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$CD4^+CD25^+$ REGULATORY T CELLS

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SUMMARY

During the past decade $CD4^+CD25^+$ regulatory T cells have emerged as key players in the development of tolerance to auto-antigens as well as to foreign antigens. This review aims to recapitulate some of the current understandings about the phenotype and function of these regulatory T cells and describe some recent findings on their interaction with bacterial antigens.

INTRODUCTION

The main role of the immune system is to protect the individual from pathogens and one of its fundamental qualities is the ability to distinguish between self and non-self, and between antigens encountered in harmful and non-harmful contexts. In the thymus potentially self-reactive T cells are deleted, resulting in the generation of a peripheral T cell repertoire that is largely self-tolerant. In spite of this, some self-reactive T cells are present in most individuals. Nevertheless, autoimmune diseases only occur infrequently, which suggests that auto-reactive T cells are controlled in the periphery. Peripheral tolerance is sustained by several mechanisms such as deletion, anergy and ignorance (*Mackay*, 2000; *Klein* and *Kyewski*, 2000). In addition there is compelling evidence for the existence of more "active" mechanisms of tolerance that operate through the generation of immune-regulating T cells. Several types of regulatory T cells have been described including, γδ T cells, NKT cells, $CD8^+$ and $CD4^+$ T cells (*Bach*, 2003). $CD4^+$ regulatory T cells can be further divided into induced regulatory cells that secrete IL-10 and TGF-β such as Tr1 cells (*Levings* et al., 2002) and Th3 cells (*Weiner*, 2001) and the so called naturally occurring $CD4^+CD25^+$ regulatory T cell ($CD25^+$Treg), which is the focus of this review.

REVIEW

T cell mediated suppression of autoimmune disease was first described by Nishizuka and Sakakura more than 30 years ago (*Nishizuka* and *Sakakura*, 1969). They discovered that thymectomy on day 3 of life (d3Tx) results in organ-specific autoimmunity. However, disease did not develop in mice thymectomized as early as day 2 or as late as day 7 of life. Further studies

showed that d3Tx animals could be rescued from disease if reconstituted with thymocytes or splenocytes from a normal adult animal but not from an adult animal that had been d3Tx (*Kojima* et al., 1976). This indicated that murine T cells that exit the thymus before day 3 of life are qualitatively different from cells that emigrate later on. However, the cells responsible for the inhibition of autoimmune disease were not discovered until the mid-1990s when Sakaguchi and co-workers identified a sub-population of $CD4^+$ T cells expressing the IL-2 receptor α-subunit (CD25) (*Sakaguchi* et al., 1995). Depletion of $CD25^+$ T cells from adult splenocytes followed by transfer of $CD25^-$ T cells to immune-deficient hosts resulted in a similar spectrum of organ-specific autoimmune diseases as d3Tx. Indeed, the frequency and severity of autoimmune disease correlated with the degree of CD25+ Treg depletion (*Ono* et al., 2006). In addition, co-transfer of $CD25^+$ T cells prevented induction of autoimmunity in the cell-transfer model as well as in the d3Tx model (*Suri-Payer* et al., 1998). Due to intense research during the past decade, $CD4^+CD25^+$ regulatory T cells ($CD25^+$Treg) has emerged as a central T cell population for preserving peripheral tolerance, not only to auto-antigens but also to foreign antigens, in mice as well as in humans (*McHugh* et al., 2002; *Viglietta* et al., 2004).

