EMERGING EVIDENCE OF AN EFFECT OF SALT ON INNATE AND ADAPTIVE IMMUNITY

Authors: Rhys D R Evans[1*], Marilina Antonelou[1], Scott Henderson[1], Stephen B Walsh[1], Alan D Salama[1]

Affiliation: [1]University College London (UCL) Department of Renal Medicine, United Kingdom

*__Corresponding author__:
Rhys D R Evans
UCL Department of Renal Medicine
Royal Free Hospital
Pond Street
London
NW3 2QG
Rhys.evans@ucl.ac.uk

Keywords: inflammation; glomerulonephritis; transplantation; T cell; macrophage

ABSTRACT

Salt intake as part of a western diet currently exceeds recommended limits, and the small amount found in the natural diet enjoyed by our Paleolithic ancestors. Excess salt is associated with the development of hypertension and cardiovascular disease, but other adverse effects of excess salt intake are beginning to be recognised, including the development of autoimmune and inflammatory disease. Over the last decade, there has been an increasing body of evidence, which demonstrates that salt affects multiple components of both the innate and adaptive immune systems. In this review, we outline the recent laboratory, animal and human data, which highlight the effect of salt on immunity, with a particular focus on the relevance to inflammatory kidney disease.

INTRODUCTION

Salt (sodium chloride; NaCl) has been a prominent feature of the human diet for a relatively short time, at least in evolutionary terms. Our ancestors survived for millions of years on a diet that contained less than 1g salt per day. Salt is required for the maintenance of extracellular, and therefore plasma volume, and given the limited salt composition of this natural diet, humans evolved under an intense evolutionary pressure for the selection of salt-conserving genes. Salt was discovered as a way of preserving food around 5000 years ago, and since then salt intake has increased rapidly to current estimates of 8g per day in the United Kingdom, with even greater intake in other parts of the world[1]. Most of this salt is found in processed foods and restaurant meals; the remaining salt is added whilst cooking and eating. Current salt intake as part of a western diet is therefore far in excess of the amount with which we are physiologically programmed to handle.

Given our ability to retain salt, in steady-state the minimum amount required to replace losses is less than 0.5g per day. There is good evidence from both observational and interventional studies that excess salt intake is associated with the development of hypertension[2,3], it is generally accepted that excess salt intake is linked to the development of cardiovascular disease[4], and there is some evidence for a role in the progression of chronic kidney disease (CKD)[5]. Based on this, the World Health Organisation recommends limiting salt intake to less than 5g per day, which it estimates could prevent 2.5 million deaths globally each year.

There are, however, perhaps less well appreciated adverse effects of excess salt intake, which are becoming apparent. These include the development of malignancy, in particular gastric cancer[6], obesity, and the metabolic syndrome[7]. Excess salt is also implicated in autoimmune and inflammatory disease. Observational studies have demonstrated that increased salt intake is associated with the development of rheumatoid arthritis[8], and correlates with disease severity in multiple sclerosis[9]. Furthermore, in renal transplantation, peri-operative salt loading worsens short-term outcomes[10], and adherence to a low salt diet improves renal outcomes and survival in the longer term[11]. In this review, we outline the recent data from laboratory, animal, and human studies that demonstrate an effect of salt on both innate and adaptive immunity, and postulate how these findings may be relevant in inflammatory kidney disease.

SALT AND INNATE IMMUNITY

Initial evidence of an effect of salt on macrophages

The mechanism by which excess salt causes hypertension is unclear, and cannot be fully explained by an effect on extracellular volume[12]. Over the last decade, however, two concepts have been described which not only provide novel insights in to the pathogenesis of hypertension, but also provide the initial evidence of salt impacting immune cells. Firstly, it has been shown, largely through the work of Titze and colleagues, that salt loading results in sodium storage at interstitial sites within muscle and skin[13,14]. This results in sodium concentrations in interstitial compartments (up to 250mM) in excess of that found in plasma, where sodium is tightly regulated due to its effect on osmolality. Secondly, is the increasing awareness of a role for inflammation in the development of hypertension.

A link between both these concepts was demonstrated in a seminal paper in 2009 which demonstrated a high salt diet in rats results in elevated skin sodium concentrations, which in turn activate resident macrophages, which through production of vascular endothelial growth factor C (VEGF-C), clear this stored sodium through the lymphatic system[13]. Macrophage depletion promoted skin sodium storage and the development of hypertension. Hence, salt loading leads to storage at interstitial sites, with NaCl concentrations in excess of plasma, resulting in immune cell activation and salt clearance via the lymphatic system. Either excess salt, or impaired immune-mediated salt clearance, can lead to hypertension.

