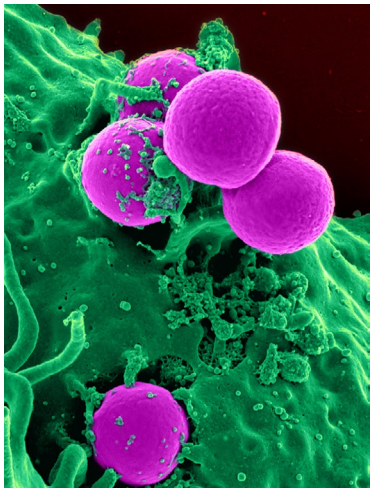
The dream of *Staphylococcal* vaccination

Insight from Patrick Schlievert

There are effective vaccines against multiple extracellular bacterial pathogens, many of which contain surface antigens or toxoids of potent secreted exotoxins. But despite successful preclinical studies and multiple human trials using *Staphylococcus aureus* surface antigens as vaccine candidates, none have protected humans against *S. aureus* infections. In 2007, the CDC published that *S. aureus* is the most significant cause of serious infections in the US, causing skin/soft tissue infections, pneumonia, infective endocarditis, septicemia, osteomyelitis, and toxic shock syndrome (TSS). Thus having an effective vaccine is highly desirable.

In this issue, Pauli et al. provide a potential explanation for why it has been difficult to develop effective immunity against *S. aureus*. The organism encodes a myriad of virulence factors, one of the best characterized of which is Protein A (SpA). SpA inhibits phagocytosis by binding the Fc of human IgGs and also acts as a B cell superantigen that expands and then ablates variable heavy 3 (VH3) idiotype B cells in mice. Pauli et al. now show that the same SpA-induced expansion of VH3 B cells occurs in humans but without ablation, reminiscent of the differential response of mice versus humans to staphylococcal T cell superantigens.

Humans naturally infected with *S. aureus* were found to develop skewed antibody responses due to SpA B cell superantigenicity. The active B cell response in these individuals was focused exclusively on SpA, despite the presence of circulating B cells reactive to many other *S. aureus* surface antigens. The data presented are superb and provide strong evidence for the misdirection of humoral immune responses in many natural *S. aureus* infections. However, Pauli et al. do not address cases in which *S. aureus*–specific antibody responses successfully protect against subsequent infection. For example, by age 13, a majority (80%) of females develop high titers of antibodies to exotoxins, such as toxic shock syndrome toxin-1 (TSST-1), and are thus protected from menstrual TSS (mTSS). The remaining 20% who are susceptible do not develop antibodies in response to initial infection. Additionally, intravenous human immunoglobulins (IVIGs), which are pools of antibodies from thousands of healthy human volunteers, contain antibodies against all *S. aureus* superantigens, such that IVIG is often used as an adjunct therapy for serious *S. aureus* diseases. These data suggest that the type of *S. aureus* infection studied in the Pauli et al. manuscript may have influenced the skewing of the immune response to SpA. This could easily be the case if the causative strains were predominantly USA300 *S. aureus*, which are skin and soft tissue pathogens that do not produce the major recognized T cells superantigens.



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Staphylococcus aureus

The data provided by Pauli et al. may help explain the failure of vaccine efforts, although it remains unclear why this large immune response to SpA is not sufficient for protection. What is less clear is how these data might inform future vaccine design. The authors suggest that neutralizing SpA function through vaccination with a nonsuperantigenic SpA may be required for the generation of a successful vaccine against *S. aureus*. However, it was recently shown that a vaccine that included a nonsuperantigenic SpA linked with two other surface antigens substantially prolonged survival in mice, but ultimately did not provide protection. One must also consider that in all *S. aureus* vaccine trials to date, humans were vaccinated against surface antigens. In the absence of SpA, vaccinees developed strong humoral responses to the vaccine antigens, yet none of these trials resulted in protection against subsequent infection, and in at least one trial, vaccinees appeared to be even more susceptible. The latter finding might be explained by the observation that *S. aureus* aggregates contribute to disease, and aggregation may be facilitated by IgG against surface antigens.

To date, no human trials have used *S. aureus* toxoids as vaccine antigens, but animal studies suggest that this may be a promising approach. Alternatively, it is possible to produce Fabs of IgG against *S. aureus* surface antigens, including SpA, coagulases, and clumping factors, and use these to passively prevent potential aggregation-associated diseases.