Antigen specificity

Both murine and human $CD25^+$Treg have as diverse TCR repertoires as $CD4^+CD25^-$ T cells as judged by the expression of various TCR α/βgene segments (*Takahashi* et al., 1998; *Kasow* et al., 2004; *Taams* et al., 2002). This suggests that $CD25^+$Treg are capable of responding to a wide spectrum of antigens. Whether they are biased towards responding to self-antigens or are as broad in their repertoire as $CD25^-$ T cells is not known. A very recent study on murine $CD25^+$Treg indicate that the TCR repertoire of $CD25^+$Treg and $CD25^-$ effector T cells although being similarly diverse, recognize only partly overlapping antigens. Furthermore, particular $CD25^+$Treg have substantially higher avidity for MHC class II bound peptides from peripheral self than the $CD25^-$ T cells (*Hsieh* et al., 2004). Additionally, a few studies of human $CD25^+$Treg have shown that they suppress proliferation and cytokine production to both self-antigens and foreign antigens, including MOG, hHSP60, *Helicobacter pylori* antigens, beta-lactoglobulin (beta-LG) and pollen extract *in vitro* (*Wing* et al., 2003; *Lundgren* et al., 2003; *Taams* et al., 2002, *Tiemessen* et al., 2002; *Ling* et al., 2004; *Grindebacke* et al., 2004). This exemplifies the diversity of the $CD25^+$Treg pool in un-manipulated individuals. Indeed, if the primary function of $CD25^+$Treg is to prevent the activation of potentially hazardous T cells then it would be an advantage to have a similarly composed TCR repertoire as the potentially reactive $CD25^-$ T cells.

Phenotype of CD25+ regulatory T cells

Naturally occurring regulatory T cells express CD25 constitutively and this marker has proven to be very useful for isolation of these cells in mice. However, CD25 is not an optimal marker as it is up-regulated upon activation of T cells. This is especially apparent when investigating human $CD25^+$Treg. In the naïve mouse, $CD4^+CD25^+$ T cells are seen as a distinct population of cells easily distinguished from $CD4^+CD25^-$ T cells that comprise between 5-10% of peripheral $CD4^+$ T cells. The isolation of murine

$CD25^+$Treg is therefore fairly straightforward unless the animal is suffering from ongoing inflammation. Among human $CD4^+$ T cells, approximately 30% express CD25. The majority of these cells express CD25 with low to intermediate intensity ($CD25^{int}$) and only between 1-3% of the $CD4^+$ T cells express CD25 with high intensity ($CD25^{high}$) (*Wing* et al., 2002). *In vitro* studies of sorted $CD25^{int}$ and $CD25^{high}$ cells have shown that it is the $CD25^{high}$ population that functions as suppressor cells (*Baecher-Allan* et al., 2001). Consequently, $CD25^{int}$ cells are most likely memory cells with CD25 expression resulting from encounter with foreign antigens. The continuous expression pattern of CD25 on $CD4^+$ T cells from adult peripheral blood has made the isolation of human $CD25^+$Treg with high purity difficult. It should also be noted that there has been several reports on $CD4^+CD25^-$ cells with regulatory properties (reviewed in *Curotto de Lafaille* and *Lafaille*, 2002). This implies that naturally occurring $CD4^+$ regulatory T cells are not necessarily confined to the $CD4^+CD25^+$ T cell population.

In mice surface expression of markers other than CD25 have been useful in isolating $CD4^+$ regulatory T cells, including CD45RB, CD38 and CD62L (*Powrie* et al., 1996; *Read* et al., 1998; *Lepault* and *Gagnerault*, 2000). Notably, *in vitro* all freshly isolated murine $CD25^+$ T cells suppressed the proliferation of $CD4^+CD25^-$ T cells irrespectively of their expression of these markers (*Thornton* et al., 2000). However this does not exclude that the suppressive property *in vivo* could be affected. Indeed, $CD4^+CD25^+CD62L^+$ but not $CD4^+CD25^+CD62L^-$ splenocytes were shown to delay the onset of diabetes in NOD mice (*Szanya* et al., 2002). However, these markers do not necessarily identify human $CD25^+$Treg. We found that $CD25^{high}$Treg in adult blood expressed intracellular cytotoxic T lymphocyte associated antigen-4 (CTLA-4/CD152) and CD122, while $CD25^-$ and $CD25^{int}$ T cells were negative for CTLA-4 and expressed low levels of CD122. Furthermore, similar $CD25^+$ T cells were identified in cord blood and in thymus (*Wing* et al., 2002). In accordance, CTLA-4 is a marker that is constitutively expressed in naïve animals only by CD25+ T cells (*Sakaguchi* et al., 1995; *Takahashi* et al 2000). In addition, $CD25^+$Treg from adults displayed a memory phenotype as they were $CD45RA^-RO^+$, $CD45RB^{low}$ and expressed both CD62L and CD38 with low intensity and this phenotype was largely shared with the $CD25^{int}$ T cells. In contrast, $CD25^+$Treg derived from cord blood had a naive phenotype and were mainly $CD45RA^+RO^-$ as were the $CD25^-$ T cells in cord blood (*Wing* et al., 2002). Notably, *Jonuleit* et al. (2001) showed that in adult peripheral blood only the $CD25^+CD45RO^+$ cells have suppressive ability. However, we found that cord blood $CD25^+$Treg, which are mainly $CD45RA^+$, were able to suppress proliferation induced by anti-CD3Ab (*Wing* et al., 2003). Furthermore, $CD45RA^+CD25^+$ T cells expressed two-fold higher levels of *FOXP3* mRNA than $CD45RA^-CD25^+$ T cells did. This suggests that expression of CD45RO is an indicator of antigen experience and of limited use for identification of $CD25^+$Treg in humans.