Further studies on the effect of salt on macrophage activation and its relevance in the clearance of cutaneous infection

Following this initial work, there have been several other studies investigating how salt affects macrophage activation. Like other cells of the immune system, macrophages display a spectrum of activation states with plasticity along this spectrum. At one end, are pro-inflammatory M1 macrophages, which secrete inflammatory cytokines IL-1, IL-6, IL-12, and TNF-α and activate effector T cells (Teff), and at the other end are reparative M2 macrophages which secrete anti-inflammatory cytokines IL10 and TGF-β, with reduction in Teff and increase in regulatory T cell (Treg) activity[15]. M2 macrophage activity is important in wound healing, and experimentally, they have been shown to reduce the development of autoimmune disease[16,17].

Salt has been shown to have a pro-inflammatory effect on macrophages, increasing M1 activation and reducing M2 activation (**Figure 1**)[18,19]. In cell culture, the addition of 40mM NaCl increases M1 macrophage activation and this is dependent on a signaling pathway involving p38-mitogen activated protein kinase (p38-MAPK) and the transcription factor nuclear factor of activated T-cells 5 (NFAT5; also known as tonicity-responsive enhancer binding protein, TonEBP)[18]. Sodium accumulates at sites of skin infection in mice and in humans, adding NaCl to cell cultures *in vitro* led to increased M1 clearance of infection, and a high salt diet in animals resulted in enhanced clearance of cutaneous leishmaniasis. By contrast, M2 activation is blunted *in vitro* in high salt culture conditions, and a high salt diet is associated with reduced wound healing and reduced M2 activity in a mouse model of peritonitis[19]. Unlike the salt effect on M1 macrophages, and indeed that on Th17 cells, which is dependent on NFAT5, the inhibitory salt effect on M2 macrophages is dependent on reduction in signaling through a pathway involving protein kinase B (PKB, also known as Akt) and the mammalian target of rapamycin (mTOR).

Taken together, these data on macrophage activation states and high interstitial NaCl concentrations provide an insight in to the potential evolutionary reasons for why the immune system responds to salt. Infection is associated with the creation of localised hypersalinity, which allows for local activation of pro-inflammatory cells whilst limiting such activation in the body more generally. A similar high salt environment is created at sites of body-environmental interface (e.g. skin, kidney/genitourinary tract) providing protection from pathogens at these locations.

The effect of salt on renal mononuclear phagocytes and the development of pyelonephritis

Further evidence for this role of hypersalinity in providing protection from infection was provided in a recent study, which assessed the role of interstitial sodium concentrations on tissue resident mononuclear phagocytes (MNPs) in the kidney[20]. Tissue MNPs include distinct subsets of macrophages and dendritic cells. *Berry et al* demonstrated that CD14+ MNPs, a subset of MNPs with enhanced phagocytic capability, were enriched in the medulla compared to the cortex of human kidneys; this positioning was dependent on NFAT5 mediated production of the chemokines CCL2 and CX3CL1 by renal tubular epithelial cells in response to hypersalinity. Medullary CD14+ MNPs demonstrated increased phagocytosis of uropathogenic E.Coli, and phagocytic activity increased with increasing extracellular sodium concentrations in an NFAT5 dependent manner. The induction of nephrogenic diabetes insipidus (NDI) resulted in increased bacteraemia and death following intravesical uropathogenic E.Coli challenge in mice; this was interpreted as being due to disruption of the medullary renal sodium gradient. Moreover, NDI in patients, either in the setting of tolvaptan use, or as a result of sickle cell disease, is associated with increased urinary tract infection risk[20]. Taken together, these data demonstrate that high interstitial salt concentrations in the kidney, primarily required for water reabsorption and the maintenance of extracellular volume, also promote medullary localisation of specific subsets of MNPs, which in turn provide protection from infection. It is tempting to speculate that autoimmune kidney disease might in part arise due to the hypertonic environment found in the kidneys, created through evolution for these volume and infectious purposes.

SALT AND ADAPTIVE IMMUNITY

CD4+ T cell polarization and the imbalance of Th17 cells and Treg in inflammatory kidney disease

Antigen is presented to naïve CD4+ T cells on MHC class II (MHC-II). This is done by a select group of antigen presenting cells, including dendritic cells, macrophages, and B cells. After antigen presentation, naïve CD4+ T cells become activated and polarize towards one of a number of CD4+ T cell subsets, including Th1, Th2, Th17, and Treg cells, each of which has different effects on the immune response, in part through the cell-specific cytokines produced.

