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The role of salt for immune cell function and disease

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Abbreviations

BMDM	-	Bone marrow-derived macrophage
BMI	-	Body mass index
CNS	-	Central nervous system
DC	-	Dendritic cell
EAE	-	Experimental autoimmune encephalitis
FoxP3	-	Forkhead box protein
GF	-	Germ-free
GM-CSF	-	Granulocyte macrophage-colony stimulating factor
GvHD	-	Graft versus host disease
HSD	-	High-salt diet
ILC2	-	Group 2 innate lymphoid cell
LCFA	-	Long-chain fatty acids
LP	-	Lamina propria
LPS	-	Lipopolysaccharide

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MS	-	Multiple Sclerosis
mTOR	-	Mammalian target of rapamycin
NaCl	-	Sodium chloride
NFAT	-	Nuclear factor of activated T cells
SCFA	-	Short-chain fatty acids
SGK	-	Serum glucocorticoid-regulated kinase
Tfh	-	Follicular follicular helper T cells
T_H1	-	T helper cell 1
T_H2	-	T helper cell 2
T_H17	-	T helper cell 17
TNF-α	-	Tumor necrosis factor-α
Treg	-	Regulatory T cell

Abstract

The immune system evolved to protect organisms from invading pathogens. A network of pro- and anti-inflammatory cell types equipped with special effector molecules guarantees efficient elimination of intruders like viruses and bacteria. However, imbalances can lead to an exceeding response of effector cells incurring autoimmune or allergic diseases. An interplay of genetic and environmental factors contributes to autoimmune diseases and recent studies provided evidence for an impact of dietary habits on the immune status and related disorders. Western societies underwent a change in lifestyle associated with changes in food consumption. Salt (sodium chloride) is one component prevalent in processed food frequently consumed in western countries. Here we summarize recent advances in understanding the mechanisms behind the effects of sodium chloride on immune cells like regulatory T cells (Tregs) and T helper (T_H) 17 cells and its implication as a risk factor for several diseases.

Introduction

Innate and adaptive immune cells are armed with a diverse arsenal of effector molecules that provide effective defence mechanism against pathogens. T cells can provide host protection via soluble mediators including cytokines. Interferon (IFN)-γ producing T helper (T_H) 1 cells contribute to the elimination of intracellular bacteria and viruses (1). In contrast T_H2 cells are characterized by interleukin (IL)-4, IL-5 and IL-13 production and play important roles to combat extracellular parasites (2). However, T_H2 cells are also associated with allergic disorders (2). IL-17 producing T helper cells have been identified as a distinct lineage and subsequently referred as T_H17 cells (3, 4). T_H17 cells provide protection from fungal infection and extracellular bacteria (5). However, exacerbating T_H17 responses are also associated with autoimmune diseases (5). Altogether, T helper cells provide essential effector molecules to fight pathogens but can also contribute to immune pathology. $CD25^+$ $FoxP3^+$ regulatory T cells (Tregs) are important modulators of immune reactions due to their capacity to suppress effector cells (6). The absence of regulatory T cells, either through deletion of the transcription factor Forkhead box protein 3 (FoxP3) or antibody mediated depletion in mice, results in severe autoimmunity. Further, mice with a mutation in the *FoxP3* gene (Scurfy mice) develop autoimmune syndroms (7). In humans, mutations in the *FoxP3* gene manifest as IPEX syndrome (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) (8, 9). Tregs are constitutively produced in the thymus and referred as natural Tregs (nTregs) (10). However, it has been demonstrated that Tregs can also be induced in peripheral tissues e.g. by TGF-β and Retinoic acid mediated FoxP3 upregulation, thus these Tregs were subsequently named induced Tregs (iTregs) (10). Furthermore, other $FoxP3^-$ $CD4^+$ T cells with suppressive potential have been identified. For instance, the so called Type 1 regulatory T (Tr1) cells can produce large amounts of the suppressive cytokine IL-10 (11, 12). The equilibrium between effector cells and Tregs is important to maintain immunity without harmful side effects. Especially at protective barriers, like the skin, the mucosal tissue of the lung and the gastrointestinal tract, a balance between tolerance to harmless environmental antigens and immunity to pathogens needs to be maintained.

