Harnessing immune regulation for treatment of human diseases : CD4+CD25+ regulatory T cells & antibody glycosylation

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CHAPTER 1

General Introduction

Rheumatoid arthritis (RA), a worldwide distributed disease with an estimated prevalence of 1-2%, is characterized by chronic systemic inflammations that primarily occur in the synovial membranes of multiple joints. Although the mechanisms underlying the development of this chronic inflammation remain largely unknown, RA is generally considered an autoimmune disease initiated by $CD4^+$ T cells because of the association with certain HLA genes and the presence of autoreactive antibodies (1-3). The chronic inflammatory process and the appearance of autoantibodies suggest that the immune regulation in RA patients is disturbed. Therefore, this thesis will focus on two aspects that are involved in immune regulations 1) $CD4^+CD25^+$ regulatory T cells and 2) antibody glycosylations.

I. IMMUNE TOLERANCE

Immune tolerance refers to the non-immunological responses to self antigens. Distinguishing self from non-self is a key trait of the immune system, which enables the body to maintain immune responses against foreign antigens while being tolerant to self-antigens. Tolerance occurs in two forms, central tolerance and peripheral tolerance.

Central tolerance is induced in the thymus and bone marrow, where developing T and B lymphocytes are selected based on self-reactivity (4). In thymus, only thymocytes bearing receptors with sufficient affinity for self MHC molecules are selected, a process called "positive selection". Next, developing T and B cells that recognize self antigens are deleted, a process called "negative selection or clonal deletion". These two mechanisms ensure a broad T cell reactivity against external antigens while excluding potentially autoreactive T cells from entering the periphery. Central tolerance is most active in fetal life, but continues throughout the whole life span.

Although most T lymphocytes with the repertoire against self are deleted in the thymus, some autoreactive T cells can escape from negative selection and enter the periphery. Because these cells have the potential to cause autoimmune diseases once reactivated, their functions need to be tightly controlled by multiple mechanisms, for example, the induction of hyporesponsiveness/anergy in cells encountering antigen in the absence of co-stimulation. These mechanisms play part in peripheral tolerance induction, and are important to prevent the development of autoimmune diseases (5).

II. DIFFERENT SUBSETS OF T CELLS

After stimulation by antigens, naive $CD4^+$ T cells proliferate and differentiate into various subsets of T cells characterized by the expression of distinct transcription factors/cytokines as well as by distinct effector functions (Figure 1). This process is dictated by the antigen (Ag, type/dose), antigen-presenting cells (APCs, type/status) as well as the local cytokine milieu during the priming of naive T cells. In general, high dose of Ag presented by mature APCs with high levels of co-stimulatory molecules will skew naive T cells towards effector cells, whereas low dose of Ag presented continuously together with low co-stimulatory signals favors the development of cells with a regulatory phenotype. Th1 differentiation occurs when naive cells are primed by APCs in the presence of high levels of IL-12 and IFN-γ, which trigger transcription factor T-bet expression *via* Stat4/1. Th1 cells produce large quantities of IFN-γ, elicit delayed-type hypersensitivity responses,

activate macrophages and are important for the eradication of intracellular pathogens. These cells are also associated with the development of autoimmune diseases and graft rejections (6). The transcription factor favoring Th2 development, GATA-3, is activated by the cytokine IL-4 *via* Stat6. Th2 cells produce IL-4/5/13 and are involved in atopic allergic reactions and the host defense against parasites (6). Th1 cells generally express the homing receptors CXCR3, CXCR5 and CCR6, while Th2 cells preferentially express CCR3, CCR4, CCR8 and the chemokine receptor-homologous CRTh2, a cognate receptor for prostaglandin D2 (PGD_2) (7).

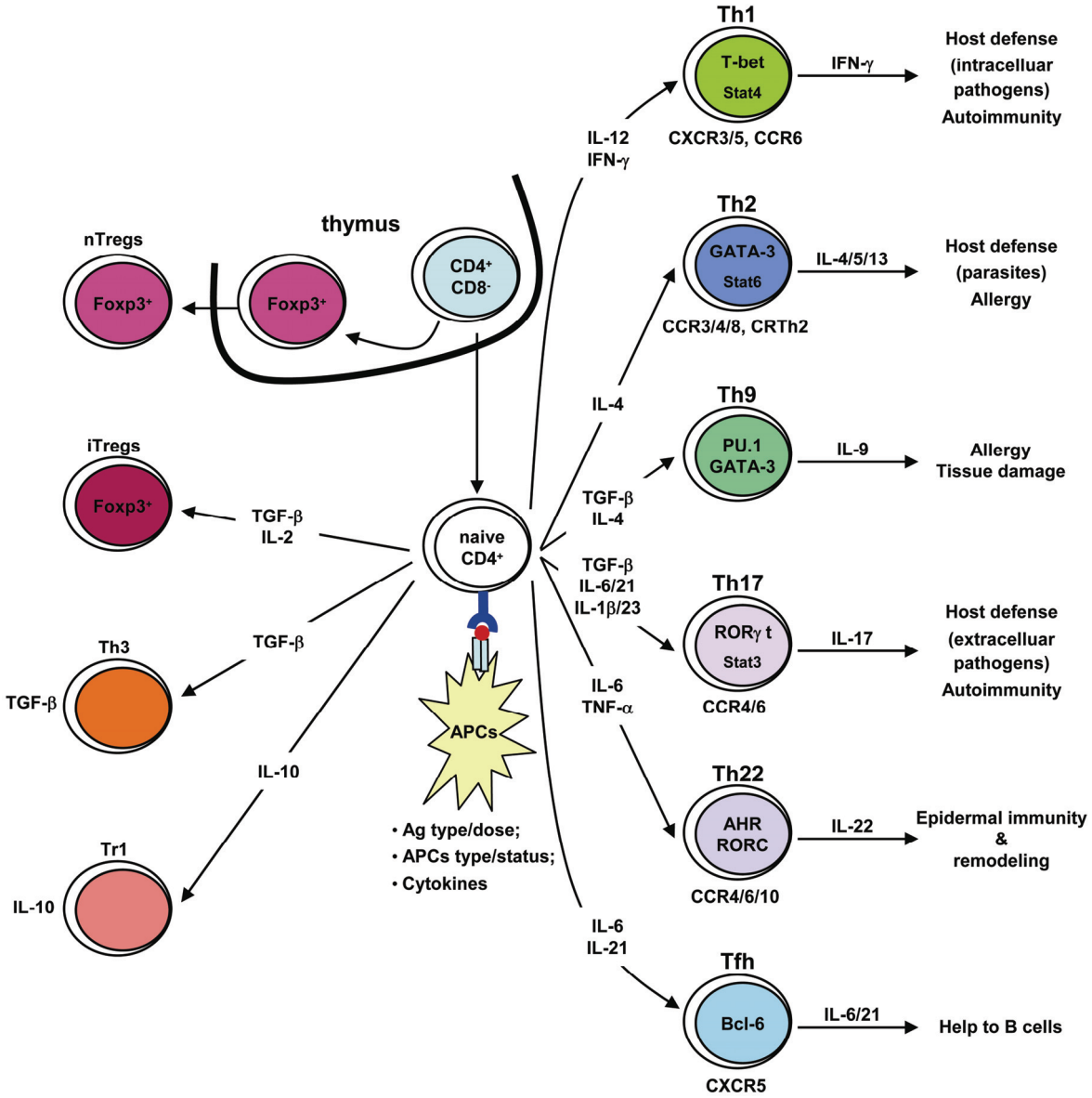

Figure 1. A schematic and simplified overview of the differentiation of $CD4^+$ T cell lineages.

Apart from the Th1 and Th2 cells that have been proposed 20 years ago, several new subsets of effector cells have been described recently. Th17 cells are a new lineage of effector T cells characterized by the expression of transcription factor RORγt/RORC and the cytokine IL-17. Murine Th17 cells are induced by the combination of TGF-β and IL-6 or IL-21 and stabilized/expanded by IL-23, whereas TGF-β, IL-6, IL-1β, IL-21 and IL-23 seem to be required to drive the differentiation of Th17 cells in humans (6,8-10). Th17 cells mainly express CCR4 and CCR6 and are important in mucosal defense against extracellular pathogens. Ample evidence has also been accumulated in animal models

supporting the idea that Th17 cells play a role in the pathogenesis of inflammatory arthritis, multiple sclerosis and inflammatory bowel disease (6,7,11,12). Although IL-22 was originally considered to be produced by Th1 or Th17 cells, a new subset of T cells, Th22 cells, has been characterized by the secretion of IL-22, but not IL-17, IFN-γ or IL-4 (13-15). These cells can be induced by plasmacytoid dendritic cells in an IL-6 and TNF-α dependent manner. Th22 cells express the chemokine receptor CCR6 and the skin-homing receptors CCR4 and CCR10, and are involved in epidermal immunity and remodeling.

Although Th1, Th2 and Th17 cells could all provide help to B cells in terms of antibody (Ab) production and/or class switching, the presence of a separate lineage of T cells with a unique ability to home to B cell follicles and to induce Ab production by B cells has been suggested (16,17). These cells, termed T follicular helper cells (Tfh), express the chemokine receptor CXCR5 and migrate towards high levels of its ligand CXCL13 in germinal centers within B cell follicles of secondary lymph nodes and promote the differentiation of B cells into antibody producing cells through the production of IL-6/21. Tfh cells express high levels of inducible T cell co-stimulator (ICOS) and their development is dependent of IL-21/6 and the transcriptional factor Bcl-6. Moreover, the combination of TGF-β and IL-4 induces a distinct IL-9-producing helper-effector T-cell population, the so called "Th9" cells (18-20). These cells are related to Th2 cells in that they require Stat6 and GATA-3 for development (18,19), but IL-9 production is regulated by the ETS-family transcriptional factor PU.1 (20). Although the function of these "Th9" cells remains largely unknown, it has been suggested that they are involved in allergic reactions and tissue damages as mice with less IL-9-producing T cells display attenuated allergic inflammation and adoptive transfer of these cells into $RAG^{-/-}$ mice induces colitis and peripheral neuritis (18,20). However, the human counterpart for murine Th9 cells has not been identified.