Th17 cells are pro-inflammatory, and provide protection against extracellular bacterial and fungal infections, particularly at epithelial cell surfaces[21]. Polarisation requires stimulation by IL-1β, IL-6, TGF-β, while full maturation requires IL-21 and IL-23. Th17 cells secrete various cytokines including IL-17, which acts on stromal cells and is important for neutrophil recruitment, and others such as IL-26 and IL-22, which act on non-immune cells, enhancing inflammation or serving to attenuate damage, depending on the tissue and environment. Tregs have opposing effects to Th17 cells. Naturally occurring and peripherally induced Tregs are characterized by expression of the transcription factor Forkhead Box P3 (FOXP3), and provide peripheral tolerance through inhibiting the function of effector CD4+ and CD8+ T cells, B cells, and cells of the innate immune system, through cell-cell contact mechanisms and release of suppressive cytokines (e.g. TGF- β, IL-10)[22].

Th17 cells and Tregs both play a major role in autoimmune kidney disease and renal transplant rejection. Th17 cells are implicated in the development of both glomerular and tubulointerstitial disease in patients and animal models including ANCA-associated glomerulonephritis[23,24], Lupus Nephritis[25], IgA Nephropathy[26], and Primary Sjögren's Syndrome associated tubulointerstitial nephritis[27]. Conversely, Treg activity limits inflammatory glomerular disease and autoimmune disease relapses, both clinically and in experimental models[28]. Furthermore, it is becoming increasingly clear that excessive Th17 and reduced Treg activity are important components in the development of acute and chronic allograft rejection[29]. Hence, excess Th17 activation and reduced Treg activity underlie a number of inflammatory kidney diseases in both the native and transplant setting. This augmented-Th17 and attenuated-Treg phenomenon is the same effect that salt has on these CD4+ T cell subtypes, as we describe below.

The effect of salt on Th17 cells and the development of multiple sclerosis

Two studies published in Nature in 2013 provided the initial evidence of an effect of salt on Th17 polarization[30,31]. *Kleinewietfeld et al* isolated naïve CD4+ T cells from healthy volunteers, and cultured these for 7 days in optimal Th17 polarising conditions in standard media and in media supplemented with varying concentrations of NaCl (0-80mM). There was a dose dependent increase in Th17 polarisation with salt up to 40mM NaCl, which provided optimal polarization and cell survival. The authors concluded on the basis of control experiments in which media was supplemented with sodium gluconate, magnesium chloride, and mannitol, that sodium, as opposed to chloride or tonicity, was mediating this pro-inflammatory effect. Further *in vitro* work showed that salt stimulation affected naïve, rather than memory CD4+ T cells, did not influence Th1 and Th2 polarisation, and not only polarized Th17 cells but also promoted a pathogenic Th17 phenotype (increased expression of IL-2, TNF-α, IL-9, and CSF2/GM-CSF). It was subsequently shown that the intracellular pathway mediating this response to hypersalinity involved up-regulation of phosphorylated p38-MAPK, NFAT5, and serum- and glucocorticoid-regulated kinase 1 (SGK1) (**Figure 2**). Knocking down any of these molecules resulted in a sharp reduction in salt mediated Th17 polarisation. The authors then investigated the salt effect on Th17 cells *in vivo*, in an animal model of multiple sclerosis (experimental autoimmune encephalitis; EAE), a Th17-mediated disease. A high salt diet exacerbated disease severity, and this was associated with increased Th17 cells in the central nervous system (CNS). The use of a p38-MAPK inhibitor abrogated both salt-induced Th17 CNS infiltration, and clinical markers of disease severity.

Wu et al focused further on the role of SGK1 in salt mediated Th17 polarisation. They described the pathway downstream of SGK1, which involves phosphorylation of FOXO1 and upregulation of the main Th17 transcription factor, RAR-related orphan receptor gamma T (RORγT; **Figure 2**). They also confirmed the polarising effect of salt on Th17 cells *in vitro*, and demonstrated this Th17 polarisation was prevented in SGK1 knock out cells. They showed that a high salt diet in mice is associated with increased gut Th17 cell frequency, confirmed that a high salt diet exacerbates EAE, and showed that this was prevented in SGK1 knock out mice. Interestingly, since this initial work, the same group has demonstrated a pivotal role of SGK1 in mediating the balance between Th17 and Treg in isotonic conditions[32]. Taken together, these initial studies demonstrate that Th17 cell polarisation is enhanced by high salt conditions both *in vitro* and *in vivo*, that this is dependent on signaling through p38-MAPK, NFAT5 and SGK1, and that salt mediated Th17 polarisation exacerbates animal models of autoimmune disease.