A large fraction of innate and adaptive immune cells resides in the lamina propria (LP) of the small intestine. Hence, it is of interest how these cells are influenced by dietary components and the gut microbiota. The gut microbiome comprises a total of $>10^{14}$ microorganisms composed of >1000 species of bacteria and fungi (13-15). These microorganisms are supportive in dietary food digestion. The gut microbiota and its metabolites have a profound impact on the local and systemic immune system (16, 17). Insights in how the gut microbiota shapes the immune system were derived from studies in germ free (GF) mice. GF mice have reduced numbers of T_H17 cells in the lamina propria of the small intestine (18). Furthermore, GF mice are protected from experimental autoimmune encephalomyelitis (EAE) a mouse model for multiple sclerosis (MS) (19). In addition, it has been observed that the suppressive capacity of Tregs isolated from GF mice is impaired (20). A critical role for the gut microbiota on Treg modulation is further underlined by the fact that the transfer of a single polysaccharide derived from *Bacteroides fragilis* was sufficient to ameliorate disease score in mice suffering from EAE due to induction of IL-10 producing Tregs (21). In line with this notion, GF mice reconstituted with a specific mixture of bacteria showed ameliorated colitis scores attributed to Treg induction by specific members of the group Clostridium (22). In contrast, reconstitution of GF mice with segmented filamentous bacteria (SFB) promote T_H17 cell development that protects against infection by pathogenic *Citrobacter rodentium* (18). A critical contribution of the microbiota to autoimmune diseases was recently underpinned by the observation that transfer of MS patients microbiota into mice could modulate EAE. GF mice reconstituted with MS patient microbiota developed more severe EAE attributed to a diminished generation of IL-10 producing regulatory T cells compared to GF mice reconstituted with microbiota derived from healthy controls (23). A finding shared by another study using GM free RR mice as recipients of MS patient microbiota. In this model, relapsing-remitting (RR) mice develop spontaneous EAE due to the expression of a transgenic MOG-specific T cell receptor. Interestingly, transfer of MS patient microbiota accelerated spontaneous EAE development in these mice as well (24).

The composition of the gut microbiota is highly influenced by the diet. Human studies have shown that the microbiota rapidly adapts to short-term dietary changes towards plant-based or animal-based nutrition (25). Specific fiber-rich diets are usually associated with enhanced generation of short chain fatty acids (SCFA), metabolized by gut commensals, that have been shown to promote immune tolerance (26, 27). For instance, feeding mice with SCFA ameliorates EAE symptoms accompanied by an expansion of Tregs and a decline of T_H17 cell numbers in the small intestine, the spleen and the CNS. In contrast, long chain fatty acids (LCFA) promote intestinal and systemic T_H17 cell induction leading to more severe disease score (26). Of note, a recent study has shown that dietary salt could affect the gut microbiota as well. Enhanced sodium chloride consumption depletes particularly *Lactobacillus murinus* in line with enhanced EAE development and induction of pathogenic T_H17 cells in mice. Interestingly, oral gavage of *L. murinus* protected high-salt diet (HSD) fed mice from exacerbated EAE, attributed to diminished T_H17 responses. This effect was suspected to be correlated to the ability of *L. murinus* to produce certain indole-metabolites that had the capability to block murine T_H17 differentiation *in vitro* (28).

Intestinal T_H17 cells are enabled to enter distant organs as recently demonstrated with the kaede reporter mouse (29, 30). Photoconversion of the kaede protein from green to a red fluorescence upon violet light exposure of the colon, allows tracking of intestinal cells by flow cytometry. Of note, kaede-red T_H17 cells were found in the spleens of arthritis mice indicating recruitment of gut-derived T_H17 cells during extraintestinal autoimmune reactions (29). A similar approach using kaede mice revealed that intestinal imprinted T_H17 cells egress the gut in a S1P-receptor-1 dependent manner and migrate to kidneys via Chemokine (C-C motif) ligand Ccl20 / chemokine receptor Ccr6 interactions. In the kidneys these gut derived T_H17 cells promote glomerulonephritis an autoimmune inflammation of the kidneys (30). In summary, the gut-immune cell axis seems to regulate systemic immune balance (31), hence it is important to know the impact of dietary habits on it. In western countries a trend toward consumption of processed food enriched in salt has been observed.

