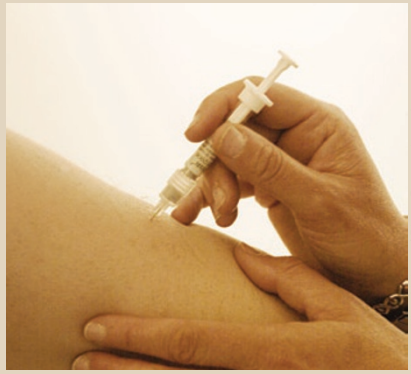
Rechallenging immunological memory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The adaptive and innate arms of the immune system coordinate to respond to a secondary infection, resulting in both antigen-specific bactericidal activities and 'bystander' killing of unrelated pathogens, according to a recent report in *The Journal of Experimental Medicine* (**204**, 2075–2087).

After a foreign pathogen is encountered in an initial infection or vaccination, long-lived immunological memory is believed to be primarily in the hands of memory T cells. Once re-exposed to that pathogen, the armed memory $CD8^+$ T cells quickly mount their killing campaign against infected cells. In an antigen-specific process, they release interferon (IFN)-γ and tumor necrosis factor (TNF)-α to control the growth and clearance of the pathogen. $CD8^+$ T cells were thought to manage this process independently.

Emilie Narni-Mancinelli *et al.* have challenged this concept by demonstrating that the response to secondary infection is not solely dependent on memory T cells. Instead, activation of innate mononuclear phagocytic cells (MPCs) by the memory T cells is the necessary step for the final elimination of bacteria.

Upon re-exposure to the pathogen, existing memory T cells released the chemokine CCL3 to activate MPCs. MPCs released TNF-α, which in turn caused neutrophils and other MPCs to produce radical oxygen intermediates (ROIs) to clear the bacteria. The memory T cells by themselves were not sufficient to clear the infection, and blocking CCL3, TNF-α or ROIs prevented bacterial clearance.

Interestingly, an unrelated pathogen that is sensitive to ROIs was also cleared following the activation of innate cells during the secondary infection. When mice were immunized with bacteria and infected with another ROI-sensitive parasite, the mice cleared the remaining bystander parasite effectively during the secondary bacterial infection.

These findings have a number of clinical applications. For instance, in the past, measurements of TNF-α and IFN-γ have been used to determine vaccine efficacy. This work suggests that CCL3, the crucial link for MPC activation and ROI production, could be a superior readout, because it better represents the activity of memory T cells. This knowledge could also change the way we think about vaccinations. Memory responses could be manipulated to eliminate microbes that have developed resistance to multiple drugs, such as *Mycobacterium tuberculosis* and *Staphylococcus aureus*. Perhaps the triggering of memory T cells specific to a previously received, unrelated pathogen could be used to activate ROI-producing MPCs to clear these or other new infections.

—Kate Jeffrey

Not so fast: adaptive suppression of innate immunity

Noah W Palm & Ruslan Medzhitov

The innate and adaptive immune systems act in concert to effectively combat infection while minimizing collateral damage caused by the host immune response. T cells of the adaptive immune system have now been shown to suppress overzealous early innate responses to infection that can lead to 'cytokine storm'–mediated death (pages 1248–1252).

Morbidity and mortality from infectious diseases can be caused either by direct damage to the host by the pathogen or by collateral damage to host tissues by the immune response to the pathogen. This collateral damage is referred to broadly as immunopathology and can result from overproduction of inflammatory signals by immune cells.

Mammalian hosts employ two interconnected systems—innate and adaptive immunity—to protect themselves from infection while minimizing immunopathology. We are only beginning to understand how these two systems are coordinated to maintain this delicate balance. It is generally thought that innate immunity combats infection immediately, whereas adaptive immunity reacts only after a delay of several days. This suggests that adaptive immunity should not influence the early innate response. In this issue of *Nature Medicine*, however, Kim *et al.*[1] reveal that T cells of the adaptive immune system actively suppress the cells of the innate immune system to prevent an overzealous early innate response and severe immunopathology.