The effect of salt on suppressor Treg function and transplant rejection

Given the interrelationship between Th17 cells and Tregs, and the effect of salt on Th17 polarisation, subsequent work investigated whether there was an effect of salt on Treg function, firstly *in vitro*, and then in mouse models of graft versus host disease (GVHD), colitis, and mismatched cardiac transplantation[33,34]. *Hernandez et al* demonstrated that addition of salt to co-culture of Tregs with effector T cells inhibited regulatory cell function in a dose dependent manner over the range 10-40mM NaCl[33]. Further analysis of purified Tregs cultured under high salt conditions demonstrated that the mechanism of this loss of suppressor function was Treg shift to a Th1 phenotype with up-regulation of pro-inflammatory molecules such as IFN-γ; knock-down of IFN-γ resulted in maintenance of suppressor function. The authors demonstrated that the same pathway, increased SGK1 activity and phosphorylation of FOXO1 and FOXO3, that is involved in Th17-Treg balance in isotonic conditions, and in the polarization of Th17 in hypertonic conditions, also mediates the salt effect on Tregs. Inhibition or knock down of SGK1 resulted in recovery of suppressor function; high salt conditions increased phosphorylation of FOXO1 and FOXO3, which resulted in impaired stabilization of the FOXP3 locus. This inhibitory effect of salt on Treg was then confirmed *in vivo*. A high salt diet was associated with increased IFN-γ secreting Tregs in the spleen and mesenteric lymph nodes, and this was associated with increased SGK1 expression. Moreover, a high salt diet increased IFN-γ secreting Tregs and worsened disease in a mouse model of GVHD, while transfer of Tregs cultured under high salt, as opposed to standard conditions, led to exacerbation of disease in an animal model of colitis[33].

Safa et al investigated the effect of high salt on the allo-immune response, using a mouse model of MHC-II mismatched cardiac transplantation, which is dependent on Treg suppressor function for graft survival[34]. Despite having no effect on blood pressure, a high salt diet reduced allograft survival, and this was associated with a reduction in Tregs within the transplant and the spleen. This high salt effect on rejection was abolished in SGK1 knock out mice. Together, these initial Treg studies demonstrate an inhibitory effect of salt on Treg suppressor function, which is mediated through an SGK1-FOXO1/3 pathway. High salt exacerbates animal models of autoimmune disease and worsens murine transplant rejection.

The effect of salt on the development of autoimmune diseases

Subsequent studies have investigated further aspects of the effect of salt on immunity in models of multiple sclerosis (EAE), colitis, lupus nephritis, and also in the setting of the progression of acute kidney injury (AKI) to chronic kidney disease (CKD). The results of this work are summarized below.

- *Animal models of multiple sclerosis*

Wilck et al proposed an alternative indirect *in vivo* mechanism by which a high salt affects Th17 cells and the development of EAE[35]. This was through alteration in the gut microbiome, and provides a link to studies which demonstrate gut derived Th17 cells traffic to, and mediate the development of extra-intestinal disease, including inflammatory kidney disease[36]. Salt loading inhibited the growth of lactobacillus sp.

in vitro, in mice, and in healthy human volunteers. In an EAE model, lactobacillus administration ameliorated Th17 CNS infiltration and the development of disease. The mechanism of the effect on Th17 polarisation was proposed to be due to reduced bacterial production of indole derivatives, which have previously been shown to associate with improvement in EAE. Hence, multiple extracellular cues may affect Th17 polarisation, and several pathways may mediate the pro-inflammatory salt effect *in vivo*.

Jorg et al investigated whether alteration of dendritic cell function occurred in response to salt and mediated the *in vitro* effects of salt on Th17 and the development of EAE[37]. They found no effect on dendritic cell function, suggesting the salt effect on Th17 is a direct effect on Th17 cells themselves. By contrast, *Barbaro et al* demonstrated that high salt conditions increased both macrophage and dendritic cell activation, and that co-culture of salt activated dendritic cells with T cells from salt sensitive mice led to increased pro-inflammatory cytokine production (IL-17 and IFN-γ) from CD4+ and CD8+ cells[38]. The intracellular pathway mediating this effect involved sodium entry in to dendritic cells through amiloride sensitive sodium channels, formation of reactive oxygen species, and the production of isolevuglandin protein adducts, with ultimately enhanced antigen presentation to T cells.

- *Animal models of inflammatory bowel disease*

Several studies have investigated the role of salt on intestinal immunity and the development of colitis, perhaps unsurprising given the abundance of Th17 cells within the gut[33,39,40]. In these studies, salt loading of mice without disease results in increased gut Th17 cells and reduced Treg suppressor function. This is associated with increased SGK1 expression, increased gut permeability and histological features of inflammation. High salt has also been shown to worsen mouse models of inflammatory bowel disease, associated with increased gut IL-17. One study investigated the source of this IL-17 and demonstrated that salt not only affects CD4+ T cells, but also innate lymphoid cells (ILCs), in particular ILC3, the innate lymphoid equivalent of Th17 cells[40]. Indeed, RAG knock out (i.e. lymphocyte deplete) mice still get IL-17 mediated gut inflammation in response to a high salt diet, highlighting that salt promotes IL-17 production from multiple immune cells.