In this review we discuss how salt as one component of typical western diets shapes the gut-immune cell axis and may thereby increases the risk for many diseases.

Effects of salt on innate and adaptive immune cells

The effects of salt on immune cells were studied *in vitro* by enriching the cell culture medium with additional 40mM NaCl. This mirrors the excess concentration detected in the interstitial fluidics of rats fed a HSD compared to rats on control diet (32). *In vitro* differentiation of naive T cells towards T_H17 cells was strongly enhanced under high-salt conditions. In line with that, high-salt induces a particularly pathogenic phenotype of T_H17 cells manifested by enhanced TNF-α and GM-CSF production, as shown for both murine and human T cells (33, 34), enhanced expression of CCR6 and the receptor for IL-23 (IL-23R). Application of small molecule inhibitors as well as short-hairpin RNA (shRNA) mediated gene silencing, revealed the involvement of p38 mitogen-activated protein kinase (MAPK), nuclear factor of activated T cells (NFAT)-5 and serum glucocorticoid-regulated kinase-1 (SGK-1) (33, 34). SGK-1 is upregulated in salt exposed naïve T cells and mediates Forkhead box protein O (FOXO)-1 phosphorylation that leads to retinoic acid related orphan receptor (ROR)-γt release from the complex with Foxo1 and subsequent ROR-γτ-dependent IL-23R expression. In line herewith, sodium effects on *in vitro* T_H17 cultures were more pronounced when IL-23 was supplied (34). It has previously been shown that IL-23 augments the pathogenicity of T_H17 cells (35, 36). Tregs are also sensitive to sodium chloride. It was demonstrated that *in vitro* exposure of human peripheral blood Tregs to increased sodium concentrations reduces their suppressive capacity, along with higher expression of pro-inflammatory cytokines. More specifically, salt-cultered Tregs particularly produce higher amounts of IFN-γ and antibody-mediated neutralization of IFN-γ restores the suppressive activity of Tregs in co-cultures with effector T cells. This was further confirmed by Treg specific IFN-γ gene silencing using lentiviral transduced shRNA (37). The effect is dependent on SGK-1; similarly to T_H17 cells,

salt-exposed human Tregs upregulate SGK-1 and show encanced phosphorylation of FOXO proteins. Silencing of *SGK1* expression in Tregs by lentiviruses containing *SGK1* targeting shRNA restores the suppressive capacity under high-salt conditions in line with reduced IFN-γ production (37). Besides its direct effects on T cells, high-salt can also alter the function of macrophages. *In vitro* experiments with murine bone marrow-derived macrophages (BMDM) and human monocyte-derived macrophages have shown that salt increased the expression of pro-inflammatory genes while decreasing anti-inflammatory markers introducing the concept of M(Na) macrophages (38). Furthermore, salt influences the polarization of murine and human macrophages *in vitro*, promoting pro-inflammatory LPS/IFN-γ induced M1-type macrophages while suppressing the function of IL-4-polarized regulatory M2-type macrophages (38-41). M1-type macrophages produce higher amounts of the pro-inflammatory cytokines IL-6, IL-8, TNF-α when differentiated under high-salt and are more efficient in bacterial clearance (38-40). In line with this, the anti-leishmania activity of M1-type macrophages is increased under excessive sodium conditions, attributed to elevated production of nitric oxid (40). In contrast, M2-type macrophage effector molecules like Fizz-1, Ym-1, Arg-1 and PD-L2 are suppressed under high-salt (38, 41). M2-type macrophages can also suppress T cell proliferation, a function that is abrogated under high-salt conditions (41). Sodium chloride may also acts as chemoattractant for macrophages. Murine macrophages are attracted by a 40mM excess NaCl concentration in *in vitro* transwell assays. Interestingly, this effect was less pronounced with 80mM excess NaCl concentration (42). Sgk-1 is required for NaCl mediated effects on T cell polarization as shown by T cell specific deletion of the *Sgk1* gene in mice (34). However, it seems to be dispensable for mediating high-salt effects on LPS stimulated macrophages, since application of a Sgk-1 specific inhibitor does not block the salt effects in BMDM (39). On the other hand, silencing or overexpression experiments for *Nfat5* revealed a critical role for this transcription factor as mediator of the salt response of murine macrophages (40). Recently, it was shown that bone marrow-derived dendritic cells react to enhanced sodium concentration in a Sgk-1- and p38-MAPK-dependent manner resulting in elevated IL-23 secretion (43). In summary, high-salt

