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Influence of Dietary Components on Regulatory T Cells

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Common dietary components including vitamins A and D, omega-3 and probiotics are now widely accepted to be essential to protect against many diseases with an inflammatory nature. On the other hand, high-fat diets are documented to exert multiple deleterious effects, including fatty liver diseases. Here we discuss the effect of dietary components on regulatory T cell (Treg) homeostasis, a central element of the immune system to prevent chronic tissue inflammation. Accordingly, evidence on the impact of dietary components on diseases in which Tregs play an influential role will be discussed. We will review chronic tissue-specific autoimmune and inflammatory conditions such as inflammatory bowel disease, type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis and allergies among chronic diseases where dietary factors could have a direct influence via modulation of Tregs homeostasis and functions.

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INTRODUCTION

Regulatory T cells (Tregs) are a heterogeneous T-cell subpopulation that regulates the immune system in various ways. Given the significance that deregulation of the immune system plays in the development and progression of chronic inflammatory processes, including inflammatory bowel disease (IBD), type 1 diabetes mellitus (T1D), asthma, arthritis and multiple sclerosis (MS), the factors influencing and influenced by Tregs may offer insight in the mechanisms underlying the pathological course of these diseases as well as provide clues to improving their management.

The mechanisms involved in the generation and maintenance of Tregs on the cellular level and in the host environment are just starting to be understood. It is even less clear how the environment-host interaction affects the immune balance and the status of Tregs. Nonetheless, a variety of model systems are available to interrogate the characteristics and function of Tregs in health and disease. Although, few studies directly look at the effect of common dietary components on Treg homeostasis, despite the important role shown for the influence of diet on inflammation and immune regulation. Accordingly, evidence on the impact of dietary components on diseases in which Tregs play an influential role may provide insight into the role of these dietary components on Tregs themselves.

This report reviews some of the evidence showing how dietary components influence Tregs. Although dietary components have been reported to influence disease outcome via regulating other cells, such as helper T cells (Th1, Th2 and Th17), important in health and the pathogenesis of immune-related diseases, in this review, we restrict our focus to Tregs. Recently published results on the influence of dietary components on Tregs, including probiotics and vitamins, are discussed. In addition, related studies on the pathways involved in the modulation of Tregs by dietary components are also examined. In addition to the role of single components such as vitamins and the consequences of probiotics, gluten and fatty acids are also discussed.

DEFINITION OF Tregs

Broadly speaking, Tregs have the capability to suppress the activity of the immune system and to regulate self-tolerance. However, it is not possible to unambiguously define Tregs as a homogeneous group, since T cells of different phenotypes have been shown to exhibit immune regulating potential (1). Currently, the most commonly known regulatory T-cell lineage is called $CD4^+CD25^{high}FoxP3^+$ regulatory T cells. The forkhead box P3 (FoxP3) transcription factor is the cell lineage–determining master transcription factor of this T-cell subtype (2). Generation of Tregs is shown to depend on the cytokine transforming growth factor (TGF)-β (3). A new population of Tregs has been described lately that is under the regulation of interleukin (IL)-35 (T_{R35}) (4). Although the first report showed them to be $FoxP3^+$, a more recent study suggests that T_{R35} cells are independent of FoxP3

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(5). Because not much information exists regarding the influences of dietary products on generation or function of this population, within this report, Tregs are defined as $CD4^+CD25^+FoxP3^+$ T cells unless otherwise stated.

Tregs have been found to suppress various immune cells such as $CD8^+$ T cells, dendritic cells (DCs), monocytes/macrophages, B cells, natural killer cells and natural killer T cells (6). Nonfunctional mutations in the *FOXP3* gene in humans cause the immune dysfunction, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, indicating an essential role of $FoxP3^+$ Tregs in the prevention of hyperimmune responses and autoimmune diseases (7,8). On the other hand, Tregs at tumor sites or in the peripheral blood are suggested to be partly responsible for tumor immune escape, making the inhibition of Treg function a favorable treatment intervention in some cases (9).

Tregs can be further divided into two groups on the basis of their origin. Natural Tregs (nTregs) are generated in the thymus through major histocompatibility complex class II–dependent T-cell receptor interactions resulting in high-avidity selection (10). nTregs are speculated to prevent autoimmunity and to generally raise the activation threshold to initiate an immune response. Adaptive or induced Tregs (iTregs), on the other hand, develop outside the thymus during subimmunogenic antigen presentation. iTregs are thought to be essential to maintain a noninflammatory environment in the gut, to suppress allergic immune responses to environmental and food antigens and to decrease chronic inflammation (11,12). The discrimination of nTregs and iTregs is mentioned here solely for the sake of completeness and is not discussed further in this review.

Nevertheless, it is noteworthy to mention that oral administration of antigens is commonly used to induce peripheral tolerance to subsequent disease induction by the same antigens (13). It has been shown that the ensuing oral tolerance depends on successful expansion of functional Tregs (14). Thus, it is important to distinguish if any substance administered orally would lead to nonspecific general Treg expansion or whether dietary components suggested in the literature have more specific effects leading to induction of molecular pathways to generate or expand Tregs.

EFFECTS OF PROBIOTIC BACTERIA ON Tregs

Probiotic Effects in Digestive Tract Disorders

Probiotics have been defined by the World Health Organization (WHO) as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (15). The rationale for beneficial effects of probiotics is that manipulation of the gut flora might have an effect on pathogenic bacteria and might also affect the formation of disease-controlling Tregs (16,17).

IBD, which includes Crohn's disease and ulcerative colitis, involves a variety of inflammatory manifestations of the gut. Existing murine models of IBD support the concept that host bacteria play an important role in the disease process. Therefore, conditions affecting the homeostasis of gut bacteria could have a decisive impact on health versus disease status. Accordingly, specific pathogen-free conditions are protective against gut inflammatory disease (18). However, mice in gnotobiotic conditions (that is, exposed to only certain strains of bacteria), exposed to *Bacteroides vulgatus*, are also protected against *Escherichia coli*–induced colitis (19). Additionally, administration of probiotics leads to reduced disease in experimental colitis, which is accompanied by an increase of $FoxP3^+$ Tregs (20).

Because the gut hosts normal and potential pathogenic bacteria, the immune responses in the intestine are tightly regulated to ensure sustained effector responses to pathogens and to avoid potentially deleterious inflammatory responses to commensal bacteria or food antigens. Therefore, while the gut is regulating efficient immune responses to pathogenic bacteria, oral administration of proteins leads to a state of peripheral (oral) tolerance to ingested proteins. The maintenance of balance between tolerance and immunity in the gut is in part dictated by specialized gut-resident DCs. DCs in the gut-associated lymphoid tissues are unique in their ability to activate naive T cells to effector T cells in response to pathogens, but under homeostatic conditions, gut DCs seem to promote noninflammatory T-cell responses, mainly composed of Tregs via production of IL-10 and TGF-β (21).

The importance of Tregs in controlling gut inflammation has long been understood. Depletion of Tregs leads to chronic inflammation in the experimental models of colitis. Similarly, the transfer of Tregs is able to control chronic inflammation in the gut. Loss of Tregs in the gut is observed during the IPEX syndrome, which leads to the manifestation of intestinal lesions (22).

