. & McMahon, C.W. Regulation of the natural killer cell receptor repertoire. *Annu. Rev. Immunol.* **19**, 291–330 (2001).
8. Janeway, C.A. Jr. Approaching the Asymptote? Evolution and Revolution in Immunology. *Cold Spring Harb. Symp. Quant. Biol.* **54**, 1–13 (1989).
9. Vivier, E. & Biron, C.A. Immunology. A pathogen receptor on natural killer cells. *Science* **296**, 1248–1249 (2002).
10. Brown, M.G. *et al.* Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science* **292**, 934–937 (2001).
11. Daniels, K.A. *et al.* Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. *J. Exp. Med.* **194**, 29–44 (2001).
12. Lee, S.H. *et al.* Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C–type lectin superfamily. *Nat. Genet.* **28**, 42–45 (2001).
13. Arase, H., Mocarski, E.S., Campbell, A.E., Hill, A.B. & Lanier, L.L. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science* **296**, 1323–1326 (2002).
14. Smith, H.R. *et al.* Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proc. Natl. Acad. Sci. USA* **99**, 8826–8831 (2002).
15. Dokun, A.O. *et al.* Specific and nonspecific NK cell activation during virus infection. *Nat. Immunol.* **2**, 951–956 (2001).
16. Mandelboim, O. *et al.* Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* **409**, 1055–1060 (2001).
17. Raulet, D.H. Roles of the NKG2D immunoreceptor and its ligands. *Nat. Rev. Immunol.* **3**, 781–790 (2003).
18. Bauer, S. *et al.* Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* **285**, 727–729 (1999).
19. Diefenbach, A., Jamieson, A.M., Liu, S.D., Shastri, N. & Raulet, D.H. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. *Nat. Immunol.* **1**, 119–126 (2000).
20. Cerwenka, A. *et al.* Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* **12**, 721–727 (2000).
21. Carayannopoulos, L.N., Naidenko, O.V., Fremont, D.H. & Yokoyama, W.M. Cutting edge: murine UL16-binding protein-like transcript 1: a newly described transcript encoding a high-affinity ligand for murine NKG2D. *J. Immunol.* **169**, 4079–83 (2002).
22. Diefenbach, A., Hsia, J.K., Hsiung, M.Y. & Raulet, D.H. A novel ligand for the NKG2D receptor activates NK cells and macrophages and induces tumor immunity. *Eur. J. Immunol.* **33**, 381–391 (2003).
23. Cosman, D. *et al.* ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* **14**, 123–133 (2001).
24. Groh, V. *et al.* Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. *Proc. Natl. Acad. Sci. USA* **96**, 6879–6884 (1999).
25. Lodoen, M. *et al.* NKG2D-mediated natural killer cell protection against cytomegalovirus is impaired by viral gp40 modulation of retinoic acid early inducible 1 gene molecules. *J. Exp. Med.* **197**, 1245–1253 (2003).
26. Groh, V. *et al.* Costimulation of CD8 αβ T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat. Immunol.* **2**, 255–260 (2001).
27. Krmpotic, A. *et al.* MCMV glycoprotein gp40 confers virus resistance to CD8+ T cells and NK cells in vivo. *Nat. Immunol.* **3**, 529–535 (2002).
28. Hamerman, J.A., Ogasawara, K. & Lanier, L.L. Cutting edge: toll-like receptor signaling in macrophages induces ligands for the NKG2D receptor. *J. Immunol.* **172**, 2001–2005 (2004).
29. Diefenbach, A., Jensen, E.R., Jamieson, A.M. & Raulet, D.H. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* **413**, 165–171 (2001).
30. Cerwenka, A., Baron, J.L. & Lanier, L.L. Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated rejection of a MHC class I-bearing tumor in vivo. *Proc. Natl. Acad. Sci. USA* **98**, 11521–11526 (2001).

31. Groh, V., Wu, J., Yee, C. & Spies, T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T- cell activation. *Nature* **419**, 734–738 (2002).

32. Sivori, S. *et al*. p46, a novel natural killer cell-specific surface molecule that mediates cell activation. *J. Exp. Med.* **186**, 1129–1136 (1997).

33. Vitale, M. *et al*. NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis. *J. Exp. Med.* **187**, 2065–2072 (1998).

34. Pende, D. *et al*. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. *J. Exp. Med.* **190**, 1505–1516 (1999).

35. Ljunggren, H.G. & Karre, K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol. Today* **11**, 237–244 (1990).

36. Medzhitov, R. & Janeway, C.A. Jr. Decoding the patterns of self and nonself by the innate immune system. *Science* **296**, 298–300 (2002).

