ADAPTIVE IMMUNITY

PD-1 blockade unblocks immune responses to vaccination

Wherry and colleagues describe how anti-PD-1 immunotherapy impacts outcomes of influenza vaccination in patients with cancer, and specifically, how it increases seroconversion and affects quantitative and qualitative aspects of antibodies and follicular T helper cell responses.

Katherine Kedzierska and Thi H. O. Nguyen

Discovery of the programmed death molecule 1, PD-1, on T cells was a major breakthrough that led to the development of immune checkpoint receptors for cancer treatment, and consequently the 2018 Nobel Prize in Physiology or Medicine for Tasuku Honjo. Anti-PD-1 immunotherapy is now widely used for the treatment of several cancers to boost patients' $CD8^+$ T cell immunity. Yet, effects of anti-PD-1 therapy on other key immune cell subsets remain understudied. Of particular interest is the effect of anti-PD-1 treatment on circulating T follicular helper (cT_{FH}) cells, classically expressing PD-1 and playing an important part in germinal center (GC) reactions within lymphoid tissues following infection and vaccination. In this issue of *Nature Immunology*, Herati et al.[1] defined quantitative and qualitative effects of

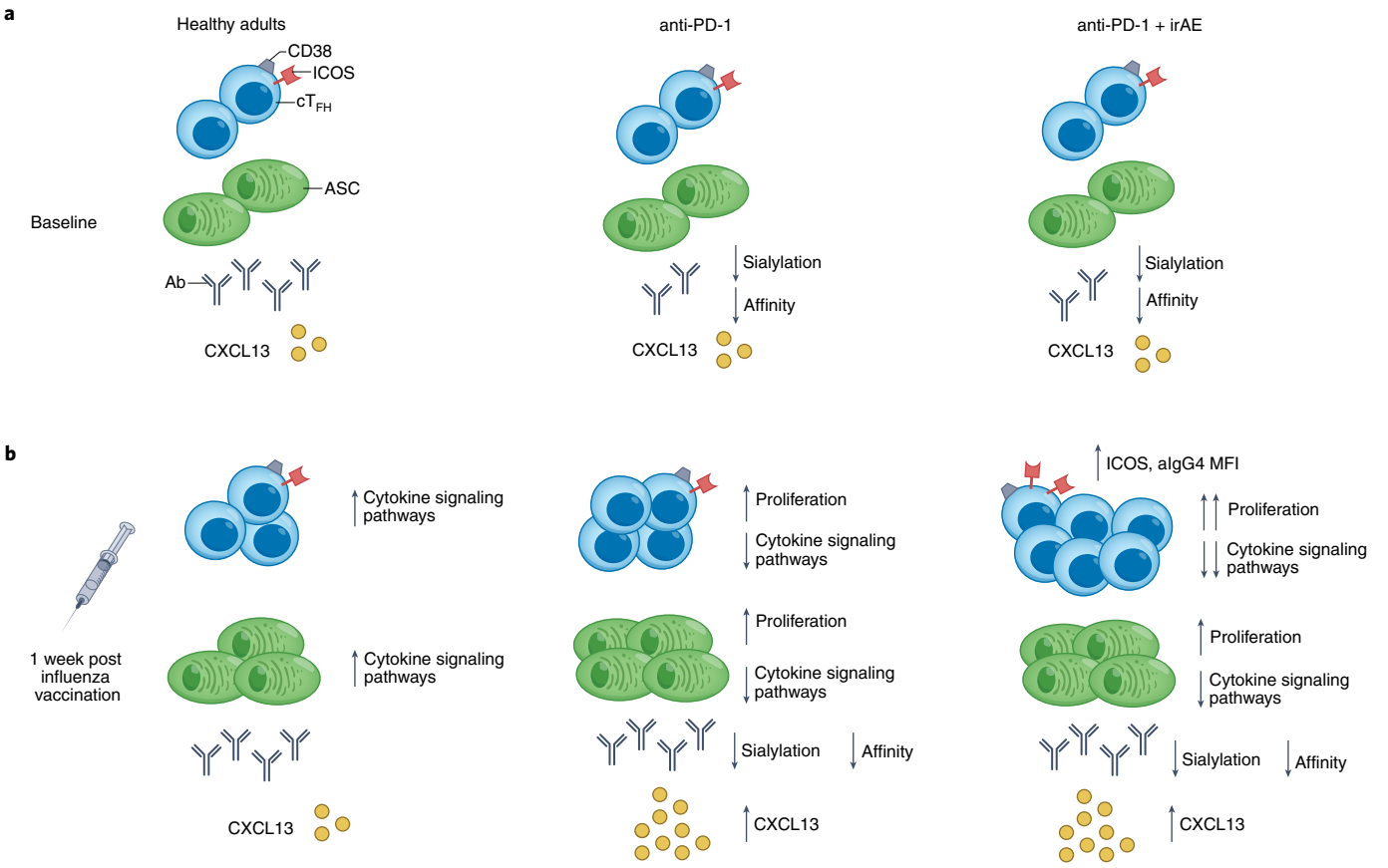

Fig. 1 | Anti-PD-1 immunotherapy impacts immune responses to influenza vaccines in cancer patients. a,**b**, Frequencies of circulating T follicular helper (cT_{FH}) cells, plasmablasts (also known as antibody-secreting cells (ASCs)) and plasma CXCL13 concentrations are increased in patients receiving anti-PD-1 immunotherapy 1 week after influenza vaccination (**b**) compared to baseline (**a**). Anti-PD-1 was also associated with altered antibody responses. In patients receiving anti-PD-1 therapy, proliferation and cell cycle genes are upregulated in cT_{FH} cells and plasmablasts, but cytokine signaling pathway genes are downregulated. These transcriptional profiles are enriched in anti-PD-1-treated patients with immune-related adverse events (irAE). aIgG4 MFI, aglycosylated immunoglobulin G4 median fluorescence intensity levels.

anti-PD-1 therapy on cT_{FH} cells and humoral responses to influenza vaccination. Robust cT_{FH} cell responses were also observed in a subset of patients on anti-PD-1 therapy with immune-related adverse events (irAE) associated with immunotherapy.