Pauli, N.T., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141404.

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A Mad(2) modification modulating megakaryopoiesis

Insight from Adam Goldfarb

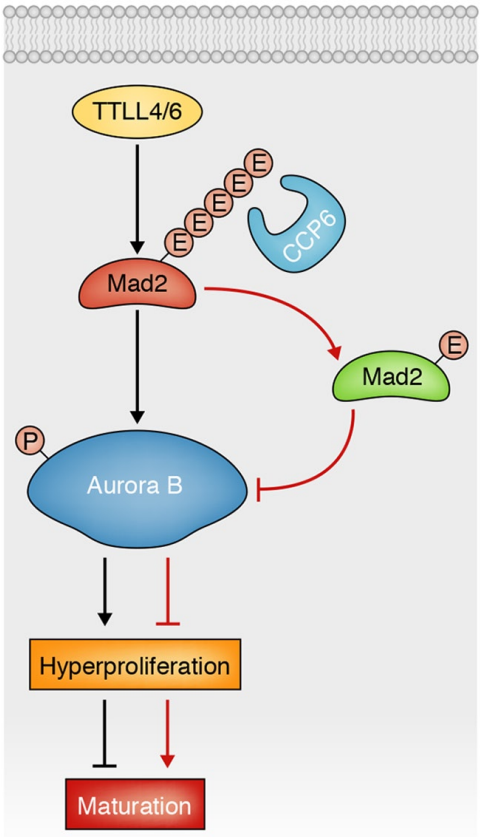
Megakaryocytes, the rare platelet-producing cells within the bone marrow, undergo an unusual developmental transition from early mitotic proliferation to endomitosis associated with polyploidization and enlargement. This transition is precisely coordinated with a lineage-specific differentiation program and equips the cell with sufficient mass to yield proplatelet extensions that shed appropriate numbers of platelets into the circulation. While the mechanisms that maintain this choreography remain obscure, the disastrous consequences of even slight miscues are evident in several diseases. A common feature of human myeloproliferative neoplasms (MPNs), for example, consists of

enhanced mitotic proliferation resulting in excessive and malformed megakaryocytes. In this issue, Ye et al. broaden our understanding of megakaryopoietic regulation with a new signaling pathway whose perturbation elicits MPN-like features.

While characterizing mutant mice deficient in cytosolic carboxypeptidase (CCP) 6, the authors documented a selective increase in platelet counts, which resulted from a ~threefold increase in megakaryocytes in marrow and in spleen. Paradoxically, the animals had prolonged bleeding times due to defective platelet aggregation. CCP6-deficient megakaryocytes displayed enhanced mitotic proliferation and decreased endomitosis, leading to impaired polyploidization and enlargement. In humans, elevated platelets with defective aggregation combined with increased marrow megakaryocytes occur in the MPNs essential thrombocythemia (ET) and chronic myelogenous leukemia (CML), and defective megakaryocytic polyploidization occurs in CML.

Starting with the known function of CCP6 in removal of polyglutamate protein modifications, the authors pieced together an entirely new megakaryopoietic regulatory cascade. Specifically, loss of CCP6 increased polyglutamylation of the spindle checkpoint protein MAD2. This MAD2 modification promoted recruitment and hyperactivation of the mitotic kinase Aurora B, which is normally inhibited by MAD2. A series of elegant rescue experiments confirmed the functional significance of each step. Thus, normal megakaryopoiesis could be restored in CCP6-deficient progenitors through either knockdown of the relevant polyglutamylase TTLL6, replacement of wild-type MAD2 with a glutamylation-acceptor mutant, or inhibition of Aurora kinase activity.

Inappropriate Aurora kinase activity likely contributes to polyploidization defects in human megakaryocytic neoplasms and represents an attractive therapeutic target. How polyglutamate modification converts MAD2 from inhibitor to activator of Aurora B remains an intriguing question with potential clinical relevance. Furthermore, this paper raises the possibility that perturbations in megakaryocytic MAD2 glutamylation, normally maintained by the balance of TTLL6 and CCP6 activities, could contribute to human MPN. Future topics of interest include the influence MPN signaling defects—e.g., JAK2 V617F—on MAD2 glutamylation and the potential role of Aurora inhibitors in MPN therapy.



