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Sodium chloride triggers Th17 mediated autoimmunity

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ABSTRACT

The detrimental effects of a high-salt diet on human health have received much attention in the past few years. While it has been well established that high dietary salt intake is related to cardiovascular diseases, there is growing evidence that excess salt also affects the immune system and might be considered as a risk factor in autoimmune diseases such as multiple sclerosis (MS). Several studies have implicated T helper 17 cells (Th17) in the pathogenesis of MS. We and others recently demonstrated that excessive salt enhances the differentiation of Th17 cells, inducing a highly pathogenic phenotype that aggravates experimental neuroinflammation. Moreover, a diet rich in sodium affects intestinal microbiota alongside increased intestinal Th17 cells, thus linking the detrimental effects of high salt consumption to the gut-immune axis. First human studies revealed an association of increased MS disease activity with elevated sodium chloride consumption, while more recent epidemiology studies in larger cohorts suggest no correlation between salt intake and MS. However, it is known that ordinary urinary sodium analyses and nutritional questionnaires do not necessarily correspond to the actual sodium load and more sophisticated analyses are needed. Moreover, studies revealed that sodium can tem- porarily be stored in the body. This review summarizes recent findings on the impact of salt on the immune system and discusses potential challenges investigating dietary salt intake as a risk factor in MS.

1. Introduction

A vast number of animal and human studies revealed a role for CD4+ T helper 17 (Th17) cells and their downstream pathways as important drivers of autoimmune diseases. Indeed, Th17 cells were already implicated in the development of inflammatory bowel disease (Gálvez, 2014), rheumatoid arthritis (Annunziato et al., 2009; Azizi et al., 2013), psoriasis (Annunziato et al., 2009) and also multiple sclerosis (MS). First studies using the murine experimental autoimmune encephalomyelitis (EAE) model revealed that interleukin (IL) 23 defi- cient mice are protected from the disease (Cua et al., 2003). Thus, IL-23 directly acts on T cells, promoting the induction of Th17 cells (Aggarwal et al., 2003). In accordance, the transfer of myelin antigen- specific Th17 cells was very potent in inducing EAE in naive recipient mice (Jäger et al., 2009; Langrish et al., 2005). Strikingly, Th17 cells

most frequently occur in the intestine, where they help to protect against extracellular pathogens and maintain the barrier function of the gut (Conti et al., 2009). Yet, depending on the modulating factors they are exposed to, Th17 cells can also have pro-inflammatory properties, characterized by the up-regulation of IL-23 receptor (IL-23R) and an increased secretion of IL-17A, granulocyte-macrophage colony-stimu- lating factor (GM-CSF), tumor necrosis factor (TNF) α and IL-2 (Ghoreschi et al., 2010). Dietary components might represent such modulating factors, implicating the diet as a potential risk factor in autoimmune diseases. The excess consumption of salt (sodium chloride) has repeatedly been associated with an increased risk of developing high blood pressure (Mozaffarian et al., 2014), disturbances in normal kidney function (Fellner et al., 2014), cancer (D'Elia et al., 2014) and chronic inflammation (Sundström et al., 2015). Moreover, there is growing evidence that excess salt affects the immune system, and first studies assume that high salt enhances MS disease activity (Farez et al., 2015). It is thus not surprising that salt restriction is widely viewed as an essential part of a healthy life style. The latest World Health Orga- nization guidelines recommend adults to consume less than 5 g salt or 2 g sodium per day (Guideline: Sodium Intake for Adults and Children, 2012). However, the estimated average salt intake is about two to three times higher as the recommended maximum level (Brown et al., 2009), rendering it a potential risk factor in various diseases, including MS.

