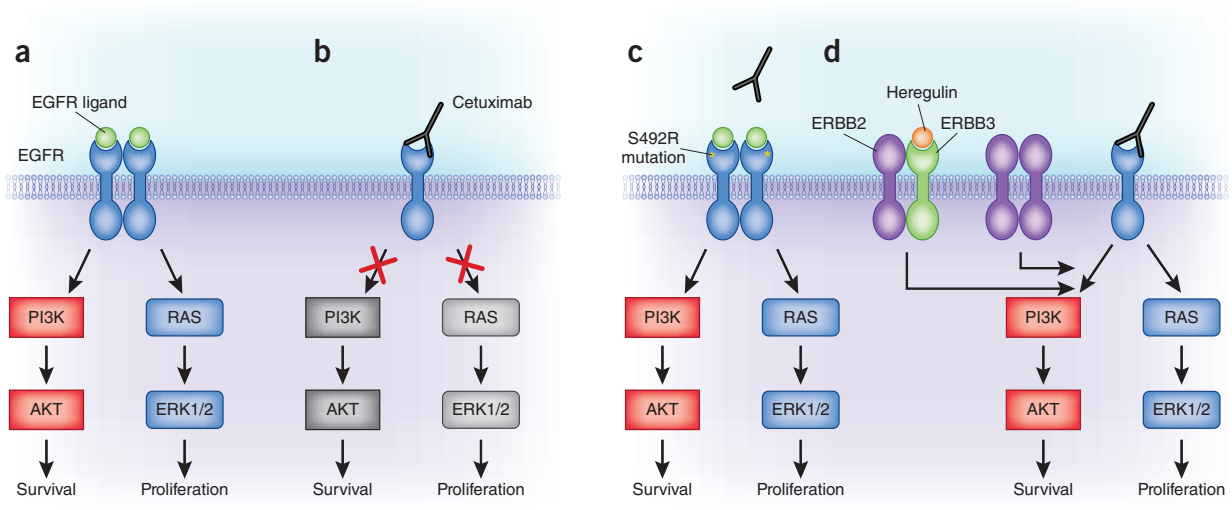

Figure 1 Cetuximab sensitivity and resistance. (**a**) EGFR ligands bind the extracellular domain of EGFR, induce receptor dimerization and activate downstream signaling pathways that are crucial for cell survival and proliferation. (**b**) Cetuximab prevents ligand binding to EGFR, thus blocking EGFR signaling. (**c**) Montagut *et al.*[1] now show that the EGFR S492R mutation inhibits cetuximab but not EGFR ligand binding to EGFR. (**d**) Cetuximab resistance can be mediated by activation of alternative signaling pathways. In this scenario, although cetuximab can still block EGFR ligand binding, it does not inhibit downstream signaling. PI3K, phosphoinositide 3-kinase; ERK, extracellular signal-regulated kinase.

discover additional mechanisms[12–14]. Once these analyses have been completed, the information could be used to develop a rational framework to guide the treatment of individuals who relapse while on EGFR-targeting antibody therapies. There are likely to be multiple mechanisms of drug resistance, each requiring a specific, and often a nonoverlapping, therapeutic approach. For example, when relapse is linked to the *EGFR* S492R mutation, the subsequent therapeutic strategy will be likely to involve alternative ways of inhibiting EGFR, including using panitumumab. In cases in which cetuximab resistance is mediated by ERBB2, combination therapies that include an EGFR inhibitor need to be employed, as more effective EGFR inhibitors, including panitumumab, are unlikely to be effective as single therapeutic agents.

In summary, understanding the mechanisms of drug resistance through both *in vitro* models and human tumor tissues can clearly lead to the development of more effective targeted therapies, new therapeutic combinations or both. It will be important to personalize such approaches on the basis of the mechanisms of resistance of each individual tumor.

COMPETING FINANCIAL INTERESTS
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CD4+ T cells limit the damage in influenza

Anne Kelso

Why do some influenza infections cause fatal disease and others barely a sniffle? Although viral virulence can vary, the immunological history of the host is also important. A new study in humans suggests that CD4+ T lymphocytes activated during previous infections can limit disease severity in the absence of specific antibodies (pages 274–280).

