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Booki Min, Robert L. Fairchild

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Commentary

Regulatory components of the immune system are critical for preventing unintended activation of immune cells. Failure to prevent this unintended activation raises the risk of developing exaggerated inflammation and autoimmunity. In this issue of the *JCI*, Binger et al. and Hernandez et al. report that salt can play an important role in undermining regulatory mechanisms of the innate and adaptive immune systems. High salt levels interfere with alternative activation of macrophages (M2), which function in attenuating tissue inflammation and promoting wound healing. High salt also impairs Treg function by inducing IFN γ production in these cells. Together, these results provide evidence that environmental signals in the presence of high dietary salt enhance proinflammatory responses by interfering with both innate and adaptive regulatory mechanisms.

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Over-salting ruins the balance of the immune menu

Booki Min and Robert L. Fairchild

Department of Immunology, Cleveland Clinic, Cleveland, Ohio, USA.

Regulatory components of the immune system are critical for preventing unintended activation of immune cells. Failure to prevent this unintended activation raises the risk of developing exaggerated inflammation and autoimmunity. In this issue of the *JCI*, Binger et al. and Hernandez et al. report that salt can play an important role in undermining regulatory mechanisms of the innate and adaptive immune systems. High salt levels interfere with alternative activation of macrophages (M2), which function in attenuating tissue inflammation and promoting wound healing. High salt also impairs Treg function by inducing IFN γ production in these cells. Together, these results provide evidence that environmental signals in the presence of high dietary salt enhance proinflammatory responses by interfering with both innate and adaptive regulatory mechanisms.

A balanced immune response requires precise regulation

One of the primary purposes of the immune system is to recognize and respond to molecular patterns expressed by pathogens. Innate and adaptive immunity must be highly regulated, as responses of increased intensity or prolonged duration may lead to collateral tissue damage and self-reactive responses that initiate autoimmune disease. Many mechanisms have evolved to regulate both the initiation and termination of innate and adaptive immune responses to maintain efficacious removal of the infection followed by resolution of tissue inflammation that avoids responses to self-antigens.

Several factors contribute to the development of a successful immune response to infection. First, the development of the innate and lymphoid cells needed for effective responses to intracellular versus extracellular infection are orchestrated by the inflammatory tissue signals generated by infectious agents within the tissue environment (1). Protective T cell responses are initiated by DCs that emigrate from the infection site to secondary lymphoid organs. These DCs not only present peptide/MHC complexes to trigger naive

T cell activation, but also produce soluble factors programmed by environmental cues delivered to the DC at the inflammatory tissue site. Once the infection is eliminated, T cell responses are attenuated by regulatory mechanisms that include apoptosis of the effector cells due to the absence of antigen and the activities of CD4⁺ Tregs. Macrophages also traffic to sites of infection and tissue inflammation, where these cells undergo functional differentiation dependent on environmental cues, as well as participate in clearance of the infection and subsequent resolution of the inflammation and tissue injury. Collectively, these processes ensure that the effector functions expressed will be those that can most efficiently destroy the cause of the infection and then resolve the inflammation to restore tissue integrity.

A second and distinct level of regulation occurs within the cell upon receptor engagement of ligands and the delivery of cytokine signals, which together promote the development of the innate and lymphoid cells that will most efficiently deal with the infection. Multiple steps occur between the triggering of receptors and the induction of gene expression, and they

include the activation of several kinases and/or phosphatases. Moreover, each of these enzymes has inherent chemical regulatory mechanisms to avoid aberrant triggering of effector functions within tissues. Activation of these intracellular enzymes is often regulated by cations, sequestered within cytoplasmic stores and/or transported into cells through membrane channels, and the availability of these cations is itself regulated.

