Vaccination programs, such as that executed in response to the 2009 swine flu outbreak, aim to exploit the ability of the immune system to mount a faster and more vigorous response against a pathogen that has been encountered previously. This memory response reflects both quantitative and qualitative changes in lymphocyte populations. After acute infection with influenza virus, high frequencies of virus-specific CD8+ T cells persist many months after virus is cleared. These CD8+ memory T cells have a lower activation threshold, more rapidly acquire effector functions and preferentially localize to infected tissues, compared to naive CD8+ cells. Our preliminary data suggest that CD8+ memory T cells also differ from naive counterparts in their ability to respond to cytokine instruction. The hypothesis we now propose to test is that an altered cytokine response pattern defines the function of CD8+ memory T cells. The information we obtain will further our understanding of the memory response to influenza and thus enable the design of refined vaccination strategies.

Preliminary data
Surviving infection requires a precise balance between the pro-inflammatory responses needed to eliminate the pathogen, and anti-inflammatory signals limiting collateral damage to the host. Mortality in both the 1918 pandemic influenza and the 2006 avian influenza outbreaks was associated with an uncontrolled inflammatory response, described as a cytokine storm. In a mouse model, primary influenza infection elicits the immunosuppressive cytokine IL-10, which is expressed by CD8+ effector T cells and is essential to prevent lethal pulmonary inflammation. However, exciting new data from our laboratory demonstrates that IL-10 is absent in the CD8+ T cell response to a second influenza infection. We determined that this decreased IL-10 reflects changes in cytokine receptor expression that render CD8+ memory T cells unable to receive the IL-27 signals necessary for IL-10 induction. We will now examine whether influenza-specific CD8+ memory lymphocytes show additional changes in cytokine responsiveness, and assess whether these alterations also characterize human CD8+ memory cells.

Aim 1
Cytokine signaling is mediated via phosphorylation of intracellular signal transducers and activators of transcription (STATs), and BD has recently developed a series of BD™ Phosflow reagents that enable the direct detection of intracellular phosphorylated STAT proteins by flow cytometry. We will use the BD Phosflow system to measure cytokine responsiveness in naive and memory CD8+ T cells from the spleens of mice previously infected with influenza virus. Our preliminary data has shown that IL-27 stimulation elicits STAT3 phosphorylation in naive but not memory CD8+ T cells. We will now extend these studies to examine other STAT proteins targeted by IL-27 (STAT1 and STAT4) and other cytokines that control the pro/anti-inflammatory function of CD8+ T cells in influenza infection (IL-12, IL-6, IL-18 and IFNγ). We will confirm the functional impact of changes in cytokine responsiveness by purifying naive and memory
lymphocytes, using BD antibodies and a BD FACS™ Vantage™ cell sorter, and stimulating each population in the presence of recombinant cytokine. Both the cytolytic potential and cytokine secretion of the stimulated cells will be assessed, the latter using ELISA and BD™ Cytometric Bead Arrays.

**Aim 2**
Peripheral blood mononuclear cells from healthy human donors with demonstrated reactivity to an influenza A epitope are commercially available. We will stimulate these cells with recombinant human cytokines and use BD Phosflow reagents to assess the capacity of naive and memory CD8+ T cells to respond. BD antibodies against the surface antigens CD8, CD45RA and CD27 will be combined with MHC multimer reagents to distinguish naive and influenza-specific memory populations. Our hypothesis is that the distinct cytokine response pattern of murine CD8+ memory T cells, including the loss of IL-27 responsiveness, will be mirrored in human memory T cells. If the hypothesis is confirmed, these data will identify specific cytokine signaling pathways as candidates for therapeutic manipulation as part of a vaccination strategy. If not, the information gained regarding the signaling capacity of naive and memory human lymphocytes will be invaluable in the appraisal of alternative targets.

**Summary**
Our preliminary data indicate that the specific loss of IL-27 responsiveness in memory T cells limits the expression of immunosuppressive IL-10 during a challenge infection. In this study, we propose to further exploit BD Phosflow techniques to define the extent and the impact of cytokine signaling in influenza-specific memory lymphocytes in mice and humans.

**Significance**
Current influenza vaccines rely on either predictive or retrospective analysis of the antigenicity of the prevailing viral strain. These approaches are prone to inaccuracy and delay, and the Center for Disease Control estimates that approximately 40,000 Americans die each year with seasonal influenza. Pandemic viruses pose a significant threat. Several laboratories are working to develop a new generation of influenza vaccines designed to elicit CD8+ memory T cell responses effective against multiple strains of influenza virus. It is imperative that this is achieved without predisposing the patient to harmful immunopathology, and an optimal vaccine may need to incorporate palliative features. The data that we will generate in this study will clarify the signals capable of regulating influenza-specific CD8+ memory T cells in vivo, thereby facilitating the rational design of successful influenza vaccines.

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