It is of interest to mention that gut flora have profound effects on the balance of $FoxP3^+$ Tregs versus proinflammatory Th17 cells in the lamina propria. When the gut flora were manipulated through cohousing of mice from different suppliers, mice from different sources that had marked differences in their basal levels of Th17 cells or were lacking Th17 cells acquired Th17 cells after introduction of bacteria from Th17 cell–sufficient mice. Of note, differentiation of Th17 cells correlated with the presence of commensal microbiota, such as Cytophaga-Flavobacterium-Bacteroidetes bacteria in the gut. This result was shown to be independent of Toll-like receptor (TLR) and IL-21 or IL-23 signaling, but it required appropriate TGF-β activation. Treatment with selective antibiotics inhibited Th17 differentiation. Of note, the absence of Th17 cell–inducing bacteria was accompanied by an increase in $Foxp3^+$ Tregs in the lamina propria (23). Furthermore, it is also suggested that Tregs regulate intestinal inflammation induced by pathogenic bacteria. In agreement, $CD25^+FoxP3^+$ Tregs regulate gastric inflammation and

Helicobacter pylori colonization *in vivo* (24), whereas commensal-induced Tregs are involved in protection against pathogen-induced inflammation (25).

Interventions with probiotics have been conducted in several models of digestive tract disorders and are summarized in Table 1.

Probiotic Effects on Extraintestinal Diseases

Probiotics, in addition to having a local effect on disease models of the digestive tract, also operate systemically in inflammatory models such as collagen-induced arthritis (CIA) (Table 2), experimental autoimmune encephalomyelitis (EAE) (Table 3), asthma and delayed-type hypersensitivity reactions (Table 4).

Lactobacillus casei (*L. casei*), a widely commercialized dairy product, is reported to reduce arthritis in CIA. Administration of this product results in lower disease scores and less complement-fixing IgGs. Treatment with *L. casei* alone had no effect on FoxP3, but an increase was detected with a combination treatment of *L. casei* and oral tolerance–inducing collagen type II (26,27). Collectively, administration of *L. casei* resulted in disease-reducing effects in several experimental models including CIA (20,28). This result is also reported to be associated with an increase in $FoxP3^+$ Tregs (20).

L. casei is also shown to exert protective effects on T cell–mediated skin inflammation, in contact hypersensitivity to the hapten 2-4-dinitrofluorobenzene, a model for allergic contact dermatitis (29), on dermatitis (20) and on delayed-type hypersensitivity responses (28), all associated with increases in $FoxP3^+$ Tregs. Taking these results together, *L. casei* appears to be a candidate probiotic agent to regulate Tregs and thereby act as a potent immunomodulator of T cell–mediated skin allergies.

Lactobacillus rhamnosus Goldin-Gorbach and *L. casei Shirota* have protective effects on EAE (30), the most commonly used rodent model for MS, an alleged autoimmune disease affecting the central nervous system (CNS). Other studies also suggest an important role for commensal bacterial antigens, in particular, *Bacteroides fragilis* expressing polysaccharide A, in protecting against CNS demyelination in EAE via modulation of Tregs (31). Similarly, oral treatment of mice with a broad spectrum of antibiotics induced significant changes in Tregs and reduced the susceptibility to EAE (32). However, literature regarding effects of probiotics in the treatment of EAE is controversial. Some authors have observed either exacerbating effects in rat EAE models, with increased Th1 immune responses, or no treatment effect after using probiotics (33–35). The plausible reasons for such controversies will be discussed later.

Possible Mechanisms of Probiotic-Induced Tolerance

Introducing living bacteria into a host likely elicits responses on numerous levels affecting the immune response. One of the major mechanisms of action of living bacteria is reported to occur by increasing number of Tregs. Others have shown mechanisms by which bacteria directly induce an increase in $FoxP3^+$ Tregs and/or their suppressive function via production of immunomodulatory cytokines. Moreover, there are several reports indicating that the major pathway of probiotic-induced tolerance seems to operate via the induction of tolerogenic antigen-presenting cells (APCs). These tolerogenic APCs consequently contribute to generation of Tregs and hence regulation of inflammatory diseases.

In support of the positive influence of oral probiotics on expansion of Tregs, administration of *L. casei* to mice affected the frequency of $CD4^+FoxP3^+$ Tregs in the skin. Whereas this ameliorated skin inflammation, it did not change the extent of *in vivo* suppressive function of nTregs (29). Accordingly, in an experimental model of colitis, live probiotics as well as their immunomodulatory DNA resulted in generation of higher numbers of $FoxP3^+$ Tregs (36). Moreover, it has also been shown that Tregs isolated after oral feeding with *L. reuteri* are more potent than Tregs from mice without previous probiotic exposure and could prevent airway inflammation caused by allergies (37).

It is suggested that probiotics have direct effects on Tregs by interfering with nuclear factor (NF)-κB degradation. In accordance, *Lactobacillus plantarum* is able to block NF-κB degradation via blockage of proteasome function. This effect is even maintained in bacteria-free conditioned medium but containing the TLR2-activating molecule lipoteichoic acid (38,39). Another chemical proteasome blocker, benzyloxycarbonyl-isoleucyl-glutamyl(O-tert-butyl)-alanyl-leucinal (PSI), was associated with the generation of $FoxP3^+$ Tregs via PSI-treated APCs (40). Additionally, oral application of the probiotic strain *B. infantis* reduced NF-κB activation and increased the numbers of $FoxP3^+$ cells in mucosa and spleen, counterbalancing the NF-κB–activating properties of pathogenic strains such as *Salmonella typhimurium* (25).

In support of the indirect affects of probiotics in regulation of Tregs, it was shown that tolerogenic DCs are playing a crucial role in conversion of T cells to Tregs. Activation of DCs by certain species of probiotics such as *L. reuteri* and *L. casei*, but not *L. plantarum*, leads to generation of Tregs. This effect is mediated by the 3-grabbing nonintegrin molecule on human DCs (41). Although the effector cells showing suppressive capabilities were called Tregs, no direct evidence for the presence of Treg markers was reported. Additionally, tolerogenic DCs were generated *in vitro* with specific species of lactobacilli. The *in vivo* transfer of these DCs resulted in generation of Tregs and reduction of IBD in mice. The protective effect of the probiotic-matured DCs induced Tregs depended the pattern recognition receptors TLR2 and non-obese diabetic (NOD)-2 (42). In support of putative influence of probiotics in regulation of tolerogenic DC-induced Tregs, NOD2/CARD15 (nucleotide-binding oligomerization domain containing 2, also known as the caspase recruitment domain family, member 15) also has a di-

Table 1. Probiotic effects on Tregs and disease models (digestive tract).

