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Editorial

Editorial: NKT *get* the 'flu: NKT cells as (*mostly*) good guys in influenza; monocytic cells as double agents

Mark A. Exley[1]

Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

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SEE CORRESPONDING ARTICLE ON PAGE 357

A CD1d-restricted subset of NKT includes conserved immune elements with positive and negative regulatory activity in immunity, including pathogen resistance. NKT cells have been shown to have physiological, protective effects in certain but not all virus infections via contributing to activation of first NK cells and subsequently, T and B cells. NKT cells can also promote the switch to a regulatory environment, limiting successful as well as pathologic adaptive responses. However, as with any immune element, excessive or chronic NKT proinflammatory activities can lead to tissue injury, for example, in myocarditis, lung diseases, and hepatitis, as a result of viral infections. The NKT role in influenza infection is only recently becoming clear. Although in certain pulmonary infections, NKT cells can promote damaging inflammation, several recent studies, including one in this issue of the *Journal of Leukocyte Biology* [1], find that NKT can limit excessive lung inflammation by monocytic-type myeloid cells.

Abbreviation: αGC=α-galactosylceramide, iNKT=invariant NK T cell, iTCR=invariant TCR, KO=knockout, MDSC=myeloid-derived suppressor(-type) cell, NKT=NK T cell, PR8 strain=A/Puerto Rico/8/34, RSV=respiratory syncytial virus

CONTRASTING ROLES OF NKT CELLS IN IMMUNOSURVEILLANCE VERSUS IMMUNOPATHOLOGY WITH DISTINCT VIRUSES

NKT populations have significant positive or negative roles in regulating immunity, including surveillance against certain pathogens [2, 3]. The major functionally defined, CD1d-restricted subset of NKT includes innate-type T cells, recognizing exogenous and partially defined endogenous lipid antigens presented by monomorphic MHC-like CD1d. There are two main sources of CD1d reactivity: the best-known iTCR-α, expressing a "Type 1" subset of NKT (iNKT) and "Type 2" (polyclonal/noninvariant) [2, 3]. iNKT make up only a small fraction of human CD1d-reactive NKT and are the minority in rodent spleen and bone marrow [2, 3]. Exogenous CD1d ligands include prototypic, high-affinity iNKT-specific αGC [2, 3]. CD1d is expressed by DCs and other APCs, as well as in certain tissues, and can be increased under inflammatory conditions, including potentially, in influenza [4].

The NKT:CD1d system is a potent and highly conserved immune component [2, 3]. The basis of NKT function is rapid secretion of cytokines accompanied by cytotoxicity [2, 3]. Regulatory cytokines (e.g., Th2 IL-4, regulatory T cell IL-10) and/or proinflammatory Th1 cytokines, such as IL-2, IL-17, and IFN-γ, can be produced, reflecting NKT capacity to suppress or stimulate immunity. Therefore, NKT can be pro- or anti-inflammatory.

The contribution of NKT to immune surveillance against pathogens is at least partly based on their capacity to mature DCs and subsequently activate potent cytotoxic NK and CD8 T cells. Upon recognition of CD1d:lipid complexes and costimulatory molecules CD80/86 on the surface of DCs, iNKT up-regulate IL-12R and CD40 molecules. Subsequently, and mediated by CD40 ligand, iNKT induce maturation and production of IL-12 in DCs. This IL-12 release in turn potently increases IFN-γ production by iNKT [2, 3], a positive-feedback loop in Th1-type acute antipathogen immunity (**Fig. 1**).

