

Contents lists available at [SciVerse ScienceDirect](#)

# Medical Hypotheses

journal homepage: [www.elsevier.com/locate/mehy](http://www.elsevier.com/locate/mehy)

## Immunoplasticity – Triggers of regulatory function

Adrian M. Moser<sup>a,\*</sup>, Helmut J.F. Salzer<sup>b,1</sup>, Robert Krause<sup>b</sup><sup>a</sup> Department of Internal Medicine, Elisabethinen Hospital Graz, A-8010 Graz, Austria<sup>b</sup> Section for Infectious Diseases, Division of Pulmonology, Department of Internal Medicine, Medical University of Graz, Austria

## ARTICLE INFO

## Article history:

Available online xxx

## ABSTRACT

Regulatory T cells (Tregs) as key players of the immune system are exposed to numerous triggers including exogenous and endogenous factors. Autonomous nerve activity, melatonin, hormones such as vitamin D and glucocorticoids as well as the exposure to sunlight and microorganisms shape our immunological profile. The complexity of this system is highlighted by the power of each single trigger but more impressive by influencing each others function and potentials directly and indirectly. However, while mono-causal correlations of single triggers on Tregs have been studied at length, there is much less known about the impact of numerous coexistent triggers on the dynamics of Treg activity. It can be hypothesized that the dynamics of Treg activity plays a crucial role for the control of our immune system. Therefore it is of the utmost importance that a new translational, multi- and interdisciplinary approach finds its way into future research efforts, which should lead to a more comprehensive and holistic view on the complex immunoregulatory mechanisms and to act in the sense of public health.

© 2011 Elsevier Ltd. All rights reserved.

### Regulatory T cells: the emerging key player of immunoregulatory mechanisms

The balance of inflammation is a critical point in many diseases. On the one hand a sufficient inflammatory response has to be created to fight microbial infections; on the other hand a sufficient inhibition of inflammatory pathways must be provided to control excessive inflammation and its harmful effects.

The immune responses are tight regulated by “upper level” regulation such as the psychoneuroendocrinological control and also the regulations in the system itself such as the impact of infections on the immune responses via antigens triggered bystander suppression. Observing that organisms have an impact on immunoregulation led to the “old friend hypothesis”, first used by Rook and colleagues, suggesting that different organisms including saprophytic mycobacteria, bifidobacteria, lactobacilli and helminthes, are recognized by the human innate immune system as innocuous. These organisms can induce specific and unusual maturation patterns of dendritic cells, which in turn can induce Treg responses [1]. In all these controlling pathways Tregs, which are able to inhibit immune responses in different ways, are crucial. But what are these Tregs? Classified into different subgroups they include natu-

rally occurring Tregs which interestingly can be transformed to IL-17 secreting pro-inflammatory Th17 cells via IL-6. In contrast, induced Tregs do not transform after exposure to IL-6 and stay in the regulatory function. However, both subgroups provide similar phenotypes and functions and it has been shown that naive CD4<sup>+</sup>CD25<sup>-</sup>FOXP3<sup>-</sup> T cells attain a transient CD25<sup>+</sup>FOXP3<sup>+</sup> state when confronted with different stimuli. FOXP3 expression and regulatory function are not exclusive features of a stable or unique lineage of T cells, but may also be a transient state attained by almost CD4<sup>+</sup> T cells and the population is considerably more flexible than previously thought. Exogenous – as well as endogenous triggers might be able to regulate the differentiation of T cell populations into a responsive Treg population in humans, but interestingly this wasn't observed in mouse effector T cells [2]. Activated FOXP3<sup>+</sup> cells have the capacity to down regulate FOXP3, which may correlate with their scope of suppression and interestingly, they can be reactivated. Could it be that these induced Tregs play the most important role in maintaining the balance of the transient regulatory function and further, which cells should be included to draw suitable conclusions by commonly used flow cytometric staining to distinguish between transient Tregs? The majority of published articles define Tregs as the CD4<sup>+</sup>CD25<sup>high</sup> population. Thus, it's generally accepted that this population is highly regulatory and prevents harmful immune responses, although a huge number of FOXP3<sup>+</sup> Tregs which are CD25<sup>intermediate</sup> are not considered and probably bias percentages of outcomes of clinical studies, especially when regarding the activation profile of CD25 indicating the high dynamic properties of these cell line [3,4]. So, which triggers lead to these transient regulatory functions? If the psychoneu-

Abbreviations: Tregs, regulatory T cells; FOXP3, forkhead box P3;  $\alpha 7nAChR$ ,  $\alpha 7$  nicotinic acetylcholine receptor.