Disease model	Bacterial strains used	Treg after probiotic treatment	Treatment effect of probiotics	Investigated effector mechanisms	Reference
Trinitrobenzene sulfonic (TNBS) acid–induced murine colitis	Mix 1 (*Lactobacillus acidophilus* and *Bifidobacterium longum*) Mix 2 (*Lactobacillus plantarum, Streptococcus thermophilus,* and *Bifidobacterium animalis* subspecies Lactis)	FoxP3 ↑in intraepithelial lymphocytes FoxP3 → in Lamina propria	IBD↓	IL-10↑ TNF-α↓ Monocyte chemotactic protein-1 (MCP1)↓	126
TNBS-induced colitis (Balb/c)	DCs pulsed with: *L. salivarius* *L. rhamnosus*	FoxP3→	IBD↓	indoleamine 2,3-dioxygenase (IDO)↑ IL-10↓ (IL-10–independent) TLR2-, NOD2- and MyD88-dependent	42
TNBS-induced colitis (SLJ mice)	VSL#3	ND	IBD↓	IL-10↑ IFN-γ ↓ Leukocyte alkaline phosphatase^{+} (LAP^{+}) cells↑	127
Pouchitis disease in ulcerative colitis (humans)	VSL#3	FoxP3↑	Pouchitis↓	IL-1β↓	128
CD4 + CD62L + T-cell–induced IBD in CB17-Prkdc^{scid}	CpG	FoxP3↑	IBD↓ IFN-γ↓	IL-10↑	48
129 Ola × C57BL/6-IL 10 KO	*L. salivarius* UCC118, Rafamycin-resistant subspecies (subcutaneous)	ND	IBD↓	TGFβ↑ TNF-α↓ IL-12↓	129
Salmonella typhimurium LPS-induced intestinal inflammation	*B. infantis* 35624	FoxP3↑	Intestinal disease score↓	IL-6↓ TNF-α↓ NF-κB activation↓	25
H. pylori–induced gastritis	*H. pylori*	FoxP3↑	Intestinal disease score↑ after anti-CD25 treatment		24

ND, not determined; ↑, increased expression; ↓, decreased expression; →, unchanged.

Table 2. Probiotic effects on Tregs and disease models (arthritis).

Disease model	Bacterial strains used	Treg after probiotic treatment	Treatment effect of probiotics	Investigated effector mechanisms	Reference
Adjuvant and tropomyosin-induced arthritis in Lewis rats	*Lactobacillus rhamnosus* GG (ATCC 53103)	ND	Arthritis↓	ND	130
CIA in Lewis rats	*L. casei*	FoxP3→	Arthritis↓	IL-10↑ Proinflammatory molecules↓	27
CIA in Lewis rats (oral tolerance)	*L. casei*	FoxP3↑	Arthritis↓	TGF-β ↑ IL-10↑ Proinflammatory molecules↓	26
CIA in dark black agouti (DBA)/1 mice	*L. salivarius* UCC118, Rafamycin-resistant subspecies (subcutaneous)	ND	Arthritis↓	ND	129
CIA in DBA/1 mice	*L. casei* Shirota	ND	Arthritis↓	IFN-γ↓ Anti–collagen type II (aCII) IgG↓ Delayed-type hypersensitivity↓	28

ND, not determined; ↑, increased expression; ↓, decreased expression; →, unchanged.

rect effect on the survival of Tregs. In agreement, the bacterial cell wall product muramyl dipeptide (MDP), which has a capacity to bind NOD2, is reported to reduce Fas-induced Treg apoptosis (43). Moreover, a clear deficiency in the quantity of FoxP3$^+$ lymphocytes was reported in patients with Crohn's disease, which was associated with disease polymorphisms in the *NOD2* gene. Subsequently, it was found or reported that the NOD2 ligand, MDP, activates NF-κB in primary human FoxP3$^+$ T cells (43). Consequently, NOD2 deficiency in the mouse also exacerbates graft versus host disease as well as experimental colitis. The NOD2 deficiency was also mirrored by a reduced number of FoxP3$^+$ Tregs (44). It would prove fruitful to test the probiotic-NOD2 axis *in vivo* and its effect on Treg expansion and function.

Genetic makeup may be important for the colonization of the gut with pathogenic bacteria and the effect of probiotics, as suggested in a study using NOD2-deficent mice (45). Such a study, extended to humans, raises a question regarding the efficacy of probiotics in patients and consumers harboring defects in pattern recognition receptors, as seen in disease-associated NOD2 polymorphisms. For example, the limited efficacy of lactobacilli in Crohn's disease could be related to NOD2 deficiency. This hypothesis was tested in mice, and it was found that *Lactobacillus salivarius* rescued mice

Table 3. Probiotic effects on Tregs and disease models (EAE/MS).

Disease model	Bacterial strains used	Treg after probiotic treatment	Treatment effect of probiotics	Investigated effector mechanisms	Reference
Spinal cord homogenate/H37Ra/Freund complete adjuvant (CFA)–induced EAE in Lewis rats	*L. casei* strain Shirota	ND	EAE↑ Body weight↓	ND	34,35
Spinal cord homogenate/H37Ra/CFA–induced EAE in Lewis rats (LEW/CrlCrlj)	*L. casei* strain Shirota (LcS) *B. breve* (BbY)	ND	EAE→ Body weight→	ND	33
EAE in rats (EAE in SLJ mice)	*L. casei* DN 114-001, *L. rhamnosus* GG *L. casei* Shirota	ND	Rat EAE↓ Mixed effects (no effect in mice)	ND	30
EAE in C57BL/6	*L. paracasei* DSM 13434, *L. plantarum* DSM 15312	FoxP3↑ in WT not in IL-10 KO mice	EAE↓	IL-10↑ TGF-β↑	131

ND, not determined; ↑, increased expression; ↓, decreased expression; →, unchanged.

Table 4. Probiotic effects on Tregs and disease models (allergy/asthma).

Disease	Disease model	Bacterial strains used	Treg after probiotic treatment	Treatment effect of probiotics	Investigated effector mechanisms	Reference
Asthma	Ovalbumin (OVA)-induced asthma model in Balb/c	*L. rhamnosus* GG (ATCC 53103) *Bifidobacterium lactis* (Bb-12)	FoxP3↑	BAL cells↓ Airway reactivity↓	Proliferation↓ IL-4, -5, and -10↓ IFN-γ↓ TGF-β→ Anti-OVA Ig↓	132
	OVA-induced asthma model in Balb/c	*L. salivarius* *L. reuteri* (ATCC 23272)	FoxP3↑ (more potent)	BAL cells↓ Airway reactivity↓ IL-10↑ (spleen) IL-10↓ (BALF) TGF-β →	Proliferation↓	37
Delayed-type hypersensitivity	OVA and 2-4-dinitrofluorobenzene–sensitized mice	*L. casei* DN-114 001	FoxP3↑	Delayed-type hypersensitivity↓	IL-10↑	29

ND, not determined; ↑, increased expression; ↓, decreased expression; →, unchanged.

from colitis via generation of $CD103^+$ DCs and $CD4^+FoxP3^+$ Tregs. This suppressive effect was abolished in NOD2-deficient mice (46).

It is noteworthy to mention that the protective effects of probiotics related to generation of direct Tregs or tolerogenic APC-mediated Tregs do not require live bacteria. In agreement, subcutaneous injection of bacterial cytosine–phosphate–guanine (CpG) motifs, in the form of synthetic immunostimulatory sequence oligodeoxynucleotides (ISS-ODNs), reduced symptoms in a model of IBD in a TLR9-mediated manner (47). The disease reduction was also observed after injection of probiotic or *E. coli*–derived DNA in a murine colitis model (36). Similar effects were shown through intraperitoneal injection of CpG-ODN. Interestingly, this treatment induced $FoxP3^+$ Tregs in germ-free mice, indicating that even without preexistence of bacterial flora, CpG-ODN induces tolerance, which suggests that CpG-ODN–induced Tregs are not bacterial antigen specific (48). Particularly, CpG-induced $CD8^+$ plasmacytoid DCs from the gut have been shown to convert T cells into Tregs, whereas spleen-derived DCs do not have the same capacity (49). Although bacterial CpGs are not strictly speaking referred to as probiotics, they nonetheless show that bacterial components have tolerizing properties via induction of tolerogenic DC–mediated Treg cell generation.