CONTRIBUTION OF NKT CELLS TO INFLUENZA AND OTHER VACCINE RESPONSES

An earlier report found that NKT were not essential for vaccine-based cross-reactive immunity to a different influenza strain [5]. However, NKT can "adjuvantize" influenza viral and other vac-

1. Correspondence: Medicine, Beth Israel Deaconess Medical Center, 330 Brookline Ave., RW 633, Harvard Medical School, Boston, MA 02215, USA. E-mail: mexley@bidmc.harvard.edu

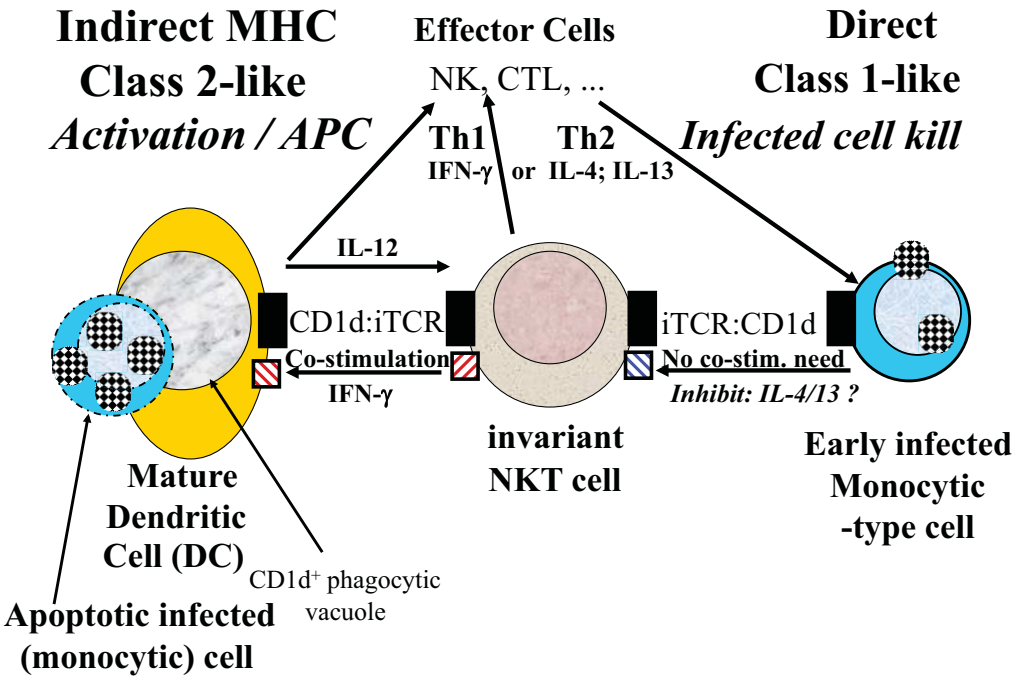

Figure 1. Diagram of iNKT cell : monocytic cell interactions and impact on influenza. On the left, an infected monocytic cell is phagocytosed by a dendritic-type cell, which either directly or indirectly activates the iNKT cell and DC via CD1d and Th1 cytokines. On the right, direct interaction of iNKT with early infected monocytic cell, which can either lead to direct killing by the iNKT cell or inhibit iNKT Th1 response via Th2 cytokines.

cines by boosting T and B cell responses [6–11] (**Table 1**). Therefore, in acute and vaccination settings, the ability to activate NKT can enhance pathogen clearance.

αGC induced some positive immune effects in cancer patients, but patient defects in iNKT led to suboptimal responses [17]. Trials in hepatitis C and B illustrated potential deleterious effects of NKT in chronic infections, by inducing transiently elevated liver enzymes. However, the low amounts of iNKT in human compared with rodent liver [2, 3] and compared with non-iNKT in human liver [3] ensured modest outcomes [17]. Therefore, αGC and relatives can have safe potential in acute infections and vaccines.

NKT cells are physiologically protective in certain, but not all, viral and other infections [2, 3]. This is supported further by numerous viral and other pathogen countermeasures directed against CD1d [2, 3], analogous to MHC targeting by pathogens. In model enterovirus infections, NKT physiologically contribute to clearance [2, 3, 18]. However, a subset of CD1d-reactive γδ T cells can subsequently respond to viral-induced cardiac CD1d up-regulation, causing myocarditis [2, 18]. As is generally the case [2, 18], iNKT pharmacological activation is therapeutic for all enteroviral disease, including myocarditis [18], presumably via viral clearance. Therefore, for myocarditis [18], postviral respiratory disease [12, 13], and analogously in chronic hepatitis C [2, 3], elements of the heterogeneous populations of NKT, instead of being protective, can promote damaging inflammation and subsequent fibrosis. In such chronic infections, therefore, inhibiting NKT could potentially suppress their contribution to immunopathology.