Posttranslational polyglutamylation of Mad2 by TTLL4/6 results in activation of Aurora B, which in turn induces hyperproliferation and impaired maturation of megakaryocytes. This pathway is normally opposed by the carboxypeptidase CCP6.

Ye, B., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141123.

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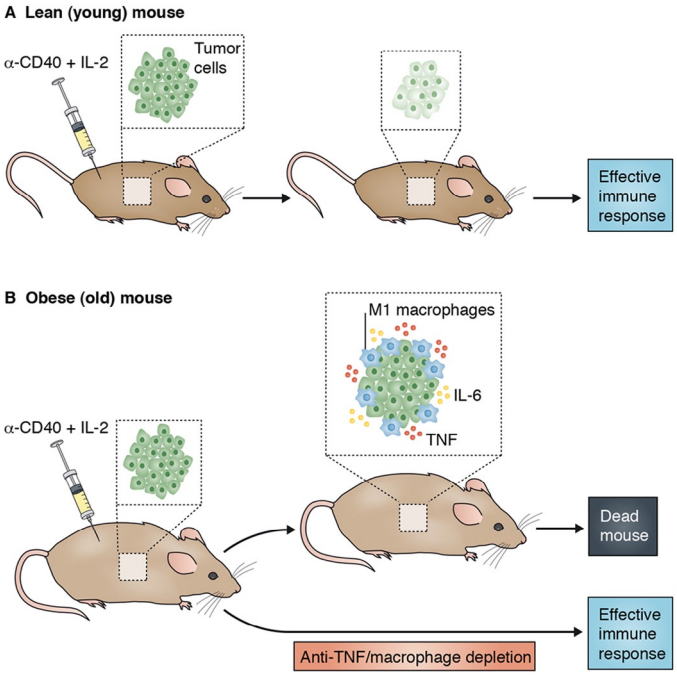
Immunotherapy and the belly of the beast

Insight from Taha Merghoub (left) and Jedd Wolchok (right)

Immunotherapy has emerged as an effective means to restore immune recognition of cancer. Numerous forms of immunotherapy are currently being explored clinically. The most common are vaccines (dendritic cell, viral, and whole tumor cell based), adoptive T cell therapy and immune checkpoint blockade. The recent successes in melanoma, renal cancer, and lung cancer have generated renewed optimism for treatment of multiple cancer types that were believed not amenable to immune-based therapies. However, immune-related events such as colitis, dermatitis, and, less frequently, endocrinopathy and pneumonitis have been reported and can be a challenge in the clinical use of these approaches. Cytokine release syndrome has been reported in the case of CAR (chimeric antigen receptor) T cell therapy and treatment with agonist antibodies. In this issue, Mirsoian et al. provide evidence that adiposity in aged mice induces a lethal cytokine storm following systemic administration of stimulatory immunotherapy consisting of anti-CD40 agonist antibody with IL-2.

This is an elegant follow up to a previous study by the same authors in which they showed that systemic immunotherapy administration in aged mice resulted in the induction of a rapid and lethal cytokine storm. In the current paper, they find that it is the fat that accumulates during aging, and mainly the visceral fat, that is associated with toxicity. This results in increased levels of proinflammatory



The Murphy group previously showed that immunotherapy with anti-CD40 and IL-2 leads to a productive immune response in young mice (A) but lethal cytokine storm in older mice (B). They now find that the visceral fat that accumulates in aged mice (or young obese mice) is the primary trigger of inflammation after immunotherapy, resulting in increased inflammatory M1 macrophages and toxic cytokine storm (B). Immunotherapy-induced lethality was prevented by blocking TNF or depleting macrophages (B). Whether the success of immunotherapy in the treatment of tumor-bearing mice will also be impaired by obesity remains to be determined.

M1 macrophages within the peritoneal cavity and visceral adipose tissues, leading to heightened production of TNF. In order to confirm that it is indeed the fat that is associated with lethal effects, the authors repeated their studies in young mice with genetic (ob/ob) or diet-induced obesity (DIO). While young obese mice also displayed severe toxicity to the therapy, it was much more severe in older mice suggesting that age is also a significant factor. Reciprocally, calorie-restricted aged mice had lower visceral body fat content and reduced cytokine levels, with increased survival following immunotherapy. Obese mice were also protected by macrophage depletion or TNF blockade. These data demonstrate the need to consider age and body fat content as variables in preclinical assessment of therapeutics and when modeling diseases such as cancer.