2. High-salt effects on immune cells

Several immune cell subtypes are potentially important in med- iating the detrimental effects of excess sodium chloride. First studies investigated a possible effect of high salt concentrations on the innate immune system. While dendritic cells do not respond to excess sodium chloride in vitro and in vivo (Jörg et al., 2016), monocytes acquire a highly pro-inflammatory state (Shapiro and Dinarello, 1995). RNA analyses revealed the induction of a set of pro-inflammatory genes, including chemokines, cytokines, and chemokine receptors, while genes associated with anti-inflammatory functions were down-regulated. More precisely, high-salt activated pro-inflammatory M1 macrophages (Müller et al., 2013; Zhang et al., 2015) while it decreased the ability of regulatory M2 macrophages to suppress effector T cell proliferation (Binger et al., 2015). Moreover, high salt had direct stimulatory effects on the migration of macrophages (Müller et al., 2013). Mechanistically, high salt-induced pro-inflammatory macrophage activation required p38/MAPK and downstream signaling (Jantsch et al., 2015), whereas the blockade of regulatory macrophage activation depended on the impairment of the protein kinase AKT and the mechanistic target of rapamycin (mTOR) (Binger et al., 2015). Further studies revealed that high salt-induced pathways also play an important role in T cell im- munology. Already two decades ago, it was shown that increasing so- dium chloride conditions by approximately 40 mM boosted IL-2 ex- pression and T cell proliferation (Coimbra et al., 1995; Junger et al., 1994). Moreover, excess salt does not only favor T cell proliferation in general, but also affects the polarization of T cells: High sodium con- centrations increased the differentiation of murine and human Th17 cells and induced a highly pathogenic phenotype, characterized by an

increased expression of the surface receptors IL-23R and chemokine receptor CCR6 and the upregulation of GM-CSF, IL-2 and TNFα (Kleinewietfeld et al., 2013; Wu et al., 2013). The osmoprotective transcription factor tonicity enhancer binding protein (TonEBP/NFAT5) mediated the pro-inflammatory response by signaling through the serum/glucocorticoid-regulated kinase 1 (SGK1). Other studies also implicated SGK1 in sodium homeostasis and revealed its expression on a wide array of immune cells, including Th17 cells (Wu et al., 2013). Indeed, most studies discussing the mechanism for the detrimental ef- fect of high salt demonstrated a pivotal involvement of pathogenic Th17 cells. In a murine model of colitis, high dietary sodium increased the level of Th17 cells in the gut and exacerbated colitis (Tubbs et al., 2017; Wei et al., 2017). Moreover, high sodium chloride intake en- hanced disease severity in mouse models of lupus nephritis by the in- duction of Th17 and Th1 cells (Yang et al., 2015). In the same study, high salt concentrations up-regulated Th17 cells in CD4+ T cells iso- lated from systemic lupus erythematosus patients in an SGK1 depen- dent manner, indicating that high salt may also affect lupus er- ythematosus in humans (Yang et al., 2015). Immunologic effects of high salt consumption have also been linked to transplantation (Safa et al., 2015): Feeding a high-salt diet in a mouse model of solid organ trans- plantation accelerated cardiac allograft rejection without affecting blood pressure or serum sodium concentrations. The accelerated re- jection was associated with a reduction of regulatory T cells (Tregs) and a significant decrease of Treg proliferation, resulting in an increase of CD4+ T cells in mice fed a high-salt diet (Safa et al., 2015). Accord- ingly, increased sodium chloride concentrations also impaired murine and human Treg cell function in vitro and in vivo (Hernandez et al., 2015). High-salt induced a pro-inflammatory Treg phenotype asso- ciated with a heightened interferon (IFN)-γ secretion and a diminished suppressive capacity (Hernandez et al., 2015). These studies provide evidence that, in addition to the increased induction of pro- inflammatory Th17 cells, excess dietary sodium intake can impact au- toimmunity by inhibiting the function of Tregs. Since it is well accepted that the autoimmune basis of MS stems from an imbalance between pro- inflammatory Th1 and Th17 cells and anti-inflammatory Tregs (Viglietta et al., 2004), salt may be added to the list of risk factors af- fecting the disease. Indeed, studies in EAE as an animal model of MS, showed that a high-salt diet augmented disease onset and severity, which was accompanied by an increased infiltration of pathogenic Th17 cells in the spinal cord (Hammer et al., 2017; Jörg et al., 2016; Kleinewietfeld et al., 2013; Wu et al., 2013).