When it comes to protection from influenza infection, antibodies are best. Neutralizing antibodies to the surface glycoproteins of influenza type A and B viruses, hemagglutinin and neuraminidase, block binding to receptors on the respiratory epithelium, preventing virus uptake and subsequent release of new virus particles (**Fig. 1**). But influenza viruses move quickly: the selective pressure of the antibody response on seasonal influenza viruses drives the emergence of escape mutants that can cause epidemics in communities immune to earlier strains[1]. This is why seasonal influenza vaccines require frequent updating.

The specificity of the antibody response also underlies the emergence of influenza pandemics. Pandemics occur when gene shuffling between animal and human influenza A viruses produces a human-transmissible virus

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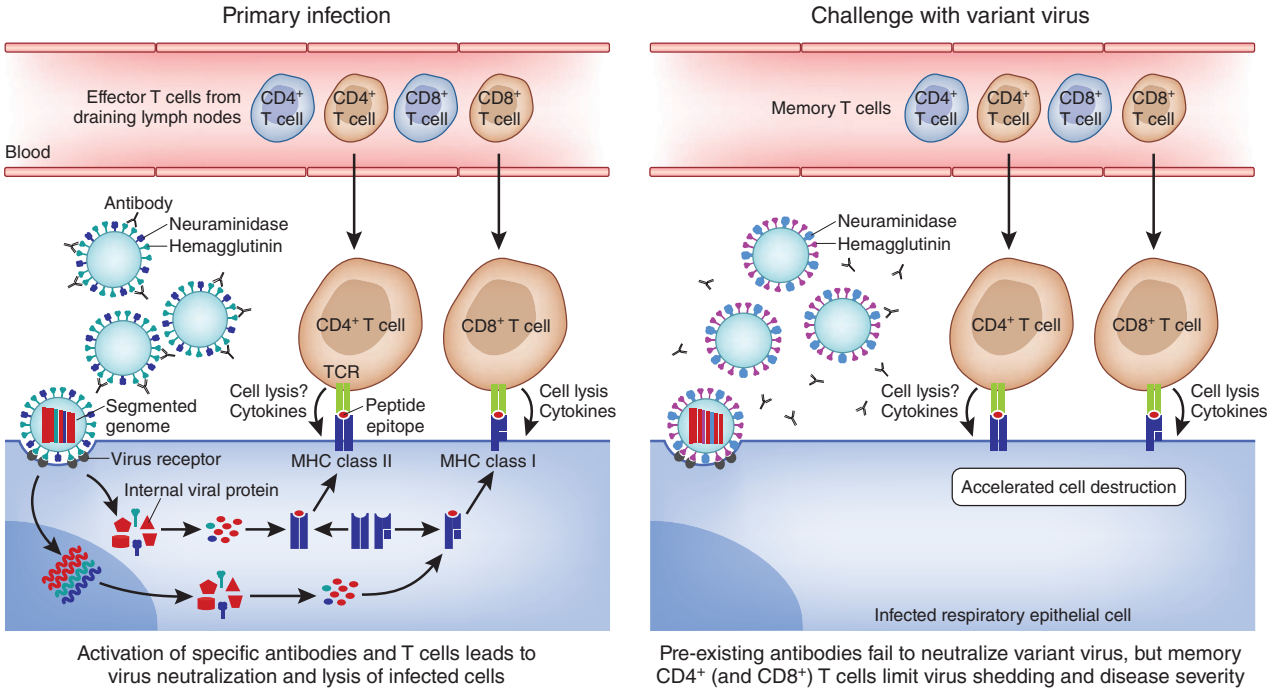