conditions could particularly affect the functionality of macrophages, dendritic cells, T_H17 cells and Tregs (Table1).

Salt mediated immune cell modulation and its role in disease

Hypertension

To decipher the effects of enhanced dietary salt *in vivo,* animals are fed with a diet supplemented with 4% or higher NaCl compared to control mice that reveive 0,5% NaCl containing food, a classical protocol used for salt-induced hypertension since decades. The first evidence for the involvement of immune cells in Na^+ sensing *in vivo* was derived from feeding rats on high-salt diet. It was shown that excessive Na^+ accumulates in the skin where it activates macrophages to release nitric oxid and vascular endothelial growth factor (VEGF)-C (44). Depletion of macrophages impairs clearing mechanism of excessive Na^+ leading to hyperplasia of lymph capillaries and increased blood pressure (hypertension) (44-46). Interestingly, there is accumulating evidence that the adaptive immune system plays a critical role in hypertension as well (47). Mice with a deficient B cell and T cell compartment (*Rag*$^{-/-}$) are resistant to angiotensin II-induced hypertension. Only T cell transfer into *Rag*$^{-/-}$ mice induces hypertension symptoms as observed in wild type controls (48). Recently, it was demonstrated that mice with $CD4^+$ T cell restricted deficiency in *Sgk1* are resistant to angiotensin II-induced hypertension and vascular inflammation (49). Similar experiments in *Il17a* deficient mice suggest involvement of T_H17 cells in promoting hypertension (50). IL-17 and IFN-γ were found to be elevated in patients with symptoms of coronary artery atherosclerosis, supporting a role for T cells as mediators of vessel damage in humans (51).

Autoimmune diseases

Experimental animal studies have provided strong evidence that excessive dietary sodium could also influence induction and severity of several autoimmune disease models for e.g. colitis, systemic lupus erythematosus (SLE) and multiple sclerosis (MS). Indeed, lupus prone MRL/lpr mice, a mouse model for SLE, develop severe disease on HSD accompanied by

elevated T_H17 responses and increased serum titers of autoantibodies (52). Furthermore, PBMCs from SLE patients cultured *ex vivo* under high-salt conditions tend to develop more T_H17 cells compared to healthy controls. The observation that HSD deteriorates lupus symptoms was confirmed by others in the same mouse model. However, this study attributes the disease outcome to an increase in the frequency of follicular helper T cells (Tfh) promoting autoantibody production by B cells. Additionally, increased numbers of $CD4^+$ T cells with a signature of Tfh cells, expressing the programmed death receptor (PD)-1 and the chemokine receptor CXCR5, were found in human PBMC cultures when supplied with excessive sodium chloride. The hydroxyltransferase TET2 was shown to mediate NaCl induced Tfh differentiation via hypomethylation of the *spn* and *STAT5b* gene (53).