- *Animal models of Systemic Lupus Erythematosus (SLE)*

Elevated salt concentrations *in vitro* have been shown to increase follicular helper T cell (Tfh) polarisation, and a high salt diet worsens lupus features in an animal model[41]. A further study demonstrated that a high salt diet leads to exacerbated experimental lupus nephritis, with more significant disease on histology, increased proteinuria and worse survival[42]. This was associated with increased Th17 and Th1 cells in the spleen, and reduced Tregs. The salt response of isolated CD4+ T cells from patients with SLE was then analysed; Th17 cell polarization increased with anti-CD3 and anti-CD28 stimulation in the presence of NaCl. This, as in healthy volunteers, was abrogated by the addition of an SGK1 inhibitor. Of note, high salt conditions don't promote increased polarization of naïve T cells to Th1 cells *in vitro*, under Th1 polarising conditions[43], but this and other studies have shown a high salt diet does promote a Th1 phenotype *in vivo*[42,43]. This may reflect CD4+ T cell plasticity, with Th17 cells or Tregs developing a pro-inflammatory Th1 phenotype with expression of IFN-γ in response to high salt conditions.

- *Animal models of acute kidney injury*

Acute Kidney Injury (AKI), particularly if recurrent, can lead to CKD, and high salt has been shown to increase the progression of AKI to CKD in animal models[44]. The mechanism behind this phenomenon is unclear, but has been proposed to be immune mediated as immunosuppression with mycophenolate mofetil abrogates the salt effect. More recent work has demonstrated that Th17 cells mediate interstitial inflammation in AKI; salt loading worsened Th17 mediated inflammation and this resulted in more significant CKD[45]. This was prevented by angiotensin receptor blockade, which was shown *in vitro* to directly affect Th17 cells.

Human studies of dietary salt modification and their effect on Th17-Treg balance

Given the growing body of laboratory and animal work that have demonstrated a polarising effect of salt on diverse immune cells (summarised in **Table 1**), using dietary modifications to limit salt intake in the management of patients with autoimmune disease provides an intriguing and potentially beneficial, low-cost, therapeutic avenue. Initial interventional studies have investigated the effect of changing salt intake on the immune response, in healthy volunteers and in patients with autoimmune disease. The results of these studies are outlined in **Table 2.** Together, these demonstrate dietary salt modification may be used to modulate the immune response to varying degrees, but whether this translates to changes in clinical outcomes, is yet to be determined. Moreover, where salt exerts its pro-inflammatory effect on immune cells *in vivo* is unknown.

CONCLUSION

The immune system consists of opposing pro-inflammatory and anti-inflammatory cells. Salt affects the activity of multiple cells of both innate and adaptive immunity, promoting predominantly pro-inflammatory subtypes. *In vitro*, salt increases activation of M1 macrophages and Th17 cells, whereas it suppresses M2 macrophage and Treg function. Intracellular molecules that mediate this effect include NFAT5 and SGK1. The *in vivo* consequences of hypersalinity are increased clearance of infection, but also the development of autoimmune disease. Whether dietary salt manipulation may be used as an adjunctive therapy in inflammatory kidney diseases or transplantation remains to be investigated.

FIGURE LEGENDS

Figure 1: The effect of salt on M1 and M2 macrophage polarisation and the intracellular pathways involved

Figure 2: Intracellular pathways involved in salt induced Th17 polarisation

TABLES

Table 1: Summary of the known effects of salt on immune cell function, the intracellular pathways involved, and the *in vivo* clinical consequences of the high salt effect