In addition to the effector cells described above, other cells with regulatory function are involved in the control of unwanted immune responses. These regulatory T cells (Tregs) are essential in maintaining immune tolerance by counteracting the function of effector cells. Foxp3-expressing naturally occurring regulatory T cells (nTregs) are generated in the thymus. Type 1 regulatory cells (Tr1) could be induced by the repetitive stimulation of naive cells by immature dendritic cells (DCs) or in the presence of IL-10. Moreover, TGF-β and IL-2 could program naive cell into $Foxp3^{+}$ induced/adaptive Tregs (iTregs), while Th3 cells are primarily induced from naive cells by orally-ingested Ag and TGF-β. Tr1 and Th3 cells exert their immune suppressive function *via* the production of IL-10 and TGF-β, respectively (6,21).

III. REGULATORY T CELLS

1. History: from suppressor to regulatory T cells

In 1970's, Gershon and Kondo discovered that irradiated mice receiving a mixture of bone marrow and thymocytes had a weaker response to antigen rechallenge than those received bone marrow only (22,23). These data indicated the presence of a special subset of T cells within the thymocyte population, which could suppress the immune responses. This notion was supported by other studies showing that day 3 thymectomy resulted in the development of autoimmune diseases, while adoptive transfer of T cells from the periphery or thymus prevented the tissue damage (24-28). This population of T cells, called "suppressor T cells", was subsequently studied intensively. However, since the hypothetical I-J molecule, which was supposed to be a key molecule involved in their

function, could not be detected by modern molecular methods developed later in the middle of 1980's, the existence of suppressor T cells came to be questioned and thereby the associated researches rapidly waned (29).

From the middle of 1980's, Sakaguchi and colleagues performed some seminal work which eventually confirmed the existence of suppressor T cells, now known as Tregs (30-33). They found that adoptive transfer of splenocytes depleted of IL-2 receptor α-chain (CD25)-expressing cells induced many kinds of autoimmune diseases in athymic nude mice, while co-administration of $CD4^+CD25^+$ T cells prevented the development of these autoimmune symptoms in a dose-dependent manner. Day 3 thymectomy prevented the appearance of this T-cell population in the periphery, thereby leading to the development of autoimmune disorders (30,32,33). These findings not only confirmed the existence of a special subset of T cells that could suppress the immune response, but also identified CD25 as a relevant surface marker. These $CD4^+CD25^+$ T cells, which only account for ~10% of $CD4^+$ T cells, are crucial in maintaining tolerance to self in the periphery, are now called Tregs.

2. Development: is Foxp3 the decider?

In mice. The aforementioned findings that day 3 thymetomy prevents the appearance of CD25-expressing cells in the periphery and thereby induces autoimmune disorders suggest that, like conventional T cells, Tregs are produced by the thymus as a functionally mature T cell subpopulation (34). Later, it was discovered that in addition to "naturally occurring Tregs", which are generated in the thymus, Tregs can also be generated in the periphery, the so-called "adaptive/induced Tregs". However, the molecular mechanisms governing their development remain largely unknown until the discovery of the role of Foxp3 (forkhead box P3), which represents another milestone in Tregs research.

Foxp3, a member of the forkhead or winger-helix family of DNA-binding transcription factors, was originally identified as the disease-causative gene for an X-linked fatal multi-organ autoimmune/inflammatory disease in Scurfy mice in 2001. These mice spontaneously develop aggressive, fatal lymphoproliferative disorders due to a single mutation in Foxp3 gene on the X-chromosome (35). Given that the disease in Scurfy mice can be transplanted into a T-cell deficient host upon the transfer of $CD4^+$ T cells (36), these immunological similarities between Scurfy and abnormalities produced by depletion of $CD4^+CD25^+$ Tregs prompted several groups to investigate a possible role for Foxp3 in the biology of $CD4^+CD25^+$ Tregs (32,33,35). Shortly later, it was shown that 1) expression of Foxp3 is highly restricted to, if not exclusively, Tregs (37-40); 2) Foxp3-deficient mice lack functional-, while mice over-expressing Foxp3 posses more Tregs (37,40); 3) Foxp3 deficiency or over-expression in mice does not have an impact on the function of effector cells (37,38); 4) ectopic expression of Foxp3 converts conventional $CD4^+CD25^-$ T cells towards a regulatory phenotype (37,39,40). Moreover, depletion of Foxp3-expressing cells in adult mice results in similar multiple autoimmune disorders as those in neonatal ones, demonstrating their crucial importance in maintaining tolerance throughout the lifespan (41). Together, these results indicate that Foxp3 is a master regulatory gene for cell lineage commitment and function of $CD4^+CD25^+$ Tregs in mice.

Recently, more insights into the roles of Foxp3 in Treg cell lineage commitments have been gained by using genetically-engineered mice, in which Foxp3 is transcribed (as indicated by the expression of inserted fluorescence protein under the control of Foxp3 promoter) but not functionally translated with the insertion of a stop codon. Studies from the groups of Rudensky and Chatila have shown that the effector function but not the lineage commitment of Tregs requires the expression of functional Foxp3 (42,43). Cells, with disrupted Foxp3 protein expression, maintain the characteristic Tregs "genetic

signature" but do not bear suppressor function. Likewise, Flavell's group has shown that attenuated Foxp3 protein expression results in the decrease in suppressive function, but does not affect thymic development, homeostatic expansion/maintenance as well as *in vitro* hyporesponsive properties of Tregs (44). Nonetheless, through modification of cell surface and signaling molecules by directly binding to DNA and/or indirectly interacting with the other transcription factors including NFAT (nuclear factor of activated T-cells) and AML1 (acute myeloid leukemia 1)/Runx1 (Runt-related transcription factor 1), Foxp3 to a larger extent amplifies and fixes pre-established molecular features of Tregs, and thereby stabilizes the Tregs lineage (42-49).

In humans. Similar to Scurfy mice, mutations in the human FOXP3 gene, the ortholog of murine Foxp3, cause the chronic wasting disease termed immune dysregulation, polyendocrinopathy, enteropthy, X-linked syndrome (IPEX) (50-52). However, unlike the Scurfy mice in which Foxp3 mutation results in the lack of functional Tregs, the phenotype and function of $CD4^+CD25^{++}$ T cells in IPEX patients depends on the type of mutations in FOXP3 gene (53,54). $CD4^+CD25^{++}$ T cells from IPEX patients with FOXP3 expression possess weak suppressive ability while those from FOXP3-negative patients do not. Moreover, in contrast to Scurfy mice in which Foxp3 deficiency does not affect the function of non-regulatory T cells, effector T cells from IPEX patients show altered cytokine profile and sensitivity to suppression. These results not only demonstrate the importance of FOXP3 in the development/function of Tregs, but also point to a possible role of FOXP3 in the function of human effector cells (53,54). Indeed, at the bulk T cell level up-regulation of FOXP3 expression has been observed in $CD4^+CD25^-$ T cells after activation, but conflicting data on the function of these cells have been reported (55-58). Likewise, ectopic expression of FOXP3 in conventional T cells does not always result in the acquisition of suppressive function (55,59). Therefore, the role of FOXP3 in human Treg cell lineage commitment and function remains to be further elucidated.

3. Modes and mechanisms of suppression

The crucial role of CD25-expressing $CD4^+$ T cells in down-regulating immune responses, as shown by *in vivo* adoptive transfer experiments in rodents (32,33), prompted the establishment of a simple and reliable *in vitro* assay to further characterize this special T-cell population, especially in humans. In 1998, two groups demonstrated that activated, murine $CD4^+CD25^+$ Tregs strongly inhibited the *in vitro* TCR-triggered proliferation of co-cultured CD4/8 T cells (60,61). Besides this active suppression of other T cells, some other important features of Tregs were also revealed by this *in vitro* model system, including 1) the inability to produce IL-2 and other pro-inflammatory cytokines; and 2) the anergy/hyporesponsiveness to TCR stimulation that can be reversed by the addition of high dose of exogenous IL-2. This simple and reliable assay, called "suppression assay", has become the gold-standard assay widely used in Tregs research.

Soon after the establishment of the *in vitro* suppression assays (60,61), it has been widely used to analyze the possible modes and mechanisms employed by Tregs to suppress (62-64). Tregs were shown to be capable of inhibiting the proliferation as well as cytokine production of co-cultured T cells in the absence or presence of APCs. Direct cell-cell contact between suppressors and effector cells seems to be essential for the function of Tregs *in vitro* because suppression is abrogated when they are separated by a semi-permeable membrane (transwell system) that allows soluble factors to cross but not cells. Activation *via* TCR ligands or anti-CD3 is essential for the initiation of Tregs function, but the effector phase of suppression is antigen-independent as Tregs can inhibit by-stander T cells both *in vitro* and *in vivo* (62,65). Multiple mechanisms have been demonstrated to

contribute to the *in vitro* function of Tregs (Figure 2). However, one should be cautious to extrapolate these data to the *in vivo* situation as increasing evidence has been accumulated indicating that Tregs may employ different modes/mechanisms to exert their functions *in vitro* as well as in different species.

