Rapid innate control of antigen abrogates adaptive immunity

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Summary
Natural killer (NK) cells provide an immediate first line of defence against viral infections. Memory responses, maintained by CD4+ T cells, require exposure to viral antigen and provide long-term protection against future infections. It is known that NK cells can promote the development of the adaptive response through cytokine production and cross-talk with antigen-presenting cells. In this paper however, we summarize a series of recent publications, in mouse models and for the first time in man, with the unifying message that rapid viral antigen control by the innate immune system limits antigen exposure to CD4+ cells thereby abrogating the development of a memory response. We discuss the significant implication of these studies on viral treatment strategies and immunization models.

Keywords: CD4+ T cells; natural killer cells; viral immunity.

Introduction
Natural killer (NK) cells form an important part of the innate immune response against viral infections. They were first described as lymphocytes, which possess cytotoxic functions without the need for previous antigen exposure. The NK responses are not only directly cytotoxic against virus-infected cells but also serve as a bridge between the innate and adaptive branches of the immune system, as they can stimulate CD4+ and cytotoxic T lymphocytes by cytokine production (Box 1 and reviewed in ref. 2). These adaptive immune responses, which confer antigen specificity, can also provide long-term protection against further infections. To develop an antigen-specific response, cognate CD4+ T cells must recognize peptide epitopes presented by MHC class II heterodimeric glycoproteins, on antigen-presenting cells (APC) such as dendritic cells (DC) and B cells. If antigen can access the peripheral lymphoid compartment, and if appropriate timely co-stimulation is given to the T cells, then this process can start within hours of infection.

Box 1 Natural killer and adaptive immune cells and their interactions
Natural killer (NK) cells are large granular lymphocytes that are characteristically CD56+ CD3-. Although they were first given their name natural killer cells based on their ability to kill 'non-self' tumour cells deficient in MHC class I, it has become apparent that they are able to release cytokines and even have a role in uterine vascular homeostasis during pregnancy. CD4+ T lymphocytes include T helper cells and are activated when peptide antigens are presented to them by MHC class II glycoproteins. Once activated CD4+ cells divide and release cytokines to stimulate and regulate the immune response. CD8+ T cells are also known as cytotoxic T cells and kill target cells by binding to specific antigens associated with MHC class I glycoproteins. Professional antigen-presenting cells (APC) display foreign antigen particles (usually bound by MHC glycoproteins) on their cell surface to T cells. Professional APC include macrophages, dendritic cells (DC) and B cells; some epithelial cell types can up-regulate MHC molecules after cytokine stimulation and act as APC.

It is established that NK cells can influence CD4+ T cells in the following ways:
- Direct – NK cells release interferon-γ (IFN-γ) and pro-inflammatory cytokines promoting antigen processing and presentation to T cells and polarizing T helper type 1 responses.
- Indirect – stimulation of DC; lysis of excess immature DC, chemokine expression (IFN-γ-induced protein-10 CCL-21) to promote DC trafficking to lymph nodes.

Recent studies described in this paper report the following mechanisms:
- Clearance of antigen before a CD4+ T-cell memory response is established.
- Perforin-mediated NK-cell reduction of antigen-bearing APC
- Perforin-mediated NK-cell reduction of activated T cells.
Stimulation by DC-produced cytokines (interleukin-12 or interleukin-18) can induce interferon-γ (IFN-γ) production by NK cells, which promotes T helper type 1 polarization. The importance of IFN-γ production by NK cells is also highlighted in a recent publication examining the role of NK cells in directing influenza-specific CD8+ T-cell responses. In this study, NK-cell depletion attenuated T-cell responses by reducing DC antigen presentation and limiting the recruitment of both DC and T cells into the draining lymph nodes. This effect of NK cells was IFN-γ-dependent. The bi-directional stimulation between NK cells and DC to promote CD4+ T cells described could be considered a potential target of viral immune evasion. Indeed, Mandaric et al. have demonstrated that mouse cytomegalovirus (MCMV)-infected interleukin-10 limits NK–DC cross-talk and suppresses CD4+ T-cell priming through reduced interleukin-12 expression.

