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# Review

# Regulatory CD4$^+$CD25$^+$ T cells and macrophages: communication between two regulators of effector T cells

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**Abstract**. Regulatory T cells (Tregs) play an essential role in the induction and maintenance of peripheral tolerance as well as prevention of autoimmunity by limiting the strength of the immune response of effector T cells. Macrophages, a heterogeneous population of phagocytes and professional antigen presenting cells (APCs), can also exert suppressive effects on effector T cells to keep the peripheral balance of immunity. The bi-directional interactions of dendritic cells (DCs) and Tregs have been cell studied. However, much less is known about the reciprocal interaction between macrophages and Tregs. In this review, we will discuss recent observations regarding the interplay of these two regulators of immunity.

**Key words:** Regulatory T cells – Macrophages – Interaction – Effector T cells

**Abbreviations:** AAM, alternatively activated macrophages; ACAID, anterior chamber–associated immune deviation; APC, antigen presenting cell; CpG-ODN, immune-stimulatory oligonucleotide with CpG sequences motifs; CTLA-4, cytotoxic T lymphocyte antigen; CNS, central nervous system; DCs, dendritic cells; DTH, delayed-type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; ECL, entorhinal cortex lesion; FoxP3, forkhead box protein 3; GITR, glucocorticoid-induced tumour necrosis factor receptor; HLA- II, leukocyte antigen class II; IBD, inflammatory bowel disease; IDO, indoleamine 2,3-dioxygenase; IFN-gamma, interferon-gamma; iNOS, inducible nitric oxide synthase; KCs: Kupffer's cells, LPS: lipopolysaccharide; MDC, macrophage-derived chemokine; MHC, major histocompatibility complex; MLR, mixed lymphocyte response; NO, nitric oxide; TARC, thymus and activation-regulated chemokine; TGF-beta, transforming growth factor-beta; TECs, thymic epithelial cells; TLR, toll like receptor; TNF-alpha, tumor necrosis factor-alpha; Tregs, regulatory T cells

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## Introduction

Proper immune responses are largely shaped by the positive and negative regulating components including cells, cytokines and genes in the immune system. We have learned a lot about the positive regulation in the past. However, the information on the negative regulation of immunity is relatively sparse. Although suppressor T cells were already been noted in the early 1970s, the concept of regulatory T cells (Tregs) has been widely accepted since 1995 when Sakaguchi's group observed that CD4$^+$CD25$^+$ T cells inhibited autoimmune diseases caused by effector T cells [1].

According to differential expression of specific markers, cytokine profile, origin and mechanisms of action, Tregs are divided into several subsets and the number of subsets is still expanding. According to their immunosuppressive function, surface markers or cytokine production, a variety of Treg subsets have been identified, including CD4$^+$Tregs (naturally arising CD4$^+$CD25$^+$Tregs, IL-10-secreting Tr1 cells, TGF-β-secreting Th3 cells), Qa-1-restricted CD8$^+$ T cells, CD8$^+$CD28$^-$ T cells, CD8$^+$CD122$^+$ T cells, γ/δT cells, and NKT cells (NK1.1$^+$CD4$^-$CD8$^-$), NK1.1$^-$CD4$^-$CD8$^-$ cells[2,3]. It is, thus far, well accepted that three main types of Tregs exist in mice and humans, namely naturally occur-

**Table 1.** The characteristics of different subsets of macrophages

| | **Classical** | **Alternative** | **Type II** |
|---|---|---|---|
| Activation induction | IFN-gamma, TNF-alpha | IL-4, IL-13, IL-10, glucocorticoid | IgG complexes<br>TLR ligation |
| Cytokine production | TNF, IL-12, IL-23, IL-1, IL-6 | IL-10, IL-1R antagonist | IL-10, TNF, IL-6 |
| Chemokine production | IP-10, MIP-1 | CCL18 | unknown |
| Killer molecules | NO, O2- | none | NO, O2- |
| Antigen presentation | efficient | not efficient | |
| Phagocytic capacity | | efficient | |
| Immune receptors | MHC-II, CD86 | CD206, CD163, CD23 | CD86, MHC-II, TLR |
| Biological functions | Kill and degrade intracellular microorganisms, support specific Th1 cell response | Suppress T cell proliferation in vitro, tissue repair and wound healing | Anti-inflammatory effect,<br>Th2 humoral immune response |
| References | [68–70] | [68–70] | [68–70] |

ring $CD4^+CD25^+$ Tregs that constitutively express cytotoxic T lymphocyte antigen (CTLA-4), glucocorticoid-induced tumour necrosis factor receptor (GITR) and forkhead box protein 3 (Foxp3), inducible Tregs including transforming growth factor-beta (TGF-beta) producing T helper 3 (Th3) cells and IL-10 secreting regulatory T1 (Tr1) cells. Recent evidence shows that $CD4^+CD25^+$ Tregs can also be induced in the periphery from naïve $CD4^+$ T cells [4, 5].