Since many cancer patients fall into the elderly category, this study brings up a critical point that aged mice respond differently to immunotherapy than younger mice. This also highlights an important issue when considering potential toxicities associated with any therapy, as most animal studies are performed in young, healthy mice. Another interesting point is the potential impact of body mass index and adipose accumulation in predicting side effects when investigating and utilizing immunotherapies. This is a very important and impactful finding for the field, and further investigations in tumor bearing mice with commonly used therapies are warranted. If the findings are confirmed in other therapeutic settings, the inclusion of supportive measures, such as TNF blockade, should continue to be considered for early management of immune-related toxicities to improve clinical outcomes.

Mirsoian, A., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20140116.

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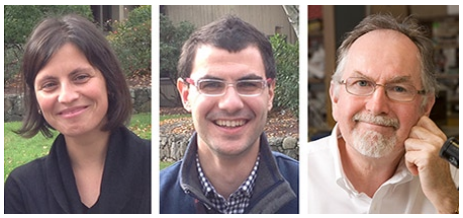
FLUshing in the bathroom



Insight from (left to right) Carolina Amezcua, Nicola Gagliani, and Richard Flavell

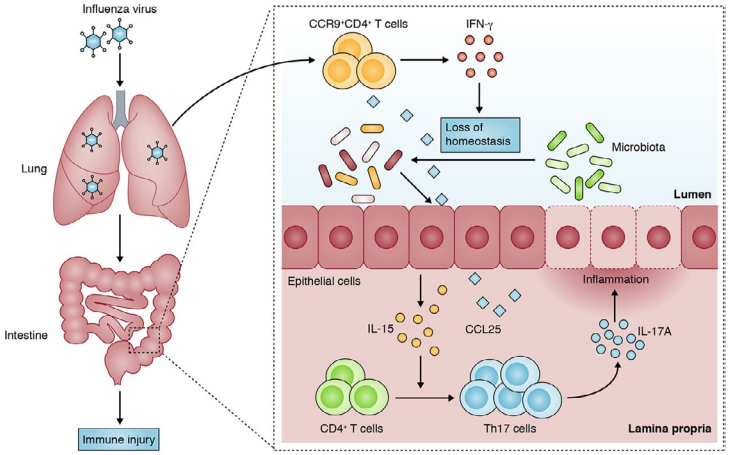
Seasonal influenza represents a contagious family of respiratory viruses that infect 5–30% of the global population yearly and account for as many as 500,000 deaths annually. Flu infection is characterized by symptoms such as fever, cough, sore throat, runny nose, body aches, and fatigue. Interestingly, some people also develop diarrhea, even though the virus tropism is for respiratory tissue. Despite being a well-studied viral infection, the underlying mechanisms involved in the development of gastroenteritis-like syndrome with flu infection are poorly understood.

Now, Wang et al. have used a mouse model to show that influenza infection not only causes lung inflammation but also causes intestinal inflammation, even though influenza virus was not detectable in the gastrointestinal tract. They showed that during intranasal flu infection, $CCR9^{+}CD4^{+}$ T cells migrate from the lung into the intestinal mucosa in a CCL25/CCR9-dependent manner and alter the composition of the gut microbiota by secreting IFN-γ. Homeostasis of the intestinal microbiota was altered, and increased numbers of *Escherichia coli* were detected after flu infection. Antibiotic treatment of intranasal flu-infected mice protected them against infection-induced diarrhea. These data suggest that the dysbiosis generated after flu infection leads to intestinal injury. The changes generated in the gut microbiota induced the production of IL-15 by intestinal epithelial cells. IL-15 induced the expansion of Th17 cells in the small intestine, which then mediated intestinal immune injury.

It is noteworthy that not all flu-infected patients develop gastroenteritis-like symptoms. Why is this? One possibility could be that the migration of pathogenic cells into the intestine depends on the severity of the infection. Consequently only highly infected patients may get diarrhea. Alternatively, the specific microbial landscape in some individuals and the existence of regulatory bacteria, could obstruct the growth of *E. coli* or promote regulatory T cells which in turn can control intestinal inflammation.