In line with this, it has been demonstrated that HSD exacerbates colitis in mice, an autoimmune inflammation of the gastrointestinal tract that can also occur in humans. This is associated with an elevated T_H17 response in the LP (43, 54, 55). Additionally, HSD induces LP innate lymphoid cells (ILCs) to produce IL-17 while small intestinal Tregs produce less IL-10 and are impaired in their suppressive activity (55). Accordingly, co-transfer of *in vitro* NaCl pre-treated Tregs and effector T cells leads to accelerated colitis in $Rag^{-/-}$ recipients (37). The inflammatory response in the colitis model is dependent on GM-CSF sensing eosinophils (56). Thus, it is possible that salt incensed T_H17 cells are the source of increased GM-CSF, however, this still needs to be experimentally proven. Disturbances of the gut-immune axis could be responsible for neuroinflammatory diseases. Hence, the hypothesis that dietary salt might have an impact on EAE progress has been tested experimentally in mice. Indeed, several studies have shown that HSD fed mice succumb to more severe EAE (33, 34, 39, 57-59). This is caused by a more pronounced CNS infiltration of pathogenic T_H17 cells (33, 34). Moreover, mice with $CD4^+$ T cell specific deletion of the *Sgk-1* gene is protected from HSD induced EAE exacerbation. These mice failed to mount a similar T_H17 response as wild type controls under HSD. The important role for T_H17 cells was further corroborated with mice genetically manipulated to deplete *Sgk-1* exclusively in IL-17 producing cells ($IL\text{-}17f^{Cre}$ $Sgk\text{-}1^{fl/fl}$). These mice were protected from severe EAE

compared to control mice (34). The observation that T_H17 cells produce GM-CSF, when differentiated *in vitro* under high-salt, argues for a role of this cytokine in exacerbating EAE. Supporting evidence for this hypothesis derives from the observation that GM-CSF activated microglia cells promote EAE development (60). This is further corroborated by the finding that mice with a deletion of the GM-CSF receptor in monocytes developed a less severe EAE phenotype (61). Moreover, high-salt diet affects monocyte recruitment and activation toward an pro-inflammatory phenotype in the CNS of EAE mice, supporting a role for the monocyte/macrophage lineage in committing augmented EAE scores in HSD mice. In agreement with that, transfer of BMDM pre-treated with 40mM NaCl in EAE mice exacerbated disease severity (39). Studies in humans also suggest a role for myeloid cells as possible mediators of MS disease severity induced by excessive salt intake (62, 63). Volunteers were subjected to constant dietary salt intake ranging from either 6,9 up to 12 gramm per day (g/d) for several months. Monocyte counts in the blood peaked after the 12g/d interval and decreased to normal levels with dietary salt adjustment to 6 g/d. Similarly, highest serum concentrations of the pro-inflammatory cytokines IL-6 and IL-23 were measured at the end of the 12g/d intervall. In line with this, serum IL-17 concentration was elevated in the 12g/d group (63). In summary, these studies in mice and humans suggest dietary salt as an environmental risk factor for autoimmune diseases (Figure1).

Transplantation

Deleterious effects of excessive salt intake were also observed in a murine allogeneic transplantation model. Cardiac transplants were faster rejected in MHC class II-mismatched reciepients kept under HSD compared to control feeding. Immune tolerance to transplants is normally committed by functional Tregs. However, Treg numbers were reduced in allograft draining lymph nodes of HSD fed recipients. Interestingly, transplant tolerance was improved in HSD fed mice with a *Sgk-1* gene-deficient $CD4^+$ T cell compartment including Tregs (64). The *in vivo* relevance for impaired function of Tregs under high-salt was further demonstrated in a xenogeneic graft-versus-host disease (xGvHD) model. In this model

human PBMCs depleted of Tregs are transferred in immune-deficient *NOD-scid IL-2Rg*^{null} mice where they cause severe host tissue destruction as evident by body weight loss. Hosts that receive human PBMCs and Tregs showed ameliorated disease score under normal diet. The suppressive effect of co-transferred Tregs was abrogated when recipient mice were kept on a high-salt diet. These mice showed severe disease score similar to control mice that received Treg depleted PBMCs, indicating that sodium diminishes the suppressive capacity of human Tregs *in vivo* (37).