Over the past ten years, accumulating data have revealed that besides autoimmune diseases [6–9], $CD4^+CD25^+$ Tregs exert regulatory functions in the control of transplantation tolerance [10], tumor immunity [11–15], and infection as well [16,17]. The extensive inhibitory effects of $CD4^+CD25^+$ Tregs involve multi-target cell communications including those with $CD4^+$ and $CD8^+$ T cells, B cells, dendritic cells (DCs), natural killer (NK) cells [18] and monocytes/macrophages.

Macrophages differentiate from myeloid precursors and form a heterogeneous population of antigen presenting cells (APCs) that link the innate and adaptive immune systems. There are three types of activated macrophages with different phenotypes and functions, i. e. classical activated macrophages, alternatively activated macrophages (AAMs) and the third type macrophages [19]. The detailed information on these three macrophages is summarized in Table 1. Tissue specific resident macrophages such as Kupffer cells (KCs) exist in the liver, microglia cells in the brain, alveolar macrophages in the lung, and osteoclasts in the bone. They all actively defend against microbial invasion and contribute extensively to the inflammatory process and tissue destruction via the elevated production of proinflammatory cytokines (tumor necrosis factor-alpha (TNF-alpha), IL-1, IL-6, IL-8, and IL-12) and via their T cell–stimulatory capacity. On the other hand, evidence suggests that macrophages can also dampen the effector T cell response as negative regulators, which is partially due to induction of $CD4^+CD25^+$ Tregs.

Up to now, much attention has been paid to the reciprocal interaction of $CD4^+CD25^+$ Tregs with effector T cells and $CD4^+CD25^+$ Tregs with DCs [20–22]. However, the crosstalk between Tregs and macrophages has not been well explored so far. In the present manuscript, we will focus on the bi-directional regulation between $CD4^+CD25^+$ Tregs and macrophages so that we may better understand their delicate modulation of the integral immune system.

## Modulation of macrophages by $CD4^+CD25^+$ Tregs

$CD4^+CD25^+$ Tregs were initially recognized as potent regulators of adaptive immune responses. Recent growing evidence has demonstrated that the suppressive effects of $CD4^+CD25^+$ Tregs are not restricted to the adaptive immune system (T and B cells) but can also affect the activation and function of innate immune cells (monocytes /macrophages, DCs, and NK cells) [20–23].

Maloy et al found that $CD4^+CD25^+$ Tregs could inhibit innate immune pathology in a murine model of inflammatory bowel disease (IBD) induced by the pathogenic bacterium, *Helicobacter hepaticus* and the inhibition was dependent on T cell–derived IL-10 and TGF-beta [24]. This study first revealed that $CD4^+CD25^+$ Tregs could negatively regulate innate immune response. Additional findings [25–27] further indicated that $CD4^+CD25^+$ Tregs might regulate the innate immune response through GITR, which is constitutively expressed at high levels on $CD4^+CD25^+$ Tregs. Although these investigations did not all focus on the cellular communication of $CD4^+CD25^+$ Tregs with their specific target cells, especially the most active player (macrophages) in these diseases, they did provide us a clue that $CD4^+CD25^+$ Tregs may affect the functions of innate immune cells.

The inhibitory function of $CD4^+CD25^+$ Tregs on macrophages in mice was observed *in vitro* and *in vivo* [28]. Mouse $CD4^+CD25^+$ Tregs modulated macrophages by inhibiting the activation of the latter, leading to reduced proinflammatory cytokine production and a downregulated effector phenotype *in vitro*. Furthermore, in a SCID mouse model of chronic renal disease, the reconstituted $CD4^+CD25^+Foxp3^{high}$ Tregs strongly reduced glomerular and interstitial injury and remarkably decreased the number of macrophages in both the glomeruli and interstitium compared with controls [28]. This protective effect of $CD4^+CD25^+$ Tregs was significantly impaired by blockade of TGF-beta [28], indicating that the inhibitory effect of $CD4^+CD25^+$ Tregs on macrophages is dependent on the presence of TGF-beta.