However, several recent studies using mouse models have thrown a different light on the relationship between NK-cell responses to infection and subsequent adaptive immune responses. The NK cells appear to be capable of shaping adaptive immune responses by rapid elimination of antigen actually preventing formation of CD4+ T-cell responses or even by direct lysis of APC and CD4+ T cells. Hence, NK cells might provide a previously unrecognized challenge for the treatment of viral infections if life-long immunity is desired.

**NK-cell depletion increases anti-viral CD4+ T-cell responses**

To study the role of NK cells in the development of adaptive T-cell responses Andrews et al. measured the effects of NK-cell depletion on CD4+ and CD8+ T-cell responses following MCMV infection. Depletion of NK cells before infection actually increased virus-specific CD4+ T cells, measured ex vivo by IFN-γ production. In vivo NK cells reduced the number and longevity of DC infected with MCMV, through a perforin-dependent mechanism, so reducing the quantity of viral antigen presented to CD4+ cells. Effective CD4+ responses were required for control of MCMV replication in the salivary glands, which are the major source of transmitted virus.

The importance of temporary viral antigen persistence in the development of a CD4+ response is further demonstrated in a mouse model using vesicular stomatitis virus (VSV) infection. VSV is effectively cleared from most tissues by type I IFN produced by an early rigorous innate response. However, a subpopulation of splenic marginal zone CD169+ macrophages is permissive to ongoing viral replication because of inherent cellular resistance to type I IFN, and hence enables persistent antigen presentation. This allows an adaptive response to develop. Depletion of these CD169+ macrophages deprives the adaptive arm of adequate exposure to antigen, and hence, although VSV is controlled and removed by the innate response, no specific adaptive memory response ensues.

There may be a host advantage to innate immunity preventing adaptive responses during a disseminated infection with high viral load whereby a robust CD4+-mediated T-cell response could induce widespread immunopathology (Fig. 1). Using lymphocytic choriomeningitis virus (LCMV) infection, Waggoner et al. demonstrated the effects of increasing the infecting dose of virus on interplay between NK cells and the adaptive immune response. Unlike MCMV infection, LCMV-infected cells appear to be resistant to direct NK-cell lysis. During high-dose LCMV infection, NK cells targeted and depleted virus-specific CD8+ T cells, facilitating CD8+ T-cell exhaustion, resulting in subclinical viral persistence but avoiding lethal immunopathology. Hence in this situation depletion of NK cells has lethal consequences.

Evidence points to NK cells controlling adaptive responses by control of antigen availability, or by directly impinging on effector lymphocytes. The function of NK cells seems to vary depending on the pathogen, infecting dose etc. This role of NK cells was explored further comparing control of MCMV and recombinant *Listeria monocytogenes* expressing ovalbumin. Narni-Mancinelli et al. demonstrated that mice bred with hyper-responsive NK cells exhibited reduced CD4+ and CD8+ T-cell responses on repeated challenge compared with wild-type mice. The NK responsiveness was enhanced in these mice because of failure to express the activating receptor NKP46. These results could be replicated by treating mice, after NK-cell depletion, with an NKP46-blocking antibody. So again the theme, which emerges, is that rapid innate control of pathogens and hence antigen supply comes at a cost to adaptive responses.

**NK-cell activity limits CD8+ T-cell immunity**

The effects of NK-cell activation on CD8+ T-cell anti-viral activity are demonstrated by Lang et al. in a mouse model of LCMV infection. LCMV infection induces increased perforin-mediated NK cytotoxicity and alters the phenotype of NK cells with increased activating receptor NKG2D expression and reduced inhibitory 2B4 expression. However, NK depletion, NKG2D blockade and perforin-deficient strains of mice exhibit increased CD8+ T-cell immunity. Importantly, depletion of NK cells prevented viral persistence and chronic viral infection by enhancing CD8+ T-cell-mediated clearance of LCMV. Furthermore, there was reduced LCMV hepatitis with a greatly reduced number of infected cells in the liver and lower levels of liver cell damage as measured by alanine aminotransferase...
ALT in NK-cell-deficient mice as CD8+ T cells cleared the virus.11 In a mouse tumour model NK-cell killing of tumour antigen-specific CD8+ T cells has been found to be perforin-dependent and NK activation receptor NKG2D-dependent. In this mouse tumour model, depletion of NK cells has also been shown to enhance a greater number of antigen-specific CD8+ T cells,12 suggesting that this effect is not restricted to viral pathogens.