3. High-salt affects the gut microbiome

In vitro studies investigating the effect of high-salt on various im- mune cells used comparable sodium chloride concentrations to those measured in the intestinal lumen after ingesting a high-salt diet. Moreover, before being absorbed and distributed to tissues, sodium chloride passes the gastrointestinal tract (Jose et al., 2016). Indeed, studies in models of inflammatory bowel disease imply the gut as re- levant organ mediating effects of salt on the immune system (Monteleone et al., 2017; Wei et al., 2017). It was shown that a high- salt diet increased the frequency of IL-17A producing cells in the in- testinal lamina propria compared to mice on a

normal salt diet (Wei et al., 2017). Moreover, ingestion of a high-salt diet significantly in- hibited the secretion of IL-10 and the suppressive function of Tregs in the small intestine, while not affecting the percentage of Treg cells (Wei et al., 2017). These results were also corroborated in human studies where exposure of human intestinal mononuclear cells to high sodium chloride concentrations enhanced the production of IL-17A, IL-23R, TNFα and RORγt (Monteleone et al., 2017). While not expecting a direct correlation between the gut and brain autoimmunity, the potential importance of the gut microbiome for the development of MS and EAE has recently been recognized (Berer et al., 2011; Jangi et al., 2016; Ochoa-Repáraz et al., 2009; Yokote et al., 2008). MS patients display an altered microbiome composition com- pared to healthy controls. Interestingly, gut microbes rapidly respond to fluctuations in dietary composition (David et al., 2014). It is thus likely that dietary factors could lead to alterations of the gut microbiome. Furthermore, diet-induced shifts in microbiome composition can have profound effects on the host immune system (Haghikia et al., 2015; Turnbaugh et al., 2006), including T cells (Arpaia et al., 2013; Monteleone et al., 2017; Wei et al., 2017). Th17 cells are particularly affected by the abundance of specific commensal bacteria (Ivanov et al., 2009). Thus, the gut and the gut microbiota represent potential targets linking high salt intake, Th17 cell induction and MS. Indeed, we re- cently aimed to unravel a link between high-salt intake, gut microbiome composition and Th17 induced central nervous system autoimmunity (Wilck et al., 2017). High-salt diet significantly increased fecal sodium concentrations, what was paralleled by alterations in the fecal meta- bolome and gut microbiome composition. Several intestinal bacteria were affected by the high-salt concentrations; particularly a Lactoba- cillus strain was suppressed. We further hypothesized that the depletion of Lactobacillus by high salt might be linked to the observed induction of intestinal and systemic Th17 cells. Indeed, treatment with Lactobacillus prevented the salt-induced increase of Th17 cells, thereby preventing the exacerbation of EAE. Mice on a high-salt diet concomitantly re- ceiving Lactobacillus displayed less severe EAE symptoms, with a re- duced infiltration of Th17 cells in the spinal cord and a decreased production of IL-17A in the intestine and spleen. Interestingly, high salt induced increase of Th17 cells was paralleled by reduced concentra- tions of the fecal tryptophan metabolite indole-3-lactic acid (ILA), a bacterial fermentation product produced by Lactobacilli (Zelante et al., 2013) that has been implicated in the suppression of central nervous system autoimmunity (Rothhammer et al., 2016). Finally, we could show that ILA inhibited Th17 cell polarization in vitro. ILA may thus link the high-salt diet induced suppression of Lactobacillus to the induction of Th17 cells. Indicating a potential relevance of these find- ings in humans, we showed that a salt challenge in healthy humans affects the abundance of intestinal Lactobacillus, alongside increased frequencies of pro-inflammatory Th17 cells in the blood. Yet, it is un- clear whether Lactobacillus mediated high-salt effects can be transferred from the EAE model to MS. These limitations may also extend to the recent controversy on more general effects of a high-salt diet in neu-roinflammation.