IL-2. In vitro, it has been shown that Tregs inhibit the transcription of IL-2 in target cells early after activation (61,66,67), and IL-2 production from effector cells seems to be important to trigger the function of Tregs during suboptimal activation (67,68). Deprivation of IL-2 by CD25 on Tregs could also induce apoptosis of target T cells, thereby contributing to the suppressive activity of murine Tregs (69). *In vivo*, IL-2 plays an essential role for the maintenance and functional integrity of Tregs because the survival and expansion of Tregs in the periphery is dependent on IL-2 (70).

CTLA-4. CTLA-4 (cytotoxic T lymphocyte antigen-4) is constitutively expressed on murine and human Tregs at high levels, however, its role for the suppressive function of Tregs has only been appreciated recently (63,71-73). The requirement of CTLA-4 on Tregs for their optimal function is illustrated by the spontaneously developed systemic and fatal T cell-mediated autoimmune disease in mice with specific CTLA-4 deficiency in $Foxp3^+$ Tregs (72). Although it remains largely unknown how CTLA-4 controls Tregs function, the following mechanisms have been proposed (70,72,74): 1) ligation of CD80/86 expressed on activated effector cells by CTLA-4 on Tregs may deliver a "outside-in" signal *via* CD80/86 and lead to suppression; 2) by competing with CD28 on effector cells for binding to CD80/86 on APCs, CTLA-4 inhibits the activation of T cells; 3) interaction between CTLA-4 on Tregs and CD80/86 on APCs blocks the up-regulation or even down-regulates effector cell-induced CD80/86 expression on APCs, thereby reducing their T-cell priming ability; 4) the receptor-ligand pair between CTLA-4 on Tregs and CD80/86 on DCs induce the production of indoleamine 2,3-dioxygenase (IDO) by DCs, which depletes the essential amino acid tryptophan and subsequently reduces T cell activation/expansion. Moreover, cross-linking of CTLA-4 on Tregs enhances the accumulation of membrane TGF-β (mTGF-β) at the site of suppressor-target cell contact, and thereby facilitates mTGF-β-mediated cell-contact dependent suppression (75,76).

Granzyme and perforin. Cytolytic activity contributes to the contact-dependent suppression of Tregs in some studies. Anti-CD3/CD46-, and to a lesser extent anti-CD3/CD28-, activated human $CD4^+CD25^+FOXP3^+$ Tregs express granzyme A and kill autologous target cells, including activated T cells, monocytes and DCs, in a perforin-dependent fashion. This cytotoxicity is independent of Fas-FasL interactions but dependent on CD18 adhensive interactions (77). Likewise, activated murine Tregs can kill target cells *via* granzyme B and/or perforin as well (78,79). The importance of this mechanism for the *in vivo* function of Tregs remains to be determined.

TGF-β and IL-10. Although Tregs produce high levels of TGF-β and IL-10 upon activation, most studies failed to identify their roles in the function of Tregs by using transwell culture system, blocking Abs and responders from TGF-β unresponsive mice (60,61,64,80-82). In contrast, many other studies suggested that mTGF-β, expressed on the surface of both activated murine and human Tregs, contributes to their *in vitro* cell contact-dependent suppression (75,76,83-89). Likewise, IL-10 has been implicated in the suppression of some subsets of Tregs (84,90). *In vivo*, TGF-β1-deficient Tregs do not protect mice from colitis in the SCID transfer model, and $IL\text{-}10^{-/-}$ Tregs fail to control the immune responses at environmental interfaces such as the colon and lungs (83,91-93).

These observations may be related to the role of TGF-β and IL-10 in maintaining Foxp3 expression and suppressive function of Tregs *in vivo* (94,95). In addition to the direct inhibition on target cells, Tregs could also generate *de novo* $CD4^+Foxp3^+$ Tregs from naive precursors *via* TGF-β and divert pathogenic T cells into IL-10-producing Tr1 cells (96,97). This phenomenon, called "infectious tolerance", may represent an important mechanism as how Tregs maintain tolerance and amplify their suppressive abilities *in vivo*.

IL-35 and TNFRII. The newly discovered cytokine IL-35 is a heterodimer of Epstein-Barr-virus-induced gene 3 (Ebi3) and the p35 subunit of IL-12. It is constitutively secreted by murine Tregs and contributes to their suppressive function, because Tregs deficient in either subunit (Ebi3 or p35) display significantly reduced regulatory activity *in vitro* and fail to control homeostatic proliferation and to cure inflammatory bowel disease *in vivo* (98). Interestingly, although activated conventional effector cells do not produce IL-35, they augment IL-35 and IL-10 production by Tregs in a cell contact-dependent manner (99). These observations indicate that direct contact with target cells is required to trigger the optimal function of Tregs, albeit that suppression could be cell-contact independent. In strikingly contrast to their murine counterparts, human Tregs do not express IL-35 (100). Additionally, given that both murine and human Tregs express higher levels of TNFRII, shedding of TNFRII that neutralizes biological activities of TNF-α, has been shown to be involved in the suppression of inflammation by Tregs (101-103).

Adenosine/cAMP. Extracellular adenosine triphosphate (ATP), released from injured cells as an indicator of trauma and cell death, functions as a "natural adjuvant" with multiple pro-inflammatory effects for the immune system. Normally ATP is rapidly hydrolyzed into adenosine monophosphate (AMP) by CD39 (ENTPD1, ectonucleoside triphosphate diphosphohydrolase-1) expressed on neutrophils, and subsequently degraded into adenosine by CD73 (ecto-5'-nucleotidases) which is expressed on the surface of endothelial cells and some subsets of T cells at inflammatory sites or damaged organs. Recently, Kobie and others showed that Tregs could also do so by degrading extracellular ATP into adenosine *via* CD39 and CD73 expressed on their surface (104-106). In turn, extra- or peri-cellular adenosine dampens immune responses by binding to adenosine type 1 purinergic G protein-coupled cell surface receptor A2A that is expressed on activated effector T cells. Notably, CD39 is only expressed on a subset of human Treg cells with effector/memory ($CD45RO^+CCR6^+$) phenotype, indicating that conversion of extracellular ATP to adenosine may represent one important mechanism for Tregs to control immune reactions at sites of inflammation where effector/memory Tregs accumulate (105,107). In addition, compared to effector cells, Tregs harbor high levels of intracellular cyclic AMP (cAMP), a known potent inhibitor of T cell growth and function. Accordingly, transfer of cAMP from Tregs to co-cultured target cells *via* cell contact-dependent gap junction formation contributes to the immune suppressive function mediated by Tregs (108).

DCs. Although Tregs could directly suppress target T cells in the absence of APCs *in vitro* through multiple mechanisms as described above, DCs seem to be essential for the control of immune responses *in vivo* because active suppression can still be observed in the absence of stable associations between Tregs and target T cells (109). Through surface expressed LFA-1 (lymphocyte function associated antigen-1) and neuropilin-1 (Nrp-1), Tregs preferentially aggregate on DCs, inhibit their maturation and the formation of stable contact with effector cells, ultimately resulting in reduced priming of target cells (68,109-111). This Treg-mediated modulation of DCs can be attributed to interactions between LFA-1 and ICAM-1 (inter-cellular adhesion molecular 1), CTLA-4 and CD80/86,

lymphocyte-activation gene 3 (LAG-3) and MHC-II (major histocompatibility complex class II) on respective Tregs and DCs, as well as multiple soluble factors produced by Tregs (68,110,112,113). Moreover, Tregs induce the production of IDO by DCs *via* CTLA-4 and GITR (glucocorticoid-induced tumor necrosis factor receptor). In addition to the depletion of the essential amino acid tryptophan for T cell function, IDO could also activate, expand and generate *de novo* Tregs, thereby forming a positive feedback loop that amplifies the suppression of Tregs (114-117).