Salt tips the balance

It is clear that increases in sodium have a detrimental impact on the vasculature, increasing blood pressure and impairing the function of the heart and kidney (2). With increased interest in the impact of environmental factors on the homeostasis and function of the immune system, several recent studies have documented increased inflammatory innate and adaptive immune function following activation in the presence of increased sodium (3, 4). High-salt diets result in interstitial hypertonic Na⁺ accumulation in the skin and muscle that activates tissue-resident macrophages to secrete endothelial growth factor-C, which in turn increases interstitial hypertonic volume retention and blood pressure (4). Within tissue inflammatory sites, elevated sodium also increases the development of proinflammatory M1 macrophages (5). Moreover, increased sodium promotes the development of IL-17-producing CD4⁺ T cells (Th17 cells), which are critical effector cells in response to extracellular bacterial infections but are also important mediators of autoimmune disease (3, 6–8). This increased Th17 response occurs via sodium enhancement of serum/glucocorticoid regulated kinase 1 (SGK1) activation (3). Thus, in addition to increasing blood pressure and the risk of stroke, increased salt intake can undermine the course and balance of the immune response by promoting the development of macrophages and T cells with proinflammatory functions.

In this issue, two articles provide strong evidence that high salt intake

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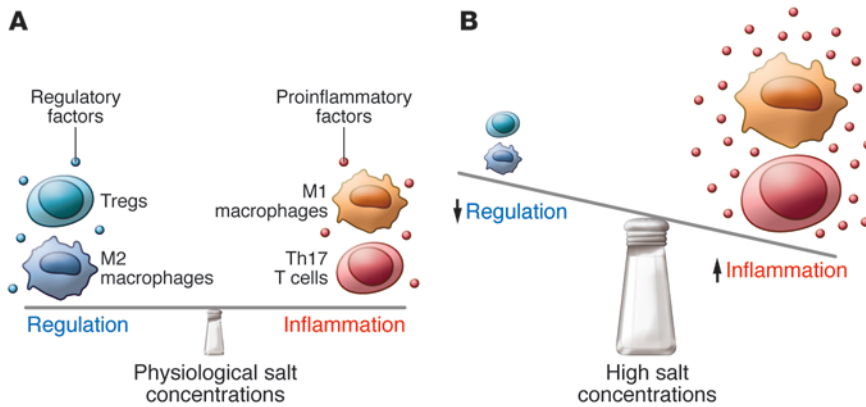


Figure 1. High salt wields a double-edged sword in promoting tissue inflammation. (A) Under physiologic salt conditions, inflammatory immune responses are counterbalanced by regulatory mechanisms, including Tregs and M(IL-4+IL-13), alternatively activated M2-type macrophages. (B) High salt concentration disrupts this balance by both enhancing proinflammatory responses and impairing the regulatory mechanisms.

undermines the function of regulatory mechanisms expressed by components of innate and adaptive immunity. Binger and colleagues (9) report that increased sodium inhibits alternative macrophage differentiation to the M2 or M(IL-4+IL-13) functional phenotype, which contribute to the attenuation of adaptive immune responses and tissue inflammation, thereby promoting wound healing. Binger et al. demonstrated that high salt levels in an alternative activation-associated environment abrogate the ability of macrophages to constrain T cell proliferation in response to polyclonal stimulation. In mice fed a high-salt diet, the development/activation of M(IL-4+IL-13) macrophages following chitin injection was inhibited, and skin wound healing was substantially delayed and incomplete. On the adaptive side of the immune response, Hernandez and colleagues (10) demonstrate that increased sodium abrogates the suppressive function of mouse and human CD4⁺ Tregs. Notably, increased sodium induced Treg production of IFN γ , which appears to underlie the abrogation of Treg function, as neutralization of IFN γ with specific antibody or with specific shRNA restored the regulatory function (10). High salt levels also interfered with the ability of Tregs to attenuate T cell-mediated inflammation in experimental models of graft versus host disease (GvHD) and colitis. Of note, in the GvHD model, host mice were maintained on a high-salt diet during the course of the experiment, while in the col-

itis model, the Tregs were preactivated in high-salt conditions and then transferred with naive T cells to immunodeficient *Rag2*^{-/-} hosts with the absence of the regulation observed by control Tregs apparent by 8–10 weeks after transfer. The absence of regulation during the development of colitis in this experiment strongly suggests that once the Treg function is abrogated by activation in a high-salt environment, the lack of regulatory function is maintained long-term.