Taams et al [29] first showed that human $CD4^+CD25^+$ Tregs could directly suppress the functions of monocytes/macrophages *in vitro*. Co-cultured with autologous $CD4^+CD25^+$ Tregs, monocytes appeared as a small and resting phenotype. In contrast to the co-culture of monocytes with $CD4^+CD25^-$ T cells, monocytes with $CD4^+CD25^+$ Tregs produced a low level of TNF-alpha, interferon-gamma (IFN-gamma) and IL-10 as detected in co-culture medium [29]. Furthermore, the response to lipopolysaccharide (LPS) of these monocytes, pretreated with $CD4^+CD25^+$ Tregs, was severely inhibited as indicated by low production of TNF-alpha and IL-6 as well as the limited up-regulation of human leukocyte antigen class II (HLA-II), CD40 and CD80, and significant down-regulation of CD86 compared with controls [29]. However, IL-10 did not appear to play an essential role in the modulation process [29].

Kryczek et al reported that human $CD4^+CD25^+$ Tregs could induce co-inhibitory molecule expression on monocytes and render monocytes suppressive in exerting their regulatory function [30]. Compared with effector T cells, $CD4^+CD25^+$ Tregs could trigger significantly higher levels of IL-10 production by monocytes *in vitro* [30]. More interestingly, high levels of IL-10 derived from monocytes stimulated B7-H4 expression on their surfaces, which finally made them tolerogenic [30]. In contrast to the work we just referred above, this study showed that IL-10 plays an essential role in the interaction of $CD4^+CD25^+$ Tregs and monocytes [30]. The most obvious difference between these two studies is the time point of detection after coculture: the latter was at 72 hours, and the former was at 40 hours. Perhaps the additional 32-hours may account for a significant increase in IL-10 secretion. Time profiles of IL-10 secretion in the coculture of $CD4^+CD25^+$ Tregs and monocytes need to be determined to explain this distinction more clearly. Another group used similar conditions [31] but found that rather than Treg-treated monocytes, it was the non-conditioned freshly isolated monocytes that suppress T cell proliferation. Kryczek attributed this difference to T cell apoptosis caused by a high density of T cells and over activation in the system that the group used.

Recently, one group reported that human Tregs can induce monocytes/macrophages to differentiate into AAMs [32]. They revealed a different view of Treg modulation of monocytes/macrophages. Consistent with previous work of other [29], they also found that after 40-hour coculture with Tregs, monocytes/macrophages produced much reduced proinflammatory cytokines (IL-6, TNF-alpha, IL-1-beta) in response to LPS stimulation. This suppression was associated with IL-10, IL-4 and IL-13 secreted by Tregs. However, instead of general inhibition of monocytes/macrophages by Tregs, the production of anti-inflammatory cytokines, such as IL-10 and IL-1Rα secreted by monocytes/macrophages, were significantly increased. Moreover, Treg-treated monocytes/macrophages expressed more macrophage mannose receptor (CD206) and hemoglobin scavenger receptor (CD163) with greater phagocytic capacity, increased CCL18 production, as well as reduced expression of HLA-DR, which are typical features of AAMs. Treg-mediated induction of CD206 is entirely cytokine-independent, whereas the up-regulation of CD163, CCL18, and phagocytosis is (partly) dependent on IL-10 but not on IL-4/IL-13 [32].

In addition to induction of co-inhibitory molecules on monocytes, $CD4^+CD25^+$ Tregs can enhance indoleamine 2,3-dioxygenase (IDO) expression and activity in monocytes, which directly results in immunosuppression. It was reported that CTLA-4 expressed on $CD4^+CD25^+$ Tregs could up-regulate IDO expression on decidual and peripheral blood monocytes by the induction of IFN-gamma production [33]. After treatment with CTLA-4/Fc fusion protein or IFN-gamma, IDO expression on decidual monocytes was up-regulated during normal pregnancy while decreased in spontaneous abortion cases [33]. The expression of CD86 on peripheral blood and decidual monocytes in spontaneous abortion cases was lower compared with those in normal pregnancy subjects [33]. These data reveal an indirect action of $CD4^+CD25^+$ Tregs to mediate maternal tolerance to the fetus through enhancing the suppressive ability of monocytes.