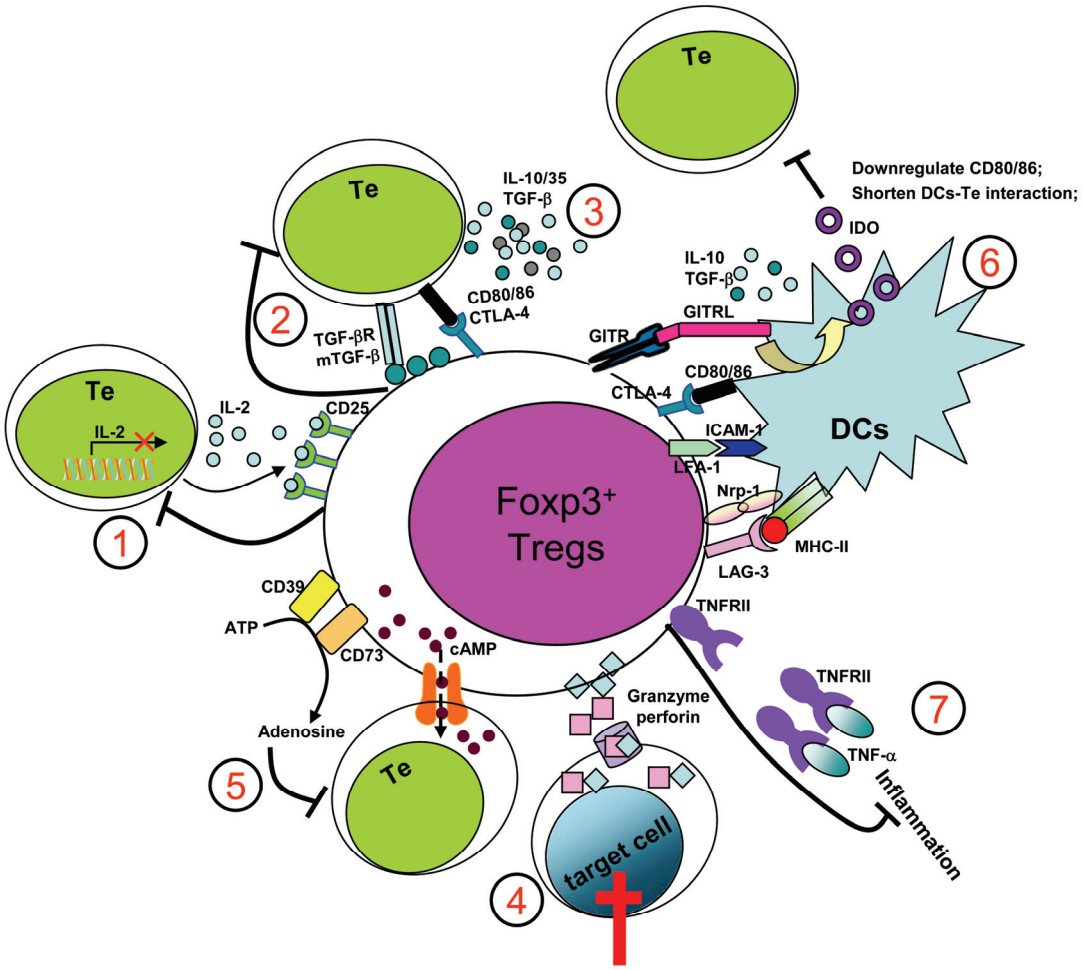

Figure 2. Proposed mechanisms for the functions of Tregs. Tregs can directly inhibit the proliferation/function of effector T cells (Te) through **1)** IL-2 inhibition/deprivation; **2)** cell-contact dependent interaction between mTGF-β/CTLA-4 on Tregs with the corresponding receptors on Te; **3)** production of soluble suppressive cytokines such as TGF-β, IL-10 and IL-35; **4)** cytolysis *via* the production of granzymes and perforins; **5)** metabolic disruption *via* the direct transfer of cAMP to target cells or the indirect hydrolysis of ATP into adenosine. **6)** Moreover, Tregs could also suppress Te through modulating DCs *via* surface molecular interactions and/or soluble factors. **7)** Furthermore, Tregs could dampen the inflammation by secretion of soluble TNFRII, which antagonize the biological activities of TNF-α.

4. Roles and therapeutic potential of Tregs in various diseases

Given the essential role and function of Tregs in immune regulation/tolerance, it has been shown that Tregs are involved in many models of autoimmune diseases, allergy, infectious diseases, tumors as well as transplantations (118-122). Data from these models indicate a strong role of Tregs in the prevention and progression of autoimmune diseases and graft rejections. Moreover, Tregs down-regulate the immune reactions against invading pathogens, thereby preventing/limiting the possible collateral tissue damage but could concurrently allow the establishment of chronic infections owing to the hampered

clearance of pathogens. Furthermore, induction/expansion of Tregs is one of the mechanisms exploited by tumors to escape immune surveillance. Accordingly, these Tregs present in tumor microenvironment represent one of the main obstacles tempering successful immunotherapy and active vaccination against tumors. In line with the data obtained from these disease models, many studies have indicated a deficit in the numbers and/or function of Tregs in patients with autoimmune disorders like RA, multiple sclerosis (MS) and type I diabetes (T1D), whereas an over-abundance of Tregs in patients with tumors and chronic infections (118-123).

Thus, Tregs-based immunotherapy represents a potential treatment for patients with disturbed immune balance. Adoptive transfer or preferential *in vivo* expansion of Tregs has been successfully used in several animal models of graft rejections and autoimmune diseases (103,124-128). Possibilities to translate these animal studies into the human situation are being intensively investigated and some clinical trials have already been ongoing or planned, in the context of both bone marrow transplantation and autoimmune diseases (118,129-132). Moreover, both in mice and humans, inactivation of Tregs by depletion or targeting molecules involved in their *in vivo* activity, such as CTLA-4, TGF-β and IL-10, has often resulted in enhanced immune reactions against infections and tumors (119,120,122,133).

IV. GLYCOSYLATIONS OF IMMUNOGLOBULING

The family of IgG Abs plays an important role in the host defense against pathogenic microorganisms, but those specific for self-antigens (i.e., auto antibodies) are also involved in chronic inflammatory processes and the destruction of tissues/organs in patients like RA and systemic lupus erythematosus (SLE). Although the specificity of Ab is determined by the variable region, antibody-mediated effector functions are crucially dependent on the interaction of its constant region (Fc part) with the complement system and Fc receptors (FcR) expressed on various innate immune cells (134). These Fc-mediated effects of IgG, such as complement activation and antibody-dependent cellular cytotoxicity (ADCC), are influenced by the amino acid sequence (i.e. Ab isotype and subclass) as well as the sugar side chain attached to the asparagine 297 (Asn297) residue in the CH2 domain of each IgG Fc portion (135).

The Asn297-linked sugar chain consists of a biantennary core sugar structure of N-acetylglucosamine (GlcNAc) and mannose residues, with variable additions of terminal and branching GlcNAc, fucose, galactose and sialic acid (Figure 3). In a single IgG molecular, the two Asn297 sites may be differently glycosylated and contain two different glycans from a family of 32. Depending on the presence/absence of galactose on one or both arms of the glycan moiety, these 32 glycans have been assigned to three most prevalent glycoforms, called IgG-G0 (no galactose), IgG-G1 (one galactose) and IgG-G2 (two galactose) (135). These carbohydrate chains are crucial for the structure and biological activity of Abs (135-137). Whereas IgG glycoforms lacking the branching fucose show an enhanced ADCC through increased interaction with human FcγRIIIα and mouse FcγRIV (138-140), glycans containing terminal sialic acid confers IgG an anti-inflammatory activity (141,142). Moreover, removal of these sugar moieties by hydrolysis or mutational deletion of the attaching site Asn297 changes the structure of Fc part, resulting in a non-immunogenic Ab unable to interact with Fcγ receptors and to trigger significant cytokine release (136,143,144).

Changes in IgG glycosylation have been shown to be associated with various physiological and pathological conditions. The degree of galactosylation shows an age dependency as evidenced by population studies: the levels of IgG-G0 decreases in normal human individuals from birth till age 25, whereas it increases thereafter (145,146). IgG galactosylation falls during pregnancy and rises rapidly after delivery in both healthy and arthritic woman, and moreover, this change is paralleled by pregnancy-induced remission and post-partum recurrence of disease in arthritic patients (147-149). Likewise, the IgG-G0 glycoforms in sera of RA patients correlate with the disease activity and reverse to normal levels in patients undergoing remission, indicating that IgG-G0 glycoform may have the potential to cause inflammation and joint damage (150-153). In line with these human correlative data, agalactosylated glycoforms of IgG displayed an increased arthritogenicity in a murine arthritis passive transfer model (154). This effect might be attributed to the sialic acid residue because recent studies suggest that Fc sialylation rather than galactosylation controls the activity of IgG by binding to a specific C-type lectin SIGN-R1 expressed on macrophages in the splenic marginal zone in mice (141,142,155).

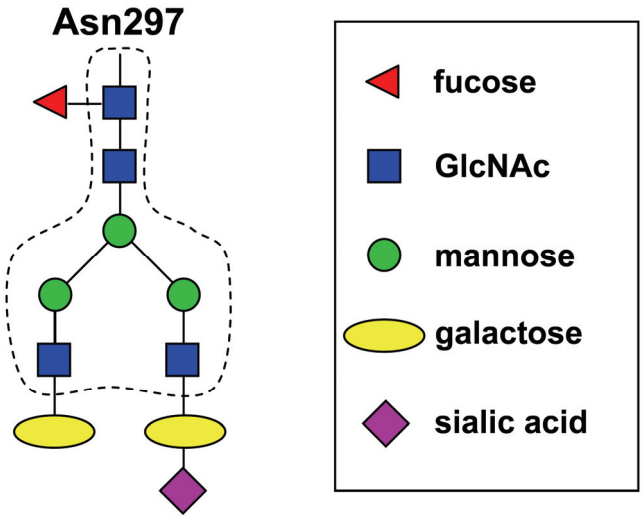

Figure 3. Schematic depiction of a monosialylated glycan chain linked to Asn297 of the IgG heavy chain. The dotted line indicates the core sugar structure of N-acetylglucosamine (GlcNAc) and mannose residues. An additional sialic acid residue can be attached to the second galactose residue, and an additional GlcNAc residue ('bisecting GlcNAc') to the central mannose (not depicted).

Collectively, these findings not only underline the significance of Fc-coupled glycan structures for antibody-mediated immune responses, but also indicate that glycosylation profiles of IgG are tightly regulated by physiological and pathological determinants. In line with the latter notion, active immunization reduces sialylation of IgG in mice, thereby switching the steady-state serum IgG from an anti-inflammatory phenotype to a pro-inflammatory state with the capacity to elicit an effective inflammatory response (142). Moreover, this effect is specific for IgG and most prominent for those targeting the antigen immunized (142). Thus, analyzing the glycosylation pattern of pathology-related IgG (i.e., autoantibodies) and unraveling the underlying regulating mechanisms could give more insights on their roles in disease induction and progression. This may also identify potential targets for novel treatments.

V. AIM AND OUTLINE OF THIS THESIS

The current thesis consists of two parts, with respectively a focus on Treg cells (*Part I*) and on IgG glycosylations (*Part II*).