Unlike invertebrates, which rely exclusively on innate immunity, mammals require both innate and adaptive immunity for an effective host response to infection. As the first line of defense, the innate immune system senses infection through pattern-recognition receptors, which recognize conserved molecular features of pathogens that are unique to microbial life forms[2]. These pattern-recognition receptors, such as the Toll-like receptors (TLRs), trigger a variety of antimicrobial responses to combat the infection. When the innate immune system is unable to contain an infection, the cells of the adaptive immune system step in as a second line of defense.

T and B lymphocytes of the adaptive immune system use randomly generated antigen receptors and, once activated, maintain a long-term memory of previously encountered pathogens[3]. These lymphocytes, however, cannot reliably distinguish 'self' from 'non-self', and so they rely on the innate immune system for instructions on when and how to respond to infection[2]. In turn, activated T and B cells further activate and direct innate defenses: T helper 1 (Th1)

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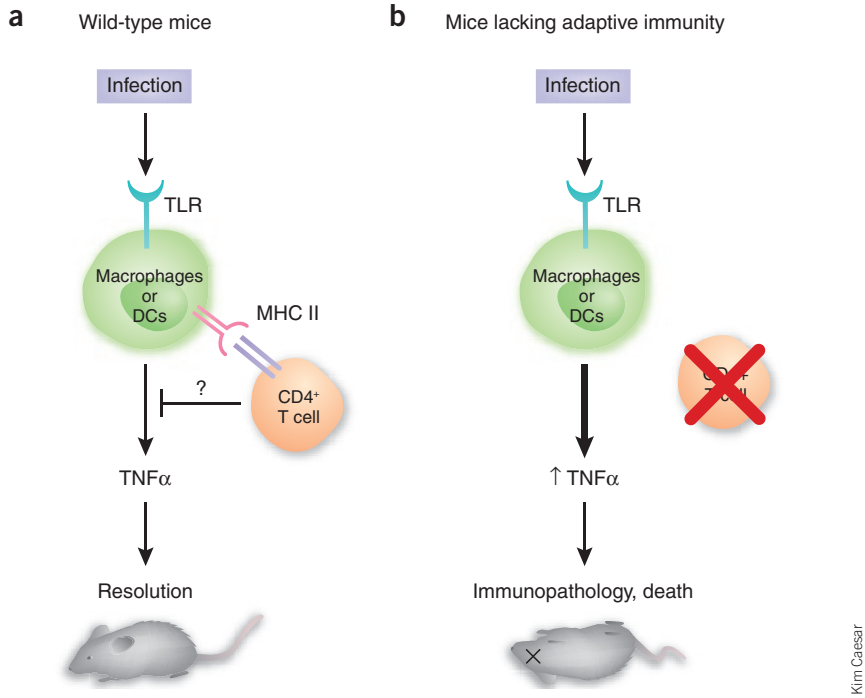
cells activate macrophages, Th2 cells activate eosinophils, and antibodies produced by B cells activate the complement pathway, phagocytosis and mast-cell degranulation.

When either the innate or the adaptive immune system is compromised, the host is unable to combat microbial infection or control endogenous microflora[4–6]. Thus, maximal immunity is achieved only when innate and adaptive immunity work together to combat infection. For instance, mice unable to mount an adaptive immune response, such as nude mice that lack T cells or Rag-deficient mice that lack all lymphocytes, succumb rapidly to infections that would normally be cleared in wild-type animals[4]. It was long-assumed that these mice died because of unchecked microbial growth in the absence of adaptive defenses. Kim *et al.*[1] set out to test this assumption and found that it does not always hold true.

Kim *et al.*[1] inoculated nude mice with a normally sublethal dose of the coronavirus mouse hepatitis virus (MHV) and observed the expected high rate of lethality. However, upon examination, these mice had only negligible increases in viral load and virus-induced tissue pathology, suggesting that they did not die from an overwhelming infection. Instead, when the authors measured cytokine levels in these mice, they found that the levels of interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα) were drastically increased, suggesting that the mice died from damage caused by the high amounts of inflammatory cytokines (cytokine storm) released by cells of the innate immune system.

To confirm that this lethality was caused by a cytokine storm and not by the infection *per se*, the authors[1] stimulated the antiviral immune response without introducing virus into the animals by injecting the synthetic TLR3 ligand poly(I:C), which mimics viral double-stranded RNA. This ligand can induce lethal immunopathology via a cytokine storm in wild-type mice, but in Rag-deficient mice, even a normally sublethal dose of poly(I:C) caused a very rapid death. Antibodies to TNFα prevented the poly(I:C)-induced death, indicating that the cytokine storm caused the lethality.

