Regulatory T Cells and Clinical Application

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Editor

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Preface

A major process of rediscovery has taken place in the field of Cellular Immunology over the past 12 years—subsets of T lymphocytes exist that are specifically dedicated to regulation or as it should be more appropriately termed suppression of all aspects of immune responses. It is certainly appropriate at this time to recall some of the history that lead to the development of the concept of immune regulation/suppression. Shortly after the term helper T cells was coined to described lymphocytes that "helped" both humoral and cell-mediated responses, studies from the laboratory of the late Professor Richard Gershon demonstrated that under certain conditions, antigen recognition by T lymphocytes also resulted in the development of cells that are able to suppress immune responses. Unfortunately, research in this field rapidly shifted from studies of the function of the suppressor T cells to studies of their soluble products that were thought to be shed or secreted T cell receptors. A number of highly complex suppressor cell pathways and cell circuits were developed and were the subjects of more than 5,000 papers during this era. In 1983–1984, this field completely collapsed as studies called into question the existence of the I-J region of the mouse major histocompatibility complex that was thought to encode one of the major chains of the suppressor T cell factors. The cloning of the T cell receptor at that time firmly established that the T cell receptor genes were completely unrelated to the genes encoding immunoglobulin heavy chains calling into question the existence of soluble T cell factors that contained immunoglobulin VH gene products. The number of papers in the literature dealing with suppressor cells fell from a high of 1,300–1,500/year in 1981 to 150–200/year by the end of the 1980s. At this point in time, most immunologists felt it was even inappropriate to use the term suppressor cell!

Although a number of workers in the period of 1970–1995 continued to focus their studies on T suppressor cells rather than soluble factors, their work was largely ignored by the immunologic community. The detailed history of their pioneering work will be covered in Chapter 1 by Professor Sakaguchi. Immunologists are somewhat obsessed with dividing what initially appears to be homogeneous population of cells, e.g, $CD4^+$ T lymphocytes, into multiple subpopulations with distinct functional properties, e.g, Th1 and Th2 cells. Ideally, most immunologists desire that each subpopulation could easily be identified and separated by the expression of a cell surface antigen unique to that subpopulation. Although immunologic

phenomena that appeared to be mediated by regulatory T cells were described in the literature in the early 1990s, what was really missing from this field was a cell surface marker that would allow immunologists to define a regulatory/suppressor cell. It was only after Prof. Sakaguchi identified the CD25 antigen in 1995 as a marker for a major population of T cells that had suppressor functions both in vitro and in vivo that the resurgence in the regulatory T cell area could begin.

The regulatory T cells field has grown dramatically over the past decade. It is now impossible to read a journal that does not contain numerous papers whose titles deal with regulatory T cells. More importantly, it is also difficult to submit a new research grant proposal in any area of immunologic research that does not include a section on analysis on the contribution of regulatory T cells to the subject matter under study. Regulatory/Suppressor T cells have come of age, again, hopefully this time to stay. Although it was initially thought that regulatory T cells functioned primarily in controlling autoreactive immune responses and several chapters in this volume are devoted to that topic, there is little doubt that the role of regulatory T cells in infection, cancer, and transplantation is just as important. Regulatory T cells even appear to play critical roles in cardiovascular disease in the pathogenesis of atherosclerosis. Many of the chapters in this volume with deal with the lineage of regulatory T cells that are defined by expression of CD25 and more importantly the transcription factor Foxp3. These cells were originally believed to be generated exclusively during T cell development in the thymus, but many recent studies indicate that they can be generated extrathymically. Cell types other than $CD4^+CD25^+Foxp3^+$ have also been shown to manifest regulatory properties and some of these unique cell types will be described in Chapters 23–30.