A number of studies have identified additional markers that are expressed at higher levels on $CD25^+$Treg compared to $CD25^-$ T cells (reviewed in *Curotto de Lafaille* and *Lafaille*, 2002). Some of these markers are inhibitory costimulatory receptors like PD1 or members of the tumour necrosis factor receptor (TNFR) superfamily, such as

GITR (glucocorticoid-induced TNFR-related protein), OX40, 4-1BB and TNFRII. Yet others are chemokine receptors, Toll-like receptors or homing receptors such as CD103 (αEβ7 integrin) and the recently discovered neuropilin (Nrp1), which is involved in axon guidance, angiogenesis and T cell activation (*Bruder* et al., 2004). However, the majority of these markers should be used with caution since most surface markers, although expressed on $CD25^+$Treg are up-regulated also on $CD25^-$ T cells after stimulation. In addition, several of these markers were identified on $CD25^+$ T cells isolated from mice and the expression has been difficult to confirm in humans. Currently, none of these molecules have proven to be fully responsible for the suppressive function of $CD25^+$Treg and questions regarding their functional significance remain.

Recent studies have revealed the gene *Foxp3* to be central in the development and function of $CD25^+$Treg (*Sakaguchi*, 2004). The importance of *Foxp3* was discovered when the underlying defect in scurfy mice was investigated. Scurfy mice suffer from a spontaneous X-linked mutation, which leads to fatal lympho-proliferative disease associated with multi-organ infiltrates and early death by 3-4 weeks of age in hemizygous males (*Godfrey* et al., 1991). Lately, the genetic defect in scurfy mice has been identified as a mutation in *Foxp3* (forkhead box p3), a gene coding for a member of the forkhead/winged-helix family of transcriptional regulators (*Brunkow* et al., 2001). *FOXP3*, the human orthologue of the murine *Foxp3*, has been found to be mutated in patients suffering from IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), a severe and fatal autoimmune/allergic syndrome, which reminds to great extent of the condition of scurfy mice. The majority of the mutations in the human patients have been found to be located to the forkhead region, showing the importance of this domain in the function of *FOXP3* (*Gambineri* et al., 2003).