37. Ljunggren, H.-G. & Karre, K. Host resistance directed selectively against H-2-deficient lymphoma variants. *J. Exp. Med.* **162**, 1745–1759 (1985).

38. Bix, M. *et al*. Rejection of class I MHC-deficient hemopoietic cells by irradiated MHC-matched mice. *Nature* **349**, 329–331 (1991).

39. Karlhofer, F.M., Ribaudo, R.K. & Yokoyama, W.M. MHC class I alloantigen specificity of Ly-49+ IL-2 activated natural killer cells. *Nature* **358**, 66–70 (1992).

40. Colonna, M. & Samaridis, J. Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells. *Science* **268**, 405–408 (1995).

41. Wagtmann, N. *et al*. Molecular clones of the p58 natural killer cell receptor reveal Ig-related molecules with diversity in both the extra- and intra-cellular domains. *Immunity* **2**, 439–449 (1995).

42. Cosman, D. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. *Immunity* **7**, 273–282 (1997).

43. Samaridis, J. & Colonna, M. Cloning of novel immunoglobulin superfamily receptors expressed on human myeloid and lymphoid cells: structural evidence for new stimulatory and inhibitory pathways. *Eur. J. Immunol.* **27**, 660–665 (1997).

44. Braud, V.M. *et al*. HLA-E binds to natural killer cell receptors CD94/NKG2A, B, and C. *Nature* **391**, 795–799 (1998).

45. Lee, N. *et al*. HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. *Proc. Natl. Acad. Sci. USA* **95**, 5199–5204 (1998).

46. Vance, R.E., Kraft, J.R., Altman, J.D., Jensen, P.E. & Raulet, D.H. Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical MHC class I molecule Qa-1^b. *J. Exp. Med.* **188**, 1841–1848 (1998).

47. Long, E. Regulation of immune responses through inhibitory receptors. *Annu. Rev. Immunol.* **17**, 875–904 (1999).

48. Valiante, N.M., Lienert, K., Shilling, H.G., Smits, B.J. & Parham, P. Killer cell receptors: keeping pace with MHC class I evolution. *Immunol. Rev.* **155**, 155–164 (1997).

49. Iizuka, K., Naidenko, O.V., Plougastel, B.F., Fremont, D.H. & Yokoyama, W.M. Genetically linked C-type lectin-related ligands for the NKRP1 family of natural killer cell receptors. *Nat. Immunol.* **4**, 801–807 (2003).

50. Carlyle, J.R. *et al*. Missing self-recognition of Ocil/Clr-b by inhibitory NKR-P1 natural killer cell receptors. *Proc. Natl. Acad. Sci. USA* **101**, 3527–3532 (2004).

51. Held, W., Roland, J. & Raulet, D.H. Allelic exclusion of Ly49 family genes encoding class I-MHC-specific receptors on NK cells. *Nature* **376**, 355–358 (1995).

52. Tanamachi, D.M., Hanke, T., Takizawa, H., Jamieson, A.M. & Raulet, D.H. Expression of natural killer cell receptor alleles at different Ly49 loci occurs independently and is regulated by major histocompatibility complex class I molecules. *J. Exp. Med.* **193**, 307–315 (2001).

53. Liao, N., Bix, M., Zijlstra, M., Jaenisch, R. & Raulet, D. MHC class I deficiency: susceptibility to natural killer (NK) cells and impaired NK activity. *Science* **253**, 199–202 (1991).

54. Johansson, M.H., Bieberich, C., Jay, G., Karre, K. & Hoglund, P. Natural killer cell tolerance in mice with mosaic expression of major histocompatibility complex class I transgene. *J. Exp. Med.* **186**, 353–364 (1997).

55. Glas, R. *et al*. Recruitment and activation of natural killer (NK) cells in vivo determined by the target cell phenotype: An adaptive component of NK cell-mediated responses. *J. Exp. Med.* **191**, 129–138 (2000).

56. Murali-Krishna, K. *et al*. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. *Immunity* **8**, 177–187 (1998).

57. Fernandez, N.C. *et al*. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses in vivo. *Nat. Med.* **5**, 405–11 (1999).

58. Ferlazzo, G. *et al*. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. *J. Exp. Med.* **195**, 343–351 (2002).

59. Gerosa, F. *et al*. Reciprocal activating interaction between natural killer cells and dendritic cells. *J. Exp. Med.* **195**, 327–333 (2002).

60. Piccioli, D., Sbrana, S., Melandri, E. & Valiante, N.M. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *J. Exp. Med.* **195**, 335–41 (2002).

61. Andrews, D.M., Scalzo, A.A., Yokoyama, W.M., Smyth, M.J. & Degli-Esposti, M.A. Functional interactions between dendritic cells and NK cells during viral infection. *Nat. Immunol.* **4**, 175–181 (2003).