As in any rapidly moving field in science, many of the concepts and theories presented here will rapidly be modified or even discarded as new studies are performed and new questions are raised. For example, there are now at least a dozen proposed cellular mechanisms for the suppressive activity of the $CD4^+CD25^+Foxp3^+$ regulatory cells. Are all of these suppressive pathways actually used? Which ones are the most important? Which ones can be manipulated for therapeutic purposes? All of these questions should be answered in the next five years. Lastly, an important focus of this book is clinical application. Although numerous studies in animal models have strongly suggested that manipulation (augmentation or downregulation) of regulatory T cell function can be used for therapy of autoimmune, neoplastic, or infectious disease, we are now just on the threshold of translating some of the approaches from animals to man. Regulatory T cells can be best thought of today as "teenagers" ready to take on all the challenges of complex immune responses. In ten years, the field will certainly be more mature, and manipulation of regulatory T cell function by cellular biotherapy, antibodies and small molecules will be routine function of the clinical immunologist.

Bethesda, USA Ethan M. Shevach

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Part I
Immunobiology of Regulatory T Cells

Chapter 1
Regulatory T Cells and the Control of Auto-Immunity: From day 3 Thymectomy to FoxP3+ Regulatory T Cells

Makoto Miyara and Shimon Sakaguchi

Abstract Regulatory T-cell population is now widely accepted as an important component of the immune system as professional suppressors of immune responses. It was shown in the late sixties that some CD4+ T cells in normal mice were capable of suppressing autoimmunity. Efforts to characterize this autoimmune-suppressive CD4+ T cell population led to the identification of CD25 as a constitutional marker. Using this marker, it became possible to separate regulatory T cells from other CD4+ T cells, to further analyze their developmental pathways, especially in the thymus, and to better describe how they suppress immune responses in vivo and in vitro. The marker was also found to be useful to identify regulatory T cells with comparable suppressive function and phenotype in humans. It was recently shown that transcription factor Foxp3 was specifically expressed by CD25+ CD4+ regulatory T cells in rodents. Anomalies in FOXP3 gene are responsible for the development of an autoimmune and inflammatory disease in humans and rodents characterized by a deficiency in the development and function of CD25+CD4+ regulatory T cells. These recent findings provide clear evidence that Foxp3+CD25+CD4+ regulatory T cells are indispensable for the establishment and the maintenance of immunologic self-tolerance and immune homeostasis. Therefore, characterization of regulatory T cell mediated immune suppression should bring new clinical tools to control pathological immune responses.

The immune system is able to mount durable efficient destructive responses against exogenous pathogenic micro-organisms like viruses, bacteria, fungi and parasites and against endogenous pathogens like tumors. These responses are potent enough to destroy not only pathogens but also the host. This implies that immunity, like the other major systems in the body machinery, maintains essential regulatory mechanisms that prevent inappropriate harmful responses. These regulatory processes constitute the immunological tolerance. Several key mechanisms have been described in the last three decades to explain how the immune system is capable of preventing

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what Ehrlich denominated the *Horror autotoxicus* i.e. auto-immunity at the end of the nineteenth century [1]. One of these major mechanisms is the clonal deletion that occurs in the thymus. Clonal deletion leads to the destruction of most self reactive T cells [2–5] and is effective under the control of the gene AIRE [6–8]. Mutation of AIRE gene leads to the development of systemic auto-immune disease APECED that is characterized by the occurrence of multiple endocrine organ autoimmunity [9]. Another key mechanism in the maintenance of tolerance is the activation induced cell death (AICD) of T cells [10] through the interaction of Fas with its ligand Fas-L [11]. The auto-immune lymphoproliferative syndrome (ALPS) [12] which is characterized by the occurrence of auto-immune cytopenia including auto-immune hemolytic anemia and idiopathic thrombopenic purpura and sometimes of a systemic lupus erythematosus resembling disease is secondary to mutations in FAS or FAS-ligand gene.

In addition, it is now widely accepted that another mechanism involving T cells that actively suppress the activation and the proliferation of other immune cells is also key in the maintenance of tolerance to self constituents and in the prevention of auto-immune diseases. Circulating T cells that have specifity to self antigens can be detected in healthy individuals but usually do not trigger clinically patent auto-immunity [13]. This suggests that a dominant inhibitory phenomenon that controls these potentially harmful cells is operating permanently. It took a very long time for investigators to convince the scientific community that a distinct subset of CD4+ T cells, initially called suppressor T cells then rebaptized regulatory T cells, was the mediator of such permanent suppression of auto-immune responses in the periphery.