On the other hand, human $CD4^+CD25^+$ Tregs could inhibit monocyte survival through a proapoptotic mechanism involving the Fas/FasL pathway *in vitro* [34]. CD14 expression on monocytes was significantly up-regulated by LPS treatment, and this effect was completely blocked by $CD4^+CD25^+$ Tregs but not by $CD4^+CD25^-$ T cells. Because loss of CD14 was considered a hallmark of monocyte apoptosis, this result suggested that $CD4^+CD25^+$ Tregs inhibited monocyte survival. Through transwell experiments and cytokine analysis, they found that this effect was independent of direct cell-cell contact and mediated through some soluble factors, neither IL-10 nor IL-4 [34]. The effect was blocked by anti-FasL mAb and reproduced by Fas agonist and recombinant soluble FasL. Furthermore, expression of FasL was much higher on Tregs than on their $CD25^-$ counterparts. Collectively, these results indicate that $CD4^+CD25^+$ Tregs could inhibit monocyte survival through Fas/FasL pathway.

Overall, because monocytes/macrophages and their mediators are known to be major contributors to chronic inflammatory conditions, these findings provide support for the inhibiting function of $CD4^+CD25^+$ Tregs on monocytes/macrophages which may be used as a potential immunotherapeutic target in clinic in the future.

## Suppression of effector T cells by subsets of macrophages

Recent studies have revealed that macrophages have the potential to curb inflammation and lymphocyte activation as a negative regulator. As a type of APCs, but different from DCs, macrophages may mainly mediate anergy in allogeneic T cells in humans and mice, supporting the concept that resting macrophages maintain peripheral immune tolerance *in vivo* [19, 35]. Several studies show that macrophages are tolerogenic in tumors and inflammatory diseases [36, 37]. The mechanism involved has three major explanations, i.e. tryptophan and arginine catabolism, nitric oxide (NO) synthesis and induction of Tregs.

In 1999, Munn et al [38] first put forward a novel mechanism that macrophages could down-regulate T cell activation via tryptophan catabolism. By combination of the T cell–derived IFN-gamma and CD40-ligand the enzyme indoleamine 2,3-dioxygenase (IDO) was induced in macrophages

and resulted in degradation of tryptophan. Seven years later, Matlack and his colleagues [35] confirmed and extended this mechanism in mice. They found that through tryptophan and arginine catabolism, peritoneal macrophages inhibited T lymphocyte activation in a cell dose-dependent and MHC-independent fashion. Similar to tolerogenic DCs, these macrophages were immature. They also found that IFN-gamma was essential in this process, while either TGF-beta or IL-10 was dispensable.

Apart from amino acid catabolism, it has been reported that NO produced by the activated macrophages can inhibit lymphocyte responses and lead to immunosuppression [39]. In mice, either disruption of the inducible nitric oxide synthase (iNOS) gene or inhibition of iNOS resulted in proliferation of pathogenic T cells and autoreactive T cells, respectively [40–42]. This mechanism was confirmed not only peripheral macrophages but also for residential macrophages in brain and liver [43–45]. Recently two reports [46, 47] further provided a clue that the B7-H1 pathway in macrophages may contribute to IFN-gamma production by naive $CD4^+$ T cells and, hence, NO production by macrophages.

## Induction of Tregs by macrophages

It is well known that Tregs have a low proliferation capacity but can expand in the presence of IL-2 and IL-15, and that Tr1 cells are induced by IL-10. Evidence shows that several kinds of cells can induce Tregs *in vitro*, including thymic epithelial cells (TECs), vascular endothelial cells, immature and semi-mature DCs, and even neurons and cancer cells [22, 48–51]. Moreover, $CD4^+CD25^+$ Tregs are capable of direct proliferation in response to microbes and parasites through toll-like receptors (TLRs) [52]. Recent evidence has demonstrated that macrophages also can induce Tregs *in vitro* and *in vivo*.