\* Corresponding author. Address: Department of Internal Medicine, Elisabethinen Hospital Graz, Seebachergasse 4, 8010 Graz, Austria.

E-mail address: [adrianm.moser@hotmail.com](mailto:adrianm.moser@hotmail.com) (AM. Moser).

<sup>1</sup> These two authors contributed equally.

roendocrinological system such as acetylcholine, vitamin D, corticosteroid systems, and melatonin has some control over the immune system, could it be that these signals lead to transient regulatory function?

### Vagus nerve as a potent real time immunoregulator

The autonomous nerve system plays a key role in the regulation of the immune system. The first studies of Tracey and colleagues showed the importance of acetylcholine in control of inflammation. Stimulation of the vagus nerve is inhibiting macrophages through the cholinergic nicotine receptor,  $\alpha 7nAChR$ , suppressing the release of pro-inflammatory cytokines, such as TNF, which has routinely been used as a drug target for rheumatoid arthritis and Crohn's disease [5,6]. Furthermore clinical studies of heart rate variability showed correlations of low vagus activity with the disease severity of rheumatoid arthritis [7]. The implementation of this mechanism is now highly discussed and nicotine therapy on its way to therapeutically use, while Tracey discussed the possible role of the nicotinic pathway in acupuncture, which has been known since thousands of years [8].

Since vagus nerve stimulation can regulate the immune system in real time, physiological stimulation should be considered as a preventive strategy and treatment option, showing a link of lifestyle and inflammatory diseases [5]. The role of Tregs in the cholinergic anti-inflammatory pathway is rather unknown, since the only study concerning this issue was conducted nearly 20 years ago before Tregs took a centre stage in immune regulation. Thereby nicotine induced a suppressive T cells population and may represent the first link of a differentiation pathway to immunoregulatory function [9].

### “Zeitgeber” as a link between endocrine- and autonomous nerve system

Melatonin and its role in the circadian rhythm is well known and evidence about the significance in immune-mediated diseases and cancer is arising. Moreover, the immune-modulating role of melatonin on T cell populations has been shown although no studies visualize the effect on Tregs indicating a future perspective of research.

Melatonin itself has miscellaneous effects on the immune system and also, interestingly, on cholinergic anti-inflammatory pathways, since Nishiyama and colleagues demonstrated that melatonin administration increased vagal tone in healthy men and elevated heart rate variability [10]. The link between melatonin and vagal activity is a direct and indirect, while melatonin induces vagal activity, but also light inhibits melatonin production and vagal activity via the “Zeitgeber”, suprachiasmatic nucleus [11]. This essential mechanism is even more interesting when looking at vitamin D, which is produced in the skin during sunlight exposure, thus inversely correlated. It could be hypothesized that the common focus of these physiological immune-modulating mechanisms is light [12]. Therefore it should be considered that also natural Tregs follow a 24 h circadian rhythm of their regulatory function and number with the highest levels during night correlating with high melatonin level and vagus activity [13].

### Vitamin D: the key hormone for a sustainable health?

In the last few years we have been confronted with a huge wave of published research articles regarding the role of vitamin D in immunological disorders. It seems that this vitamin presents a major link between the body and the environment. Regulatory function and other immunomodulatory effects of vitamin D such as