The Origins (1969–1982)

One major milestone discovery in immunology was made in 1966 by Claman et al. who showed that the cooperation of two distinct subsets of lymphocytes, one originating from the bone marrow and the other one derived from the thymus, was necessary for the production of antibodies [14]. Thymus derived lymphocytes named T cells were defined as the cells that highly responded to antigenic stimuli by mitosis and protein production [15]. Lymphocytes derived from the bone marrow were identified as the cells that produced antibodies [16]. As T cells were shown to be necessary for the induction of immune responses, it became critical to know whether the very same population could also dampen immune responses and how important T cells were in the induction or in the prevention of auto-immunity.

Two major types of experimental approaches were then developed to break or to induce tolerance in normal animals by manipulating T cells. Experiments conducted in the late 1960s by Nishizuka and Sakakura [17] consisted in the observation of mice that were thymectomized at day 3 of life whereas the other kind of experiments designed by Gershon and Kondo in 1970–1971 was meant to study the effects of cells derived from the thymus transferred into mice after they were immunized with foreign antigens [18, 19]. In these experiments, lymphocytes that

were isolated from the thymus could inhibit the cooperation between T and B cells and inhibit the production of antibodies against foreign antigens. In their experiments, athymic mice were first irradiated and then reconstituted 10 days later with autologous hematopoietic stem cells and with autologous thymocytes. These mice were then immunized with sheep red blood cells (RBC). As expected, they produced allo-antibodies against sheep RBC. However, mice that received a second injection of autologous thymocytes before a second challenge with sheep RBC did not increase their allo-antibodies secretion [18]. Gershon and Kondo therefore concluded that induction of both immune responses and tolerance in bone marrow derived cell population required the co-operation of thymus derived T cells. One year later, Gershon and Kondo showed that the transfer of splenocytes in athymic mice that were isolated from tolerized mice could induce a similar tolerance in the recipient [19]. Hence, they termed this phenomenon "infectious tolerance" and the tolerizing thymocytes "suppressor T cells" [20].

In parallel, Nishizuka and Sakakura performed their experiments of day 3 neonatal thymectomy in mice at a period when it was not clearly demonstrated that thymus had an exclusive immunologic function. Thymus had "long been considered to be an endocrine organ somehow related to sexual physiology" because thymectomy at 3 days of age in certain strains was followed by an impaired development of mammary glands and to a reduction in the frequency of mammary cancers [17]. They also observed that female mice that were thymectomized at day 3 of life were sterile because of the destruction of their ovaries. Interestingly, this ovarian disease was not induced when the thymectomy was executed after the 7th day of life and it was also observed that the disease could be prevented by thymus grafting [17]. It came to light that the pathological mechanism that led to ovarian failure was not the lack of sexual hormone, supposedly secreted by the thymus, but rather due to auto-immune inflammation [21]. Moreover, depending on the mice strain, other auto-immune features were observed in day 3 thymectomized mice. For example, in BALB/c strain, about 25% of mice developed auto-immune oophoritis and one third developed gastritis. In A strain, oophoritis was more prevalent (90%) and gastritis was observed in 10% of mice. Thyroiditis was also found in 6% of mice and orchitis developed in 16% of male mice. However, in other strains such as C57BL/6, mice did not develop auto-immunity [22]. Altogether these findings raised the possibility that particular lymphocytes produced in the thymus from day 3 of life in mice were capable of preventing the emergence of auto-immune diseases.

Penhale et al. also confirmed in 1973 that thymectomy could induce organ specific auto-immunity. They showed that normal rats that were thymectomized at 6 weeks of life developed auto-immune thyroiditis with the production of anti-thyroglobulin antibodies, after they received four sublethal doses of X-irradiation every 2 weeks [23]. Following the same procedure in other strains, they confirmed several years later that thymectomy could also induce other autoimmune features such as diabetes mellitus [24].

Therefore, the bases of the concept of T cells that suppress immune responses against either foreign or self antigens were set.