Several studies showed that TGF-beta-treated antigen-presenting $F4/80^+$ peritoneal macrophages could induce antigen-specific Tregs in both naive and primed mice [53–55]. Pretreated with TGF-beta 2 and antigen *in vitro*, the mouse $F4/80^+$ peritoneal macrophages were injected i.v. and led to the generation of Tregs *in vivo*. Furthermore, they found that in naive mice most of the Tregs induced by TGF-beta-treated macrophages were $CD4^+$ Tregs and this involved the production of TGF-beta, while in primed mice the Tregs induced were $CD8^+$ Tregs and involved Fas-mediated deletion of effector T cells. Another study showed that $Gr\text{-}1^+CD115^+F4/80^+$ myeloid suppressor cells from tumor-bearing mice and cancer patients could suppress T cell proliferation *in vitro* and induce the development of $Foxp3^+$ Tregs *in vivo*. Upon IFN-gamma stimulation, $Gr\text{-}1^+CD115^+F4/80^+$ myeloid suppressor cells produced more IL-10 and TGF-beta. The development of Tregs depended on the presence of IFN-gamma and IL-10, and was independent of the NO-mediated suppressive mechanism [56].

Using $F4/80^{-/-}$ mice and anterior chamber–associated immune deviation (ACAID) model, Lin et al found that the mouse macrophage-restricted F4/80 protein was required for generation of antigen-specific $CD8^+$ Tregs that could suppress antigen-specific immunity [57]. Either in the in-vivo or the in-vitro ACAID model, $F4/80^{-/-}$ mice or $F4/80^{-/-}$ macrophages could not effectively induce Tregs and suppress delayed-type hypersensitivity (DTH) responses. Peripheral tolerance was restored in $F4/80^{-/-}$ mice by adoptive transfer of $F4/80^+$ APCs. Therefore, the F4/80 molecule seems indispensable in the generation of efferent $CD8^+$ Tregs in this model.

Using entorhinal cortex lesion (ECL) to induce axonal degeneration in the hippocampus, Kwidzinski et al found that Myelin-phagocytosing microglia expressed MHC-II and the costimulatory molecule CD86, but lacked CD80, which is found only on activated APCs. The authors considered that immature macroglia may lead to T cell anergy and/or differentiation of regulatory/Th3-like cells due to insufficient costimulation and presence of high levels of TGF-beta and IL-10 in the central nervous system under the pathological conditions [58].

In humans, macrophages derived from peripheral monocytes and co-cultured with naive T cells, could induce IL-10-producing $CD4^+CD25^+$ Tregs [19]. Using the model of allogeneic mixed lymphocyte response (MLR), Hoves et al found that naive T cells did not proliferate in co-culture with macrophages even in the presence of anti-CD28 mAb and IL-2. Meanwhile, a high level of IL-10 was detected in the co-culture medium. Furthermore, only minimal T cell proliferation was observed in a secondary MLR when T cells were rescued from primary MLR with macrophages and re-stimulated with DCs of the same donor, or DCs of an unrelated donor (the third party). Among the T cells that were rescued from co-culture with macrophages, IL-10-producing anergic $CD4^+CD25^+$ Tregs were found [19]. Interestingly, macrophages could not induce T cell anergy and Tregs when they were co-cultured with pre-activated T cells. This result indicates that resting macrophages may play a role in the early events of inducing $CD4^+CD25^+$ Tregs and exert immune surveillance in "peace time".

## Regulation of $CD4^+CD25^+$ Tregs by macrophages

Up to now, there are few papers related to the direct effect of macrophages on $CD4^+CD25^+$ Tregs. Christiane Wiegard et al [59] first showed that mouse KCs, the resident macrophages in the liver, could stimulate $CD4^+CD25^+$ Treg proliferation *in vitro*. They found that when KCs and $CD4^+CD25^+$ Tregs were co-cultured in the presence of anti-CD3 antibody, a fraction of the $CD4^+CD25^+$ Treg population divided twice within 4 days. Moreover, the expanded $CD4^+CD25^+$ Tregs had suppressive characteristics, though their ability to suppress was lower than that of freshly isolated $CD4^+CD25^+$ Tregs[59]. From this point, KCs seemed able to enhance $CD4^+CD25^+$ Treg function by increasing the number of effective $CD4^+CD25^+$ Tregs. However, when KCs and $CD4^+CD25^+$ Tregs were co-cultured in the presence of LPS or immune-stimulatory oligonucleotide with CpG sequence motifs (CpG-ODN), the suppressive effect exerted by $CD4^+CD25^+$ Tregs on effector T cells was decreased[59]. These results indicate that KCs are active players in maintaining tolerance in the liver under physiological conditions and contribute to inflammatory responses. Interestingly, IL-6 produced by KCs did not play a central role in this process. Which molecules mediate the regulation of $CD4^+CD25^+$ Tregs by KCs is still unknown.