Cell type	**Salt Effect**	**Intracellular signaling molecules and pathways mediating salt effect**	**Clinical consequences of high salt effect *in vivo***
		INNATE IMMUNE SYSTEM	
M1 Macrophage[18]	Activation	p38-MAPK, NFAT5	Enhanced clearance of cutaneous infection
M2 Macrophage[19]	Inhibition	Akt, mTOR	Reduced wound healing
Monocytes[46]	Expansion of CD14++CD16+ (intermediate) monocytes	Formation of reactive oxygen species	Renal hypoxia and inflammation
Tissue resident mononuclear phagocytes (MNPs)[20]	Increased phagocytic activity of CD14+ MNPs	NFAT5	Protection against pyelonephritis
Dendritic Cell[38]	Activation	NADPH Oxidase (reactive oxygen species formation), formation of isolevuglandin protein adducts	Hypertension
Neutrophils[40]	Activation as a consequence of increased IL-23 production (direct effect unknown)	Unknown	Exacerbated gut inflammation
Innate lymphoid cells (ILC)[40]	Activation of ILC3	Unknown	Exacerbated gut inflammation
		ADAPTIVE IMMUNE SYSTEM	
Th17[30,31,39,40,42,45]	Activation	p38-MAPK, NFAT5, SGK1, FOXO1	Exacerbated EAE, colitis, and lupus nephritis. Increased progression AKI to CKD.
Treg[33,34]	Inhibition	SGK1, FOXO1/3	Exacerbated transplant rejection, EAE.
Th1[42]	Increased activity *in vivo*, possibly conversion from	N/A	Exacerbated lupus nephritis

	Th17 or Treg (no direct effect *in vitro*)		
Th2	No effect[30]	N/A	N/A
Tfh[41]	Activation	TET2	Exacerbated SLE
CD8+ T cell[38]	Increased IFN-γ and IL-17 from CD8+ cells from salt sensitive mice when cultured with salt primed dendritic cells	Unknown	Unknown
B cells	Unknown	Unknown	Unknown

Table 2: Summary of studies investigating the immunological effects of dietary salt modification in healthy human volunteers and in patients with autoimmune disease

Study Reference	**Subjects included**	**Dietary Modification**	**Main results**
Zhou et al, 2013[46]	Healthy volunteers (n=20)	3-day run in on normal diet, then fixed daily salt intake of 15g (high salt) for 7 days, then 5g (low salt) for 7 days. Food provided to subjects.	Increase in CD14++CD16+ (intermediate) monocytes; decrease in CD14++CD16- (classical) and CD14+CD16++ (non-classical) monocytes with high salt intake.
Yi et al, 2015[47] *(part of Mars flight simulation study)*	Healthy volunteers (n=6)	Fixed daily salt intake of 12g, 9g, then 6g for 1-2 months, then reverted back to 12g for 1 month. Food provided to subjects.	Serum cytokines analyzed after each intervention period: increased pro-inflammatory cytokines (IL-6 and IL-23) and reduced anti-inflammatory cytokines (IL-10) with high salt intake
Luo et al, 2016[48]	Healthy volunteers (n=15)	3-day run in on normal diet, then fixed daily salt intake of 15g (high salt) for 7 days, then 5g (low salt) for 7 days. Food provided to subjects.	T cell subset analysis: increased Th17 and reduced Treg populations with high salt intake. Associated with increased expression of NFAT5 and SGK1.
Wen et al, 2016[49]	Healthy volunteers (n=49)	Fixed daily salt intake of 3g (low salt) for 7 days, then 7 days high salt (18g), then 7 days high salt (18g) plus potassium supplementation (4.5g KCl; 60mmol K)	Serum IL17 and PBMC IL17 mRNA increased with high salt. Associated with increased SGK1. High salt effect abolished with potassium supplementation of diet.

Scrivo et al, 2017[50]	Patients with rheumatoid arthritis (RA; n=15) and SLE (n=15)	Dietary counseling used to limit daily salt intake to 5g for 3 weeks. Then reverted back to standard diet for 2 weeks. 24-hour urine Na measurement used to assess adherence to diet.	RA patients: no effect in Th17 or Treg subsets. SLE patients: increase in Treg after salt restriction; no effect on Th17 cells

REFERENCES

1. Powles, J. *et al.* Global, regional and national sodium intakes in 1990 and 2010: a systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. *BMJ Open* **3,** e003733 (2013).
2. Intersalt: an international study of electrolyte excretion and blood pressure. Results for 24 hour urinary sodium and potassium excretion. Intersalt Cooperative Research Group. *BMJ* **297,** 319–328 (1988).
3. Sacks, F. M. *et al.* Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group. *N. Engl. J. Med.* **344,** 3–10 (2001).
4. Whelton, P. K. *et al.* Sodium, blood pressure, and cardiovascular disease: further evidence supporting the American Heart Association sodium reduction recommendations. *Circulation* **126,** 2880–2889 (2012).
5. Smyth, A. *et al.* Sodium intake and renal outcomes: a systematic review. *Am. J. Hypertens.* **27,** 1277–1284 (2014).
6. Joossens, J. V. *et al.* Dietary salt, nitrate and stomach cancer mortality in 24 countries. European Cancer Prevention (ECP) and the INTERSALT Cooperative Research Group. *Int. J. Epidemiol.* **25,** 494–504 (1996).
7. Lanaspa, M. A. *et al.* High salt intake causes leptin resistance and obesity in mice by stimulating endogenous fructose production and metabolism. *Proc. Natl. Acad. Sci. U. S. A.* **115,** 3138–3143 (2018).
8. Sundström, B., Johansson, I. & Rantapää-Dahlqvist, S. Interaction between dietary sodium and smoking increases the risk for rheumatoid arthritis: results from a nested case-control study. *Rheumatol. Oxf. Engl.* **54,** 487–493 (2015).