Because Rag-deficient mice lack both T and B cells, Kim *et al.*[1] went further to show that it is the T cells that suppress inflammatory cytokine production. Mice that had been depleted of T cells also showed high levels of cytokines after having been given poly(I:C), and nude mice into which lymphocytes had been adoptively transferred had reduced levels of cytokines. In total, these results revealed an unexpected negative regulation of the early innate response by the adaptive immune system and suggested that T lymphocytes are necessary and sufficient to suppress an overzealous innate immune response (**Fig. 1**).



Kim Caesar

Figure 1 Conventional T cells suppress overzealous early innate responses, thus preventing severe immunopathology. In response to infection or to purified pathogen-associated molecular patterns, TLRs on macrophages and dendritic cells (DCs) of the innate immune system are activated, inducing the production of inflammatory cytokines, such as TNFα. (**a**) In wild-type mice, conventional T cells of the adaptive immune system suppress early inflammatory cytokine production by innate cells in a contact- and MHC class II–dependent manner; regulatory T cells can also suppress innate cytokine production similarly (not shown). The precise mechanism of suppression, however, is unclear. (**b**) Nude mice, which are deficient in T cells, or Rag-deficient mice, which lack all adaptive immunity, are unable to control the early innate response to infection or to pathogen-associated molecular patterns. In the absence of T-cell–mediated regulation of innate immunity, an overzealous early innate response characterized by the overproduction of TNFα can lead to severe immunopathology and death.

Regulatory T cells (T_{reg} cells) inhibit both innate and adaptive immune responses[7] and are the obvious candidates for the suppressors of the lethal cytokine storm. However, both T_{reg} cells and conventional T cells were able to repress poly(I:C)-induced cytokine production by cells of the innate immune system *in vitro*[1]. This suppression was dependent on direct contact between T cells and cells of the innate immune system, as well as on the antigen-presenting molecule major histocompatibility complex (MHC) class II (**Fig. 1**). Nonetheless, the precise mechanism by which conventional T cells suppress the lethal cytokine storm, including whether or not they use the same mechanisms used by T_{reg} cells to regulate innate immune responses, will need to be investigated further.

The realization that adaptive suppression of early innate immunity is necessary to maintain the balance between immunity and immunopathology has important implications for our understanding of immune regulation in health and disease. In spite of this, some of the most interesting implications of the study by Kim *et al.*[1] relate not only to the current mammalian immune system but also to the evolutionary history of modern mammalian immunity.

Innate immunity alone is sufficient for host defense in invertebrates, yet mammals require both innate and adaptive immunities, indicating that the advent of adaptive immunity may have altered the innate immune system in several ways. The evolution of an adaptive immune response has allowed vertebrate animals to minimize immunopathology by specifically targeting host defenses to pathogens, and it has prevented repeated infection with commonly encountered pathogens through the formation of immune memory[4].

Because it provided these distinct advantages, the vertebrate development of adaptive immunity probably caused drastic changes in the way immune tasks were both delegated and executed. The study by Kim *et al.*[1] suggests that one such change is the temper-

ing of the early innate response by adaptive lymphocytes—an alteration that might have arisen to maximize the benefits gained from engaging the adaptive immune system.

Kim *et al.*[1] have unmasked one reason why mice lacking adaptive immunity do not survive a normally sublethal pathogen challenge, revealing a new regulatory relationship between adaptive and innate immunity. Because the innate immune response precedes the adaptive immune response to infection by several days, one would assume that adaptive immunity should not affect the early innate response, but the findings of Kim *et al.*[1] show that even the earliest innate response requires adaptive regulation.

It seems that the coevolution of innate and adaptive immunity is a story that began with cooperation and has ended in codependence. The adaptive immune system appears to have evolved ways to regulate the early innate immune response in an effort to minimize immunopathology and maximize host defense. Now accustomed to this level of control, the innate immune system can no longer properly regulate its own response in the absence of adaptive suppression. In the future, it will be fascinating to learn how conventional T cells suppress innate immunity and to determine the importance of this suppression in the proper regulation of immune response and resolution of pathogen threats.

