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Phenotypic and functional characterization of CD56brigh CD16neg natural killer cell subsets within the context of influenza vaccination

Kay, Patti Sheryl

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ABSTRACT

Natural killer cell (NK) heterogeneity is attributable to the presence of multiple NK cell receptors and co-receptors and whose expression is shaped by genetics and unique antigen exposure during developmental and differentiation pathways. NK cells with bright expression of CD56 but lack CD16 ($CD56^{bright}CD16^{neg}$) are widely distributed throughout various tissues including the lymph nodes which is the site of dendritic cell, NK and T cell interactions and the liver, where they play a role in maintaining liver homeostasis. NK cells can develop an antigen "trained" phenotype which respond differently upon a secondary antigen encounter, and as $CD56^{bright}CD16^{neg}$ NK cells play an important immunoregulatory role during immune responses, understanding antigen mediated alterations of $CD56^{bright}CD16^{neg}$ NK subphenotypes provides insight into those cells that may influence overall immune responses. Furthermore, human $CD56^{bright}CD16^{neg}$ NK cells are believed to undergo further development and maturation giving rise to $CD56^{dim}CD16^{pos}$ NK cells, but this paradigm has been recently challenged. If indeed, $CD56^{dim}$ NK cells arise from $CD56^{bright}CD16^{neg}$ NK cells then an understanding of what constitutes a "normal" antigen response by $CD56^{bright}CD16^{neg}$ NK cells is warranted.

Using an influenza vaccination model, this thesis explored i) $CD56^{bright}CD16^{neg}$ NK subphenotypes which may be associated with a trained immune response, ii) receptors that may be associated with co-regulation (co-stimulation), iii) functional differences between circulating $CD56^{bright}CD16^{neg}$ NK cells with differing migration patterns, as well as iv) developing post-vaccination NK cell responses in comparison to those of CD4 and CD8 T cells. A phenotyping study explored differences in receptor and co-receptor expression patterns between $CD56^{bright}CD16^{neg}$ hepatic and NK cells circulating in the periphery.

A 12 colour flow cytometry panel was developed that simultaneously phenotyped and determined functional ($CD107a^{+}$ and IFNγ) responses of NK and T cells. The panel allowed for the definition of the 4 major NK subsets and co-expression patterns of NKp46, CD27 and CD62L subphenotypes. CD4 and CD8 T cells were phenotyped into central memory (Tcm), effector memory (Tem), effector cells and naive cells. Cytokine responses were determined by intracellular staining for IFNγ and staining for CD107a expression was used to detect degranulation responses. Using an influenza vaccination model, functional and trained immune responses were investigated for 12 healthy volunteers who received a standard 0.5 ml dose of the 2009- Influvac® subunit inactivated influenza vaccine. Venous blood was collected pre-vaccination, 3 days, 6 months and 1 year following vaccination and using the same vaccine preparation with which participants had been vaccinated, influenza antigen responding cells, were determined by comparing *ex vivo* stimulated cells to CD28/CD49d co-

stimulated cells and responses attributable to vaccination were measured by comparing antigen stimulated pre-vaccination cells to post-vaccination cells. As the thesis was focused on the $CD56^{bright}CD16^{neg}$ NK cell subset, all data presented refers to this subset.

Analysis of receptor co-expression patterns revealed that CD27 is predominantly expressed only on $NKp46^{+}$ cells and that NKp46 surface density is greater with CD27 co-expression. $NKp46^{+}$ cells were further defined by CD62L expression as, ($CD62L^{+}$) lymph homing ($lhNKp46^{+}CD27^{+}$ and $lhNKp46^{+}CD27^{-}$) and, ($CD62L^{-}$) peripheral ($pNKp46^{+}CD27^{+}$ and $pNKp46^{+}CD27^{-}$) subphenotypes. Following vaccination, dynamic alterations of subphenotype proportions developed, peaking at 6 months and which by 1 year returned to levels similar to those observed prior to vaccination. Lymph homing NKp46 subphenotype proportions expanded and peripheral NKp46 subphenotype proportions contracted, which suggests that with vaccination, certain $CD56^{bright}CD16^{neg}$ subphenotypes were altered or "trained either through up- or downregulation of co-receptors, or changes in migratory patterns. In addition, the extreme inter-individual diversity in proportions of lymph homing and peripheral $NKp46^{+}$ cells suggested a long term influence on the activating NKp46 receptor, possibly as a consequence of unique antigen exposures.

Co-expression of CD27 functionally delineated $NKp46^{+}$ cells, as $pNKp46^{+}CD27^{+}$ cells were more cytolytic than $pNKp46^{+}CD27^{-}$ cells and both lymph homing subphenotypes (P<0.05). Although a significant loss of $pNKp46^{+}CD27^{-}$ IFNγ producing cells occurred after vaccination (P=0.037 by 6 months), $pNKp46^{+}CD27^{-}$ cells produced more IFNγ than both lymph homing subphenotypes (P<0.05) and $pNKp46^{+}CD27^{+}$ cells, prior to vaccination (P=0.0005) and 1 year post-vaccination (P=0.016). Functional responses could therefore also be delineated by lymph homing properties, as the magnitude of peripheral $NKp46^{+}$ functional ($CD107a^{+}$ and $IFN\gamma^{+}$) responses were greater than those of lymph homing cells.

Monitoring of T cells prior to and following influenza vaccination revealed no changes in CD4 T cell memory proportions, but 3 days following vaccination, both CD8 Tcm and Tem proportions increased whereas naive and effector cells decreased (P<0.05). IFNγ responses remained unchanged following vaccination, for both CD4 and CD8 T cells. However, 6 months after vaccination CD4 T cells degranulated ($CD107a^{+}$) significantly more than pre-vaccination cells (P<0.05), as did CD8 naive and Tem subsets.

$CD56^{bright}$ cells are the predominant NK subset found in the liver and the importance of CD27 in hepatic pathology has been established. To investigate if hepatic perfusate (obtained during liver transplant procedures) provides a suitable source of liver NK cells, hepatic perfusate derived (hpNK) cells from deceased liver donors (n=9) were immunophenotyped and compared to circulating NK cells from healthy age and sex matched donors (n=9). Hepatic NK

cells were enriched for $CD27^+$ cells and CD27 was only expressed on $NKp46^+$ hpNK cells. However, unlike circulating $CD56^{bright}CD16^{neg}$ NK cells, NKp46 surface density remained unchanged and co-expression patterns for $CD27^+CD69^+$ and $CD27^+CD161^+$ subphenotypes differed between hpNK and circulating (P<0.05) NK cells. Immunophenotyping of hpNK cells from an acute liver failure (ALF) patient revealed that, similar to healthy controls, NKp46 expression was unchanged and surface expression of CD27 was confined to $NKp46^+$ cells. However, the percentage $CD56^{bright}CD16^{neg}$ hepatic cells that expressed $CD27^+$ cells was extremely low and few $NKp46^+$ cells expressed CD27. The majority of $NKp46^+$ cells were therefore of a $NKp46^+CD27^-$ subphenotype. Aberrant expression of the T cell memory marker, CD45RO, was found on NK cells from the ALF patient but which was not observed on NK cells from healthy donors.

Overall, given the global emergence of novel respiratory infections, and the subsequent severe impact on human health mediated by “cytokine storms” during infection, it is important to understand what constitutes a ‘normal’ functioning $CD56^{bright}CD16^{neg}$ response. Data from this thesis therefore adds to our understanding of $CD56^{bright}CD16^{neg}$ cNK cells that respond differentially to influenza antigen and which is determined by migratory potential and expression of co-receptors.