Figure 1 Role of virus-specific memory T cells in protection from influenza virus challenge. During primary infection of the respiratory tract, influenza viruses bind receptors on the epithelial cell surface via their surface hemagglutinin and are internalized (left). The eight segments of the viral RNA genome are transcribed and translated to produce viral proteins, including the highly variable hemagglutinin and neuraminidase and the more conserved internal proteins, enabling the production of new virions. The host produces antibodies mainly directed against hemagglutinin and neuraminidase that neutralize the virus and prevent further infection. Intracellular degradation of newly synthesized or endocytosed viral proteins produces peptide fragments, some of which (T cell epitopes) bind host class I or class II major histocompatibility complex (MHC) molecules and can be recognized by class I–restricted $CD8^+$ or class II–restricted $CD4^+$ effector T cells bearing T cell receptors (TCRs) of appropriate specificity. These effector T cells contribute to viral clearance, for example, by lysing infected cells and secreting soluble mediators. On challenge with a variant (seasonal or pandemic) influenza virus that is not neutralized by pre-existing antibodies, memory T cells specific for conserved viral epitopes shared between the priming and challenge viruses are recruited to the site of infection (right). The data of Wilkinson *et al.*[3] suggest that these pre-existing $CD4^+$ T cells can reduce virus shedding and the severity of infection, possibly by direct lysis of infected epithelial cells.

with a new hemagglutinin against which most of the population lacks immunity. This has happened four times in the last century. On each occasion, antibodies against contemporary seasonal viruses failed to protect against the new virus subtype: A(H1N1), A(H2N2), A(H3N2) and a new A(H1N1) in 1918, 1957, 1968 and 2009, respectively.

The unexpected nature of the 2009 A(H1N1) pandemic and the recent human-to-human transmission of a variant A(H3N2) virus of swine origin in the US[2] remind us that new influenza viruses are a continuing and unpredictable threat. In an ideal world, vaccines would induce durable broad-based immunity that would minimize the impact of these viruses. In this issue of *Nature Medicine*, Wilkinson *et al.*[3] present evidence that $CD4^+$ T cells activated by earlier influenza virus infections reduced the severity of disease when seronegative volunteers were challenged with seasonal influenza viruses. These observations point to $CD4^+$ T cells as potential targets for activation by vaccines that would protect not only against new seasonal influenza variants but also against future pandemic viruses.

There is evidence that T cells can mediate cross-protective (heterosubtypic) immunity. In classic studies, it was shown that adults who had previously experienced an A(H1N1) infection were protected from the pandemic A(H2N2) virus when it emerged in 1957 (ref. 4) and that specific cytotoxic T lymphocyte activity was associated with low virus shedding in infected seronegative volunteers[5]. T cells cannot prevent virus infection but they can sense infected cells by recognizing fragments of viral protein (epitopes) complexed to human leukocyte antigen (HLA) molecules on the surface of infected epithelial cells or antigen-presenting cells (**Fig. 1**). As T cells preferentially see epitopes derived from the conserved internal proteins of the influenza virus, cross-protective immunity has been attributed to pre-existing cytotoxic $CD8^+$ T cells that kill virus-infected cells presenting these conserved epitopes. In this way, $CD8^+$ T cells might reduce the duration and severity of infection with a seasonal variant or pandemic virus that escapes the protective antibody barrier. Indeed, many of the $CD8^+$ T cell epitopes identified in internal proteins of A(H3N2) or pre-2009 A(H1N1) influenza viruses are shared with the highly pathogenic avian A(H5N1), the 2009 pandemic (A(H1N1)pdm09) or both influenza viruses, and $CD8^+$ T cells specific for some of these epitopes have been detected in the blood of healthy donors[6–8].

Cross-protection by $CD4^+$ T cells has received less attention, perhaps because mouse studies have suggested they function mainly to promote antibody responses[9]. Nevertheless, conserved $CD4^+$ T cell epitopes have been identified in internal and surface proteins of human and avian influenza viruses. Some of these epitopes, at least, are naturally generated in infected cells, and $CD4^+$ T cells from seronegative donors can respond to them[7,8,10,11].

Wilkinson *et al.*[3] have now shown that virus-specific memory $CD4^+$ T cell numbers predict the outcome of human influenza infection. They counted virus-specific T cells in the blood of volunteers before and after challenge with recent seasonal viruses of the A(H3N2) subtype or the former seasonal A(H1N1) subtype, which circulated until it was replaced by A(H1N1)pdm09 in 2009. Their key finding was that pre-existing virus-specific T cell numbers were inversely related to the severity of illness after challenge[3].

Remarkably, it was the CD4+, rather than the CD8+, T cell counts that correlated with lower virus shedding and shorter illness in response to A(H3N2) challenge and with lower symptom scores in response to both challenge viruses. By contrast, virus-specific T cell numbers after challenge correlated positively with viral load and symptoms[3].