The fact that scurfy mice are hyperresponsive to TCR stimulation and that over-expression of *Foxp3* in cells induce poor proliferation and limited production of IL-2 prompted the investigation of the relationship between *Foxp3* expression and $CD25^+$ Treg (*Hori* et al., 2003). These studies showed that both mRNA and protein levels of *Foxp3* were confined to the $CD4^+CD25^+$ subset (*Hori* et al., 2003; *Fontenot* et al., 2003; *Khattri* et al., 2003). In mice Foxp3 mRNA is detected in peripheral $CD4^+CD25^+$ T cells and in $CD25^+CD4^+CD8^-$ thymocytes, whereas other thymocytes/T cells or B cells do not express *Foxp3*. In contrast to the previously mentioned markers for $CD25^+$Treg, *Foxp3* was not induced in T cells after TCR stimulation (*Hori* et al., 2003). Importantly, retroviral transduction of both murine and human naïve $CD25^-$ T cells with *Foxp3* converts them to regulatory cells with similar functional characteristics as naturally occurring $CD25^+$Treg. Interestingly, as a result of the transduction, cell-surface molecules associated with $CD25^+$Treg were upregulated, including CD25, CTLA-4, GITR and CD103 (*Hori* et al., 2003; *Yagi* et al., 2004). Also, adoptive transfer of wild type $CD25^+CD4^+$ T cells but not $CD25^-CD4^+$ T cells rescued scurfy mice from disease, which shows that *Foxp3* is required for the development of $CD25^+$Treg (*Fontenot* et al., 2003). Investigations of *FOXP3* in humans have shown that it is expressed by all CD4+ $CD25^{high}$ T cells, a minority of the CD4+ $CD25^{int}$ T cells and of the CD4+$CD25^{neg}$ T cells, but not by CD8+ T cells (*Gavin* et al., 2006).

$CD4^+CD25^+$*FOXP3*$^+$ T cells have been identified in thymus as well as in adult peripheral blood and cord blood (*Yagi* et al., 2004; *Walker* et al 2003; *Cosmi* et al., 2003; *Godfrey* et al., 2005; *Takahata* et al., 2004). In contrast to the murine studies, human $CD4^+CD25^-$ T cells have been reported to express *FOXP3* and to acquire suppressive ability after stimulation *in vitro* with plate-bound anti-CD3 and anti-CD28 mAbs (*Walker* et al., 2003). $CD4^+CD25^-$ T cells in human intestinal lamina propria were reported to contain *FOXP3*$^+$ cells (*Makita* et al., 2004). These cells were anergic upon TCR stimulation *in vitro* but did not display suppressive function when cultured in a one to one ratio with responder T cells, which possibly was due to a low frequency of *FOXP3*$^+$ cells. Of note, TGF-β, which is abundant in the intestine, has been shown to induce *FOXP3* expression in both murine and human $CD4^+CD25^-$ T cells (*Chen* et al., 2003; *Fantini* et al., 2004). Conclusively, co-stimulation with TGF-β might be a key for development and maintenance of peripheral regulatory T cells (*Marie* et al., 2005) Overall, *Foxp3* plays a vital part in the generation $CD25^+$Treg and is the most specific marker currently available. Since Foxp3 is a nuclear protein, it is of limited value as a tool for isolation of $CD25^+$Treg *ex vivo,* why we are still confined to the surface molecule CD25 that shows the best correlation to the expression of FOXP3.

Activation of CD25+ regulatory T cells and target cells

Freshly isolated $CD25^+$Treg are not able to suppress T cell responses and only exert inhibitory function after stimulation via the TCR. Antigen-specific as well as polyclonal TCR stimulation activates $CD25^+$Treg and induces suppressive function *in vitro*, whereas irrelevant antigens do not (*Takahashi* et al., 1998; *Thornton* et al 1998). $CD25^+$Treg are very sensitive to stimulation with antigen and are suppressive at antigen doses 10-100 times lower than those needed to activate $CD25^-$ T cells (*Takahashi* et al., 1998). They have a diverse TCR repertoire and are therefore able to respond to many different antigens, including food, microbial, allo- and auto-antigens (*Wing* et al., 2003; *Sakaguchi* et al., 2004; *Lundgren* et al., 2003; *Dieckmann* et al., 2001). In contrast to the lack of proliferation *in vitro*, $CD25^+$Treg actively proliferate to antigen stimulation *in vivo* in non-lymphopenic normal hosts and persist without antigen stimulation for long periods of time (*Gavin* et al., 2002; *Fisson* et al., 2003; *Walker* et al., 2003; *Klein* et al., 2003). Therefore, the anergic behaviour *in vitro* is probably due to lack of appropriate stimulation by for example IL-2.