9. Farez, M. F., Fiol, M. P., Gaitán, M. I., Quintana, F. J. & Correale, J. Sodium intake is associated with increased disease activity in multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* **86,** 26–31 (2015).

10. Adwaney, A., Randall, D. W., Blunden, M. J., Prowle, J. R. & Kirwan, C. J. Perioperative Plasma-Lyte use reduces the incidence of renal replacement therapy and hyperkalaemia following renal transplantation when compared with 0.9% saline: a retrospective cohort study. *Clin. Kidney J.* **10,** 838–844 (2017).

11. Osté, M. C. J. *et al.* Dietary Approach to Stop Hypertension (DASH) diet and risk of renal function decline and all-cause mortality in renal transplant recipients. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* (2018). doi:10.1111/ajt.14707

12. Wenzel, U. O., Bode, M., Kurts, C. & Ehmke, H. Salt, inflammation, IL-17 and hypertension. *Br. J. Pharmacol.* (2018). doi:10.1111/bph.14359

13. Machnik, A. *et al.* Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat. Med.* **15,** 545–552 (2009).

14. Kopp, C. *et al.* 23Na magnetic resonance imaging-determined tissue sodium in healthy subjects and hypertensive patients. *Hypertens. Dallas Tex 1979* **61,** 635–640 (2013).

15. Sica, A. & Mantovani, A. Macrophage plasticity and polarization: in vivo veritas. *J. Clin. Invest.* **122,** 787–795 (2012).

16. Jiang, H.-R. *et al.* IL-33 attenuates EAE by suppressing IL-17 and IFN-γ production and inducing alternatively activated macrophages. *Eur. J. Immunol.* **42,** 1804–1814 (2012).

17. Parsa, R. *et al.* Adoptive transfer of immunomodulatory M2 macrophages prevents type 1 diabetes in NOD mice. *Diabetes* **61,** 2881–2892 (2012).

18. Jantsch, J. *et al.* Cutaneous Na+ storage strengthens the antimicrobial barrier function of the skin and boosts macrophage-driven host defense. *Cell Metab.* **21,** 493–501 (2015).

19. Binger, K. J. *et al.* High salt reduces the activation of IL-4– and IL-13–stimulated macrophages. *J. Clin. Invest.* **125,** 4223–4238

20. Berry, M. R. *et al.* Renal Sodium Gradient Orchestrates a Dynamic Antibacterial Defense Zone. *Cell* **170,** 860-874.e19 (2017).

21. Gaffen, S. L., Jain, R., Garg, A. V. & Cua, D. J. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat. Rev. Immunol.* **14,** 585–600 (2014).

22. Kleinewietfeld, M. & Hafler, D. A. The plasticity of human Treg and Th17 cells and its role in autoimmunity. *Semin. Immunol.* **25,** 305–312 (2013).

23. Gan, P.-Y. *et al.* Th17 cells promote autoimmune anti-myeloperoxidase glomerulonephritis. *J. Am. Soc. Nephrol. JASN* **21,** 925–931 (2010).

24. Nogueira, E. *et al.* Serum IL-17 and IL-23 levels and autoantigen-specific Th17 cells are elevated in patients with ANCA-associated vasculitis. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. - Eur. Ren. Assoc.* **25,** 2209–2217 (2010).

25. Rother, N. & van der Vlag, J. Disturbed T Cell Signaling and Altered Th17 and Regulatory T Cell Subsets in the Pathogenesis of Systemic Lupus Erythematosus. *Front. Immunol.* **6,** 610 (2015).

26. Peng, Z. *et al.* Increased number of Th22 cells and correlation with Th17 cells in peripheral blood of patients with IgA nephropathy. *Hum. Immunol.* **74,** 1586–1591 (2013).

27. Evans, R., Zdebik, A., Ciurtin, C. & Walsh, S. B. Renal involvement in primary Sjögren’s syndrome. *Rheumatol. Oxf. Engl.* (2015). doi:10.1093/rheumatology/kev223

28. Paust, H.-J. *et al.* CXCR3+ Regulatory T Cells Control TH1 Responses in Crescentic GN. *J. Am. Soc. Nephrol. JASN* **27,** 1933–1942 (2016).