4. Linking excess salt to human disease

Recently published studies investigating high dietary salt intake as a potential risk factor in MS yielded controversial results. In an ob- servational study of two independent cohorts of 70 MS patients, high dietary salt intake resulted in higher relapse rates as well as increased numbers of new MRI lesions compared to patients with moderate dietary salt intake (Farez et al., 2015). Accordingly, high salt has been implicated to influence human immune responses in general. Long-time salt intake positively correlated with the amount of monocytes and was paralleled by a significant increase of IL-6 and IL-23 production while the secretion of IL-10 was decreased (Yi et al., 2015). Intriguingly, IL-6 and IL-23 are major inducers of Th17 cells, also in humans. Although T cell numbers in the blood did not correlate with the amount of dietary salt consumed, plasma levels of IL-17 decreased with a reduction in dietary salt consumption (Yi et al., 2015). The results from a short-duration study on changes in dietary salt intake in humans were largely consistent with these findings reporting salt-related monocyte varia- tions (Zhou et al., 2013). In contrast to this positive correlation between excess salt consumption and MS, two studies of pediatric MS revealed no association between high dietary sodium intake and the disease (McDonald et al., 2016; Nourbakhsh et al., 2016). The investigators correlated an estimated sodium intake with time to relapse (Nourbakhsh et al., 2016) or an increased risk of developing MS (McDonald et al., 2016) and found no association. However, in both studies, sodium intake was estimated using a basal food frequency questionnaire (FFQ), which may be unreliable and underestimate the daily sodium intake (Shim et al., 2014). Furthermore, the questionnaire was administered only at baseline and thus could not account for changes in diet during the period of investigation. General limitations of FFQs might be the long period of investigation of more than 12 months and the missing consideration of salt intake from „dining out “ and takeaway food. It would thus be necessary to establish a good quality FFQ with shorter duration of less than 12 months and the in- clusion of regional dietary habits.

Further controversial findings regarding the risk of high salt intake and MS come from novel epidemiological studies investigating the ef- fect of high salt intake on adult MS disease course or activity (Cortese et al., 2017; Fitzgerald et al., 2017). Large-scale epidemiologic studies based on the Nurses' Health Study and Nurses' Health Study II did not show any correlation between salt intake and the risk of MS (Cortese et al., 2017). Sodium intake was assessed from food frequency ques-tionnaires at baseline and every 4 years during follow-up. The study thus relies on a large dataset. However, as already mentioned above, food frequency questionnaires represent a retrospective method that relies upon the respondent's memory. Participants were asked for their average portion size and the frequency of intake of foods covering the main dietary sodium sources. Total sodium intake was therefore only calculated by summing the sodium content of ingested foods. It is thus likely that the estimated sodium intake by food questionnaires is not consistent with the actual total sodium intake, especially in the face of “hidden sodium” in processed food. Furthermore, another study also found no association between excess salt and MS. Based on multiple assessments of sodium in spot urine samples and standardized clinical and multi-resonance imaging (MRI) follow-up, Fitzgerald and