DIFFERENTIAL ROLES OF NKT CELLS IN VIRAL LUNG DISEASE

In general, NKT can contribute to pulmonary inflammation in acute viral and other infections, which can result in pathogen clearance, but in some cases, leads instead to pathology (Fig. 1 and Table 1). Indeed, the first report of NKT direct involvement in viral lung disease showed that NKT cells could contribute to RSV-dependent bronchiolitis in a mouse model [12]. The basis of the pathological effect appeared to be production of the Th2 cytokine, particularly IL-4, leading to pulmonary eosinophilic infiltration/fibrosis [12]. Other pulmonary infections can lead to postviral lung pathology. Postviral lung pathology, as a result of Sendai virus, was shown to be dependent on iNKT cells [13]. Another Th2 cytokine also produced by iNKT cells, IL-13, contributed to pulmonary macrophage recruitment and/or activation, leading to lung tissue damage [13].

Infection with high-dose pathogenic influenza virus H3N2 leads to pulmonary LN myeloid-type CD103$^+$ DC-type inflammation, followed by CD8 T cell lung infiltration [14]. Resistance (such

TABLE 1. NKT Cells can Adjuvantize Influenza Viral and Other Vaccines by Boosting T and B Cell Responses

Virus infection	NKT KO/Rx viral load	Lung pathology	Infiltrate	NKT phenotype	NKT "good" or "bad"	Reference
Inactive RSV to RSV	Similar to WT	Bronchiolitis	Eosinophils	Th2 ?	Bad	[12]
Sendai	Similar to WT	COPD	Macrophages	IL-13$^+$	Bad	[13]
Influenza H3N2	Severe (>WT)	Influenza	CD103 DC	CD8 help	Good	[14]
Influenza PR8 H1N1	Severe (>WT)	Influenza	CD11bGr1	Anti-MDSC	Good	[15]
Influenza PR8 H1N1	Severe (=WT)	Influenza	Monocytes	Anti-inflammatory monocytic	Good	[1]
Influenza + αGC	αGC < control	αGC < control	Innate	Innate-driving	Good	[16]

Vaccine + NKT ligand	Challenge	Lung pathology	Protection	NKT role	NKT "good" or "bad"	Reference
Influenza A vax	Influenza A, B	Similar to WT	Influenza A, B	N/A	Neutral	[5]
Influenza A vax H3N2 or H1N1	Influenza A	αGC < control	Homologous challenge	Memory T and B help	Good	[6]
Influenza nasal vax inactive PR8 H1N1	Influenza A PR8 H1N1	αGC < control	Homologous challenge	Memory CD8 help	Good	[7]
Influenza nasal vax	Influenza A's H1/H3/H5 ?	αGC < control	Cross-strain A (incl. H5)	NKT IL-4 IgA help	Good	[8]
Influenza nasal vax inactive PR8 H1N1	Influenza A H3N2	αGC < control	Cross-strain A	Memory CD8 help	Good	[9]
Influenza A PR8 H1N1 attenuated vax	Influenza A PR8 H1N1	αGC < control	Homologous challenge	Memory T and B help	Good	[10]
Influenza rM2e vax	H5N1	αGC = none	N/A	T and B help	Good	[11]

COPD, Chronic obstructive pulmonary disease; vax, vaccine; Rx viral load, treatment effect on viral load.

as it is in this model!) depends on these cell types limiting viral replication, leading to their being eventually "stood down" in successful responses. Regulatory control of inflammation is absent in Jα18 KO mice lacking iNKT cells. iNKT-deficient mice also lack the robust, influenza-specific CD8 T cell response required for clearance, implying that iNKT provide some form of T cell help, as in other viral infections [2]. Importantly, reconstitution of such mice with iNKT cells restored control of inflammation, indicating that iNKT were necessary and sufficient [14].