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Where lies the blame for resistance—tumor or host?

Charles L Sawyers

Cytokine signaling from the tumor microenvironment can allow leukemia cells to survive targeted imatinib therapy, in the first report of a non-autonomous resistance mechanism.

Targeted cancer therapy, as showcased by the Abelson murine leukemia viral oncogene homolog-1 (ABL) kinase inhibitor imatinib in the treatment of chronic myeloid leukemia (CML)[1], has captivated the attention of the cancer world. Because they specifically attack the molecular underpinnings of a tumor, such as an oncogenic mutant kinase, these targeted drugs promise remarkable efficacy with minimal toxicity. The good news is that the number of successes in the development of such drugs, which could be counted on one hand just a few years ago, continues to grow. But we know that these drugs are not cures. Many individuals only partially respond to the drugs or relapse with drug-resistant disease within months of beginning treatment. This has led to intensive efforts to define the mechanisms of acquired drug resistance in the hope that this knowledge will shed light on combinations of drugs that could lead to durable remissions.

These studies have already unveiled a common resistance mechanism: relapsed tumors often have secondary mutations in the oncogenic kinase that impair drug binding (**Fig. 1**). First shown in CML[2,3], this mechanism has now been implicated in nearly all kinase inhibitor–sensitive cancers, including gastrointestinal stromal tumor, lung cancer and other leukemias[4,5]. The silver lining here is that next-generation compounds are designed to be active against these drug-resistant mutants, a concept nicely illustrated clinically in CML by the second-line ABL inhibitor dasatinib[6,7].

In a recent issue of *Genes & Development*, Williams *et al.*[8] report an exception to this cell-autonomous model of drug resistance. Using a mouse model of Philadelphia chromosome–positive acute lymphoid leukemia (Ph+ ALL), they found that cytokine signaling from the microenvironment can allow tumor cells to overcome drug treatment.

Both CML and Ph+ ALL arise from the expression of the breakpoint cluster region (BCR)-ABL fusion protein, a product of the Philadelphia chromosome translocation. In CML, the translocation originates in the hematopoietic stem cell, whereas in Ph+ ALL it occurs in committed lymphoid progenitors such as pre–B cells. In earlier work, Sherr and colleagues[9] showed that leukemic transformation of pre–B cells by BCR-ABL requires the loss of Arf, a tumor suppressor functionally linked to p53 that protects against oncogene-induced stress. Arf deletion is not required in CML, presumably because Arf is not expressed in hematopoietic stem cells[10,11]. The combination of BCR-ABL and Arf loss in pre–B cells is explosive. These cells have a tremendous capacity to initiate leukemia, and they essentially behave like cancer stem cells after only two genetic hits.

In their most recent study, Williams *et al.*[8] report that mice with BCR-ABL–expressing, Arf-null ALL are resistant to imatinib treatment—despite efficient inhibition of BCR-ABL kinase activity in the tumor cells. This unexpected result led the authors to ask the following question: is the BCR-ABL insult that initiated the leukemia no longer relevant to the tumor's survival, or is there more to the story? In a clever series of experiments, Williams *et al.*[8] showed that tumor cells isolated from the mice remained exquisitely sensitive to imatinib *in vitro*. This result indicated that BCR-ABL still had a function but suggested that some host-derived factor must confer imatinib resistance *in vivo*.

The authors found that the answer, at least in mice, lies in the hematopoietic microenvironment. There, cytokines such as interleukin (IL)-7 protect tumor cells from the antiproliferative and proapoptotic effects of imatinib treatment[8]. Williams *et al.*[8] elegantly demonstrated this mechanism by regenerating the Ph+ ALL model in a genetic background that lacks the common γ chain required by several cytokine receptors, so that the signaling of IL-7 (and other cytokines) is crippled in tumor cells. In this system, imatinib sensitivity was restored, providing formal proof that the microenvironment can cause resistance to targeted therapy.

Going forward, a number of immediate questions arise. Is the cytokine-rescue mechanism relevant in patients with Ph+ ALL? If so, can the hematopoietic microenvironment be safely targeted without losing the magical therapeutic index conferred by targeted therapy? One concern is that the

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