$CD25^+$Treg have been most thoroughly studied with regard to their effects on T cells. Once $CD25^+$Treg have been activated with specific antigen and IL-2, they inhibit the IL-2 transcription by their target cells and suppress both $CD4^+$ and $CD8^+$ T cell responses of proliferation and cytokine production in an antigen non-specific manner (*Thornton* et al., 2000; *Takahashi* et al., 1998; *Thornton* and *Shevach*, 1998; *Piccirillo* and *Shevach*, 2001). $CD25^+$Treg have also been found to down-regulate the expression of co-stimulatory molecules and reduce the stimulatory capacity of both human and murine DC (*Cederbom* et al., 2000; *Misra* et al., 2004; *Serra* et al., 2003). Others have reported that $CD25^+$Treg act directly on the target cells since suppression can be detected using *in vitro* culture systems devoid of APC (*Thornton* and *Shevach*, 2000; *Piccirillo* and *Shevach*, 2001; *Ng* et al.,

2001). This does, however, not exclude that $CD25^+$ Treg influence the stimulatory capacity of APC *in vivo* and it was recently shown using two-photon laser scanning microscopy of lymph nodes *in vivo* that CD25+ Tregs interacted with antigen-presenting DCs which resulted in prevention of T cells priming (*Tang* et al., 2006). $CD25^+$ Treg also suppress B cells directly and in addition both natural killer T cells (NKT) and natural killer (NK) cell functions have been reported to be down-regulated by $CD25^+$Treg (*Lim* et al., 2005; *Azuma* et al., 2003; *Tronkowski* et al., 2004).

The role of IL-2

A distinctive feature of $CD25^+$Treg *in vitro* is that they are hypo-responsive to stimulation and do not proliferate and produce either no or low levels of cytokines (*Sakaguchi* et al., 2001; *Shevach*, 2002). Accordingly, they are dependent upon the cytokines that the effector cells produce. IL-2 seems to be particularly important since mice deficient for IL-2, IL-2Rα or IL-2Rβ have very few or no $CD25^+$Treg and prematurely succumb to severe lymphoproliferative and autoimmune syndromes. Administration of IL-2 or transfer of IL-2 producing cells to IL-2 deficient animals restores the production of $CD25^+$Treg and lymphoid homeostasis. Further, thymic expression of IL-2Rβ in the thymus of IL-2Rβ$^{-/-}$ mice restores the production of $CD25^+$Treg and prevents lympho-proliferation and lethal autoimmunity (*Malek* and *Bayer*, 2004). This indicates that IL-2 has an important role in the generation of $CD25^+$Treg in the thymus and is crucial for peripheral homeostatic maintenance (*Setoguchi* et al., 2005). Studies of $CD25^+$Treg activation *in vitro* have shown that IL-2 is also needed for induction of suppressive ability. Murine $CD4^+CD25^+$ T cells cultured with plate-bound CD3 Ab in absence of IL-2 resulted in both poor recovery and suppressive ability (*Thornton* et al., 2004). More importantly, the addition of anti-IL-2 completely abrogated the suppressive effect of $CD25^+$Treg on IL-2 mRNA transcription.

The role of CTLA-4

Cytotoxic T lymphocyte associated antigen-4 (CTLA-4; CD152) is a CD28 homologue, which also binds to CD80/86. CTLA-4 is induced upon T cell activation and then functions as a negative regulator of activation. Interestingly, the only cells in naïve animals or human cord blood that express CTLA-4 in the absence of activation are $CD4^+CD25^+$ T cells (*Wing* et al., 2002; *Takahashi* et al., 2000). This raises questions regarding the role of CTLA-4 for the inhibitory mechanism and induction of suppressive capability in $CD25^+$Treg. The addition of CTLA-4 Ab or Fab fragments to *in vitro* co-cultures of murine $CD4^+CD25^-$ and $CD25^+$ T cells neutralizes the inhibitory effect (*Takahashi* et al., 2000) and administration of CTLA-4 mAb abolished the protective ability of $CD25^+$Treg in the murine inflammatory bowel disease (IBD) model (*Read* et al., 2002). With regard to human *in vitro* studies it was shown that suppression by $CD4^+CD25^+$CTLA-4^+ T cells was partly inhibited by blocking CTLA-4 (*Birebent* et al., 2004). However, the majority of investigations have not been able to establish a role for CTLA-4 in the suppressive function (*Baecher-Allan* et al., 2001; *Jonuleit* et al., 2001; *Levings* et al., 2001). CTLA-$4^{-/-}$ mice develop a fatal lymphoproliferative disease but $CD25^+$Treg development and homeostasis appear normal and $CD25^+$Treg displayed un-compromised suppressive ability *in vitro*.