Tracking Suppressor T Cells (1982–1995)

The fact that auto-immune diseases could only be induced when the thymectomy was performed between day 3 and day 7 suggests that a distinct population of thymus derived cells which can prevent auto-immunity arises starting from day 3 of life [17]. Importantly, auto-immunity did not occur when the mice were thymectomized after day 7. This suggests that tolerogenic T cells accumulate in the periphery between day 3 and 7, which would be sufficient for a long-term prevention of auto-immunity. Sakaguchi in Nishizuka's laboratory demonstrated in 1982 that auto-immunity provoked by neonatal thymectomy could indeed be prevented by the injection of normal adult mice T cells when performed within 2 weeks after thymectomy [21]. These findings suggested that a distinct population of T cells persisted in adult life and could prevent auto-immunity. Therefore, several groups attempted to better characterize the phenotype of the suppressor T cell subpopulation by identifying specific surface markers. They also attempted to prove the relevance of in vivo suppression by studying the consequences of the elimination of certain T-cell subpopulations in mice.

First, Sakaguchi et al. showed in 1985 that athymic BALB/c nude mice reconstituted with splenocytes without CD4+Lyt^{high} (CD5^{high}) cells developed multi-organ auto-immunity that included gastritis, thyroiditis, sialadenitis, diabetes, adrenalitis, oophoritis and testicular inflammation. They also observed that the co-transfer of all CD4+ T cells together with CD4+CD5^{low} T cells could prevent auto-immunity [22]. Five years later, Powrie and Mason demonstrated that athymic rats reconstituted with splenocytes without CD4+CD45RB^{low} T cells developed a graft versus host reaction like disease. They also observed a multiple auto-immune syndrome that included liver, lung, stomach, thyroid and endocrine pancreas autoimmune inflammation [25]. McKeever et al. also showed in 1990 that the transfer in PVG nude rats of RT6.1+ T cell depleted splenocytes led to the development of thyroiditis and diabetes mellitus [26]. In 1993, Powrie et al. on one hand and Morrissey et al. on the other hand showed that the adoptive transfer of CD4+CD45RB^{high} T cells in SCID BALB/c mice led to the development of exudative enteropathy [27]. The same year, Fowell and Mason demonstrated that the adoptive transfer of CD4+CD45RC^{low} RT6+ Thy-1- OX-40- T cells in thymectomized irradiated rats prevented the emergence of diabetes mellitus [28].

Meanwhile, in the late 1980s, two major discoveries and a scientific fiasco made the contribution of suppressor T cells meaningless in the explanation of tolerance mechanisms. First, the clonal deletion of auto-reactive T cells was shown to be effective in the thymus by Kappler in 1987 [3]. Therefore, the key role of the thymus in the prevention of autoimmunity was assumed to be exclusively related to the clonal deletion phenomenon. Second, Mosman and Coffman introduced the T helper 1 and 2 subsets dichotomy [29]. It has rapidly been shown that TH1 subset defined by TH1 cytokine secretion (interleukin 2, interferon-gamma, GM-CSF and IL-3) after stimulation by APC [30] could suppress TH2 cytokines secretion such as IL-4 [31] by TH2 cells through their cytokine secretion and vice-versa [32]. Thus, it was assumed that peripheral tolerance was rather due to the balance between TH1 and TH2 cells termed "immune deviation" rather than secondary to the action of

a specific suppressor subset of T cells. At last, it had been first assumed that the mechanism of suppressor T cells mediated inhibition of other immune cells was secondary to the production of a soluble suppressor T-cell factor called "I-J protein" and I-J locus was thought to be located between I-A and I-E [33, 34]. However, when the DNA sequence of murine MHC was molecularly cloned, it appeared that such locus did not even exist [35, 36].

As a result, despite convincing results brought by independent laboratories, the concept of suppressor T cells had eventually become taboo [37].

