In influenza virus infection, antibodies, memory CD8+ T cells, and CD4+ T cells have all been shown to mediate immune protection, but how they operate and interact with one another to mediate efficient immune responses against virus infection is not well understood. In this issue of the JCI, McKinstry et al. have identified unique functions of memory CD4+ T cells beyond providing “help” for B cell and CD8+ T cell responses during influenza virus infection.

Efficient control and clearance of viral infections requires coordinated interactions of several components of the immune system. Over the past 10 years, Susan Swain and colleagues have elucidated several functions of memory CD4+ T cells during influenza A virus (IAV) infection. They demonstrated the role of memory CD4+ T cells in innate immune responses (1, 2), in the enhancement of B cell responses by follicular helper T (T FH) cells via Signaling Lymphocyte Activation Molecule (SLAM)-associated protein (SAP) expression (3), and in direct antiviral effects via a perforin–mediated cytocidal mechanism (4, 5). In this issue of JCI, the Swain group, led by Kai McKinstry, systematically transferred memory CD4+ T cells into mice deficient in specific lymphocyte populations and elegantly dissected the mechanisms by which memory CD4+ T cells protect against IAV infection in mice (6). They report three new findings (Figure 1). First, the innate antiviral functions of memory CD4+ T cells are IFN-γ dependent and independent of the pathogen recognition receptor (PRR) pathway (Figure 1A). Second, memory CD4+ T cells enhance B cell responses independently of T FH cells and germinal center formation (Figure 1B). Third, in addition to mediating effector functions via a perforin-dependent pathway (Figure 1C), memory CD4+ T cells use the same pathway to drive selection of escape mutants for a process that was known to occur in CD8+ T cells (Figure 1D). These new findings are discussed below.

In addition to the above findings, several reports have highlighted the role of memory CD4+ T cells in immune protection from IAV infection. Memory CD4+ T cells protect mice that lack T or B cells, though CD8+ T cells are needed between days 6 and 10 after infection for viral clearance in B cell–deficient mice (6, 9). McKinstry et al. demonstrated that the protection conferred by memory CD4+ T cells in mice that lack both T and B cells is incomplete;
mice can be protected against low-dose viral challenge but not against high-dose challenge. Memory CD4+ T cells mediated protection against low-dose influenza infection via IFN-γ production independently of other lymphocytes. The authors also found that to clear infection following a high dose of challenge virus, memory CD4+ T cells interacted with naive B cells and CD8+ T cells. The reduction in morbidity and mortality in the recipient mice was convincing, but the reduction in viral titers (expressed as polymerase [PA] gene copies) was very modest, though statistically significant (6). A relatively small change in pulmonary virus titer can be associated with remarkable differences in mortality in IAV infection in mice (10). In agreement with this study, preexisting memory CD4+ T cells have also been shown to correlate with disease protection against influenza challenge in humans (11–13).

**Direct role of memory CD4+ T cells in virus infection**

Although the most well-characterized function of memory CD4+ T cells during viral infection is the maintenance of B cell and CD8+ T cell responses, several other roles of memory CD4+ T cells have been elucidated in IAV infection (14). Recent studies have shown that memory CD4+ T cells, but not naive CD4+ T cells, enhance the production of multiple innate inflammatory cytokines and chemokines in the lungs of infected mice and lead to early control of influenza virus infection. Interestingly, McKinstry et al. show, in agreement with previous publications, that innate immune responses mediated by memory CD4+ T cells are PRR independent (1, 6). This is important because influenza viruses can evade immune protection by antagonizing key components of the PRR pathway (15).

**Memory CD4+ T cells synergize with other lymphocytes**

The CD4+ T cells that enter B cell follicles and provide help to B cells are referred
to as T FH cells. Following viral infection, T FH cells express SAP to direct the formation of germinal centers (16), where they promote the formation of memory B cells and long-lived antibody-producing plasma cells. Memory CD4+ T cells are superior to naive T cells in providing help to B cells; they promote earlier B cell proliferation, higher antibody levels, and earlier antibody class switching (17–19). Interestingly, McKinstry et al. show that, unlike naive CD4+ T cells, enhancement of B cell responses by memory CD4+ T cells is not dependent on a T FH-associated pathway.

Priming of CD8+ T cells by memory CD4+ T cells during IAV infection has been studied extensively (20–22), but unlike naive CD4+ T cells, enhancement of proliferation, higher antibody levels, and are superior to naive T cells in providing plasma cells. Memory CD4+ T cells mediate cytotoxicity by a perforin-dependent mechanism (4). Here, McKinstry et al. extended these findings by showing that memory CD4+ T cells select for influenza escape mutants and that this selection requires perforin. This mechanism is similar to that by which CD8+ T cells select for escape variants (23).

Questions and future challenges

While the work by McKinstry et al. provides novel insights into cellular mechanisms by which memory CD4+ T cells contribute to immune protection against influenza, several questions remain. Areas that warrant further study include the PRR-independent protection elicited by memory CD4+ T cells via production of IFN-γ in the absence of other lymphocytes and the synergy of memory CD4+ T cells with B cells independent of T FH cells. Interestingly, Th17 cells also provided protection in this study, but their function was not well characterized. The precise role of Th17 in this complex network should be explored further. Although the authors discuss the role of memory CD4+ T cells in heterosubtypic immunity (24), the experiments in the current study only evaluated protection from homologous viral challenge. Further study of the role of memory CD4+ T cells in heterosubtypic immunity is needed. The authors close with the suggestion that vaccines that elicit memory CD4+ T cell responses in addition to antibodies may offer universal protection against seasonal and pandemic influenza viruses. An exploration of approaches to achieve this result, e.g., by administration of influenza vaccines with oil-in-water adjuvants (25), would be of interest. Last but not least, while it is clear that such reductionist approaches are of great value in dissecting the pathways, it is not clear how translatable the findings will be from mice to humans.

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Address correspondence to: Kanta Subbarao, Emerging Respiratory Viruses Section, Laboratory of Infectious Diseases, NIAID, NIH, Bldg. 33, Room 3E13C.1, 33 North Drive, MSC 3203, Bethesda, Maryland 20892-3203, USA. Phone: 301.451.3839; Fax: 301.480.4749; E-mail: KSUBBARAO@niaid.nih.gov.