### Recruitment of Tregs via chemokines from macrophages

Attracted by chemokines secreted by APCs and effector T cells, $CD4^+CD25^+$ Tregs migrate to appropriate sites where regulation is required to exert their immunosuppressive function. In terms of their trafficking potential, it has recently been shown that $CD4^+CD25^+$ Tregs can be subdivided into (at least) two distinct populations [60,61]. One subset expresses L-selectin, CCR4, and CCR7 and preferentially homes to secondary lymphoid organs (naive-like $CD4^+CD25^+$ Tregs) and the other expresses E/P-selectin ligands, CCR2, CCR4, CCR5, and CCR6 that enable their migration to inflammatory sites (effector/memory-like $CD4^+CD25^+$ Tregs).

Macrophage-derived chemokine (MDC/CCL22) and its receptor CCR4 have been implicated in chronic inflammatory processes and in the homing of monocytes, Th2 cells and $CD4^+CD25^+$ Treg subsets. CCR4 and CCR8 are specifically expressed in $CD4^+CD25^+$ Tregs. One report showed that CCL22 secreted by mature DCs guided human bloodborne $CD4^+CD25^+$ Tregs to secondary lymphoid tissues and inflamed areas. The authors only observed the chemokine profile of DCs in this process [62]; therefore, we can not exclude the possibility that as a producer of CCL22 macrophages may also play a role as well. CCL22 produced by tumor cells and microenvironmental macrophages mediated trafficking of $CD4^+CD25^+$ Tregs to the tumor in human ovarian carcinoma. $CD4^+CD25^+$ Tregs preferentially moved to and accumulated in tumors and ascites, but rarely entered draining lymph nodes in later cancer stages. This synergistic effect of specific recruitment of memory-like $CD4^+CD25^+$ Tregs by macrophages promotes the development of the tumor [63].

In a mouse model of experimental autoimmune encephalomyelitis (EAE), CCL22 was secreted by parenchymal microglia and central nervous system (CNS)-infiltrating leukocytes, whereas CCR4 was expressed on some invading leukocytes. Upon in-vitro activation, mouse microglia expressed CCL22 transcripts and secreted bioactive CCL22 that induced chemotaxis of Th2, but not Th1 cells. Based on these observations, Columba-Cabezas et al speculated that CCL22 produced by microglia could regulate Th1-mediated CNS inflammation by facilitating the homing of Th2 and, possibly, $CD4^+CD25^+$ Tregs into the lesion site [64].

Bystry et al described another chemokine CCL4 attracting $CD4^+CD25^+$ Tregs secreted by macrophages. By using gene expression profiling, they found that CCL4 was the most potent chemoattractant of a $CD4^+CD25^+$ Treg population, which is a characteristic phenotype of naive-like $CD4^+CD25^+$ Tregs. $CD4^+CD25^+$ Tregs constituted of the majority of the trafficking population in response to CCL4 instead of any other kind of $CD4^+$ T cells, which was different from the response to CCL22. Interestingly, they didn't detect any CCL22 in macrophages stimulated by LPS *in vitro* and found that CCL22 was poor at attracting $CD4^+CD25^+$ Tregs compared with CCL4. The difference between this study and the former two we have just referred is that the formers are investigated under different pathological conditions (tumor and EAE respectively) *in vivo*. Maybe some other factors exist that can exert effects on chemokine release *in vivo*. In any case, the recruitment of $CD4^+CD25^+$ Tregs by macrophages via CCL4 may be one of the important regulatory mechanisms for appropriate T cell responses, and failure to do this may lead to autoimmune activation [65].

### Summary

Although a few investigations have revealed the interaction of $CD4^+CD25^+$ Tregs and macrophages, a detailed mechanism, especially the role of cytokines produced, remains controversial. Much more effort should be given to the study of these areas. Immunity comprises the integrated functions of numerous components which are more or less connected with each other. The tripartite interaction among $CD4^+CD25^+$ Tregs, effector T cells, and macrophages may better account for immune responses occurring *in vivo*. Several advanced studies have explored observation of $CD4^+CD25^+$ Tregs in their native environment *in vivo* [66,67]. Real-time observation will help to elucidate the interaction of $CD4^+CD25^+$ Tregs and macrophages and the complex network *in vivo*. Understanding the interaction of $CD4^+CD25^+$ Tregs and macrophages may significantly impact clinical therapy for autoimmune diseases, tumors and graft rejection.

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