Additional antiinflammatory mechanism of probiotics could be mediated via the regulation of exogenous bacterial adenosine 5′-triphosphate (ATP) levels as an inflammatory mediator. In support of this, a report described the beneficial role of exogenous ATP, which increases FoxP3 transcription after A2 adrenergic receptor ($A_{2A}R$) engagement, also triggering an increase in TGF-β and a decrease of IL-6 levels (50). Furthermore, human $FoxP3^+$ Tregs are shown to convert ATP via CD39 and CD73 to immunosuppressive adenosine (51). In contrast, ATP was reported to induce inflammatory Th17 cells (52). This finding might shed light on differential effects of probiotics by engaging different subsets of gut-associated DCs and hence leading to a change in the inflammatory balance depending on the bacterial composition.

Although several different mechanisms of action have been reported to be attributed to the probiotic-mediated Treg generation, and elevated Treg-suppressive functions, there is more consistent agreement on how the probiotic-induced Tregs exert their antiinflammatory effects (that is, via production of IL-10 and or in an IL-10–dependent fashion). Accordingly, it was reported that *L. casei* treatment promoted the activation of antigen-experienced Tregs and increased their ability to produce IL-10 and hence inhibit skin inflammation (29). Moreover, suppressive mechanism of Tregs induced by *L. casei in vitro* was shown to be IL-10 dependent (41). Nevertheless, *L. salivarius* Ls33 induced Tregs, and amelioration of colitis in mice was correlated with a local production of IL-10 (46).

Although the protective effects of probiotics by regulating the generation of Tregs, functions of Tregs and prevention of local inflammation in the gut and gut-associated diseases are more rational, it is an interesting question as to how oral administration of probiotics could exert protective effects to modulate inflammatory diseases of organs other than the gut (such as the antiinflammatory effects reported in CIA, EAE and allergic airway inflammation). Such favorable results might not be too surprising in arthritis, since it was observed that the gut and joint share lymphocyte homing receptors that might redirect regulatory cells from the gut to the joint (53). This scenario is interesting and requires further investigation to determine if other tissues, such as brain and skin, share similar Treg homing receptors, allowing gut-generated Tregs

Table 5. Different mechanisms of regulatory T-cell induction by probiotics.

Direct effects in T cells	Reference	Indirect effects on T cells	Refernce
Higher potency of Tregs	37	Conversion of gut DC to induce Tregs	88
MDP activates NOD2, which prevents Treg from Fas-induced apoptosis	43	TLR2- and NOD2-dependent induction of maturation of tolerogenic DC (IDO)	42
NOD2 KO mice have increased graft versus host disease and TNBS-induced colitis (less FoxP3)	44	CpG-induced $CD8^+$ plasmacytoid gut DC convert T cells into Tregs	49
Suppression of bacterial ATP, which prevents conversion to Th17 cells	52	$CD103^+$ and $CD103^-$ lamina propria DC induce FoxP3 Tregs	133
Exogenous ATP activates Treg and FoxP3 expression	50,51	Induction of $FoxP3^-$ Tregs by $CD103^+$ ML-DC (ATRA)	134
		Proteasome block induces tolerogenic APCs	25,38,39

to home these tissues during the course of inflammation. Table 5 summarizes how probiotics directly or indirectly influence Tregs.

Caveats

Despite the promising signs for beneficial effects of probiotics, caution is advised in light of a study showing that a commonly used probiotic (*Bifidobacterium animalis*) can actually cause duodenitis and mild colonic inflammation in IL-10 knockout mice (54). It is noteworthy to mention that IL-10 knockout mice are reported to develop spontaneous IBD and transfer of Tregs leading to inhibition of inflammation. Such findings, extended to humans, might be especially relevant for immunosuppressed or certain IBD patients (55).

Furthermore, different strains of lactobacilli elicit diverse activation of the suppressor of cytokine pathway 2 and 3 (SOCS2 and SOCS3) on a human gastric carcinoma cell line (56). It will be interesting to see if SOCS3 is also activated in Tregs under these conditions, which might in this case lead to reduced suppressor functions as seen during an artificial SOCS3 increase (57). This result would fall in line with the observation that some "probiotic" strains (for example, *Bifidobacterium bifidum* BI-98, BI-504 and *Lactobacillus acidophilus*) block the suppressive activity of Tregs in an *in vitro* suppression model (58).

Additionally, there are several conflicting reports on the protective role of probiotics in the inflammatory disease models discussed (30,35). Such differences could be because of use of different strains of probiotics, different doses and/or different routes of administration. Moreover, different genetic backgrounds of the experimental models could affect how probiotics exert their immunomodulatory properties. This result could be influenced by the host's intrinsic immune composition and the predominance of a specific type of immune response. For example, some strains of mice are predominantly mounting Th2 type of immune responses, whereas others are prone to mount Th1 and/or Th17 type of immune responses. Nevertheless, the controversies could partly be caused by overriding *in vitro* results by complexity of *in vivo* applications. Hence, precautions should be considered to avoid generalization of the protective effects exerted by probiotics.

It might be especially difficult to claim a Treg-inducing effect to a unique "probiotic" property rather than a general response toward bacteria. In support of this, not only nonpathogenic bacteria seem to increase the presence of Tregs. The expansion of $FoxP3^+$ Tregs also seems to be promoted by the otherwise pathogenic bacterium *Listeria monocytogenes* in the mouse (59). Additionally, gastritis inducing *Heliobacter pylori* increase TGF-β1 and $FoxP3^+$ Treg populations in the guts of mice and humans (24,60).

Even though in many cases an increase of $FoxP3^+$ T cells could be seen in probiotic-treated models and diseases, the mechanism of action still remains to be demonstrated. It will also be important to dissect the quality of a specific probiotic strain from general properties of bacterial components including DNA or cell wall components. In summary, it seems likely that the generation, survival and homing of $FoxP3^+$ Tregs all play a role in probiotic-mediated effects.

Vitamin A and D Derivatives in Treg Function

Vitamin A (VitA) and vitamin D (VitD) are both inactive precursors of their biologically active derivatives, which need to be enzymatically modified. These modified bioactive vitamin derivatives play a fundamental role in many physiological processes such as night vision and calcium homeostasis. Furthermore, these vitamin derivatives are also involved in the regulation of the immune system (61,62) and tissue inflammation (63,64). This section focuses on their role in the formation and function of $FoxP3^+$ Tregs.

VitA Derivatives and Treg Function

The fat-soluble VitA, found in abundance in foods such as liver, carrots and sweet potatoes, has been shown to have a variety of immunomodulatory properties. Different derivatives of VitA are provided through food and vitamin supplements, such as pro-VitA from plant sources or as enzymatically modified forms from animal products (for example, retinol and retinyl ester) (65). Retinol is absorbed by intestinal epithelial cells

and esterified with fatty acids forming, among other products, less toxic stearate or oleate derivatives. These retinyl esters are further transported in chylomicrons and stored in the liver. Dietary retinol can also be directly converted to the biologically active all-*trans* retinoic acid (ATRA) by gut-associated lymphoid tissue DCs.

