which is often detrimental to the cell in diseases such as heart failure. GRK-mediated desensitization may also be the basis for the waning of the therapeutic effects of continually administered drugs acting as agonists at GPCRs.

Increased GRK2 expression may also have led to another aspect of heart failure, as observed by Lymperopoulos *et al.*[2]—decreased expression of α_2AR. This decrease could be due to agonist-promoted receptor degradation[6] because of GRK2 acting with β-arrestin. α_2ARs also undergo desensitization by agonist-triggered decreases in the cellular expression of their cognate G protein, G_i (ref. 7), which was not examined in this report.

The researchers next asked whether they could improve heart failure by targeting GRK2. Injection of a GRK2-minigene inhibitor into the adrenal medulla, akin to gene therapy, reversed the overly desensitized α_2ARs, providing for regulation of adrenal catecholamine release. The therapy resulted in lower circulating epinephrine and norepinephrine levels, improved resting cardiac performance and increased contractile reserve in response to acute exposure of the heart to catecholamines. These improvements were associated with reductions in cardiac GRK2 expression, reflecting a cardiac β_1AR signaling module with improved efficacy and adaptability.

So, adjusting the control on the amplifier, in this case the part of the circuit that amplifies the signal from the receptor to the G protein (GRK2), normalized the α_2AR module in the adrenal medulla. This adjustment altered the serial interconnectivity between the adrenal gland and the heart via a change in catecholamine release.

What is not clear from the current work is why GRK2 expression in the adrenal gland was altered in the models of heart failure. Interestingly, the expression of the kinase is not typically upregulated by a receptor-specific agonist. However, there are many neurohumoral signaling and hemodynamic abnormalities in heart failure—and so multiple GPCR signals, altered blood flow or other factors could boost GRK2 expression in the adrenal gland.

Nevertheless, the altered relationship between the heart and the adrenal gland that occurs during heart failure appears to be amenable to therapy aimed at adrenal gland GRK2—one specific control of the α_2AR signaling module. Given the complexity of the module, other critical points for adjustment may be potential therapeutic targets as well.

COMPETING INTERESTS STATEMENT
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Vaccine leads to memory loss

John B A G Haanen & Ton N M Schumacher

A promising experimental vaccine strategy, just entering clinical trials, displays a deleterious effect in mice. Use of antibodies to CD40 seems to clamp down on the long-term ability of T cells to respond to antigen (pages 354–360).

Can a vaccine be 'too efficient'? Can it provide such a strong stimulus to the immune system that the response is ultimately abortive rather than productive? In this issue, Berner *et al.*[1] use mouse models to show that a widely heralded approach to vaccine adjuvants, targeting of the CD40 receptor, can have an unexpected deleterious side effect—it abolishes long-term T-cell responsiveness toward tumor antigens. These findings dovetail with recent work by Bartholdy *et al.*[2], who came to similar conclusions in a viral infection model.

Together, these data show that our understanding of the pathways that control immune responsiveness is still far from complete and underscore the requirement for comprehensive preclinical testing of vaccine adjuvants before clinical use.

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Vaccination is arguably the most successful medical intervention conceived to date; pathogens such as polio and smallpox virus, equated in our parents' generation with suffering and death, have been all but forgotten by our children. However, effective prophylactic vaccines against major human pathogens such as malaria, mycobacterium tuberculosis and HIV are still lacking. Likewise, therapeutic vaccines that can promote immune responsiveness in case of chronic infections or cancer have not made a substantial impact on human health to date.

It is generally expected that the development of effective vaccination strategies in such highly demanding settings will be made possible by our improved understanding of the molecular interactions that control the induction of immune responses. Analogous to the rational design of small molecule drugs, such rationally designed vaccines should target specific receptors on lymphocytes or on the antigen-presenting cells (APCs) that activate them.

The utility of vaccination is readily explained by the way our adaptive immune system detects foreign pathogens. Rather than having a preformed repertoire of lymphocytes with receptors that are specific for different pathogens, our body produces a highly diverse and random collection of lymphocytes that have the capacity to recognize essentially any potential foreign antigen. The value of this system is hard to overestimate, as it allows the body to detect pathogens that evolve rapidly. But the ability of our adaptive immune system to recognize such an immense variety of antigens has one less pleasant consequence: the frequency of lymphocytes that recognize a given antigen is extremely low.