The Rebirth of Suppressor-Regulatory T Cells (1995–2000)

Sakaguchi et al. showed in 1995 that a distinct population of CD4+ T cells which expresses the alpha chain of IL-2 receptor (CD25) that represents from 5 to 10% of CD4+ T cells could prevent the emergence of autoimmune diseases [38]. The injection of normal BALB/c mice splenocytes that were depleted of CD25+ CD4+ T cells in BALB/c nude mice was sufficient to induce a multiple organ auto-immune disease that included gastritis with anti-parietal cell auto-antibodies and oophoritis in almost all cases and in some cases thyroiditis, sialadenitis, glomerulonephritis, adrenalitis, insulitis and arthritis. Moreover, when injected within a limited period of time, autologous CD25+CD4+ T cells could prevent the development of diseases in nude mice reconstituted with CD25–CD4+T cells. The following year, Asano and Sakaguchi made the relationship between CD4+CD25+ suppressor T cells and neonatal day 3 thymectomy clearer [39]. They demonstrated that the inoculation of syngeneic CD4+ CD25+ T cells could prevent auto-immunity in thymectomized mice but more importantly, they brought the evidence that the emergence of CD25+CD4+ T cells in the periphery of normal mice started immediately after day 3, and then rapidly increased within 2 week to reach levels that were close to the ones observed in adult mice. They also showed that day 3 thymectomy was sufficient to eliminate CD25+ CD4+ T cells from the periphery for several days.

In 1998, Shevach's group and Sakaguchi's group made one step forward to better characterizing the function of suppressor CD4+ CD25+ T cells in vitro. They demonstrated that CD25+ CD4+ T cells, that were anergic upon stimulation, could suppress the proliferation and the production of IL-2 of activated CD4+ T cells in vitro in a contact-dependent manner [40, 41]. Thus, the characterization of CD25 as a reliable surface marker and the possibility to assess their function in vitro definitively pushed suppressor T cells thereafter called regulatory T cells (Tregs) out from oblivion.

The Hunt for CD25+ CD4+ Regulatory T Cells in Mice and Humans (2000–2003)

One key issue to address to better defining the Treg subset is the lack of a specific marker that allows the isolation of a pure homogeneous Treg population even upon activation. Although most CD25+ CD4+ T cells have regulatory properties in naïve mice, the isolation of a pure Treg population according to the expression of

CD25 is challenging in diseased conditions or after activation in vitro because activated CD4+ T cells upregulate their expression of CD25. Therefore, several groups attempted to find new additional molecules that would have allowed a more precise definition of Treg phenotype.

It was first reported that CD4+ T cells that expressed the adhesion molecule L-selectin (CD62-L) were capable of preventing the emergence of auto-immune diabetes in NOD mice [42, 43]. Then, it was reported that the transfer of CD25+ CD4+T cells in NOD mice prevented diabetes [44]. Among these cells, those that expressed CD25 and the highest levels of CD62-L were shown to be the most potent suppressor of the disease [45, 46]. Nevertheless, it was clear that CD62-L could not be used as a single marker to isolate Tregs because CD62-L was also expressed on other T cells subsets.

One relevant way to find Treg specific markers is to elucidate the molecular mechanisms of suppression by identifying molecules that are involved in cell-cell contact mediated suppression or in the regulation of suppression. Co-stimulatory molecule Cytotoxic T Lymphocyte Antigen 4 (CTLA-4) is a major molecule in the attenuation of immune responses. Although CTLA-4 was first described to be involved in the termination of costimulatory signals in T cells once their CD28 molecules had interacted with CD80-CD86 on APCs [47], it was questioned whether CTLA-4 could also be involved in Treg mediated suppression. CTLA-4 knock-out mice develop a fatal lymphoproliferative disease with systemic auto-immune features [48] that resemble the ones observed in nude mice reconstituted with splenocytes depleted of CD25+ T cell. Sakaguchi's group and Powrie's group showed that CTLA-4 was constitutively expressed by CD4+ CD25+ T cells [49, 50]. Moreover, the latter showed that the suppression mediated by CD4+CD25+CD45RB^{low} T cells, the depletion of which was responsible for the induction of the colitis described in the middle of the 1990s, required the signalling of CTLA-4 to be effective. Nevertheless, CTLA-4 is also expressed on activated cells, making it difficult to distinguish activated T cells from genuine Tregs.