The importance of a balanced VitA concentration for the immune system is exemplified by the following findings: VitA deficiency can cause Treg imbalance with excess Th1 and insufficient Th2 function (66). On the other hand, high concentrations of VitA enhances *in vitro* development of Th2 cells via the retinoid × receptor (RXR) pathway (67). The major VitA metabolite, ATRA, was reported to convert naive FoxP3$^-$CD4$^+$ T cells into a unique, tissue-specific FoxP3$^+$ Treg subset in both human and mouse T cells. Histone acetylation at the FoxP3 gene promoter is induced upon binding of ATRA to the nuclear retinoic acid receptor α, which as a consequence, leads to the prolonged expression of FoxP3 protein in CD4$^+$ T cells and stability of Tregs. The resulting Tregs efficiently suppress effector T cells and show an additional upregulation of the mucosal tissue-homing receptors C-C chemokine receptor type 9 (CCR9) and integrin β7. These retinoid-induced FoxP3$^+$ cells were highly responsive to the CCR9 ligand, chemokine (C-C motif) ligand 25 (CCL25), a chemokine specifically expressed by intestinal epithelial cells (68). ATRA is also reported to be able to prevent the IL-6–induced conversion of Foxp3$^+$ Tregs into inflammatory Th17 cells. These *in vitro* TGF-β-plus ATRA-generated FoxP3$^+$ Tregs were also more protective in a colitis model than Tregs generated with TGF-β alone (69). The FoxP3-inducing and IL-17–suppressing effects of ATRA are likely mediated through the retinoic acid receptor α (70) (Figure 1).

The increased Treg stability is in line with findings that ATRA treatment increases histone acetylation of the Foxp3 promotor region in T cells, a process consistent with increased FoxP3 mRNA and protein expression (68). CNS-derived Tregs are capable of suppressing EAE (12), whereas in a separate study, CNS-recovered Tregs did not effectively suppress CNS effector T cells *in vitro*, even though they were able to suppress naive 2D2-Tg or splenic effector T cells. The high amounts of IL-6 and tumor necrosis factor (TNF)-α produced by CNS effector T cells were suggested to be the probable cause of preventing the suppressor func-

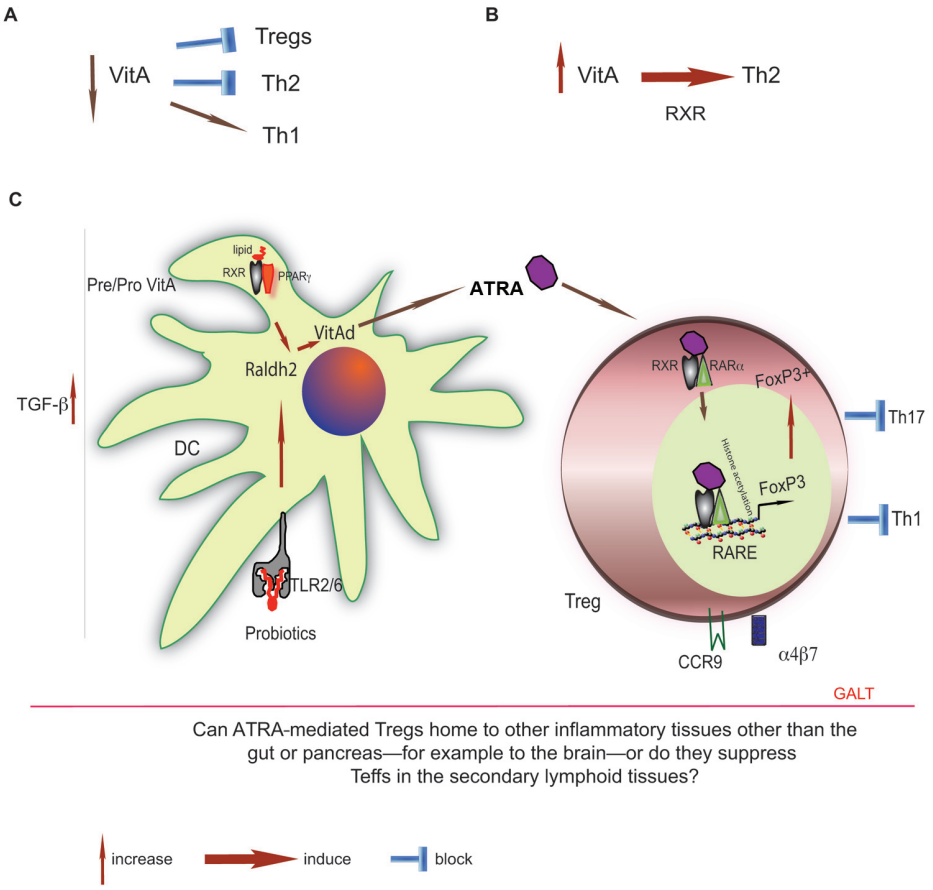

Figure 1. Overview of the effects of VitA in gut-associated lymphoid tissue. (A) Insufficient VitA can cause Treg imbalance with excess Th1 and insufficient Th2 function. (B) On the other hand, when there are elevated levels of VitA, enhanced development of Th2 cells via RXR pathway promotion occurs. (C) When there are normal physiological levels, homeostatic functions of VitA are present in tissue. The pre- or proforms of VitA found in food are absorbed by intestinal epithelial cells and esterified with fatty acids forming less toxic derivatives, which are further transported to liver for storage. Dietary retinol is also directly converted to the biologically active ATRA, the major VitA metabolite, by gut-associated lymphoid tissue DCs. This process is enhanced by TLR2/6-mediated effects of probiotics to induce Raldh2, which is important in conversion of VitA derivatives (VitAd) to ATRA. ATRA can convert naive FoxP3$^-$CD4$^+$ T cells into unique, tissue-specific FoxP3$^+$ Tregs. TGF-β exerts synergistic effect on this process. Histone acetylation at the FoxP3 gene promoter is induced upon binding of ATRA to the nuclear retinoic acid receptor α (RARα), which as a consequence, leads to the prolonged expression of FoxP3 protein in CD4$^+$ T cells and stability of Tregs. The resulting Tregs can efficiently suppress effector T cells and show an additional upregulation of the mucosal tissue-homing receptors CCR9 and integrin β7. GALT, gut-associated lymphoid tissue; RARE, retinoic acid response element.

tion of CNS Tregs (71). Nevertheless, additional study revealed that the sensitivity of CNS Tregs for inactivation could be modified by ATRA, which makes FoxP3$^+$ Tregs more resistant to IL-6–induced Th17 conversion (69). This protection from IL-6–induced conversion could indeed explain in part previously observed disease-modifying activities of ATRA and retinoid derivatives in EAE (72–74), although these early works did not address Tregs. However, more recent experiments that included Treg markers could show that the treatment resulted in decreased IL-6Rα expression and increased numbers of FoxP3$^+$ Tregs (75). It would be interesting to investigate if a systemic treatment with retinoids could further increase the stability of the CNS Treg population in the IL-6–rich environment of an inflamed brain.

The Treg-inducing effects of ATRA, generation of FoxP3$^+$ Tregs and increased gut-homing α4β7-integrin and CCR9 have been shown to be mediated by a subpopulation of intestinal DCs. Dietary VitA can be converted to ATRA by CD103$^+$ small intestine DCs, a feature not shared by splenic DCs. Blockade of the retinoic acid receptor with the retinoid inhibitor LE540 in mice on a standard diet leads to a strong reduction in FoxP3$^+$ Tregs. Peroxisome proliferator–activated receptor γ (PPARγ) activation by certain lipids induces the expression of ATRA-synthesizing enzymes in DCs. Interestingly, the PPARγ is built up as a heterodimer with the RXR. Usage of the RXR is shared by different receptor heterodimers, making it interesting to speculate whether activation of PPARγ may be a mechanism by which dietary components such as linolenic acids and probiotic bacteria affect the ATRA-mediated induction of tolerogenic DCs (76).