This is where vaccination comes in. Vaccination molds our adaptive immune system by increasing the frequency and activity of those lymphocytes that we consider useful. This requires two components: the antigen against which one wishes to increase reactivity, plus an adjuvant that conveys a sense of danger.

Traditionally, such adjuvants were natural products for which the immune-stimulating effects were established empirically. Now our increased knowledge of the cellular interactions

that control immune responses makes it possible to target specific pathways. For instance, this targeting may involve administration of ligands for activating receptors, such as CD40 or Toll-like receptors, present on antigen-presenting cells, or administration of cytokines such as interleukin (IL)-12 or IL-15.

Of these targeted adjuvants, the administration of ligands for CD40 has been considered particularly attractive, as previous work has demonstrated that signaling through CD40 forms a crucial switch in the induction of B-cell responses and cytotoxic T-cell responses[3,4]. On the basis of these data, a first set of clinical trials using either recombinant CD40 ligand or antibodies to CD40 has been performed[5]. In addition, small molecule ligands for CD40 are currently in development[6].

Berner *et al.*[1] now provide evidence for an unexpected and highly unwelcome long-term effect of this targeted vaccine adjuvant. They show that vaccination of mice with antibody to CD40, plus IL-2 (the latter for maximal T-cell expansion) can actually interfere with the long-term capacity for T-cell reactivity. Furthermore, the authors suggest that the same deleterious effect may occur with other targeted vaccination strategies that, like antibody to CD40, share a capacity to induce an interferon (IFN)-γ-dependent apoptosis of helper T cells.

The authors build their case on three pieces of data in a mouse tumor model. First, whereas administration of a tumor vaccine that contained IL-2 and antibody to CD40 enhanced tumor recognition in the short term, it reduced reactivity at later time points—even below the level observed in mice that had not been vaccinated. This finding suggests that rather than promoting recognition of tumor-associated antigens, vaccination actually leads to some level of tolerance.

Second, administration of CD40 antibody and IL-2 resulted in substantial apoptosis of $CD4^+$ (helper) T cells in the days following vaccination.

Third, both the impaired tumor recognition and the increased $CD4^+$ T-cell apoptosis induced by CD40 antibody and IL-2 administration were not observed in animals in which IFN-γ signaling was disrupted. These data fit well with a series of studies that show that IFN-γ production during natural infections contributes to the contraction of both antigen-specific $CD4^+$ and $CD8^+$ T-cell responses[7,8]. But whereas this earlier work suggested that IFN-γ signaling could be important to convert a markedly high T-cell response to a physiological memory T-cell response, the current work suggests that vaccine adjuvants such as antibodies to CD40 that induce very strong IFN-γ signaling can actually deplete the memory T-cell compartment.