Two further papers describe physiological effects of iNKT cells on modulating influenza lung inflammation. These further emphasize monocytic, DC-type pulmonary inflammation [1, 15], similar to pure human influenza, rather than later neutrophilic infiltrates of human influenza pneumonia, the latter typically involving secondary bacterial infection superimposed. Overall, the conclusions are similar—that iNKT suppressed myeloid-derived cell inflammation (Fig. 1)—although there were some different observations. De Santo et al. [15] showed that mouse pathogenic PR8 strain H1N1 induced pulmonary infiltration of CD11b$^+$Gr-1$^+$ MDSC, which demonstrated appropriate activity in vitro. The MDSC produced arginase and iNOS, which reduced influenza-specific T cell levels and led to higher viral levels. iNKT were able to "suppress the suppressors", and their absence led to more rapid lethality. Again, adoptive transfer of WT iNKT into iNKT-deficient mice reduced inflammation and levels of MDSC. Transfer into CD1 KO mice had no effect, demonstrating that CD1 recognition was involved, not a given, as iNKT can be activated by cytokines alone [2, 3]—of which, there are plenty in influenza! Inevitably circumstantial, but compelling, evidence for similar responses in humans was provided from in vitro studies of PBMC from influenza-infected donors, in which, similar MDSC were found. Pharmacological activation with αGC, various TLR ligands, or viral infection induced human iNKT lines to overcome PBMC-derived, MDSC-type cells and again, suppressed the suppressors [15].

In the current report, Kok et al. [1] studied an even more pathogenic influenza model, also using mouse-adapted PR8 H1N1. Similarly to De Santo et al. [15], iNKT deficiency resulted in worse inflammation [1]. Also, the inflammatory cells were of a phenotype, which can represent TNF and iNOS-producing myeloid cell precursors. However, the phenotype described was of CD11B$^+$ Ly6C$^+$ CX3CR1$^+$ but M-CSFR CD115-negative inflammatory monocytic-type cells. As described by Kok et al. [1] and De Santo et al. [15], murine monocytes have recently become better understood (e.g., ref. [19]). However, their role in influenza has been described only partially. Direct infection of monocytic cells themselves by the virus leads to their death, but not before they secrete type 1 IFNs, IL-1β, TNF-α, and CC chemokines MCP-1, MIP-1α, and RANTES, which in turn, recruit more monocytes to act as APCs for adaptive immunity before also potentially be-

ing infected [1]. IL-8 was not detected, suppressing neutrophil recruitment and further ensuring a monocyte-dominated infiltrate [1].

Kok et al. [1] found in an exhaustive survey that only MCP-1 was elevated in iNKT-deficient mice, suggesting that this was critical for the monocytic infiltrate and possibly supported by suggestive partial (but not statistical) effects of MCP-1 mAb on infection (MCP-1 is also required for resistance, for which a balance was aimed, with modest levels of mAb used). How iNKT reduce monocytic infiltration remains unclear, but a direct cytotoxic effect was proposed as possibly contributing, as in uninfected DCs. Clearly, iNKT are activated physiologically in such infections, as well as by cytokines alone [2, 3], so their cytotoxic potential could also be available in humans, as well as in mice. Indeed, human iNKT could lyse influenza-infected monocytes in a CD1d-dependent manner without αGC stimulation in vitro [1]. Whether additive infection of some monocytic cells and killing of others by iNKT or synergistic cytotoxicity could be involved in vivo remains to be determined. Consistent with many previous observations of therapeutic effects [2, 3, 18], iNKT activation with αGC is curative in influenza infection [16].

In conclusion, NKT have physiological, mostly (but not exclusively) protective, roles in influenza, based at least partly on their suppression of excessive monocytic infiltrates, as well as on other pulmonary and diverse infections. This, combined with positive effects on various influenza and other vaccines, suggests that the ability to control NKT positively or negatively could provide an optimal ability to exploit these potent cells, while minimizing any possible side-effects.

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