Stimulation of TLR2 and TLR6, the receptors involved in recognition of conserved bacterial molecules, on splenic DCs leads to induction of the retinol-metabolizing enzyme retinaldehyde dehydrogenase type 2 (Raldh2) and hence stimulates the formation of FoxP3$^+$ Tregs (77). Such findings underscore the significance of bacteria in the causation of immune modulation also via modification of dietary products, as discussed above.

VitD3 Derivatives and Treg Function

VitD3 can be generated in the skin through processes using ultraviolet B (UVB)-mediated photolysis of a cholesterol derivative and a spontaneous isomerization step. VitD3 needs to be enzymatically active to its hormonal form (78). Because endogenous production of VitD3 depends on UVB exposure, the concentration is influenced by environmental factors such as seasonal sunshine length and latitude, behavioral factors such as time and surface area of exposed skin, skin pigmentation and age.

VitD3 is also taken up through the diet. These factors lead to great variations in circulating VitD3 and calcidiol, spurring an ongoing discussion about "natural," "normal" and "desired" levels of VitD3 and its derivatives. A daily intake of 100 μg (4,000 IU) is suggested as a safe dose, elevating the 25-(OH)-D3 levels to desired concentrations in most humans (79). This number is higher than the recommended nutrient intake of between 5 and 15 μg/day (200–600 IU) by WHO.

This section deals with the role of VitD3 and its derivatives on the immune system, focusing on their effects on FoxP3$^+$ Treg generation and function in different diseases. UVB light exposure as well as topical calcipotriol treatment can lead to vitamin D receptor (VDR)-dependent induction of FoxP3$^+$ Tregs in the skin (80,81). VitD3 supplementation is reported to reduce the CNS autoimmune disease EAE in females but not in males or ovariectomized female mice. The disease protection correlated with high local 1,25-$(OH)_2D_3$ (active form) levels in the inflamed spinal cord, but not in serum. The authors suggested a slowed degradation of 1,25-$(OH)_2D_3$ process in the CNS, mediated through a suppression of the enzyme CYP24A1, a 24-hydroxylase with 1,25-$(OH)_2D_3$–inactivating properties (82,83). The protective effect in females could be restored in ovariectomized females through estrogen supplementation (84).

An additional pathway for the treatment effect of 1,25-$(OH)_2D_3$ has been shown by induction of high numbers of FoxP3$^+$ Tregs in combination with IL-2 (85). In support of this, dietary administration of 1a-(OH)-D_3, an active VitD3 analog, led to reduced diabetes in nonobese diabetic mice and increased FoxP3$^+$ T cells (86). Earlier, it was shown that a 1,25-$(OH)_2D_3$ analog also had a therapeutic effect in a spontaneous autoimmune nonobese diabetic model, resulting in an increased protective CD25$^+$ Treg population (87).

A combination of 1,25-$(OH)_2D_3$ and the antiinflammatory corticosteroid dexamethasone synergistically induced primarily IL-10–producing FoxP3$^-$ Tregs (88,89). Nevertheless, in an experimental IBD model, 1,25-$(OH)_2D_3$ and dexamethasone had a FoxP3 expression–promoting effect in the gut (90). Not only was the amount of FoxP3 expression increased by 1,25-$(OH)_2D_3$ treatment, but the potency of FoxP3$^+$ Tregs was accentuated, even in a Th2-driven *in vivo* asthma model (91).

The 1,25-$(OH)_2D_3$ precursor calcidiol [25-$(OH)D_3$] is mainly 1α-hydroxylated in the kidney to its active form, but also it has been shown to be processed in immune cells (92). CD3- and CD28-activated T cells as well as DCs are able to produce 1,25-$(OH)_2D_3$ from its precursor, but T cells could not directly produce precursor from VitD3 under these conditions (93). In support of this, it is shown that 1α-hydroxylase is highly upregulated in activated human T cells (94). Furthermore, 1,25-$(OH)_2D_3$ upregulates VDR expression, which has been shown to correlate with the protective effects of 1,25-$(OH)_2D_3$ in EAE (95). Nevertheless, it is reported that 1,25-$(OH)_2D_3$ induces FoxP3$^+$ Treg conversion. The authors also showed that MS patients have lower levels of calcidiol and 1,25-$(OH)_2D_3$ during active disease, suggesting that VitD3 metabolites might play a role in controlling Treg formation and the inflammatory process. This role is further supported by findings about positive

correlations of serum calcidiol and Treg function in MS patients (94,96). These studies also received support in a retrospective analysis of serum samples preceding disease onset and from epidemiological studies showing an inverted correlation of sun exposure and MS prevalence (97). Although CNS-specific conversion of encephalitogenic T cells into $FoxP3^+$–induced Tregs is described (12), its correlation to local VitD levels is still obscure. The instability of pancreas-retrieved Tregs has been suggested (98), but it is not reported if this is correlated to levels of VitD derivatives in pancreas of diabetic mice. Studying the endogenous levels of VitD derivatives in other target tissues of immune attacks, such as in gut, joint and lung, and their role in induction of tissue-induced Tregs could be valuable.

VitA and VitD Crosstalk to Maintain Immune Homeostasis

It is noteworthy to highlight that VitA and VitD could be tightly connected to regulate immunity, since their signaling receptors are interconnected. It is reported that the retinoic acid receptor and the VDR must form heterodimers with RXR to signal. Additionally, another derivative of VitA, 9-*cis* retinoic acid, increases the affinity of VDR/RXR heterodimers to its DNA recognition site, induces recruitment of coactivators by the DNA-bound heterodimer and potentiates VitD-dependent transcriptional responses. Consistent with this hypothesis, both VitD and VitA are shown to be involved in regulation of TGF-β production and TGF-β signaling molecules (including Smad3), FoxP3 and hence Treg generation and suppressive functions, inhibition of effector T helper cells by inhibiting IL-2 production, as well as inhibiting effector Th1 and Th17.

GLUTEN AND TREGS

Gluten, a protein composite of glutenin and gliadin, is found in grains such as rye, wheat and barley. Its ubiquitous presence in processed food makes selection of diet for individuals who suffer from gluten sensitivity a particularly onerous task. Celiac disease is a genetically based autoimmune disorder causing small-intestine injury elicited by the ingestion of gluten (99). The standard treatment of celiac disease is a gluten-free diet, which leads to a poor response in up to 30% of patients (99). The influence of this therapy on $CD4^+CD25^+FoxP3^+$ Tregs was investigated in a limited number of celiac disease patients. The percentage of circulating $CD4^+CD25^+FoxP3^+$ Tregs significantly decreased in patients who had received a gluten-free diet. Although, the mean FoxP3 expression levels quantified by fluorescence-activated cell sorter analysis in circulating $CD4^+CD25^+FoxP3^+$ Tregs were significantly lower in patients who had abstained from gluten compared with individuals on a standard diet, their *in vitro* suppressive functions were preserved (100). These results indicate that ingested gluten might influence the generation of Tregs. However, these observations could also be interpreted as the immune system's intrinsic attempt to control the ongoing immune response against gluten and the intestinal inflammation by generating Tregs in patients with a standard diet.