In 2008, Wrammert and colleagues[2] first reported the rise and fall of plasmablasts, also called antibody-secreting cells (ASCs), in response to influenza vaccination in blood samples obtained prior to vaccination, at 1 week post-vaccination (peak of plasmablast response) and 1 month later (peak of antibody response). Since then, studies have shown that plasmablast and cT_{FH} cell frequencies concurrently peaked at 1 week, and correlated with antibody and memory B cell responses following influenza vaccination[3–5]. The role of cT_{FH} cells is also evident in influenza virus infection[6] and more recently, SARS-CoV-2 infection and/or mRNA vaccination[7–10]. Herati and colleagues[1] have now used the influenza vaccination model with similar blood sampling timepoints in two independent anti-PD-1 immunotherapy cohorts, including patients with renal cell or urothelial carcinoma (cohort 1 includes cancer patients with and without anti-PD-1 treatment) and melanoma patients (cohort 2 also includes cancer patients with anti-PD-1 treatment and healthy adults for comparative analyses).

Activated memory $CXCR5^+$ cT_{FH} cells are typically characterized by their co-expression of ICOS and PD-1. Since PD-1 expression is lacking in anti-PD-1-treated patients, activated cT_{FH} cell populations were characterized by their co-expression of ICOS and CD38; $ICOS^+PD\text{-}1^+$ and $ICOS^+CD38^+$ cT_{FH} subsets have been used interchangeably with similar outcomes in the previous infection and/or vaccination studies mentioned above. Herati and colleagues showed that patients with anti-PD-1 immunotherapy had higher seroconversion and numerically higher fold-change increases in $ICOS^+CD38^+$ cT_{FH} responses at 1 week post-vaccination compared to patients not treated with anti-PD-1 or to healthy control adults. Robust cT_{FH} responses in anti-PD-1-treated patients coincided with numerical increases in plasmablasts as well as plasma concentrations of CXCL13, a biomarker of GC activity in lymphoid tissue (Fig. 1). However, functional analyses of the antibody response revealed qualitative impairments in anti-PD-1-treated patients, with lower affinity and less galactosylated/sialylated hemagglutinin-specific immunoglobulin G1 (IgG1) antibodies found at baseline compared to healthy controls. Fc glycosylation events are of importance as they can regulate antibody function.