collea- gues suggested that salt intake does not influence the conversion from clinically isolated syndrome to MS (Fitzgerald et al., 2017). 465
participants in the BENEFIT (Betaferon/Betaseron in Newly Emerging Multiple Sclerosis for Initial Treatment) study provided 14 spot-urine samples over 5 years that were used to estimate the 24-h urinary so- dium excretion with the mathematical 'Tanaka equation'. However, spot urine collections may not represent a valuable tool to assess total salt intake (Dougher et al., 2016; Zhou et al., 2017). Comparing the known 24-h sodium excretion and the estimated from morning spot urine collections by 'Tanaka equation' revealed that sodium excretion was about 3 g different from those measured using 24-h sodium urine collections (Zhou et al., 2017). Moreover, every second accurately collected 24-h sodium excretion sample fails to detect a 3-g difference in sodium intake. In line with data from earlier dietary intervention studies (Luft et al., 1982) and more recently published clinical trials (Dougher et al., 2016; Lerchl et al., 2015; Zhou et al., 2017), these findings suggest that multiple 24-h collections are necessary to assess salt intake and single spot urine collections may thus not be a valuable tool. Strikingly, there is a considerable day-to-day variation in 24-h sodium excretion, even when dietary salt intake is known and fixed over several weeks or month (Rakova et al., 2013). Rather, it was shown that an equilibration between sodium intake and sodium ex- cretion follows weekly or even monthly rhythms of accumulated and secreted sodium independent of dietary salt intake (Rakova et al., 2013). Moreover, the prevailing notion that excess dietary sodium leads to direct urinary excretion has been challenged by evidence of periodic sodium tissue storage in both rodents and human. Already 4 decades ago, Ivanova et al. reported that huge amounts of sodium can be stored in the body (Ivanova et al., 1978). This finding has recently been confirmed by others. Long-term studies showed that large amounts of sodium accumulate without commensurate changes in body weight, indicating a sodium storage without water retention (Heer et al., 2009; Rakova et al., 2013; Titze et al., 2002). Novel strategies to determine the body's sodium content revealed a sodium accumulation in the skin interstitium and the muscle, where sodium binds to negatively charged glycosaminoglycans (Titze et al., 2004; Titze et al., 2003). Initial studies in humans, measuring the sodium content by 23Na-multi resonance imaging (23Na-MRI), confirmed this specific accumulation in the skin (Kopp et al., 2013; Linz et al., 2015). While the mechanisms that drive dietary sodium accumulation in the skin remain elusive, it is known that the clearance is regulated by the immune system. Sodium accu- mulation in the skin interstitium activates NFAT5 in macrophages, leading to the secretion of mediators acting on the local lymphatic system (Machnik et al., 2009). Upon chemotactic stimuli, macrophages infiltrate the skin, where they secrete a soluble growth factor for lym- phatic vessels, vascular endothelial growth factor-c (VEGF-C), leading to VEGFR-3 induced cutaneous lymph capillary hyperplasia (Joukov et al., 1996; Karkkainen et al., 2004). Disturbances in this mechanism have already been linked to hypertension and elevated blood pressure (Machnik et al., 2010). Whether increased salt concentrations in the skin are directly linked to central nervous system autoimmunity re- mains to be elucidated. Considering the salt-effects on macrophage immunology during MS and the persisting challenge to accurately de- termine the salt intake via salt excretion, the

skin may represent an important organ for further investigations on salt and MS risk. In summary, current standard methods may be inappropriate to estimate sodium intake precisely. It is thus important to develop novel measures or more defined FFQs or a combination of both to better account for the above mentioned limitations.

5. Conclusion

A major challenge for all studies addressing the effect of dietary habits in MS is that it is not possible to account for all potential con- founders: sodium intake may be linked to healthy or unhealthy food that may exaggerate the effect of sodium intake. In fact, processed foods or fast foods are one of the main sources of salt, containing up to 100- times more sodium than homemade meals (Brown et al., 2009). Besides salt, fast food contains high amounts of fat, and it was recently shown that both ingredients together might represent a risk factor in neu- roinflammation (Hammer et al., 2017). While no definite association between dietary restrictions and the modulation of autoimmune dis- eases have been established yet, a large proportion of patients already consider special diets as alternative therapeutic options. In general, the goal of a nutritional intervention in MS must be the control of in- flammation. In part, this may be achieved by modulating the gut mi- crobiome composition and the systemic immune responses.

Observations in animal models of MS, colitis, lupus, transplantation and others argue for a potential role of salt as a risk factor in diseases associated with a Treg an Th17 cell imbalance. Yet, human studies are still sparse or have a limited technical approach to accurately define the association between excess salt intake and MS severity. It is thus ne- cessary to establish new methods to accurately measure the sodium intake and storage in the body. Alternative techniques such as direct non- invasive measurements of tissue sodium content in humans by 23Na-MRI may provide more concise information on the relation be- tween salt and disease.

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