The data presented in this paper corroborate the hypothesis that the intestine is a suitable place to defuse an immune response. The abundance of antiinflammatory cytokines, such as IL-10 and TGF-β, regulatory cells and continuous regeneration of the tissue predispose the intestine with an ability to control effector cells. Moreover, if effector T cells escape all possible regulatory mechanisms, diverting them to the lumen—in essence flushing them away—could still be less dangerous than allowing them to remain in situ and risking lung tissue damage.

Wang, J., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20140625.



Working model of intestinal injury induced by respiratory influenza virus infection

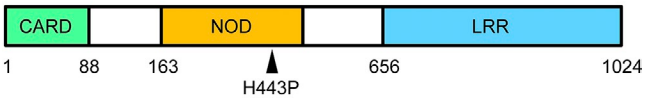
Carolina Amezcua, Nicola Gagliani, and Richard Flavell; Howard Hughes Medical Institute, Yale School of Medicine: richard.flavell@yale.edu, caro.amezcua@yale.edu, and nicola.gagliani@yale.edu

Too much of a good thing: NLRC4 and autoinflammation

Insight from Mark Anderson

NOD-like receptor (NLR) family members are intracellular molecules that play a key role in innate sensing. Upon ligand binding in the cytosol, NLRs assemble a multimeric complex that includes the adaptor protein ASC and procaspase-1. This assembled complex, referred to as the inflammasome, drives the production of an active form of caspase-1 that then activates IL-1β and IL-18. Activating mutations in members of the NLR family are associated with sterile inflammatory syndromes referred to as cyropyrin-associated periodic syndromes (CAPS). Previous studies have shown that mutations in the NLR family member *NLRP3* are associated with CAPS and the overproduction of IL-1β. This raises the question of whether CAPS may be caused by mutations in other NLR family members.

In this issue, Kitamura et al. describe a Japanese family with a sterile autoinflammatory syndrome. Affected family members developed a clinical syndrome characterized by intermittent episodes of fever and cold-induced urticaria and arthralgias. Inheritance of this syndrome was autosomal dominant, and this led the investigators to search for a genetic cause using whole exome sequencing (WES). Interestingly, the results of WES showed no identifiable mutation in the *NLRP3* gene. So the team searched the WES data for other rare variants that could explain the syndrome. WES in a single autosomal dominant family can be quite challenging, as a large number of rare variants will track with disease within a family. Here, the investigators used a combination of genetic linkage and gene function analyses to home in on *NLRC4*. The mutation (H443P) in the affected patients lies in a highly conserved portion of the nucleotide binding domain of NLRC4, and the mutant form of NLRC4 is associated with spontaneous oligomerization, enhanced activation of caspase-1, and hyper-activation of IL-1β. The team also developed an in vivo mouse model of the disease by expressing a mutant form of *NLRC4* under the control of the invariant chain (Ii) promoter in a transgene. The transgenic mice developed dermatitis, arthritis, and splenomegaly—features that overlap to some extent with the clinical features of the patients.



Structure of NLRC4 indicating the histidine to proline mutation identified at position 443 in the nucleotide-binding oligomerization domain (NOD). CARD, caspase recruitment domain; LRR, leucine rich repeat.

A number of important questions remain to be answered. First, are there distinct features of the *NLRC4* mutation–associated syndromes? Interestingly, in two other recent reports of families with *NLRC4* mutations, the phenotype was more severe and included gastrointestinal complications that were not observed in the patients in this study. NLRC4 is known to be involved in bacterial sensing through detection of flagellin or components of the bacterial type 3 secretion system, and it may be the case that more severe mutations in *NLRC4* may provoke a phenotype in the gut related to the sensing of bacterial flora. Second, how do the rare mutations in *NLRC4* lead to autoactivation at a molecular level?

This study, along with two other recently published reports, firmly establishes that mutations in the NLR family member *NLRC4* can provoke an autoinflammatory syndrome. The identification of these disease-causing mutations will be a rich resource for further unraveling how NLRC4-related assembly occurs and is controlled.

Kitamura, A., et al. 2014. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20141091.

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