*Part I (**Chapter 2-5**)*. Given the conflicting data on the function of $CD25^+$ or $FOXP3^+$ cells in patients with autoimmune diseases and limited numbers of Tregs for Treg-based immunotherapy, this part is mainly dedicated to a better identification/characterization and generation of human $CD4^+CD25^+$ Tregs with potent suppressive activities *in vitro*. In **Chapter 2**, we investigated the dynamics of endogenous FOXP3 expression and its relation to the suppressive function in activated human $CD4^+$ T cells at a single-cell level. **Chapter 3** describes the relation between membrane-bound TNFα (mTNFα) and the suppressive ability of $CD4^+CD25^{++}$ T cells as well as the effect of anti-TNFα therapy on the cell composition of the $CD4^+CD25^{++}$ T cell compartment in RA patients. In **Chapter 4**, we developed an efficient approach to generate large numbers of Tregs with potent and stable suppressive function by conversion of effector cells or expansion of isolated $CD4^+CD25^{++}$ T cells in a consistent manner across human donors. The underlying mechanisms responsible for the donor-to-donor variation in Treg conversion were unraveled as well. **Chapter 5** is a follow-up of **Chapter 4**, and describes a simple and efficient strategy to consistently obtain functional $Foxp3^+$ Tregs from total $CD4^+$ T cells in three commonly used mouse strains through manipulation of the pathways responsible for the strain-related differences.

*Part II (**Chapter 6-7**)*. This part aims to analyze the glycosylation pattern of anti-citrullinated peptide antibodies (ACPA), which are highly specific for RA patients, and to obtain more insights on how glycosylation is regulated during an active immune response. **Chapter 6** describes a method for the microscale purification and Fc-glycosylation analysis of ACPA. In **Chapter 7**, by using an *in vitro* culture system resembling the *in vivo* T cell-dependent antibody production from B cells, we identified some factors that can influence the gylcan pattern of secreted IgG during the activation and differentiation of B cells.

Chapter 8. Results obtained from **Chapters 2-7** are summarized and discussed in this chapter.

VI. REFERENCE LIST

1. Sakaguchi, S., and N. Sakaguchi. 2005. Animal models of arthritis caused by systemic alteration of the immune system. *Curr. Opin. Immunol.* 17: 589-594.
2. Buckner, J. H., and G. T. Nepom. 2002. Genetics of rheumatoid arthritis: is there a scientific explanation for the human leukocyte antigen association? *Curr. Opin. Rheumatol.* 14: 254-259.
3. Feitsma, A. L., E. I. van der Voort, K. L. Franken, B. H. el, B. G. Elferink, J. W. Drijfhout, T. W. Huizinga, R. R. de Vries, R. E. Toes, and A. Ioan-Facsinay. 2010. Identification of citrullinated vimentin peptides as T cell epitopes in HLA-DR4-positive patients with rheumatoid arthritis. *Arthritis Rheum.* 62: 117-125.
4. Hogquist, K. A., T. A. Baldwin, and S. C. Jameson. 2005. Central tolerance: learning self-control in the thymus. *Nat. Rev. Immunol.* 5: 772-782.
5. Guerder, S., and R. A. Flavell. 1995. Costimulation in tolerance and autoimmunity. *Int. Rev. Immunol.* 13: 135-146.
6. Bettelli, E., M. Oukka, and V. K. Kuchroo. 2007. T(H)-17 cells in the circle of immunity and autoimmunity. *Nat. Immunol.* 8: 345-350.
7. Acosta-Rodriguez, E. V., L. Rivino, J. Geginat, D. Jarrossay, M. Gattorno, A. Lanzavecchia, F. Sallusto, and G. Napolitani. 2007. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. *Nat. Immunol.* 8: 639-646.
8. Manel, N., D. Unutmaz, and D. R. Littman. 2008. The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgammat. *Nat. Immunol.* 9: 641-649.
9. Volpe, E., N. Servant, R. Zollinger, S. I. Bogiatzi, P. Hupe, E. Barillot, and V. Soumelis. 2008. A critical function for transforming growth factor-beta, interleukin 23 and proinflammatory cytokines in driving and modulating human T(H)-17 responses. *Nat. Immunol.* 9: 650-657.
10. Yang, L., D. E. Anderson, C. Baecher-Allan, W. D. Hastings, E. Bettelli, M. Oukka, V. K. Kuchroo, and D. A. Hafler. 2008. IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. *Nature* 454: 350-352.
11. Aujla, S. J., P. J. Dubin, and J. K. Kolls. 2007. Th17 cells and mucosal host defense. *Semin. Immunol.* 19: 377-382.
12. Conti, H. R., F. Shen, N. Nayyar, E. Stocum, J. N. Sun, M. J. Lindemann, A. W. Ho, J. H. Hai, J. J. Yu, J. W. Jung, S. G. Filler, P. Masso-Welch, M. Edgerton, and S. L. Gaffen. 2009. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J. Exp. Med.* 206: 299-311.
13. Duhen, T., R. Geiger, D. Jarrossay, A. Lanzavecchia, and F. Sallusto. 2009. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat. Immunol.* 10: 857-863.
14. Eyerich, S., K. Eyerich, D. Pennino, T. Carbone, F. Nasorri, S. Pallotta, F. Cianfarani, T. Odorisio, C. Traidl-Hoffmann, H. Behrendt, S. R. Durham, C. B. Schmidt-Weber, and A. Cavani. 2009. Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J. Clin. Invest* 119: 3573-3585.
15. Trifari, S., C. D. Kaplan, E. H. Tran, N. K. Crellin, and H. Spits. 2009. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. *Nat. Immunol.* 10: 864-871.
16. King, C. 2009. New insights into the differentiation and function of T follicular helper cells. *Nat. Rev. Immunol.* 9: 757-766.
17. Nurieva, R. I., Y. Chung, G. J. Martinez, X. O. Yang, S. Tanaka, T. D. Matskevitch, Y. H. Wang, and C. Dong. 2009. Bcl6 mediates the development of T follicular helper cells. *Science* 325: 1001-1005.
18. Dardalhon, V., A. Awasthi, H. Kwon, G. Galileos, W. Gao, R. A. Sobel, M. Mitsdoerffer, T. B. Strom, W. Elyaman, I. C. Ho, S. Khoury, M. Oukka, and V. K. Kuchroo. 2008. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. *Nat. Immunol.* 9: 1347-1355.
19. Veldhoen, M., C. Uyttenhove, S. J. van, H. Helmby, A. Westendorf, J. Buer, B. Martin, C. Wilhelm, and B. Stockinger. 2008. Transforming growth factor-beta 'reprograms' the differentiation of T helper 2 cells and promotes an interleukin 9-producing subset. *Nat. Immunol.* 9: 1341-1346.

20. Chang, H. C., S. Sehra, R. Goswami, W. Yao, Q. Yu, G. L. Stritesky, R. Jabeen, C. McKinley, A. N. Ahyi, L. Han, E. T. Nguyen, M. J. Robertson, N. B. Perumal, R. S. Tepper, S. L. Nutt, and M. H. Kaplan. 2010. The transcription factor PU.1 is required for the development of IL-9-producing T cells and allergic inflammation. *Nat. Immunol.*
21. Wan, Y. Y., and R. A. Flavell. 2006. The roles for cytokines in the generation and maintenance of regulatory T cells. *Immunol. Rev.* 212: 114-130.
22. Gershon, R. K., and K. Kondo. 1970. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. *Immunology* 18: 723-737.
23. Gershon, R. K., and K. Kondo. 1971. Infectious immunological tolerance. *Immunology* 21: 903-914.
24. Nishizuka, Y., and T. Sakakura. 1969. Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. *Science* 166: 753-755.
25. Penhale, W. J., A. Farmer, R. P. McKenna, and W. J. Irvine. 1973. Spontaneous thyroiditis in thymectomized and irradiated Wistar rats. *Clin. Exp. Immunol.* 15: 225-236.
26. Penhale, W. J., W. J. Irvine, J. R. Inglis, and A. Farmer. 1976. Thyroiditis in T cell-depleted rats: suppression of the autoallergic response by reconstitution with normal lymphoid cells. *Clin. Exp. Immunol.* 25: 6-16.
27. Sakaguchi, S., T. Takahashi, and Y. Nishizuka. 1982. Study on cellular events in post-thymectomy autoimmune oophoritis in mice. II. Requirement of Lyt-1 cells in normal female mice for the prevention of oophoritis. *J. Exp. Med.* 156: 1577-1586.
28. Sakaguchi, S., T. Takahashi, and Y. Nishizuka. 1982. Study on cellular events in postthymectomy autoimmune oophoritis in mice. I. Requirement of Lyt-1 effector cells for oocytes damage after adoptive transfer. *J. Exp. Med.* 156: 1565-1576.
29. Bloom, B. R., P. Salgame, and B. Diamond. 1992. Revisiting and revising suppressor T cells. *Immunol. Today* 13: 131-136.
30. Asano, M., M. Toda, N. Sakaguchi, and S. Sakaguchi. 1996. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J. Exp. Med.* 184: 387-396.
31. Sakaguchi, S., K. Fukuma, K. Kuribayashi, and T. Masuda. 1985. Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. *J. Exp. Med.* 161: 72-87.
32. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* 155: 1151-1164.
33. Sakaguchi, S., N. Sakaguchi, J. Shimizu, S. Yamazaki, T. Sakihama, M. Itoh, Y. Kuniyasu, T. Nomura, M. Toda, and T. Takahashi. 2001. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol. Rev.* 182: 18-32.
34. Itoh, M., T. Takahashi, N. Sakaguchi, Y. Kuniyasu, J. Shimizu, F. Otsuka, and S. Sakaguchi. 1999. Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. *J. Immunol.* 162: 5317-5326.
35. Brunkow, M. E., E. W. Jeffery, K. A. Hjerrild, B. Paeper, L. B. Clark, S. A. Yasayko, J. E. Wilkinson, D. Galas, S. F. Ziegler, and F. Ramsdell. 2001. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* 27: 68-73.
36. Blair, P. J., S. J. Bultman, J. C. Haas, B. T. Rouse, J. E. Wilkinson, and V. L. Godfrey. 1994. CD4+CD8- T cells are the effector cells in disease pathogenesis in the scurfy (sf) mouse. *J. Immunol.* 153: 3764-3774.
37. Fontenot, J. D., M. A. Gavin, and A. Y. Rudensky. 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4: 330-336.
38. Fontenot, J. D., J. P. Rasmussen, L. M. Williams, J. L. Dooley, A. G. Farr, and A. Y. Rudensky. 2005. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity.* 22: 329-341.
39. Hori, S., T. Nomura, and S. Sakaguchi. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299: 1057-1061.

