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RESEARCH HIGHLIGHT

Sodium-activated macrophages: the salt mine expands

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High sodium consumption has been raising interest as a putative environmental factor linking Western lifestyle to the growing epidemic of autoimmune and inflammatory diseases. Now Zhang and colleagues show that high sodium drives macrophage to acquire a new proinflammatory effector phenotype with a distinct signature, paving the path to assess the role of salt-activated macrophages in human disease.

In contemporary Western societies, medical advances and improvement in living conditions have dramatically lowered the burden of infectious diseases idling the immune system, previously engaged with responding to a wide variety of pathogens. This has led to the "hygiene hypothesis" ascribing a pathogenic role of dysregulated immune responses to a growing number of diseases "of the rich world" [1]. These include not only autoimmune diseases but also obesity and cardiovascular diseases, displaying elevated pro-inflammatory mediators and tissue infiltration of leukocytes of the innate and adaptive immune arms [2]. These observations have prompted the search for links between lifestyle changes in the Western world and immune dysfunction.

The increase in consumption of processed food rich in sugar, fat and salt has recently raised interest as a putative risk factor for autoimmunity. Two reports identified remarkable effects of slightly high salt concentrations on boosting T cell inflammatory responses [3, 4]. In both studies, feeding mice a high-salt diet exacerbated the clinical severity of the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, a chronic autoimmune disease of the central nervous system. Upon exposure to elevated sodium concentrations *in vitro*, CD4 T cells displayed a significant increase in the production of pro-inflammatory cytokines. High-sodium condition positively regulated the p38/MAPK pathway, leading to the activation of the transcription factor NFAT5 and the increased expression of the NFAT5 target gene *SGK-1*, both involved in driving the response to osmotic stress. Remarkably, these reports chose *in vitro* NaCl concentrations comparable to those measured in the intestinal lumen after a meal rich in salt. While these studies demonstrate that high sodium concentrations can induce a pro-inflammatory, pathogenic phenotype in T cells, they do not establish whether the effect on T cells is the only mechanism driving more severe autoimmunity in animals fed a high-salt diet, or whether other immune arms could be involved.

Macrophages are a heterogeneous population with crucial roles in pathogen clearance and tissue repair. While they are directly activated by microbial components as part of the innate immunity, their functions are mediated by cytokines produced by CD4 T cells. In order to understand the pleiotropic functions of macrophages, it is therefore important to characterize the mechanisms by which different stimuli trigger different effector phenotypes. In this regard, a number of observations have linked sodium balance to macrophage function. The long-standing paradigm of direct urinary excretion of excess dietary sodium has been challenged by evidence of periodic sodium tissue storage in both rodents and human. Sodium accumulation in the skin interstitium activated NFAT5 in local macrophages, leading to secretion of mediators acting on the endothelium to reduce hypertonic volume and ensure blood pressure homeostasis [5]. Another report links high-salt diets to organ inflammation through an effect on monocytes, the circulating precursors of macrophages. Short-term increases in salt intake expanded a population of proinflammatory monocytes and favored the formation of monocyte-platelet aggregates [6]. These effects appeared unrelated to variations in blood pressure. It appears therefore of interest to elucidate what activation state and effector functions are acquired by macrophages exposed to high sodium, and whether other stimuli might synergize with sodium to tip the balance between promoting inflammation and ensuring tissue homeostasis.

The study by Zhang and colleagues recently published in *Cell Research* sets out to explore the direct impact of high sodium exposure on macrophage activation and identifies a transcriptional and translational profile distinct from those induced by other stimuli [7]. Moreover, they assess the effect of high-salt diet on pulmonary macrophage activation *in vivo* in a condition of acute pulmonary inflammation. Initially, they treated human monocyte-derived macrophages with an increased sodium concentration comparable to the one used in human T cells [3]. RNAseq and qPCR identified induction of a set of pro-inflammatory genes, including chemokines, cytokines, receptors for pathogen-associated molecules, and chemokine receptors, while genes associated with anti-inflam-

matory and scavenging functions were downregulated. Similar results were obtained in corresponding macrophage population in mice. The activation state induced by sodium, M(Na), appears to be distinct from those induced by LPS, M(LPS), or by the T-cell cytokine IFNγ, M(IFNγ). While there is considerable overlap in the sets of pro-inflammatory genes upregulated in all three states, it is remarkable that LPS also induces a set of anti-inflammatory genes important for self-limitation, while this was not the case for sodium. Moreover, when macrophages were treated with high sodium and LPS, the sodium appeared to potentiate LPS stimulation. The skewing towards a pro-inflammatory phenotype in M(Na) was dependent on cFos, with evidence of a role for both p38 and Erk1/2 kinases in cFos activation and specifically for p38 in potentiation of the LPS effect. Erk1/2 was also associated with repression of anti-inflammatory and pro-endocytic genes in M(Na) through a negative post-translational regulation of STAT6 involving Erk1/2. Finally, the authors assessed whether feeding mice a high-salt diet had an impact on the outcome of acute lung inflammation induced by LPS challenge. In fact, a pathogenic role for monocyte recruitment has been described in acute lung injuries of different etiology [8]. Mice fed a high-salt diet displayed worsened inflammation and edema of the lungs, and increased infiltration of monocytes with higher production of pro-inflammatory cytokines.

In this study, a key mediator of the high-sodium pro-inflammatory effect is p38. Remarkably, the p38 pathway was also implicated in the effect of elevated sodium concentrations in T cells. While the p38 pathway is known for its role in the response to hypertonic stress, in this paper as well as in the previous studies on T cells the high sodium effect is proven to be independent from hypertonicity, as well as the chloride anion, but specific to the sodium cation. Therefore, future work will need to assess what are the sensors of the increase in sodium intracellular concentration in macrophages that signal to the p38 pathway.

As previously discussed, evidence supports a protective role for macrophage sodium buffering that helps maintain extracellular volume and blood pressure homeostasis. Data from the current study, instead, indicate a pathogenic role for macrophages in tissue integrity in the lung when mice are fed a high-salt diet. The authors reconcile these observations by stressing the importance of the synergy between high-salt sensitization and other pro-inflammatory stimuli, the LPS challenge in their model, which was also observed *in vitro* when sodium enhanced the phenotype of M(LPS).

Finally, a recent report suggests that dietary salt could interact with sex and genetics in establishing EAE severity [9]. Moreover, a positive correlation between dietary sodium levels and clinical disease activity in patients affected by multiple sclerosis has been recently described [10]. Higher sodium intake was also found to double the risk of developing rheumatoid arthritis, also an autoimmune disease, specifically among smokers [11]. Thus, there may be clinical links to data from both animal and human systems on the influence of salt on biologic function. This study, the first report of the acquisition of a distinct effector phenotype in macrophages activated with high sodium concentrations, suggests that dietary sodium influences multiple arms of the immune system. Comparing the transcriptional profile of M(Na) with that of pathogenic macrophages from inflammatory diseases for which a role for a high-salt diet has been proposed will allow a better understanding of how excessive sodium intake affects the immune system.

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