Infection

Sodium-induced amplification of effector cell activation has been proved to be a critical factor in pathogen clearance in infectious diseases. It has been demonstrated that excessive sodium triggers inflammasome activation in macrophages *in vitro,* initiating a caspase-1 dependent release of pro-inflammatory cytokines. Mice with a targeted deletion of the *caspase-1* gene were compared to wild-type mice after LPS/OVA immunization under HSD. *Caspase-1* deficient mice failed to mount a similar OVA-specific T_H17 response as wild-type mice. Therefore sodium might act as an additional stimuli to amplify Toll-like receptor signalling induced by pathogen associated moleculare patterns (PAMPs) (60). Accordingly, independent research data proved that HSD mice controlled *Leishmania major* infection more efficiently than control diet fed mice. This study further demonstrates that *L. major* infection leads to an accumulation of Na^+ in infected skin lesions. The local Na^+ accumulation enhances M1 macrophage activation and promoted parasite clearance (40). In contrast, skin wound repair responses are delayed under HSD that is associated with decreased M2-type macrophage activation at wound sites. NaCl excess leads to epigenetic modifications at the transcription start sites of M2-type macrophage prototype genes. It has been observed that H3K4me3 marks are reduced under high-salt conditions, suppressing IL-4/IL-13 induced M2 gene expression. Moreover, high-salt promotes metabolic reprogramming of M2-type macrophages manifested by reduced glycolysis and mitochondrial metabolic output (41).

Obesity & Cancer

Obesity rates have increased in the last decades in developed countries worldwide (65). Obesity is a consequence of high caloric diet intake, enriched in sugar and saturated fatty acids (SFA), that causes diseases like metabolic syndrome characterised by insulin resistance and hypertension (66). Salt intake has been determined as a risk factor for obesity in humans (67, 68). It has been shown that elevated dietary sodium intake correlates with a high body mass index (BMI) and waist circumference (67). This was supported by the observation that patients with metabolic syndrome tend to have a higher salt intake compared to healthy controls (66). Furthermore, studies in rats have shown that HSD feeding promotes an increase of adipose tissue mass (69). Metabolic homeostasis is regulated by an immune cell circuitry involving Tregs and cells associated with a type 2 immune response e.g. M2-type macrophages, eosinophils and ILC2 cells (70-75). Interestingly, adiposity is accompanied by a decline and phenotypical changes of these cells in fat tissue (70, 72, 76-79). Obesity is associated with a shift toward a pro-inflammatory environment indicated by the dominance of M1-type macrophages (76, 78). Mice fed a HSD were shown to have higher serum levels of the pro-inflammatory cytokines TNF-α and IL-6 (80). Additionally, HFD fed mice also showed a bias to develop T_H17 cells that manifests in more severe EAE (81). Thus, it is tempting to speculate that high-salt intake could further enhance the inflammatory environment and subsequently worsens obesity and associated diseases. However, this has to be analysed in detail in future studies.

Obesity also correlates with the development of cancer (82). People with a high BMI have a 1,5 to 1,6-fold higher risk to die from cancer (83). This was further confirmed in animal studies. Sodium chloride was shown to be a risk factor for colonic cancer development (84, 85). Gerbils that were fed a HSD succumbed to *Helicobacter pylori* induced Gastric Carcinogenesis. The authors found higher levels of *Helicobacter pylori* pro-cancerous factor cagA in HSD mice (86). Moreover, feeding mice on HSD results in pronounced gastric colonization with *Helicobacter pylori* along with alterations in mucus production favouring a pro-carcinogenic environment (87). Additive effects of dietary salt on tumor growth were also

described for chemically induced tumors in gerbils (88). The findings that HSD favors tumor growth are surprising, since sodium chloride increases the pathogenicity of helper T cells as well as Tregs, and given that T cells contribute to tumor rejection by production of cytokines e.g. TNF-α and IFN-γ (89, 90).