40. Khattri, R., T. Cox, S. A. Yasayko, and F. Ramsdell. 2003. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol.* 4: 337-342.
41. Kim, J. M., J. P. Rasmussen, and A. Y. Rudensky. 2007. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat. Immunol.* 8: 191-197.
42. Gavin, M. A., J. P. Rasmussen, J. D. Fontenot, V. Vasta, V. C. Manganiello, J. A. Beavo, and A. Y. Rudensky. 2007. Foxp3-dependent programme of regulatory T-cell differentiation. *Nature* 445: 771-775.
43. Lin, W., D. Haribhai, L. M. Relland, N. Truong, M. R. Carlson, C. B. Williams, and T. A. Chatila. 2007. Regulatory T cell development in the absence of functional Foxp3. *Nat. Immunol.* 8: 359-368.
44. Wan, Y. Y., and R. A. Flavell. 2007. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature* 445: 766-770.
45. Curiel, T. J. 2007. Regulatory T-cell development: is Foxp3 the decider? *Nat. Med.* 13: 250-253.
46. Ono, M., H. Yaguchi, N. Ohkura, I. Kitabayashi, Y. Nagamura, T. Nomura, Y. Miyachi, T. Tsukada, and S. Sakaguchi. 2007. Foxp3 controls regulatory T-cell function by interacting with AML1/Runx1. *Nature* 446: 685-689.
47. Wu, Y., M. Borde, V. Heissmeyer, M. Feuerer, A. D. Lapan, J. C. Stroud, D. L. Bates, L. Guo, A. Han, S. F. Ziegler, D. Mathis, C. Benoist, L. Chen, and A. Rao. 2006. FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell* 126: 375-387.
48. Zheng, Y., and A. Y. Rudensky. 2007. Foxp3 in control of the regulatory T cell lineage. *Nat. Immunol.* 8: 457-462.
49. Zheng, Y., S. Z. Josefowicz, A. Kas, T. T. Chu, M. A. Gavin, and A. Y. Rudensky. 2007. Genome-wide analysis of Foxp3 target genes in developing and mature regulatory T cells. *Nature* 445: 936-940.
50. Bennett, C. L., J. Christie, F. Ramsdell, M. E. Brunkow, P. J. Ferguson, L. Whitesell, T. E. Kelly, F. T. Saulsbury, P. F. Chance, and H. D. Ochs. 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* 27: 20-21.
51. Chatila, T. A., F. Blaeser, N. Ho, H. M. Lederman, C. Voulgaropoulos, C. Helms, and A. M. Bowcock. 2000. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. *J. Clin. Invest* 106: R75-R81.
52. Wildin, R. S., F. Ramsdell, J. Peake, F. Faravelli, J. L. Casanova, N. Buist, E. Levy-Lahad, M. Mazzella, O. Goulet, L. Perroni, F. D. Bricarelli, G. Byrne, M. McEuen, S. Proll, M. Appleby, and M. E. Brunkow. 2001. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* 27: 18-20.
53. Bacchetta, R., L. Passerini, E. Gambineri, M. Dai, S. E. Allan, L. Perroni, F. Dagna-Bricarelli, C. Sartirana, S. Matthes-Martin, A. Lawitschka, C. Azzari, S. F. Ziegler, M. K. Levings, and M. G. Roncarolo. 2006. Defective regulatory and effector T cell functions in patients with FOXP3 mutations. *J. Clin. Invest* 116: 1713-1722.
54. Le, B. S., and R. S. Geha. 2006. IPEX and the role of Foxp3 in the development and function of human Tregs. *J. Clin. Invest* 116: 1473-1475.
55. Allan, S. E., L. Passerini, R. Bacchetta, N. Crellin, M. Dai, P. C. Orban, S. F. Ziegler, M. G. Roncarolo, and M. K. Levings. 2005. The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. *J. Clin. Invest* 115: 3276-3284.
56. Morgan, M. E., J. H. van Bilsen, A. M. Bakker, B. Heemskerk, M. W. Schilham, F. C. Hartgers, B. G. Elferink, L. van der Zanden, R. R. de Vries, T. W. Huizinga, T. H. Ottenhoff, and R. E. Toes. 2005. Expression of FOXP3 mRNA is not confined to CD4+CD25+ T regulatory cells in humans. *Hum. Immunol.* 66: 13-20.
57. Walker, M. R., D. J. Kasprowicz, V. H. Gersuk, A. Benard, L. M. Van, J. H. Buckner, and S. F. Ziegler. 2003. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- cells. *J. Clin. Invest* 112: 1437-1443.
58. Walker, M. R., B. D. Carson, G. T. Nepom, S. F. Ziegler, and J. H. Buckner. 2005. *De novo* generation of antigen-specific CD4+CD25+ regulatory T cells from human CD4+CD25- cells. *Proc. Natl. Acad. Sci. U. S. A* 102: 4103-4108.
59. Yagi, H., T. Nomura, K. Nakamura, S. Yamazaki, T. Kitawaki, S. Hori, M. Maeda, M. Onodera, T. Uchiyama, S. Fujii, and S. Sakaguchi. 2004. Crucial role of FOXP3 in the development and function of human CD25+CD4+ regulatory T cells. *Int. Immunol.* 16: 1643-1656.
60. Takahashi, T., Y. Kuniyasu, M. Toda, N. Sakaguchi, M. Itoh, M. Iwata, J. Shimizu, and S. Sakaguchi. 1998. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and

suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol.* 10: 1969-1980.

61. Thornton, A. M., and E. M. Shevach. 1998. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J. Exp. Med.* 188: 287-296.
62. Thornton, A. M., and E. M. Shevach. 2000. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. *J. Immunol.* 164: 183-190.
63. Thornton, A. M., C. A. Piccirillo, and E. M. Shevach. 2004. Activation requirements for the induction of CD4+CD25+ T cell suppressor function. *Eur. J. Immunol.* 34: 366-376.
64. Shevach, E. M. 2002. CD4+ CD25+ suppressor T cells: more questions than answers. *Nat. Rev. Immunol.* 2: 389-400.
65. Wright, G. P., C. A. Notley, S. A. Xue, G. M. Bendle, A. Holler, T. N. Schumacher, M. R. Ehrenstein, and H. J. Stauss. 2009. Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. *Proc. Natl. Acad. Sci. U. S. A* 106: 19078-19083.
66. Sojka, D. K., A. Hughson, T. L. Sukiennicki, and D. J. Fowell. 2005. Early kinetic window of target T cell susceptibility to CD25+ regulatory T cell activity. *J. Immunol.* 175: 7274-7280.
67. Thornton, A. M., E. E. Donovan, C. A. Piccirillo, and E. M. Shevach. 2004. Cutting edge: IL-2 is critically required for the *in vitro* activation of CD4+CD25+ T cell suppressor function. *J. Immunol.* 172: 6519-6523.
68. Tran, D. Q., D. D. Glass, G. Uzel, D. A. Darnell, C. Spalding, S. M. Holland, and E. M. Shevach. 2009. Analysis of adhesion molecules, target cells, and role of IL-2 in human FOXP3+ regulatory T cell suppressor function. *J. Immunol.* 182: 2929-2938.
69. Pandiyan, P., L. Zheng, S. Ishihara, J. Reed, and M. J. Lenardo. 2007. CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. *Nat. Immunol.* 8: 1353-1362.
70. von, B. H. 2005. Mechanisms of suppression by suppressor T cells. *Nat. Immunol.* 6: 338-344.
71. Flores-Borja, F., E. C. Jury, C. Mauri, and M. R. Ehrenstein. 2008. Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U. S. A* 105: 19396-19401.
72. Wing, K., Y. Onishi, P. Prieto-Martin, T. Yamaguchi, M. Miyara, Z. Fehervari, T. Nomura, and S. Sakaguchi. 2008. CTLA-4 control over Foxp3+ regulatory T cell function. *Science* 322: 271-275.
73. Sojka, D. K., A. Hughson, and D. J. Fowell. 2009. CTLA-4 is required by CD4+CD25+ Treg to control CD4+ T-cell lymphopenia-induced proliferation. *Eur. J. Immunol.* 39: 1544-1551.
74. Shevach, E. M. 2008. Immunology. Regulating suppression. *Science* 322: 202-203.
75. Oida, T., L. Xu, H. L. Weiner, A. Kitani, and W. Strober. 2006. TGF-beta-mediated suppression by CD4+CD25+ T cells is facilitated by CTLA-4 signaling. *J. Immunol.* 177: 2331-2339.
76. Nakamura, K., A. Kitani, and W. Strober. 2001. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J. Exp. Med.* 194: 629-644.
77. Grossman, W. J., J. W. Verbsky, W. Barchet, M. Colonna, J. P. Atkinson, and T. J. Ley. 2004. Human T regulatory cells can use the perforin pathway to cause autologous target cell death. *Immunity.* 21: 589-601.
78. Gondek, D. C., L. F. Lu, S. A. Quezada, S. Sakaguchi, and R. J. Noelle. 2005. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J. Immunol.* 174: 1783-1786.
79. Zhao, D. M., A. M. Thornton, R. J. DiPaolo, and E. M. Shevach. 2006. Activated CD4+CD25+ T cells selectively kill B lymphocytes. *Blood* 107: 3925-3932.
80. Jonuleit, H., E. Schmitt, M. Stassen, A. Tuettenberg, J. Knop, and A. H. Enk. 2001. Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. *J. Exp. Med.* 193: 1285-1294.
81. Valencia, X., G. Stephens, R. Goldbach-Mansky, M. Wilson, E. M. Shevach, and P. E. Lipsky. 2006. TNF downmodulates the function of human CD4+CD25hi T-regulatory cells. *Blood* 108: 253-261.
82. Piccirillo, C. A., J. J. Letterio, A. M. Thornton, R. S. McHugh, M. Mamura, H. Mizuhara, and E. M. Shevach. 2002. CD4(+)CD25(+) regulatory T cells can mediate suppressor function in the absence of transforming growth factor beta1 production and responsiveness. *J. Exp. Med.* 196: 237-246.