62. Ferlazzo, G. *et al*. The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells. *Eur. J. Immunol.* **33**, 306–313 (2003).

63. Mocikat, R. *et al*. Natural killer cells activated by MHC class I(low) targets prime dendritic cells to induce protective CD8 T cell responses. *Immunity* **19**, 561–569 (2003).

64. Carbone, E. *et al*. Recognition of autologous dendritic cells by human NK cells. *Eur. J. Immunol.* **29**, 4022–4029 (1999).

65. Wilson, J.L. *et al*. Targeting of human dendritic cells by autologous NK cells. *J. Immunol.* **163**, 6365–6370 (1999).

66. Spaggiari, G.M. *et al*. NK cell-mediated lysis of autologous antigen-presenting cells is triggered by the engagement of the phosphatidylinositol 3-kinase upon ligation of the natural cytotoxicity receptors NKp30 and NKp46. *Eur. J. Immunol.* **31**, 1656–1665 (2001).

67. Chiesa, M.D. *et al*. The natural killer cell-mediated killing of autologous dendritic cells is confined to a cell subset expressing CD94/NKG2A, but lacking inhibitory killer Ig-like receptors. *Eur. J. Immunol.* **33**, 1657–1666 (2003).

68. Vivier, E. & Anfossi, N. Inhibitory NK-cell receptors on T cells: witness of the past, actors of the future. *Nat. Rev. Immunol.* **4**, 190–198 (2004).

69. Groh, V., Bruhl, A., El-Gabalawy, H., Nelson, J.L. & Spies, T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA* **100**, 9452–9457 (2003).

70. Westwood, J.A. *et al*. Cutting Edge: Novel priming of tumor-specific immunity by NKG2D-triggered NK cell-mediated tumor rejection and Th1-independent CD4+ T cell pathway. *J. Immunol.* **172**, 757–761 (2004).

71. Jamieson, A.M. *et al*. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity* **17**, 19–29 (2002).

72. Ho, E.L. *et al*. Costimulation of Multiple NK Cell Activation Receptors by NKG2D. *J. Immunol.* **169**, 3667–3675 (2002).

73. Wu, J., Cherwinski, H., Spies, T., Phillips, J.H. & Lanier, L.L. DAP10 and DAP12 form distinct, but functionally cooperative, receptor complexes in natural killer cells. *J. Exp. Med.* **192**, 1059–1067 (2000).

74. Gilfillan, S., Ho, E.L., Cella, M., Yokoyama, W.M. & Colonna, M. NKG2D recruits two distinct adapters to trigger natural killer cell activation and costimulation. *Nat. Immunol.* **3**, 1150–1155 (2002).

75. Diefenbach, A. *et al*. Selective associations with signaling molecules determines stimulatory versus costimulatory activity of NKG2D. *Nat. Immunol.* **3**, 1142–1149 (2002).

76. Zompi, S. *et al*. NKG2D triggers cytotoxicity in mouse NK cells lacking DAP12 or Syk family kinases. *Nat. Immunol.* **4**, 565–572 (2003).

77. Billadeau, D.D., Upshaw, J.L., Schoon, R.A., Dick, C.J. & Leibson, P.J. NKG2D-DAP10 triggers human NK cell-mediated killing via a Syk-independent regulatory pathway. *Nat. Immunol.* **4**, 557–564 (2003).

78. Andre, P. *et al*. Comparative analysis of human NK cell activation induced by NKG2D and natural cytotoxicity receptors. *Eur. J. Immunol.* **34**, 961–971 (2004).

79. McMahon, C.W. *et al*. Viral and bacterial infections induce expression of multiple NK cell receptors in responding CD8+ T cells. *J. Immunol.* **169**, 1444–1452 (2002).

80. Anfossi, N. *et al*. Expansion and function of CD8+ T cells expressing Ly49 inhibitory receptors specific for MHC class I molecules. *J. Immunol.* (in the press).

81. Moser, J.M., Gibbs, J., Jensen, P.E. & Lukacher, A.E. CD94-NKG2A receptors regulate antiviral CD8+ T cell responses. *Nat. Immunol.* **3**, 189–195 (2002).

82. Ugolini, S. *et al*. Involvement of inhibitory NKRs in the survival of a subset of memory-phenotype CD8+ T cells. *Nat. Immunol.* **2**, 430–435 (2001).

83. Hayakawa, Y., Berzins, S.P., Crowe, N.Y., Godfrey, D.I. & Smyth, M.J. Antigen-induced tolerance by intrathymic modulation of self-recognizing inhibitory receptors. *Nat. Immunol.* **5**, 590–596 (2004).