Allergic Diseases

Allergic asthma is another example of an imbalanced immune system with high incidence in the western world that incurs high costs for health insurances (91). It has been shown that western diet rather accelerates allergic diseases, whereas a Mediterranean diet has a protective effect (92, 93). Allergies are driven by an amplified type 2 immune response including T_H2 cells, ILC2s, Immunoglobulin (Ig) E producing B cells, eosinophils and basophils and M2-type macrophages. It was shown that T cells are the critical source of the disease promoting IL-4/13 in the Alum/OVA mouse model of asthma (94). T cell-derived IL-4/13 is further non-redundant in protecting mice from fatal schistosomiasis (95). However, a type 2 response can also have a protective effect ameliorating autoimmunity symptoms (96). Research data related to the effect of salt on type 2 responses are limited. A recently published study shows that T_H2 cell differentiation is impaired *in vitro* under high-salt conditions (57). However, *in vivo* data are not available to our knowledge yet.

Conclusions

Recent research gave insights on how diet may influence the immune system. A deeper understanding of how single dietary components affect subtypes of immune cells is the milestone for developing dietary based therapies to efficiently treat diseases. Salt, a component enriched in the western diet turned out to be harmful in several aspects. We now know that salt has an impact on the function of immune cells with negative effects in onset and progression of diseases like hypertension, obesity, cancer, metabolic syndrome and autoimmune diseases (Figure1). Animal studies support a role for dietary salt intake as risk factor for EAE. Human studies linking dietary salt intake to MS are contradictory. It was

shown that MS patients with the highest salt intake have 3,95 fold higher disease scores and a 3,4 fold increased risk to develop new MS lesions (97). However, other studies failed to link dietary salt intake to increased risk of MS (98-100). Conclusions derived from questionaries regarding the dietary habits of MS patients compared to healthy controls. Furthemore, Na^+ excretion was measured from 12-16 spot urine samples over 5 years to monitor dietary sodium intake. Recent data from long-term studies with volunteers subjected to constant sodium intake have shown that urine Na^+ concentrations oscillates between day to day measurements (101, 102). Studies using multi resonance imaging (MRI) revealed the skin interstitium and muscles as a Na^+ depot (40, 103-105). Hence, the methods to be applied for precisely measuring sodium intake are under debate. Moreover, the dogma that high sodium intake is balanced by enhanced fluidic intake as counterbalance mechanism has been challenged (106, 107). It has been demonstrated that high-salt feeding incurs metabolic reprogramming in liver, muscle and blood vessels tissue representing a complex mechanism to produce osmolytes for body water conservation (107). Immune cells are equipped with molecular mechanism to sense increasing salt concentrations *in vitro,* leading to cell type specific alterations of effector functions (Table1). Most of the *in vivo* studies linking high-salt intake to immune cell mediated diseases focused on T_H17 dependent models. We further need to acquire more knowledge of the effects of salt on immune reactions during infections. HSD has an advantegous effects in containing *L. major* spread attributed to an amplified M1-type macrophage activation (Figure1 & Table1). T_H1 responses against viral infection have not been studied under high-salt conditions to our knowledge yet.

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Table 1: Effects of sodium chloride on different immune cells

celltype	*effector function under high-salt*	*molecular mechanism*	*reference*
M1-type macrophage	⇧	p38MAPK, NFAT-5	(38, 40)
M2-type macrophage	⇩	mTOR, Akt	(41)
Treg	⇩	Sgk-1	(37)
T_H17	⇧	p38MAPK, NFAT-5, Sgk-1	(33, 34)
T_H2	⇩	N.D.	(57)
T_H1	⇧	N.D.	(33)
Tfh	⇧	TET2	(53)
DC	⇧	p38MAPK, Sgk-1	(43)

N.D. not determined

Figure legend

Figure1: Effects of increased sodium chloride consumption on health. High-salt diet (HSD) is considered a risk factor for increased blood pressure, atherosclerosis (blood vessel damage), obesity, cancer, and autoimmunity, while it could negatively impact wound healing processes. In contrast, HSD could enhance protection against parasite infections.