In nonobese diabetic mice, a gluten-free diet has been correlated with a decreased incidence of T1D (101–103). A recent hypothesis addressing the possibility of a direct influence of gluten on the development of Tregs analyzed differential effects of a gluten-free diet in an autoimmune genetic model (104). Nonobese diabetic and BALB/c mice were fed either a standard diet or a gluten-free diet. As expected, the standard-fed group showed a significantly higher incidence of diabetes than the gluten-free–fed group. The levels of $FoxP3^+CD4^+$ Tregs in relation to the overall amount of $CD4^+$ T cells were found to be higher in the Peyer patches of gluten-free–fed mice than in the standard-fed mice. Keeping in mind that Tregs are involved in suppressing Th1 effector cells, which are reactive toward pancreatic β cells (105), it is reasonable that a gluten-free diet can help avoid T1D pathogenesis in genetically predisposed individuals. The mechanisms by which dietary gluten modification of T1D occurs are poorly understood, even more so in how this is related to the regulation of gut-associated Tregs. However, mounting evidence suggests that the gut could play an important role as a defective barrier and a possible source of activated immune cells. Additionally, it is possible that protective gluten-free diets not only dampen immune activation, but also enhance islet function (102).

Despite these findings, the aforementioned studies do not resolve whether gluten has a direct effect on Tregs or whether any changes observed in the Treg population is a result of a gluten-induced inflammation of the intestine. One study suggests that gluten directly affects the development of Tregs (106). Peripheral blood mononuclear cells were isolated from healthy children and children suffering from celiac disease and stimulated *in vitro* with gluten. FoxP3 mRNA expression was found to be increased in gluten-stimulated cells, indicating that the glutenic stimulation of the Treg population does not depend on an intestinal inflammation. Interestingly, the extent of the FoxP3 induction was significantly higher in children with celiac disease than in reference children. It was hypothesized that memory T cells directed against an antigen closely associated with celiac disease were responsible for this effect (106).

The understanding of the basic molecular mechanisms of gluten action is currently inadequate. Its influence on generation of Tregs and on regulation of other inflammatory diseases, except for celiac disease, some allergies and partially T1D, is rather minimal and requires further investigation to allow conclusions to be drawn regarding the influences of gluten on regulation of Tregs directly and on immune regulation in general. Indeed, an earlier study investigating the effect of gluten-free diet on EAE reported that

gluten-free diet resulted in exacerbated chronic EAE (107). Interestingly, a recent study reported that ingestion of a gluten derivate induced T-cell proliferation predominantly in the spleen but little in mesenteric lymph nodes. The gluten-reactive T cells secreted much interferon (IFN)-γ but also IL-10 and had regulatory functions, which could prevent delayed-type hypersensitivity response (108).

DOCOSAHEXAENOIC ACID AND Tregs

Over the last two decades, a novel group of health-promoting food has been developed called "functional foods." Foods defined as functional were satisfactorily shown to beneficially affect one or more target functions in the body with an improvement in health and well-being and/or reduction in disease risk. Currently, functional foods, for example, cholesterol-lowering margarines, are part of many leading food companies' product lines. A typical group of ingredients of functional foods is omega-3 fatty acids, which is supplemented in products such as bread and margarine to give them a health-promoting effect. Docosahexaenoic acid (DHA) is an omega-3 fatty acid that consists of 22 carbon atoms that build six *cis* double bonds. The panoply of conditions in which DHA has been investigated run the gamut from depression (109), to neural cell death (110), to cancer (111–114) and neurodegeneration, including Alzheimer's disease.

DHA has been reported to reduce suppressive and migratory functions of Tregs (115). It is shown that coculturing $CD4^+CD25^-$ T effector cells (Teffs) with $CD4^+CD25^+$ Tregs leads to a suppression of Teff proliferation (10,116); the inhibitory capacity of Tregs on Teff proliferation was found to be decreased when Tregs were first incubated with DHA. The effect of DHA was shown to be dose dependent; a concentration of 100 μmol/L was sufficient to completely reverse the inhibitory effect of Tregs. Interestingly, the same observations were made when Teffs instead of Tregs were preincubated with DHA, indicating a general inhibitory capacity of DHA, likely by affecting the negative T-cell signaling via upregulation of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). The effects observed *in vitro* were confirmed by *in vivo* studies in mice. Tregs isolated from mice fed a DHA diet failed to suppress proliferation of Teffs isolated from mice fed a standard diet when cocultured. Surprisingly, this was not the case if both T-cell types were isolated from mice fed a DHA diet. Further effects of DHA treatment both *in vitro* and *in vivo* were an increased expression of FoxP3, CTLA-4 and TGF-β mRNA, whereas IL-10 mRNA expression decreased in Tregs. It is noteworthy that classically Treg-suppressive activity was partially attributed to IL-10 and CTLA-4 (117). In Teffs, DHA induced the expression of CTLA-4, IL-10 and FoxP3, which means that these cells obtained a Treg-like phenotype. However, these Treg-like cells were still not capable of suppressing the proliferation of untreated Teffs. In support of earlier studies, a recent report shows a similar protective effect of DHA on EAE associated with reduced numbers of IFN-γ– and IL-17–producing Teffs in both spleen and CNS, as well as increased Treg markers without increase in the suppressive function of Tregs (118). In summary, the effects of DHA treatment seem to be rather complex: on one hand, this fatty acid exerts an inhibitory effect on migratory and Treg function; on the other hand, it promotes the expression of the typical Treg markers TGF-β and FoxP3. Additionally, it should be mentioned that earlier studies have shown that FoxP3 and CTLA-4 expression alone were not sufficient to convert a T cell into a Treg capable of suppressing Teff proliferation (119). Other factors are required to interact with FoxP3 to assign Treg properties to a T cell.

HIGH-FAT DIET AND Tregs

Tregs are key players in hepatic immune regulation (120,121). A high-fat diet in mice induced hepatic steatosis, a model for nonalcoholic steatohepatitis. The high-fat diet caused a gradual decrease in hepatic Tregs. In contrast, splenic Treg levels were not affected by this dietary change. The decrease of hepatic Tregs in high-fat diet–fed mice was linked to an increased susceptibility of these Tregs to apoptosis (122). Treg apoptosis in high-fat diet–induced steatosis is probably induced by local reactive oxygen species, which are involved in T-cell apoptosis (123). Tregs, in contrast to $CD25^-$ Teffs, were found to exhibit low expression levels of Bcl-2, a protein that protects cells from reactive oxygen species–induced apoptosis (124).

Deregulation of Tregs as a result of oxidative stress and other processes might be involved in the progression of fatal steatohepatitis. Mn(III) tetrakis (4-benzoic acid) porphyrin (MnTBAP) is an antioxidant that has been shown to decrease free radical generation in ob/ob mice (125). When high-fat diet–fed mice were treated with MnTBAP, Treg apoptosis was reduced and Treg depletion was reversed. In untreated mice, Treg depletion in high-fat diet–induced steatosis led to increased expression of the proinflammatory cytokine TNF-α, which could be reversed upon passive transfer of Tregs. Briefly described, increased oxidative stress in fatty liver caused the apoptosis of Tregs, reduced the number of hepatic Tregs and led to a lowered suppression of inflammatory responses (122).

The relevance of high-fat diet to obesity-related disorders such as type 2 diabetes mellitus has received major attention. Recently, a distinct subset of Tregs was identified as an adipose tissue–resident population that seems to affect metabolic parameters, in particular, insulin resistance secondary to obesity. Such cells accumulated with age in lean mice to eventually represent more than half of the $CD4^+$ T cells residing in the visceral fat. Interestingly, Tregs were not enriched in subcutaneous adipose depots. Of relevance, the changes in visceral but not in subcutaneous fat were associated with insulin resistance. On the contrary, the percentage and number of fat Tregs were substantially reduced in obese mice compared with lean controls.