Herati and colleagues very clearly and elegantly delved further to transcriptionally profile cT_{FH} cell and ASC populations in healthy adults and anti-PD-1-treated patients using bulk mRNA sequencing methods. Gene ontology and gene set enrichment analyses of the rich dataset revealed upregulation of proliferation and cell cycle genes in cT_{FH} cells and ASCs at 1 week after influenza vaccination in anti-PD-1-treated patients compared to healthy controls, which aligned with the numerical fold-increases by flow cytometry. However, genes associated with leukocyte activation and cytokine signaling pathways were downregulated in anti-PD-1-treated patients but were enriched in the healthy adults, perhaps representing an "exhausted" phenotype similarly observed in $CD8^+$ T cells of cancer patients on anti-PD-1 immunotherapy. Therefore, it seems that PD-1 blockade may actually dysregulate cT_{FH} cell and humoral responses by unblocking their proliferative response, while dampening the overall immune response such as downregulating key cT_{FH} cytokine circuits to adequately facilitate humoral responses.

Defining cellular or molecular biomarkers for predicting better or worse outcomes in clinical settings has been the holy grail for physicians, immunologists, and the like. One major drawback of PD-1 checkpoint blockade in cancer immunotherapy is the development of irAE, which leads to morbidity and discontinuation of therapy. Differentially expressed genes were indeed already identified in $ICOS^+CD38^+$ cT_{FH} cells at baseline between anti-PD-1-treated patients who developed irAE and non-irAE patients[1]. Baseline cT_{FH} cells from irAE patients were more activated and proliferative but were blunted in cytokine signaling pathways compared to non-irAE patients, with higher cell surface ICOS and aglycosylated immunoglobulin G4 (aIgG4) median fluorescence intensity levels, suggesting that a higher cT_{FH} activation state was associated with the development of irAE. However, since only half of irAE patients had higher fold-change increases in $ICOS^+CD38^+$ cT_{FH} cells at 1 week following influenza vaccination, questions remain about whether the other ~50% of patients were transcriptionally similar to non-irAE patients on anti-PD-1 immunotherapy, although the numbers are too small for such comparisons. Therefore, these observations need to be confirmed in larger cohorts and future studies. But for now, these highly activated cT_{FH} cell populations in the context of influenza vaccination serve as a very promising tool for predicting development of irAE in cancer immunotherapy patients.

Circulating $CXCR5^+$ T_{FH} cells can be further characterized by expression of CXCR3 and/or CCR6 to define type 1 $cT_{FH}1$ ($CXCR3^+$), type 2 $cT_{FH}2$ ($CXCR3^-CCR6^-$) and interleukin-17-expressing $cT_{FH}17$ ($CCR6^+$) subsets. Influenza vaccination, typically with protein-based formulations, and influenza virus infection can elicit more robust $cT_{FH}1$ cell responses compared to $cT_{FH}2$ and $cT_{FH}17$ subsets[3,5,6]. Enrichment of SARS-CoV-2-specific $cT_{FH}1$ responses over $cT_{FH}2$ and $cT_{FH}17$ have also been observed following SARS-CoV-2 infection and mRNA vaccination[9], as well as following COVID-19 vaccination with a protein-based Spike-clamp vaccine candidate from an earlier phase I clinical trial[11]. Tucked away nicely in Extended Data Fig. 1, Herati and colleagues showed that at 1 week after influenza vaccination, frequencies of $CXCR3^+ICOS^+CD38^+$ $cT_{FH}1$ cells were equally increased in both healthy adults and anti-PD-1-treated patients, whereas $cT_{FH}2$ and $cT_{FH}17$ frequencies decreased. Numerically, if $ICOS^+CD38^+$ $cT_{FH}1$ cells were compared, would there have been fold-change differences between healthy and anti-PD-1-treated cohorts experiencing irAE or not? If cell numbers and sequencing costs were not an issue in this day and age, it would be tantalizing to further tease out these cT_{FH} cell subsets transcriptionally to see whether the re-wiring effects of anti-PD-1 immunotherapy was preferentially affecting type 1 responses, particularly as the authors report downregulation of more type 1 cytokine signaling circuits in the parent cT_{FH} populations (that is, IFNγ, IL-2/STAT5, TNF/NF-κB, IL-6/JAK/STAT3).