The search for monoclonal antibodies that would break the suppression mediated by Tregs led to the identification of the Glucocorticoid-Induced Tumor necrosis factor Receptor family-related gene (GITR) in 2002. Shimizu et al. in Sakaguchi's lab demonstrated that the anti-GITR antibody they produced could deliver an active signal in Tregs through GITR that could attenuate suppression in vitro. Moreover, mice injected with anti-GITR antibodies developed gastritis with anti-parietal cells antibodies [51]. At the same time, another group led by Byrne and Shevach also identified GITR using DNA microarray as a gene that was specifically expressed by Tregs [52]. However, it rapidly appeared that GITR was also expressed on activated effector cells making it difficult to isolate Tregs from activated T cells using this marker alone [53].

In human research field, several groups (Baecher-Allan in Hafler's group, Jonuleit et al., Dieckmann et al., Levings et al. in Roncarolo's group, Taams et al. in Akbar's group and Ng et al. in Lechler's group) confirmed in 2001 that CD4+ CD25+ regulatory T cells were also prevalent in humans [54–59]. However, although it was demonstrated in healthy donors that the 1–2% of CD4+ T cells that expressed

the highest levels of CD25 had the best suppressive capacity in vitro, it rapidly come to light that CD25 could not be used as a reliable marker for human Tregs, especially in inflammatory diseases in which circulating activated T cells expressing CD25 are also found. A specific marker for Tregs was indeed awaited until...

The Modern Age: FoxP3+ Regulatory T Cells (2003-now)

The Immune dysregulation Polyendocrinopathy Enteropathy X linked syndrome also known as IPEX is a severe multisystemic autoimmune and inflammatory disease which onset usually occurs in the neonatal period. The first description of the disease was made in 1982 by Powell et al. [60]. The murine equivalent of IPEX is the scurfy mouse that was described by Godfrey et al. [61]. Three teams simultaneously brought the evidence in 2001 that IPEX and scurfy were the consequence of a deficiency in the gene expression of the transcription factor Foxp3 (Forkhead box protein 3) [62–64]. The following year, Schubert et al. showed that Foxp3 was a transcription inhibitor that regulated T cell activation [65]. Three independent teams demonstrated in 2003 that the expression of the transcription factor FoxP3 was required for the development and the function of regulatory T cells [66–68]. Not only Foxp3 was found to be specifically expressed in CD4+CD25+ Tregs in mice but also the forced expression of FoxP3 in naive CD4+T cells was sufficient to make them suppressive in vitro and in vivo. Moreover, several genes preferentially expressed by CD4+CD25+ Treg cells such as CD25, CTLA-4, and GITR were shown to be directly controlled by FoxP3 [67]. Therefore, these findings supported the role of Foxp3 as a master control gene in the development and the function of the Treg population. In 2005, using a GFP reporter mouse expressing a fusion GFP-FoxP3 protein, Fontenot et al. in Rudensky's group showed that the expression of Foxp3 could be detected in the thymus starting from day 3 of life. They also showed that the peripheral colonization by FoxP3+ cells was seen starting from day 3 too [69]. These findings ultimately demonstrated that the induction of autoimmunity by day 3 thymectomy was indeed caused by the depletion of Foxp3 expressing natural regulatory T cells.

The most recent studies are mainly focused on the relationship between FoxP3 and the other transcription factors that are involved in the functions of T cells. Several groups showed that Foxp3 acts as a repressor of the expression of IL-2, IL-4 and IFN-γ through direct physical interactions with transcription factors NF-κB and NF-AT on one hand [70, 71] and AML1/Runx1 on the other hand. Furthermore, it has been demonstrated that the suppressive function of Tregs was dependent on the interaction of FoxP3 with NF-AT and/or AML1/Runx1 proteins. Other recent studies have dealt with the genes that are controlled by FoxP3 [72–75] in order to figure out the molecular mechanisms of suppression and to find other molecules that are specifically expressed by Tregs. Despite those efforts, it is still unclear how Tregs suppress immune responses and it is still not determined which surface proteins can be used as a mere marker for Tregs.