Suppressive mechanisms *in vitro*

The mechanism of suppression by $CD25^+$ Treg is poorly understood. The majority of murine and human *in vitro* studies have concluded that $CD25^+$ Treg mediate suppression by a yet unknown cell-contact dependent mechanism, which is cytokine independent. Suppression cannot be abrogated by neutralizing IL-4, IL-10 or TGF-β and $CD25^+$Treg cultured with $CD25^-$ T cells in a transwell system are unable to suppress the proliferation of the responder cells (*Jonuleit* et al., 2001; *Takahashi* et al., 1998; *Thornton* et al., 1998). Interestingly, human $CD25^+$Treg fixed with paraformaldehyde remained suppressive as long as they had been activated before fixation (*Dieckmann* et al., 2002; *Jonuleit* et al., 2002). Collectively, this suggests the involvement of a surface-bound molecule that is upregulated on $CD25^+$Treg upon activation and mediates a suppressive signal to the responder cell. However, no such agent has yet been identified even though CTLA-4 has been proposed as a candidate (see previous paragraph). Another suggested mechanism of cell-contact dependent suppression is by TGF-β bound to the cell surface of $CD25^+$ Treg (*Nakamura* et al., 2001; Nakamura et al 2004). These findings have been corroborated by some, as at least suppression by human thymic $CD25^+$ Treg seem to be partly dependent on TGF-β *in vitro* (*Annunziato* et al., 2002). In contrast, others have had difficulties reproducing the results by Nakamura and co-workers (*Piccirillo* et al., 2002). The potential role of TGF-β remains controversial as $CD25^+$Treg from TGF-β1 deficient mice suppress $CD25^-$ T cells *in vitro* (*Piccirillo* et al., 2002), while adoptive transfer of TGF-β1 deficient $CD25^+$Treg did not protect recipients from colitis in the SCID transfer model *in vivo* (*Nakamura* et al., 2004). These results suggest that TGF-β produced by $CD25^+$ Treg is of particular importance in regulation of intestinal inflammation.

Suppressive mechanisms *in vivo*

There is a marked contrast with regard to the importance of immunosuppressive cytokines *in vivo* as compared to $CD25^+$Treg suppression *in vitro* and several cytokines have been implicated as mediators of inhibition. In the murine model of IBD, neutralizing Ab to IL-10 or TGF-β were shown to abolish the protective effect of the $CD4^+CD45RB^{low}$ cells (*Powrie* et al., 1996; *Asseman* et al., 1999). Similar results have been obtained using adoptive transfer of $IL\text{-}10^{-/-}$ $CD25^+$Treg. In contrast, IL-10 deficient $CD25^+$Treg are able to inhibit development of gastritis (*Suri-Payer* et al., 2001). One important difference between autoimmune gastritis and IBD is the requirement for intestinal bacteria for induction of IBD, as transfer of $CD25^-$ T cells to germ-free mice does not result in disease (*Singh* et al., 2001). *In vivo* it is likely that cell-contact dependent suppression by $CD25^+$Treg is needed but, during inflammation in the intestine, IL-10 and TGF-β are also required to control the response. Still, in conditions less dependent on bacterial presence, for instance in autoimmune thyroiditis, protection from disease is reversed by neutralizing antibodies to IL-4 and TGF-β (*Seddon* et al., 1999). Similarly, the protection of NOD mice by transferred $CD4^+CD25^+CD62L^+$ is abrogated after treatment with anti-TGF-β Ab (*Lepault* et al., 2000). Overall these data indicate that more than one mechanism of $CD25^+$Treg suppression is operating *in vivo*. One possibility is that there are different subsets of $CD25^+$Treg that either suppress by cell-contact dependent mechanisms or via production of cytokines. Alter-

natively, one $CD25^+$Treg might suppress by more than one mechanism depending on the local environment.