83. Nakamura, K., A. Kitani, I. Fuss, A. Pedersen, N. Harada, H. Nawata, and W. Strober. 2004. TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. *J. Immunol.* 172: 834-842.
84. Ito, T., S. Hanabuchi, Y. H. Wang, W. R. Park, K. Arima, L. Bover, F. X. Qin, M. Gilliet, and Y. J. Liu. 2008. Two functional subsets of FOXP3+ regulatory T cells in human thymus and periphery. *Immunity.* 28: 870-880.
85. Tran, D. Q., J. Andersson, R. Wang, H. Ramsey, D. Unutmaz, and E. M. Shevach. 2009. GARP (LRRC32) is essential for the surface expression of latent TGF-beta on platelets and activated FOXP3+ regulatory T cells. *Proc. Natl. Acad. Sci. U. S. A* 106: 13445-13450.
86. Wang, R., Q. Wan, L. Kozhaya, H. Fujii, and D. Unutmaz. 2008. Identification of a regulatory T cell specific cell surface molecule that mediates suppressive signals and induces Foxp3 expression. *PLoS. One.* 3: e2705.
87. Wang, R., L. Kozhaya, F. Mercer, A. Khaitan, H. Fujii, and D. Unutmaz. 2009. Expression of GARP selectively identifies activated human FOXP3+ regulatory T cells. *Proc. Natl. Acad. Sci. U. S. A* 106: 13439-13444.
88. Stockis, J., D. Colau, P. G. Coulie, and S. Lucas. 2009. Membrane protein GARP is a receptor for latent TGF-beta on the surface of activated human Treg. *Eur. J. Immunol.* 39: 3315-3322.
89. Tran, D. Q., J. Andersson, D. Hardwick, L. Bebris, G. G. Illei, and E. M. Shevach. 2009. Selective expression of latency-associated peptide (LAP) and IL-1 receptor type I/II (CD121a/CD121b) on activated human FOXP3+ regulatory T cells allows for their purification from expansion cultures. *Blood* 113: 5125-5133.
90. Baecher-Allan, C., E. Wolf, and D. A. Hafler. 2006. MHC class II expression identifies functionally distinct human regulatory T cells. *J. Immunol.* 176: 4622-4631.
91. Asseman, C., S. Mauze, M. W. Leach, R. L. Coffman, and F. Powrie. 1999. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med.* 190: 995-1004.
92. Rubtsov, Y. P., J. P. Rasmussen, E. Y. Chi, J. Fontenot, L. Castelli, X. Ye, P. Treuting, L. Siewe, A. Roers, W. R. Henderson, Jr., W. Muller, and A. Y. Rudensky. 2008. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity.* 28: 546-558.
93. Suri-Payer, E., and H. Cantor. 2001. Differential cytokine requirements for regulation of autoimmune gastritis and colitis by CD4(+)CD25(+) T cells. *J. Autoimmun.* 16: 115-123.
94. Marie, J. C., J. J. Letterio, M. Gavin, and A. Y. Rudensky. 2005. TGF-beta1 maintains suppressor function and Foxp3 expression in CD4+CD25+ regulatory T cells. *J. Exp. Med.* 201: 1061-1067.
95. Murai, M., O. Turovskaya, G. Kim, R. Madan, C. L. Karp, H. Cheroutre, and M. Kronenberg. 2009. Interleukin 10 acts on regulatory T cells to maintain expression of the transcription factor Foxp3 and suppressive function in mice with colitis. *Nat. Immunol.* 10: 1178-1184.
96. Andersson, J., D. Q. Tran, M. Pesu, T. S. Davidson, H. Ramsey, J. J. O'Shea, and E. M. Shevach. 2008. CD4+ FoxP3+ regulatory T cells confer infectious tolerance in a TGF-beta-dependent manner. *J. Exp. Med.* 205: 1975-1981.
97. Mekala, D. J., R. S. Alli, and T. L. Geiger. 2005. IL-10-dependent infectious tolerance after the treatment of experimental allergic encephalomyelitis with redirected CD4+CD25+ T lymphocytes. *Proc. Natl. Acad. Sci. U. S. A* 102: 11817-11822.
98. Collison, L. W., C. J. Workman, T. T. Kuo, K. Boyd, Y. Wang, K. M. Vignali, R. Cross, D. Sehy, R. S. Blumberg, and D. A. Vignali. 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 450: 566-569.
99. Collison, L. W., M. R. Pillai, V. Chaturvedi, and D. A. Vignali. 2009. Regulatory T cell suppression is potentiated by target T cells in a cell contact, IL-35- and IL-10-dependent manner. *J. Immunol.* 182: 6121-6128.
100. Bardel, E., F. Larousserie, P. Charlot-Rabiega, A. Coulomb-L'Hermine, and O. Devergne. 2008. Human CD4+ CD25+ Foxp3+ regulatory T cells do not constitutively express IL-35. *J. Immunol.* 181: 6898-6905.
101. Chen, X., J. J. Subleski, H. Kopf, O. M. Howard, D. N. Mannel, and J. J. Oppenheim. 2008. Cutting edge: expression of TNFR2 defines a maximally suppressive subset of mouse CD4+CD25+FoxP3+ T regulatory cells: applicability to tumor-infiltrating T regulatory cells. *J. Immunol.* 180: 6467-6471.
102. Chen, X., J. J. Subleski, R. Hamano, O. M. Howard, R. H. Wiltrout, and J. J. Oppenheim. 2010. Co-expression of TNFR2 and CD25 identifies more of the functional CD4(+)FoxP3(+) regulatory T cells in human peripheral blood. *Eur. J. Immunol.*

103. van Mierlo, G. J., H. U. Scherer, M. Hameetman, M. E. Morgan, R. Flierman, T. W. Huizinga, and R. E. Toes. 2008. Cutting edge: TNFR-shedding by CD4+CD25+ regulatory T cells inhibits the induction of inflammatory mediators. *J. Immunol.* 180: 2747-2751.
104. Kobie, J. J., P. R. Shah, L. Yang, J. A. Rebhahn, D. J. Fowell, and T. R. Mosmann. 2006. T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5'-adenosine monophosphate to adenosine. *J. Immunol.* 177: 6780-6786.
105. Borsellino, G., M. Kleinewietfeld, M. D. Di, A. Sternjak, A. Diamantini, R. Giometto, S. Hopner, D. Centonze, G. Bernardi, M. L. Dell'Acqua, P. M. Rossini, L. Battistini, O. Rotzschke, and K. Falk. 2007. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* 110: 1225-1232.
106. Deaglio, S., K. M. Dwyer, W. Gao, D. Friedman, A. Usheva, A. Erat, J. F. Chen, K. Enjyoji, J. Linden, M. Oukka, V. K. Kuchroo, T. B. Strom, and S. C. Robson. 2007. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J. Exp. Med.* 204: 1257-1265.
107. Kleinewietfeld, M., F. Puentes, G. Borsellino, L. Battistini, O. Rotzschke, and K. Falk. 2005. CCR6 expression defines regulatory effector/memory-like cells within the CD25(+)CD4+ T-cell subset. *Blood* 105: 2877-2886.
108. Bopp, T., C. Becker, M. Klein, S. Klein-Hessling, A. Palmetshofer, E. Serfling, V. Heib, M. Becker, J. Kubach, S. Schmitt, S. Stoll, H. Schild, M. S. Staege, M. Stassen, H. Jonuleit, and E. Schmitt. 2007. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. *J. Exp. Med.* 204: 1303-1310.
109. Tang, Q., J. Y. Adams, A. J. Tooley, M. Bi, B. T. Fife, P. Serra, P. Santamaria, R. M. Locksley, M. F. Krummel, and J. A. Bluestone. 2006. Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat. Immunol.* 7: 83-92.
110. Onishi, Y., Z. Fehervari, T. Yamaguchi, and S. Sakaguchi. 2008. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells *in vitro* and actively inhibit their maturation. *Proc. Natl. Acad. Sci. U. S. A* 105: 10113-10118.
111. Sarris, M., K. G. Andersen, F. Randow, L. Mayr, and A. G. Betz. 2008. Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition. *Immunity.* 28: 402-413.
112. Bayry, J., F. Triebel, S. V. Kaveri, and D. F. Tough. 2007. Human dendritic cells acquire a semimature phenotype and lymph node homing potential through interaction with CD4+CD25+ regulatory T cells. *J. Immunol.* 178: 4184-4193.
113. Liang, B., C. Workman, J. Lee, C. Chew, B. M. Dale, L. Colonna, M. Flores, N. Li, E. Schweighoffer, S. Greenberg, V. Tybulewicz, D. Vignali, and R. Clynes. 2008. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. *J. Immunol.* 180: 5916-5926.
114. Puccetti, P., and U. Grohmann. 2007. IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. *Nat. Rev. Immunol.* 7: 817-823.
115. Baban, B., P. R. Chandler, M. D. Sharma, J. Pihkala, P. A. Koni, D. H. Munn, and A. L. Mellor. 2009. IDO activates regulatory T cells and blocks their conversion into Th17-like T cells. *J. Immunol.* 183: 2475-2483.
116. Chung, D. J., M. Rossi, E. Romano, J. Ghith, J. Yuan, D. H. Munn, and J. W. Young. 2009. Indoleamine 2,3-dioxygenase-expressing mature human monocyte-derived dendritic cells expand potent autologous regulatory T cells. *Blood* 114: 555-563.
117. Sharma, M. D., B. Baban, P. Chandler, D. Y. Hou, N. Singh, H. Yagita, M. Azuma, B. R. Blazar, A. L. Mellor, and D. H. Munn. 2007. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs *via* indoleamine 2,3-dioxygenase. *J. Clin. Invest* 117: 2570-2582.
118. Bacchetta, R., E. Gambineri, and M. G. Roncarolo. 2007. Role of regulatory T cells and FOXP3 in human diseases. *J. Allergy Clin. Immunol.* 120: 227-235.
119. Belkaid, Y., and B. T. Rouse. 2005. Natural regulatory T cells in infectious disease. *Nat. Immunol.* 6: 353-360.
120. Belkaid, Y. 2007. Regulatory T cells and infection: a dangerous necessity. *Nat. Rev. Immunol.* 7: 875-888.
121. Paust, S., and H. Cantor. 2005. Regulatory T cells and autoimmune disease. *Immunol. Rev.* 204: 195-207.