High salt dampens regulatory functions

There are two important and common themes conveyed by these reports. First, the effects of high sodium directly abrogate regulatory function in macrophages and Tregs rather than promote alternative differentiation to a proinflammatory functional phenotype. The increased Th17 and M1 macrophage activation previously observed in rodents and humans fed high-sodium diets thus occurs separately from the inhibition of Tregs, macrophages, and the resulting dissolution of mechanisms that constrain immune responses and tissue inflammation. Therefore, the exacerbation of inflammation and autoimmune disease observed in response to high-sodium diets likely arises from a double punch: (i) sodium-induced increases in macrophage and T cell inflammatory functions, and (ii) sodium-induced inhibition of macrophage and T cell differentiation to regulatory and/or neutralization

of regulatory function by the regulatory cells (Figure 1). A second common point in these two reports is that the dissolution of regulatory function in macrophages and Tregs is mediated through sodium-dependent effects on the intracellular enzymatic machinery that direct regulatory function development. In macrophages, high sodium inhibits the mTOR and AKT signaling needed for differentiation to the regulatory/pro-wound healing M(IL-4+IL-13) functional phenotype (9). In Tregs, high salt-induced IFN γ secretion appears to be mediated by SGK1, as shRNA-mediated SGK1 knockdown markedly abrogated IFN γ production and restored Treg suppressive function (10). Whether SGK1 activation is directly responsible for IFN γ production and Treg dysregulation remains to be examined. Utilization of animals with Treg-specific SGK1 deficiency is one such tool to directly test this question. Lastly, whether high salt-induced IFN γ production in Tregs is a direct cause of their dysfunction needs further examination. Previous studies have shown that IFN γ production by Tregs is essential for preventing allogeneic GvHD and virus-induced encephalomyelitis (11, 12). Notably, the high salt levels decrease Treg expression of the genes encoding the two IFN γ receptor chains, raising the possibility that high sodium-induced Treg IFN γ production does not inhibit Treg function via an autocrine mechanism but through IFN γ -mediated signals expressed by other cells in the environment. Alternatively, IFN γ signaling may interfere with mechanisms involved in directing Tregs to target organs in the inflammatory models (13).

Conclusions

In conjunction with previous studies, the current reports demonstrate the powerful effects a high-sodium diet has on promoting the inflammatory potential of innate and adaptive immune responses. Not only does high sodium increase the inflammatory function of macrophages and T cells that are activated in response to infection and/or tissue trauma, but high salt also neutralizes the inherent regulatory mechanisms that have evolved to limit the levels of immune-mediated inflammation and promote resolution of tissue injury. Importantly, the studies by Binger et al.

and Hernandez et al. suggest that reversing the salt-induced abrogation of these regulatory mechanisms does not readily occur once salt is removed. This observation indicates a need to identify strategies that either reverse the impact of high sodium on dysregulated regulatory macrophages and Tregs or rapidly promote the generation of new regulatory cells once more physiological levels of sodium are attained. The results of these studies predict that high-salt diets should accelerate development of autoimmune responses with enhanced intensity in animal models of spontaneous autoimmune inflammation, such as the NOD model of type 1 diabetes. As mice and humans with absent or defective Tregs develop autoimmune manifestations, particularly in the endocrine glands and in the gastrointestinal tract, WT animals placed on continual high-salt diets should recapitulate these autoimmune pathologies. Together, the studies by Binger et al. and Hernandez et al. imply that individ-

uals exposed to high sodium levels may be highly prone to chronic problems with wound healing and autoimmune diseases.

Acknowledgments

Address correspondence to: Robert L. Fairchild, NB3-59, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, Ohio, 44195, USA. Phone: 216.444.3146; E-mail: fairchr@ccf.org.

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