29. Sullivan, J. A., Adams, A. B. & Burlingham, W. J. The emerging role of TH17 cells in organ transplantation. *Transplantation* **97,** 483–489 (2014).

30. Kleinewietfeld, M. *et al.* Sodium Chloride Drives Autoimmune Disease by the Induction of Pathogenic Th17 Cells. *Nature* **496,** 518–522 (2013).

31. Wu, C. *et al.* Induction of pathogenic TH17 cells by inducible salt-sensing kinase SGK1. *Nature* **496,** 513–517 (2013).

32. Wu, C. *et al.* SGK1 Governs the Reciprocal Development of Th17 and Regulatory T Cells. *Cell Rep.* **22,** 653–665 (2018).

33. Hernandez, A. L. *et al.* Sodium chloride inhibits the suppressive function of FOXP3+ regulatory T cells. *J. Clin. Invest.* **125,** 4212–4222 (2015).

34. Safa, K. *et al.* Salt Accelerates Allograft Rejection through Serum- and Glucocorticoid-Regulated Kinase-1–Dependent Inhibition of Regulatory T Cells. *J. Am. Soc. Nephrol.* ASN.2014090914 (2015). doi:10.1681/ASN.2014090914

35. Wilck, N. *et al.* Salt-responsive gut commensal modulates TH17 axis and disease. *Nature* **551,** 585–589 (2017).

36. Krebs, C. F. *et al.* Autoimmune Renal Disease Is Exacerbated by S1P-Receptor-1-Dependent Intestinal Th17 Cell Migration to the Kidney. *Immunity* **45,** 1078–1092 (2016).

37. Jörg, S. *et al.* High salt drives Th17 responses in experimental autoimmune encephalomyelitis without impacting myeloid dendritic cells. *Exp. Neurol.* **279,** 212–222 (2016).

38. Barbaro, N. R. *et al.* Dendritic Cell Amiloride-Sensitive Channels Mediate Sodium-Induced Inflammation and Hypertension. *Cell Rep.* **21,** 1009–1020 (2017).

39. Wei, Y. *et al.* High salt diet stimulates gut Th17 response and exacerbates TNBS-induced colitis in mice. *Oncotarget* **8,** 70–82 (2017).

40. Aguiar, S. L. F. *et al.* High-Salt Diet Induces IL-17-Dependent Gut Inflammation and Exacerbates Colitis in Mice. *Front. Immunol.* **8,** 1969 (2017).

41. Wu, H. *et al.* High salt promotes autoimmunity by TET2-induced DNA demethylation and driving the differentiation of Tfh cells. *Sci. Rep.* **6,** (2016).

42. Yang, X. *et al.* Exacerbation of lupus nephritis by high sodium chloride related to activation of SGK1 pathway. *Int. Immunopharmacol.* **29,** 568–573 (2015).

43. Hammer, A. *et al.* Impact of combined sodium chloride and saturated long-chain fatty acid challenge on the differentiation of T helper cells in neuroinflammation. *J. Neuroinflammation* **14,** 184 (2017).

44. Spurgeon-Pechman, K. R. *et al.* Recovery from acute renal failure predisposes hypertension and secondary renal disease in response to elevated sodium. *Am. J. Physiol. Renal Physiol.* **293,** F269-278 (2007).

45. Mehrotra, P., Patel, J. B., Ivancic, C. M., Collett, J. A. & Basile, D. P. Th-17 cell activation in response to high salt following acute kidney injury is associated with

progressive fibrosis and attenuated by AT-1R antagonism. *Kidney Int.* **88,** 776–784 (2015).

46. Zhou, X. *et al.* Variation in dietary salt intake induces coordinated dynamics of monocyte subsets and monocyte-platelet aggregates in humans: implications in end organ inflammation. *PloS One* **8,** e60332 (2013).

47. Yi, B. *et al.* Effects of dietary salt levels on monocytic cells and immune responses in healthy human subjects: a longitudinal study. *Transl. Res. J. Lab. Clin. Med.* **166,** 103–110 (2015).

48. Luo, T. *et al.* Th17/Treg Imbalance Induced by Dietary Salt Variation Indicates Inflammation of Target Organs in Humans. *Sci. Rep.* **6,** 26767 (2016).

49. Wen, W. *et al.* Potassium supplementation inhibits IL-17A production induced by salt loading in human T lymphocytes via p38/MAPK-SGK1 pathway. *Exp. Mol. Pathol.* **100,** 370–377 (2016).

50. Scrivo, R. *et al.* The role of dietary sodium intake on the modulation of T helper 17 cells and regulatory T cells in patients with rheumatoid arthritis and systemic lupus erythematosus. *PloS One* **12,** e0184449 (2017).