In humans the study of innate and adaptive immune responses to viral infections is largely limited to sampling peripheral blood and using serum viral loads as substitutes for true antigen availability. We have previously demonstrated successful clearance of hepatitis C virus (HCV) in a subject lacking T-cell and B-cell responses, suggesting an important role for innate mechanisms.13 We extended this study to a cohort of 33 patients being treated for HCV with type I IFN. For the first time in a human ‘model’ we have demonstrated that during the IFN-α-based treatment, the most rapid reduction in viral load (a strong predictor of treatment success) was associated with a striking absence or paucity of CD4+ T-cell responses measured in vitro.14 Tellingly, the rapid viral clearance was associated with an increased potential of NK cells to be activated by strong stimuli (high-dose IFN-α/K562 cells) compared with weaker stimuli (low-dose IFN-α/Huh7.5 cells). It is intriguing that a lower NK-cell expression of NKp46 pre-treatment was associated with this successful rapid viral clearance, reflecting the findings of Narni-Mancinelli et al. on the importance of this NK-cell activating marker (Fig 2). This study suggests that similar principles may apply to the human system reflecting the results emerging from mouse models.7–12

Figure 1. Models of immune response to viral pathogens and development of adaptive immunity. (a) A moderate natural killer (NK) cell response to viral infection allows antigen-presenting cell (APC) priming of CD4+ T cells. The adaptive immune response may result in fatal immunopathology (Waggoner et al.9). (b) An excessive NK cytotoxic response may clear viral antigen without appropriate APC priming of CD4+ T cells, leaving the host at risk of future infection (Andrews et al.7 and Narni-Mancinelli et al.10). (c) CD169+ macrophages, interferon-α (IFN-α) resistant and permissive to viral replication, enable on-going priming of adaptive immune responses during rapid innate viral control (Honke et al.8). APC, antigen-presenting cell; IFN, interferon; NK, natural killer.
Box 2 Implications for immunotherapies and vaccination strategies

A major aim of vaccination is to provide individuals with a long-lasting pool of memory cells that can quickly respond to secondary exposure to a pathogen or tumour antigen and elicit a powerful immune effector response. The ability of the innate immune system to remove antigen without priming an adaptive immune response poses a challenge to this goal. Possible strategies to avoid this outcome include:

- Use of vaccine adjuvants to induce antigen-presenting cell (APC) uptake (and CD4+ T-cell priming) without natural killer (NK) cell activation
- Maintenance of vaccine antigen to allow optimal CD4+ T-cell exposure
- Depletion of NK cells during vaccination
- Vaccination of individuals who have been exposed to and cleared viruses without successfully mounting an adaptive immune response

Conclusions

Taken together these studies demonstrate that in both mouse and human models robust innate activity, including NK-cell antiviral responses, may clear viral infections without the immediate need for CD4+ T-cell responses. However, increased exposure to antigen seems to be required for optimal adaptive responses, and these studies above suggest that NK cells influence adaptive responses by the control of antigen. In evolutionary terms, a balance may have been sought to ensure that NK cells and/or the anti-viral effects of IFN-α are moderated, to allow such long-term protective responses to develop. Hyper-responsive innate immune responses may provide a benefit to the host (i.e. rapid pathogen removal with reduced duration of illness) but at the cost of no protective herd immunity and an easy path to pathogen re-infection. There is also the possibility that NK cells have evolved a function to prevent T-cell immunopathology by direct killing of APC and activated T cells, at the cost of subclinical persistent viral infections; this has not been demonstrated convincingly in humans.

The above results also offer potential for optimizing therapeutic strategies (Box 2). To induce enhanced adaptive memory responses after vaccination, transient manipulation of NK cells may be required in a similar manner to depletion of regulatory T cells.15–17 It is also important to consider during treatment of chronic viral infections such as HCV that successful treatment does not necessarily confer an adaptive protective memory response and post-treatment vaccination, when available, should be considered as part of the treatment protocol to subjects at risk of re-exposure.

References

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