122. Zou, W. 2006. Regulatory T cells, tumour immunity and immunotherapy. *Nat. Rev. Immunol.* 6: 295-307.
123. Roep, B. O., and M. Peakman. 2010. Surrogate end points in the design of immunotherapy trials: emerging lessons from type 1 diabetes. *Nat. Rev. Immunol.* 10: 145-152.
124. Golshayan, D., S. Jiang, J. Tsang, M. I. Garin, C. Mottet, and R. I. Lechler. 2007. *In vitro*-expanded donor alloantigen-specific CD4+CD25+ regulatory T cells promote experimental transplantation tolerance. *Blood* 109: 827-835.
125. Morgan, M. E., R. Flierman, L. M. van Duivenvoorde, H. J. Witteveen, E. W. van, J. M. van Laar, R. R. de Vries, and R. E. Toes. 2005. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cells. *Arthritis Rheum.* 52: 2212-2221.
126. Mottet, C., H. H. Uhlig, and F. Powrie. 2003. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J. Immunol.* 170: 3939-3943.
127. Tang, Q., K. J. Henriksen, M. Bi, E. B. Finger, G. Szot, J. Ye, E. L. Masteller, H. McDevitt, M. Bonyhadi, and J. A. Bluestone. 2004. *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J. Exp. Med.* 199: 1455-1465.
128. Webster, K. E., S. Walters, R. E. Kohler, T. Mrkvan, O. Boyman, C. D. Surh, S. T. Grey, and J. Sprent. 2009. *In vivo* expansion of T reg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. *J. Exp. Med.* 206: 751-760.
129. Allan, S. E., R. Broady, S. Gregori, M. E. Himmel, N. Locke, M. G. Roncarolo, R. Bacchetta, and M. K. Levings. 2008. CD4+ T-regulatory cells: toward therapy for human diseases. *Immunol. Rev.* 223: 391-421.
130. Brusko, T. M., A. L. Putnam, and J. A. Bluestone. 2008. Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. *Immunol. Rev.* 223: 371-390.
131. Roncarolo, M. G., and M. Battaglia. 2007. Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. *Nat. Rev. Immunol.* 7: 585-598.
132. Tang, Q., and J. A. Bluestone. 2006. Regulatory T-cell physiology and application to treat autoimmunity. *Immunol. Rev.* 212: 217-237.
133. Zhang, H. H., M. H. Mei, R. Fei, W. J. Liao, X. Y. Wang, L. L. Qin, J. H. Wang, L. Wei, and H. S. Chen. 2010. Regulatory T cell depletion enhances tumor specific CD8 T-cell responses, elicited by tumor antigen NY-ESO-1b in hepatocellular carcinoma patients, *in vitro*. *Int. J. Oncol.* 36: 841-848.
134. Nimmerjahn, F., and J. V. Ravetch. 2006. Fcgamma receptors: old friends and new family members. *Immunity.* 24: 19-28.
135. Arnold, J. N., M. R. Wormald, R. B. Sim, P. M. Rudd, and R. A. Dwek. 2007. The impact of glycosylation on the biological function and structure of human immunoglobulins. *Annu. Rev. Immunol.* 25: 21-50.
136. Krapp, S., Y. Mimura, R. Jefferis, R. Huber, and P. Sondermann. 2003. Structural analysis of human IgG-Fc glycoforms reveals a correlation between glycosylation and structural integrity. *J. Mol. Biol.* 325: 979-989.
137. Yamaguchi, Y., M. Nishimura, M. Nagano, H. Yagi, H. Sasakawa, K. Uchida, K. Shitara, and K. Kato. 2006. Glycoform-dependent conformational alteration of the Fc region of human immunoglobulin G1 as revealed by NMR spectroscopy. *Biochim. Biophys. Acta* 1760: 693-700.
138. Nimmerjahn, F., and J. V. Ravetch. 2005. Divergent immunoglobulin g subclass activity through selective Fc receptor binding. *Science* 310: 1510-1512.
139. Shields, R. L., J. Lai, R. Keck, L. Y. O'Connell, K. Hong, Y. G. Meng, S. H. Weikert, and L. G. Presta. 2002. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. *J. Biol. Chem.* 277: 26733-26740.
140. Shinkawa, T., K. Nakamura, N. Yamane, E. Shoji-Hosaka, Y. Kanda, M. Sakurada, K. Uchida, H. Anazawa, M. Satoh, M. Yamasaki, N. Hanai, and K. Shitara. 2003. The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. *J. Biol. Chem.* 278: 3466-3473.
141. Anthony, R. M., F. Nimmerjahn, D. J. Ashline, V. N. Reinhold, J. C. Paulson, and J. V. Ravetch. 2008. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. *Science* 320: 373-376.
142. Kaneko, Y., F. Nimmerjahn, and J. V. Ravetch. 2006. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 313: 670-673.

143. Bolt, S., E. Routledge, I. Lloyd, L. Chatenoud, H. Pope, S. D. Gorman, M. Clark, and H. Waldmann. 1993. The generation of a humanized, non-mitogenic CD3 monoclonal antibody which retains *in vitro* immunosuppressive properties. *Eur. J. Immunol.* 23: 403-411.

144. Woodle, E. S., D. Xu, R. A. Zivin, J. Auger, J. Charette, R. O'Laughlin, D. Peace, L. K. Jollife, T. Haverty, J. A. Bluestone, and J. R. Thistlethwaite, Jr. 1999. Phase I trial of a humanized, Fc receptor nonbinding OKT3 antibody, huOKT3gamma1(Ala-Ala) in the treatment of acute renal allograft rejection. *Transplantation* 68: 608-616.

145. Parekh, R., I. Roitt, D. Isenberg, R. Dwek, and T. Rademacher. 1988. Age-related galactosylation of the N-linked oligosaccharides of human serum IgG. *J. Exp. Med.* 167: 1731-1736.

146. Shikata, K., T. Yasuda, F. Takeuchi, T. Konishi, M. Nakata, and T. Mizuochi. 1998. Structural changes in the oligosaccharide moiety of human IgG with aging. *Glycoconj. J.* 15: 683-689.

147. Alavi, A., N. Arden, T. D. Spector, and J. S. Axford. 2000. Immunoglobulin G glycosylation and clinical outcome in rheumatoid arthritis during pregnancy. *J. Rheumatol.* 27: 1379-1385.

148. Pekelharing, J. M., E. Hepp, J. P. Kamerling, G. J. Gerwig, and B. Leijnse. 1988. Alterations in carbohydrate composition of serum IgG from patients with rheumatoid arthritis and from pregnant women. *Ann. Rheum. Dis.* 47: 91-95.

149. Rook, G. A., J. Steele, R. Brealey, A. Whyte, D. Isenberg, N. Sumar, J. L. Nelson, K. B. Bodman, A. Young, I. M. Roitt, and . 1991. Changes in IgG glycoform levels are associated with remission of arthritis during pregnancy. *J. Autoimmun.* 4: 779-794.

150. Parekh, R. B., I. M. Roitt, D. A. Isenberg, R. A. Dwek, B. M. Ansell, and T. W. Rademacher. 1988. Galactosylation of IgG associated oligosaccharides: reduction in patients with adult and juvenile onset rheumatoid arthritis and relation to disease activity. *Lancet* 1: 966-969.

151. Malhotra, R., M. R. Wormald, P. M. Rudd, P. B. Fischer, R. A. Dwek, and R. B. Sim. 1995. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement *via* the mannose-binding protein. *Nat. Med.* 1: 237-243.

152. Matsumoto, A., K. Shikata, F. Takeuchi, N. Kojima, and T. Mizuochi. 2000. Autoantibody activity of IgG rheumatoid factor increases with decreasing levels of galactosylation and sialylation. *J. Biochem.* 128: 621-628.

153. Parekh, R. B., R. A. Dwek, B. J. Sutton, D. L. Fernandes, A. Leung, D. Stanworth, T. W. Rademacher, T. Mizuochi, T. Taniguchi, K. Matsuta, and . 1985. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature* 316: 452-457.

154. Rademacher, T. W., P. Williams, and R. A. Dwek. 1994. Agalactosyl glycoforms of IgG autoantibodies are pathogenic. *Proc. Natl. Acad. Sci. U. S. A* 91: 6123-6127.

155. Anthony, R. M., F. Wermeling, M. C. Karlsson, and J. V. Ravetch. 2008. Identification of a receptor required for the anti-inflammatory activity of IVIG. *Proc. Natl. Acad. Sci. U. S. A* 105: 19571-19578.