induction of Tregs, production of IL-4, IL-10, decreasing cytotoxicity, and IL-17 levels and furthermore elevated proliferation of macrophages and production of the antimicrobial peptide cathelicidin, has been shown [14]. The induction of FOXP3<sup>+</sup> T cells might indicate a central role in immunoregulation, reconsidering also the fact that macrophages express the enzyme 1- $\alpha$ -hydroxylase, which converts constitutively the inactive 25-hydroxyvitamin D3 into the active state of 1, 25-dihydroxyvitamin D3 [15]. In sarcoidosis the link between high vitamin D levels in blood of patients, hypercalcemia and high amount of FOXP3<sup>+</sup> T cells in granulomas could eventually show this pathway of activation between macrophages and T cells [16,17]. Furthermore it has also been reported that respiratory epithelial cells induce cathelicidin in a 1, 25-dihydroxyvitamin D3 dependent manner and poses the potential effects on host defense in the lung [15]. These findings are underlined by translational studies where patients with low levels of vitamin D or vitamin D receptor polymorphisms were more susceptible to mycobacterial infections and showed even a more severe course of the disease. Furthermore cathelicidin is known to play an important role in the innate immunity. Could it be that cathelicidin plays a vital role for pulmonary infections and furthermore may demonstrate the link between colonization versus infection?

Animal models of vitamin D and autoimmune diseases show its immunoregulative properties such as prevention of experimental allergic encephalomyelitis or inhibition of cutaneous contact hypersensitivity [14]. Epidemiological studies show that living at higher latitude increases risk of autoimmune diseases, colon- and others cancers, cardiovascular diseases, depression and impaired lung function [12]. Although these correlations are of particular importance, vitamin D supplementation studies showed incoherent data and reflect the complexity of intervention in immunological regulations. Recent studies on cardiovascular health and vitamin D supplementation demonstrated no improvement of cardiovascular risk factors in the intervention group [18]. The problem of our time now is to find out how to implement these findings into daily clinical work and to act in the sense of public health.

### Glucocorticoids orchestrate Treg function

Since Professor Hench was awarded to Nobel Prize of Medicine for the treatment of rheumatoid arthritis with glucocorticoids, this treatment revolutionized our modern medicine and is now established as the most effective anti-inflammatory treatment. However, molecular mechanisms of glucocorticoid resistance have been reported and even the application of high steroid doses is insufficient for treatment. In these patients vitamin D re-established Treg function and secretion of IL-10 showing links between those pathways [19]. Glucocorticoid treatment mediates its effect through different anti-inflammatory pathways including the induction of Tregs or restoration of Treg function [20]. These facts that the glucocorticoid system and vitamin D act in such entangled ways shows, that different anti-inflammatory pathways maintain Treg function. This gets even more interesting when looking at the circadian rhythm profile of the hormone system including glucocorticoids, which is also controlled by the suprachiasmatic nucleus and is reset on a daily basis by light inputs [21]. Therefore, light plays again in both regulatory mechanisms a key role, since sunlight induces vitamin D production and modulates glucocorticoid secretion.

### Targeted infections: potential for treatment?

As mentioned above infections have a huge capacity in modulating some autoimmune and allergic disorders. Hence, Tregs

mediate immunoregulatory mechanisms, since it has been shown that various autoimmune disease patients have impaired Treg function which can be positively influenced by presence of helminths such as *Trichuris suis*. Therefore, the complex relationship between Tregs and especially the long-term presence of pathogens is of the utmost interest. The number of immunocompromised patients is continuously raising and poses the risk of a re-activation of long-term innocuous colonization to a potential life-threatening disease. Indicating that Tregs possess Janus-faced properties [22]. This aspect should be taken into account, when discussing issues where colonization versus infection is not fully elucidated including for example the pathogenetic role of *Candida* species in the lower respiratory tract. Therefore modern lifestyle and improved sanitation is linked to immune-mediated diseases and the reduced exposure to infectious agents. Tregs are again standing in the focus.

Tregs represent the latest trend in immunology, since they show possibilities of regulating harmful actions of the immune system and while maintaining peripheral tolerance. The induction of Tregs is of huge interest regarding autoimmune and allergic diseases, but also atherosclerosis and the maintenance of tolerance in non-autoimmune diseases including infectious diseases. The discussed issues reveal on the one hand the plasticity of T cells gaining regulatory functions and on the other hand mechanisms which preserve and influence this plasticity. The exciting results that glucocorticoids, vitamin D and obviously other stimuli as mentioned induce Tregs and that these stimuli underly day- and year-round rhythms with a common focus brings new insights of how to implement these physiological pathways into daily clinical practice. Circadian rhythms as well as influences of hormones by sleep and light exposure seems to be of the utmost importance regarding disease management.