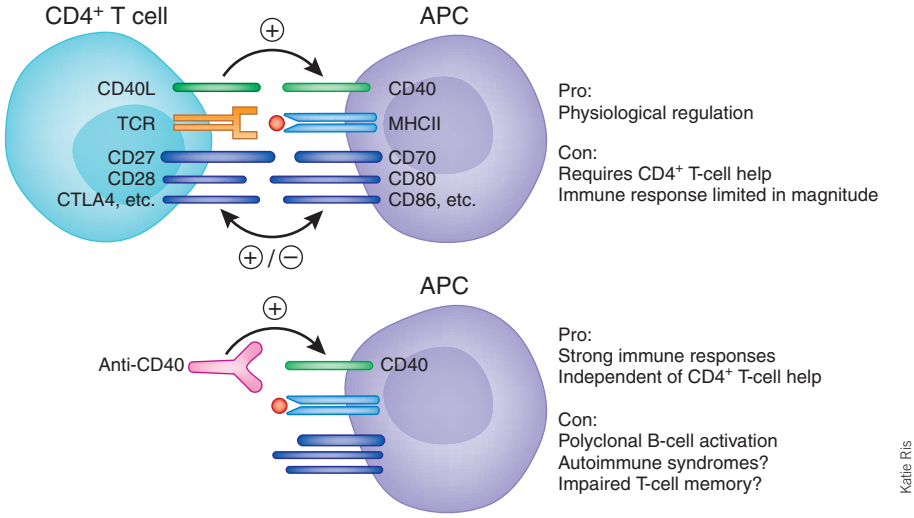

Figure 1 Pros and cons of artificial CD40 triggers. (**a**) APC activation upon encounter with an antigen-specific $CD4^+$ T cell. Interactions that control the outcome of $CD4^+$ T cell–APC encounter are exemplified by—but by no means limited to—the receptor-ligand pairs indicated. CD40L, CD40 ligand; TCR, T-cell antigen receptor; CTLA4, cytotoxic T lymphocyte–associated protein 4; MHCII, major histocompatibility complex type II protein. (**b**) APC activation upon triggering with artificial CD40 ligands, such as an antibody to CD40 (anti-CD40). The use of artificial CD40 ligands obviates the need for antigen-specific $CD4^+$ T-cell help and can overcome tolerance in mouse model systems[3,11]. However, artificial CD40 triggering has been associated with polyclonal B-cell activation and may induce auto-aggression by self-reactive T cells[12]. In addition, the data from Berner *et al.*[1] and Bartholdy *et al.*[2] suggest that—at least under some conditions—T-cell memory formation may be impaired.

Notably, the mechanism through which IFN-γ signaling interferes with long-term T-cell responsiveness remains largely obscure. Furthermore, although the authors showed a correlation between CD4 T-cell apoptosis and lack of tumor recognition in animals treated with CD40 antibody and IL-2, formal evidence for a cause-effect relationship is lacking. To further resolve this issue, it will be important to determine to what extent vaccinated animals have lost $CD4^+$ T-cell reactivity toward vaccine-encoded antigens and whether resupply of such reactivity can rescue the ability to control tumor growth.

In addition, it may be useful to extend this analysis to the tumor-specific $CD8^+$ T-cell repertoire. Specifically, whereas the authors demonstrate that treatment with CD40 antibody has no detrimental effect on total $CD8^+$ counts, it clearly remains possible that a selective loss of the tumor-specific $CD8^+$ T cells that are activated by vaccination does occur, and such a loss could play a role in the subsequent immune failure. Interestingly, this latter model would fit well with the work of Bartholdy *et al.*[2], which describes a marked loss of virus-specific $CD8^+$ T cells upon treatment with antibody to CD40.

Why was this deleterious effect of CD40 antibody as a vaccine adjuvant not noted before? All too frequently, researchers lack the stamina for long-term analyses and this may lead to an under-reporting of long-term side effects. Perhaps more importantly, adjuvant CD40 antibody treatment may have different effects depending on the antigen or pathogen involved. In support of this idea, whereas CD40 antibody treatment is highly detrimental in chronic lymphocytic choriomeningitis virus infection, this negative effect does not occur in certain other models of viral infection[2].

Given such caveats, how worried should we be? Exhaustion of long-term T-cell responsiveness may perhaps be most worrisome in clinical settings where pre-existing T-cell reactivity contributes to disease control. Specifically, naturally occurring T-cell responses are not considered a major factor in limiting the rate of progression for most human cancers. A vaccine approach that would inadvertently destroy a low-level pre-existing immune response may, in that case, not be beneficial; however, it would also not hasten disease progression. In contrast, in the case of chronic viral infections and virus-induced tumors, a delicate balance can exist between viral or tumor load and immune reactivity. In such cases, a vaccine that would accidentally reduce T-cell reactivity may well induce rapid disease progression.

The data from Berner *et al.*[1] and Bartholdy *et al.*[2] add a cautionary note to the clinical use of CD40 ligands as vaccine adjuvants (**Fig. 1**). On a more general note, the current data highlight how interventions that target the interactions of immune cells can sometimes have dramatic and unintended effects—as also evidenced by the ill-fated CD28 antibody trial in the UK last year[9]. It has recently been proposed that, because of their potency and pharmacokinetics, new guidelines may be required to assess the risk of this class of compounds before human administration[10]. Clearly, the type of studies reported here by Berner *et al.*[1] form an essential part of such analyses.