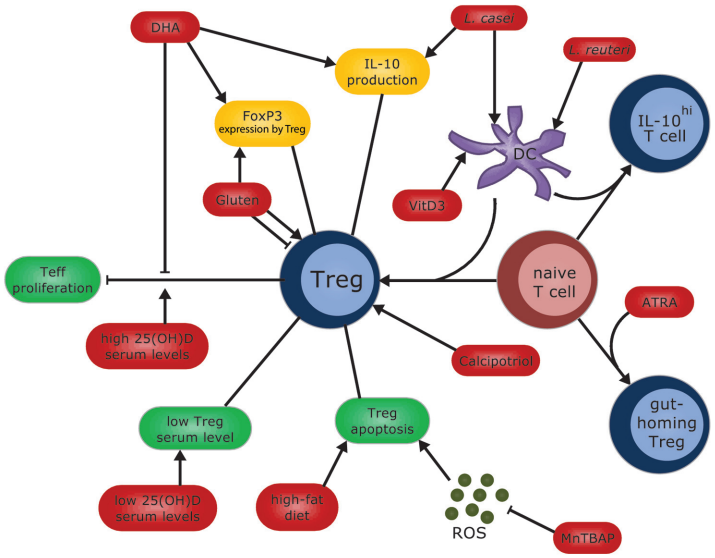

Figure 2. Overview of dietary components and conditions (represented by red objects) influencing regulatory T cells. The Treg-modulating effects of dietary components could be direct by mediating expansion and/or suppressive function of Tregs or indirect via modulation of DCs and their subsequent actions on Tregs. Additionally, the indirect influence of dietary components could be mediated by regulation of naive T cells to convert to Tregs or by blocking the function of inflammatory Teffs. The positive antiinflammatory effects of Tregs are mediated by expression of FoxP3 and production of IL-10 (indicated by yellow color). ROS, reactive oxygen species. IL-10^{hi}, high expression of IL-10.

This result was also accompanied by increased proinflammatory innate and adoptive immune cells (137). The actual reasons for the decrease in the Treg fraction in abdominal adipose tissue during obesity are still not well understood; however, it is interesting to speculate that increased oxidative stress in abdominal adipose fat could contribute to apoptosis of fat-resident Tregs. This result consequently could lead to increased attraction of proinflammatory cells to change the immune balance.

Figure 2 and Table 6 summarize how the above-mentioned dietary components could affect Treg properties and their function.

DO TISSUE-SPECIFIC FACTORS INFLUENCE Tregs?

As discussed, over the last few years, different food components such as vitamins and fatty acids have been identified to influence the development and character of Tregs *in vivo* and *in vitro*. This

Table 6. Overview of substances and conditions affecting Tregs in different models.

Substance/condition	Effects on Tregs	Model	Reference
Gluten	Decrease in % $CD4^+$ $FoxP3^+$ T cells	Mouse (NOD)	104
Gluten	Increase in circulating $CD4^+$ $CD25^+$ $FoxP3^+$ T cells and FoxP3 expression within these cells	Human (celiac disease)	100
Gluten	Increased FoxP3 expression in PBMC cultures	Human (*in vitro*)	106
DHA	Decrease of Tregs' inhibitory capacity on Teff proliferation; increased expression of FoxP3, CTLA-4, TGF-β; decreased IL-10 expression	Mouse (*in vitro*)	115
High-fat diet	Hepatic Treg depletion due to increased Treg apoptosis	Mouse	122
MnTBAP	Decrease in Treg apoptosis due to reduced levels of reactive oxygen species	Mouse	122
High 25(OH)D serum levels	Increase in the ability of Tregs to suppress T responder cells; IFN-γ/IL-4 ratio (Th1/Th2 balance) directed toward IL-4 (Th2)	Human (MS)	96
High 25(OH)D serum levels	Low blood Treg percentages	Human (MS)	135
Calcipotriol (topical)	Induction of Tregs that prevent the priming of cytotoxic T cells in response to locally administered antigen	Mouse	80
VitD3	VitD3-primed DCs can convert T cells to Tregs	Human (*in vitro*)	136
ATRA	Conversion of naive $CD4^+$ $FoxP3^-$ T cells into gut-homing $CD4^+$ $FoxP3^+$ $CCR9^+$ T cells	Human and mouse (*in vitro*)	68
VitD3	EAE reduced in females	Mouse	82

25(OH)D, 25-hydroxyvitamin D.

finding raises not only questions about the influence food components have on the immune system, but also which role different tissue environments might play in the formation of specific tissue-induced Tregs. A Treg generated in the liver that is exposed to high concentrations of vitamins, such as VitD, as well as free radicals might be different than those generated in other local conditions (for example, in brain or pancreatic tissue). Therefore, these cells might have different characteristics than iTregs generated in the spleen or mesenteric lymph nodes.

It is tempting to speculate that the exposure of T cells to cell-type specific fatty acid components, vitamins derivates and other factors in different tissues could contribute to the generation of differential tissue-specific iTregs. Indeed, the brain milieu in general and neurons in particular have been shown to support generation of Tregs from encephalitogenic Th1 cells, mediated by neuronal production of TGF-β and B7.1 costimulatory signaling (12). Additionally, a distinct subset of Tregs was identified to reside in adipose tissue, and this subset has an effect on insulin resistance (137). How these tissue-specific Tregs are generated are not yet completely understood, and further investigation is needed to understand how tissue-specific Tregs in general are generated and controlled and how they influence homeostasis of different tissues. More importantly, what are the tissue-specific cells, factors and environmental requirements to generate and maintain the function of tissue-specific Tregs? It is also important to point out that lack of stability of some tissue-specific iTregs has been reported. It was shown that a population of Tregs expressing Foxp3 are unstable *in vivo*; these so-called exFoxp3 cells with shared ontogeny with $Foxp3^+$ Tregs and conventional T cells become significantly higher in percentage in the autoimmune diabetes setting, but surprisingly they are capable of transferring diabetes instead of protecting (98). The tissue factors that facilitate generation of more stable iTregs such as VitA and TGF-β have been reported; however, the full understanding is still lacking. Hence, it is valuable to understand how Tregs are influenced by tissue-specific factors, some of which may include similar dietary derivatives. Understanding the nature of trans exchange of VitA and VitD derivatives or fatty acids such as DHA between tissue-specific cells such as neurons, β islet cells and tissue-infiltrating inflammatory T cells will prove fruitful to identify if such factors contribute to the generation, functionality and stability of Tregs to prevent chronic tissue damage.

CONCLUSION

$CD4^+CD25^+FoxP3^+$ Tregs play a central role in maintaining peripheral immune tolerance. As such, it is not surprising that proper functioning of Tregs is important in gut-associated immunity. Even more understandable is why the critical gut-associated immune cell population, Tregs, adapts itself to the influence of recurrent ingested dietary components such as VitA and VitD, gluten and fatty acids. Additionally, as functional food containing probiotics become more widely available and popular, understanding the mode of action of these dietary factors on regulation of Treg homeostasis could prove valuable to control many chronic inflammatory conditions directly affecting the gut (IBD, Crohn's disease) or indirectly influencing other chronic tissue inflammatory conditions (T1D, MS and RA) regulated by Tregs.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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