The presence of pre-existing virus-specific T cells was not surprising, as the subjects were old enough to have experienced several influenza infections (range 18–45 years). Subjects lacked detectable baseline serum antibodies to the challenge virus, suggesting that they had not recently been exposed to this subtype. As antibodies were not measured to the alternative subtype or the A(H1N1)pdm09 virus (which emerged shortly before the A(H1N1) challenge), T cell numbers might have been boosted by recent exposure to one of these viruses.

If memory CD4+ T cells are responsible for the reduced disease severity, how do they exert their effects? There are several possibilities. Given that they are primed and elevated in number, they might accelerate the development of specific antibody and/or CD8+ T cell responses, though perhaps not quickly enough to ameliorate symptoms in the first few days after challenge. Memory CD4+ T cells might produce or induce mediators that enhance recruitment and activation of innate cells that contribute to viral clearance[12]. Alternatively, they might exert direct cytotoxic activity against virus-infected cells. The authors favor the last possibility, as they found that memory CD4+ T cells showed specific granule-mediated cytotoxicity *in vitro*[3]. Whether this activity is relevant *in vivo* is not yet known and will be difficult to evaluate directly in humans.

Whatever their mechanism of action, the relevant CD4+ T cells must either reside in or be rapidly recruited to sites of viral antigen presentation in the respiratory tract or lymphoid tissue. They must also recognize viral peptides presented with class II HLA molecules at those sites, either on respiratory epithelium or on other antigen-presenting cells. Although neither of these requirements was assessed in the infected subjects, the authors showed expression of the class II molecule HLA-DR in explanted normal lung and cultured primary bronchial epithelial cells; expression was elevated in the latter cells after influenza virus infection *in vitro*[3].

Do these new data mean that pre-existing cross-reactive CD8+ T cells do not contribute to influenza immunity? It would be premature to draw this conclusion for several reasons. For example, the study may have lacked power to test this possibility, as the number of subjects in this study was small. Additionally, cross-protective cells may be a minority of virus-specific memory CD8+ T cells, too few to influence the relationship between cell number and symptoms, or they may reside predominantly in the respiratory tract rather than the circulation.

If memory CD4+ T cells do play an important part in cross-protection, how long do they persist after infection? T cell immunity declines markedly over the first 1–2 years[13,14], so maintenance of cross-protection must rely on regular re-exposure to influenza virus. Conventional inactivated influenza vaccines produced by most global manufacturers protect by inducing subtype-specific antibodies to hemagglutinin and do little to boost cross-protective T cell responses. However, vaccines that use live attenuated viruses or some new antigen delivery or adjuvant technologies can activate both antibody and T cell responses and offer the prospect of some cross-protection[15]. These vaccines will have to prime a more durable cross-protective response than natural infection to remove the need for regular vaccination against the latest circulating viruses. The study by Wilkinson *et al.* suggests that activation of long-lived cross-protective CD4+ T cells should be one of the goals of future vaccine development.

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The author declares competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/.

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Building bones by knocking down genes

Clifford J Rosen

New strategies for selectively stimulating bone formation without promoting bone resorption are required, as all currently approved agents for osteoporosis act on both of these aspects of the bone remodeling process. A recent study describes an approach that specifically delivers therapeutic siRNAs to bone-forming surfaces without affecting bone resorption (pages 307–314).

Remarkably, every 10 years, each human's entire skeleton is completely replaced through the tightly coupled process of bone remodeling. Skeletal turnover occurs in bone multicellular units (BMUs) that are composed of bone-resorbing osteoclasts, bone-forming osteoblasts that arise from mesenchymal stromal cells (MSCs) and osteocytes, which are former osteoblasts embedded within the bone matrix[1] (**Fig. 1**). A fundamental principle of bone biology purports that resorption and formation are tightly coupled through an array of signals that originate from all three of these cell types[2]. This coupling can even be seen in disease states such as post-menopausal osteoporosis, during which accelerated resorption leads to rapid bone loss and bone formation is upregulated in a futile attempt to maintain bone mass[3]. Within BMUs, the balance between resorption and formation is also evident during the administration

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