COMPETING INTERESTS STATEMENT
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Regulatory T-cell development: is Foxp3 the decider?

Tyler J Curiel

Regulatory T cells (Tregs) have gone from obscurity to rock-star status in the past decade, prompting intense scrutiny—but what exactly are they? Four studies examine this question, delving deeply into the role of the transcription factor Foxp3 in governing Treg differentiation and function.

Tregs play essential roles in immune homeostasis and protection against autoimmunity, but also contribute to immunopathology in cancers, infections and other diseases[1]. The recent discovery that the nuclear transcription factor forkhead box P3 (Foxp3) appeared necessary and sufficient for Treg development[2] electrified investigators by providing the first specific marker identifying these enigmatic cells.

Four studies in *Nature*[3–6] cleverly capitalize on existing tools arising from this discovery, and add powerful new tools to demonstrate the genomic and functional consequences of Foxp3 expression in ways recently unimaginable. The studies show how Foxp3 protein and *Foxp3* genetic regulatory elements contribute to Treg development by integrating environmental and intracellular signals. They also resolve contradictory previous results about Treg differentiation and function and yield unpredicted insights, including hints of novel lineage differentiation strategies.

History of suppression

T cells capable of inhibiting immune responses were described almost 40 years ago, but work languished owing to lack of scientific acceptance and specific techniques to identify, isolate and study suppressive cells. Sakaguchi *et al.*[7] rekindled interest in what we now call Tregs in 1995 as thinking evolved, and as technology progressed to permit their isolation.

The staggering diversity of T-cell antigen receptor recognition derives from an ingenious genetic ploy: small DNA snippets in thymocytes—nascent T cells undergoing differentiation in the thymus—are randomly scrambled and reassembled, yielding more than 10^{10} distinct variations. This random process generates a population of T-cell receptors capable of recognizing practically anything—but an unfortunate consequence is production of self-reactive T cells. Negative selection in fetal thymus clonally deletes many self-reactive T cells, permanently removing them as potential sources of autoimmune attack. Self-reactive T cells slipping past thymic defenses to seed peripheral lymphoid organs require a different strategy to tame their autoimmune potential[8].

Key agents for this second strategy are natural $CD4^+CD25^+$ Tregs that arise in the thymus through homeostatic processes (**Fig. 1a–c**). Natural Tregs curb autoreactive T cells by subduing their function, without killing them, through incompletely understood contact-dependent mechanisms[1,3–6,8]. Adaptive Tregs (**Fig. 1d**), in contrast, arise in pathologic inflammatory conditions[1] such as cancers and infections. Natural and adaptive Tregs are phenotypically indistinguishable but functionally heterogeneous. Further, even natural Tregs in thymic versus peripheral compartments can differ owing to microenvironmental influences.

Reduced Treg function contributes to autoimmune diseases including diabetes, whereas increased numbers aggravate tumor and pathogen immunopathology[1]. Thus, understanding differentiation pathways and functional attributes of these pivotal immune cells is a major goal of tolerance investigators.

Fatal autoimmune disease in scurfy mice provided an important clue. These mice lack detectable Treg function owing to a defective nuclear transcription factor, Foxp3 (originally called scurfin in mice). The human autoimmune disease IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked)[3–6] is likewise characterized by defective Treg function due to malfunctioning FOXP3, establishing this nuclear transcription factor as critical to Treg development, and finally identifying a surrogate for Treg identity in a field long bedeviled by lack of specific markers.

Outfoxing Foxp3

But how precisely does Foxp3 contribute to Treg differentiation, and which Treg population is best suited for studies? To address fundamental issues regarding Foxp3 effects on Treg development, two groups[3,4] focused on DNA binding and gene regulation and two[5,6] focused on functional consequences of Foxp3 protein. Each used a distinctive approach studying natural Tregs in different compartments, but all contribute complementary and reinforcing data.

To prove that Foxp3 is a classic nuclear transcription factor, and that genes purportedly

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