Overall, Herati and colleagues have proven that measuring real-time human immune responses to influenza vaccination remains a highly valuable tool, not only to measure immune protection from severe influenza disease with annual influenza vaccine updates, but also to decipher key mechanisms underlying the re-wiring immunological effects of anti-PD-1 treatment in cancer immunotherapy patients. It remains to be seen whether dysregulation of high activation/proliferation and blunted cytokine pathways on cT_{FH} cells and plasmablasts, and ultimately GC reactions, will persist long-term after anti-PD-1 treatment has ceased. ❐

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Competing interests
The authors declare no competing interests.

NEUROIMMUNOLOGY

Stress and immunity — the circuit makes the difference

Specific brain circuits recruited during stress contribute to differential immune responses and affect how the immune system handles viral and autoimmune challenges.

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For the past two years, the global pandemic has made us extremely aware of how our bodies respond to viral infection. Within this pandemic is another pandemic — a level of heightened stress that is affecting our behaviors, mental health and potentially our immune systems. The intersection between immune and stress systems has been studied extensively, but our understanding of how stress-specific brain circuits affect discrete elements of the immune system, and how this could impact the body's ability to respond to various immune challenges is very limited. A new study by Poller et al.[1] published in *Nature* provides mechanistic insights into how acute stress uses distinct brain circuits to regulate leukocyte dynamics and contribute to differential disease susceptibility in response to either autoimmune challenge or viral infection.

The idea that stress orchestrates the movement of immune cells to peripheral targets has been explored previously[2]. Although key stress hormones such as norepinephrine and glucocorticoids have been implicated in these processes, a direct link between the brain cells that coordinate the neuroendocrine stress response has remained elusive. Poller et al.[1] now provide insights into distinct signaling mechanisms that control the rapid mobilization of neutrophils into the circulation, followed by a slow movement of monocytes and lymphocytes from peripheral organs to the bone marrow after acute stress[1] (Fig. 1). Consistent with previous work[2], the slow transit of monocytes and lymphocytes from peripheral organs into the bone marrow requires the activation of the canonical controllers of the neuroendocrine response to stress, the corticotropin-releasing hormone neurons in the paraventricular nucleus of the hypothalamus (CRH^{PVN}). These cells release CRH to initiate a cascade of peripheral signals that culminate in an increase in circulating glucocorticoids. Poller et al.[1] propose that glucocorticoids act in a leukocyte-autonomous fashion to enhance the function of CXC chemokine receptor 4 (CXCR4). CXCR4 has previously been described as a key player in the homing of cells to the bone marrow. This increase in leukocyte sequestration into the bone marrow has opposing effects on how the body responds to an autoimmune challenge versus a viral challenge.

By subjecting acutely stressed and unstressed mice to experimental autoimmune encephalomyelitis (EAE), Poller et al.[1] show that stressed mice have lower clinical severity scores. These effects, which suggest mice are protected from disease initiation and progression, require the activation of CRH neurons and the actions of circulating corticosterone. Importantly, they showed that mice that lack CRH are more susceptible to EAE. Simply put, acute stress prevents the acquisition of autoimmunity.

The results are quite different when the system is challenged with a virus. Given the pandemic, this is particularly topical, so Poller et al.[1] examined the effects of acute stress on viral infections. In comparison to age- and sex-matched controls exposed to SARS-CoV-2, stressed mice exposed to SARS-CoV-2 had higher viral titers. These effects were also dependent on corticosterone. Furthermore, this attenuation of the response to virus is not specific to SARS-CoV-2, as stress also increases viral titers after exposure to influenza A virus. The main lesson is that acute stress during the early phase of virus exposure impairs host adaptive immunity against infections.

In addition to the movement of monocytes and lymphocytes from organs to bone marrow, the authors provide information about the rapid neutrophilia that is triggered by acute stress. This neutrophilia has been primarily linked to noradrenergic signaling[2], but Poller et al.[1] find that the sympathetic nervous system and specifically, adrenergic signaling does not have a role in stress-induced neutrophilia. Instead, they used optogenetics to reveal a circuit that requires projection neurons in the motor cortex, spinal projections and binding of CXC chemokine ligand 1 (CXCL1) to CXC chemokine receptor 2 (CXCR2) specifically in skeletal muscle. This involvement of descending motor pathways and muscle is very intriguing, and probably a consequence of the initiation of a defensive behavior. Whether other stressors would initiate a similar response is unclear, but two of the key defensive behaviors used by mice (freezing and escape) rely on intense contraction of the skeletal muscle. Whether