The scientific world is now highly interested in translational and clinical approaches to contribute, not only to a better scientific and academic understanding of the balance of the immune regulations, but also to foster a holistic approach including population-based health- and disease related determinants to set evidence based priorities in terms of public health.

#### Conflict of interest statement

Authors declare no conflict of interests.

#### Authors' contributions

All of the authors have made substantive intellectual contributions and helped to draft the manuscript. Authors read and approved the final manuscript.

#### References

- [1] Rook GA, Adams V, Hunt J, Palmer R, Martinelli R, Brunet LR. Mycobacteria and other environmental organisms as immunomodulators for immunoregulatory disorders. *Springer Semin Immunopathol* 2004;25:237–55.
- [2] Pillai V, Ortega SB, Wang CK, Karandikar NJ. Transient regulatory T-cells: a state attained by all activated human T-cells. *Clin Immunol* 2007;123:18–29.
- [3] Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med* 2006;203:1693–700.
- [4] Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *J Exp Med* 2006;203:1701–11.
- [5] Tracey KJ. Physiology and immunology of the cholinergic antiinflammatory pathway. *J Clin Invest* 2007;117:289–96.
- [6] Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 2003;421:384–8.
- [7] Evrengül H, Dursunoglu D, Cobankara V, Polat B, Selecı D, Kabukçu S, et al. Heart rate variability in patients with rheumatoid arthritis. *Rheumatol Int* 2004;24:198–202.
- [8] Oke SL, Tracey KJ. The inflammatory reflex and the role of complementary and alternative medical therapies. *Ann N Y Acad Sci* 2009;1172:172–80.
- [9] Menard L, Rola-Pleszczynski M. Nicotine induces T-suppressor cells: modulation by the nicotinic antagonist  $\alpha$ -tubocurarine and myasthenic serum. *Clin Immunol Immunopathol* 1987;44:107–13.
- [10] Nishiyama K, Yasue H, Moriyama Y, Tsunoda R, Ogawa H, Yoshimura M, et al. Acute effects of melatonin administration on cardiovascular autonomic regulation in healthy men. *Am Heart J* 2001;141:E9.
- [11] Mutoh T, Shibata S, Korf HW, Okamura H. Melatonin modulates the light-induced sympathoexcitation and vagal suppression with participation of the suprachiasmatic nucleus in mice. *J Physiol* 2003;547:317–32.
- [12] Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266–81.
- [13] Bollinger T, Bollinger A, Skrum L, Dimitrov S, Lange T, Solbach W. Sleep-dependent activity of T cells and regulatory T cells. *Clin Exp Immunol* 2009;155:231–8.
- [14] Moro JR, Iwata M, von Andriano UH. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol* 2008;8:685–98.
- [15] Hansdotter S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J Immunol* 2008;181:7090–9.
- [16] Idali F, Wahlström J, Müller-Suur C, Eklund A, Grunewald J. Analysis of regulatory T cell associated forkhead box P3 expression in the lungs of patients with sarcoidosis. *Clin Exp Immunol* 2008;152:127–37.
- [17] Tafllin C, Miyara M, Nochy D, Valeyre D, Naccache JM, Altare F, et al. FoxP3+ regulatory T cells suppress early stages of granuloma formation but have little impact on sarcoidosis lesions. *Am J Pathol* 2009;174:497–508.
- [18] Jorde R, Sneve M, Torjesen P, Figenschau Y. No improvement in cardiovascular risk factors in overweight and obese subjects after supplementation with vitamin D for 1 year. *J Intern Med* 2010;267:462–72.
- [19] Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest* 2006;116:146–55.
- [20] Xu L, Xu Z, Xu M. Glucocorticoid treatment restores the impaired suppressive function of regulatory T cells in patients with relapsing–remitting multiple sclerosis. *Clin Exp Immunol* 2009;158:26–30.
- [21] Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 2005;437:1257–63.
- [22] Bufford JD, Gern JE. The hygiene hypothesis revisited. *Immunol Allergy Clin North Am* 2005;25:247–62.