The possibility for a third mechanism of action was raised by two groups who simultaneously showed that human $CD25^+$Treg are able to induce suppressive properties in $CD4^+CD25^-$ T cells when cultured *in vitro*. This "infectious tolerance" rendered the $CD25^-$ T cells anergic and they subsequently started to produce TGF-β (*Jonuleit* et al., 2002) or IL-10 (*Dieckmann* et al., 2002). The primary culture of $CD25^+$Treg together with $CD25^-$ T cells required cell contact for induction of anergy. However, when the anergized T cells were transferred to fresh cultures they were shown to suppress naïve T cells in a cytokine dependent and cell-contact independent manner. This mechanism of infectious tolerance could clarify the discrepancy in the *in vivo* data and might also explain how a small population of cells can regulate a much larger population of responder T cells *in vivo*.

Microbial stimulation

For the immune response to clear microbes, the suppressive effect of the CD25+ Treg must be inhibited. One possibility is to make the effector cells refractory to suppression, which occurs when the stimulatory signal is strong and leads to maturation of DC (*George* et al., 2003). Recent reports show that $CD25^+$ Treg express several members of the Toll-like receptor (TLR) family, such as TLR2, TLR5, and TLR8 and signalling through these receptors is linked to modulation of function of the regulatory cells (*Peng* et al., 2005; *Sutmuller* et al., 2006; *Crellin* et al., 2005). Signalling through TLR-4 has also been proposed to have a similar role but later studies have not been able to replicate these findings (*Caramalho* et al., 2003; *Peng* et al., 2005; *Sutmuller* et al., 2006; *Crellin* et al., 2005). TLRs recognize certain components, so called pathogen-associated molecular patterns that are shared by most microbes, and also certain endogenous molecules that are released during inflammation. Thus, TLR stimulation of $CD25^+$ Tregs may induce extensive proliferation of the regulatory cells, enhanced their survival and suppressive capacity and help to downregulate the immune response. Several epidemiological studies have shown a correlation between improved hygienic conditions and the development of IBD, allergies and autoimmune diseases (reviewed in *Bach* et al., 2002). It is possible that a reduced exposure to microbes affects the development of tolerance and also the homeostasis of the $CD25^+$ Treg pool.

CD25+ regulatory T cells and chronic infection

$CD25^+$ Treg have been shown to interfere with viral as well as bacterial and parasitic infections (*Rouse* and *Suvas*, 2004). Adoptive transfer of $CD25^+$Treg prevents lethal pneumonia in recombinant-activating gene-2 deficient mice infected with *Pneumocystis carinii*, but at the expense of deficient protective response and microbial clearance (*Hori* et al., 2002). Similarly, $CD25^+$ Treg were shown to suppress Th1 responses in mice infected with *Helicobacter pylori*, thereby limiting the mucosal inflammation but with a higher bacterial load as a result (*Raghavan* et al., 2003). Furthermore, $CD25^+$ Treg prevent sterilizing immunity to *Leishmania* infection (*Belkaid* et al., 2002) and the persistence of low numbers of microbes proved essential for the development of T cell memory and prevention of reinfection. This indicates that the prevention of complete eradication of microbes may sometimes be beneficial for the host. Few

attempts have been made to study $CD25^+$Treg and infection in humans. However, it was shown that $CD25^+$Treg from carriers of *Helicobacter pylori* suppressed responses to *H. pylori* antigens *in vitro* (*Lundgren* et al., 2003) and that *H. pylori*-infected individuals have increased frequencies of $CD25^{high}$ T cells in both the stomach and the duodenal mucosa relative to healthy controls (*Lundgren* et al., 2005). Together this indicates that $CD25^+$ Treg may actively inhibit the eradication of the bacteria which contributes to the persistence of infection.

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