Tregs and Auto-Immunity

Regulatory T cells can downregulate a wide spectrum of physiological and pathological immune responses and many studies raised the possibility that Tregs could be involved in the evolution of diseases that have immune components such as infection, cancer, transplantation, allergy and inflammatory/autoimmune diseases. However, what has been shown in the last 30 years is that the primary key role of Tregs is to control auto-immune responses.

Day 3 thymectomy can be considered the first model of Treg depletion in vivo. Most mice that are thymectomized develop, depending on their strain, oophoritis and sometimes gastritis or thyroiditis. Of note, these auto-immune diseases are mainly concerning endocrine organs [17]. In the model of Sakaguchi in which nude mice were reconstituted with CD25-T cells, it was also observed that most mice developed auto-immune gastritis and that other endocrine glands were diseased such as ovaries, thyroid, adrenal glands, endocrine pancreas but also exocrine glands i.e. salivary glands [38]. Taken together, these auto-immune features is reminiscent of human diseases APECED and type II autoimmune polyglandular syndrome. The first syndrome is rather seen in young children whereas the second is prevalent in middle age adults. APECED (autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy) disease is usually characterized by the association of several endocrine autoimmune disorders such as type I diabetes, thyroiditis, gonadic deficiency and pernicious anaemia due to autoimmune gastritis. The cause of APECED syndrome has been recently identified as a mutation in the gene AIRE (autoimmune regulator) [6, 9] which is necessary for the presentation of self antigens to thymocytes in order to eliminate self reactive ones. It has been recently demonstrated that Aire was also involved in the selection of Tregs in the thymus [76]. Moreover, Treg function has been shown to be deficient in patients with APECED [77]. In type II APS, one major feature is the appearance of auto-immune gastritis which is associated with other glandular diseases such as adrenalitis, parathyroiditis, thyroiditis and type I diabetes. A deficiency in Treg suppressive function has also been described in patients with the disease [78]. Taken together, those experimental findings in mice and translational studies in human diseases strongly suggest that Tregs play a key role in the prevention of polyglandular auto-immune syndromes.

The role of Regulatory T cell subset has also been extensively studied in the pathophysiology of type I diabetes. CD25+CD4+ Treg can prevent the disease especially if NOD mice are transferred with CD25+CD4+ T cells that have high levels of CD62-L [43–45]. Studies in humans suggest that Tregs might play a role in type I diabetes. Several groups have raised the possibility that Treg suppressive function could be impaired [79, 80] or that the number of Tregs could be diminished [81]. Nevertheless, due to the lack of a reliable marker for human Treg, the conclusions on the function or the quantity of Tregs are likely to change in the next years. For example, according to the expression of FoxP3, a recent report concluded that the proportion of Treg was not modified in type I diabetes patients [82]. But again, these conclusions might be someday obsolete since FoxP3 has recently been shown to be also upregulated in activated CD4+ T cells, exactly like CD25 or GITR [83, 84].

Powrie's group showed in the 1990s that the adoptive transfer of CD4+ CD45RB^{high} T cells in SCID mice led to the development of a severe wasting disease that was secondary to colitis [25]. They could demonstrate that Tregs could prevent the disease but they also brought the evidence that Treg could cure the colitis [85]. In humans, Maul et al. showed that patients with inflammatory bowel disease including Crohn's disease and ulcerative colitis had less circulating CD4+CD25+ T cells when compared to healthy donors. However, they did not notice any impairment in the suppressive function of Treg in vitro [86].

In some nude mice reconstituted with CD25– T cells, in addition to polyglandular syndrome, one could observe systemic auto-immune features such as arthritis that is reminiscent of rheumatoid arthritis (RA), or glomerulonephritis and the presence of anti-double strand DNA antibodies that are characteristic of systemic lupus erythematosus(SLE) [38]. Several groups observed that the proportion of circulating Treg was decreased among CD4+ T cells in patients with SLE during flares whereas no modification was noted when the disease was inactive [87–89]. In RA, both quantitative deficiency [90] or functional impairment [91] in Treg cell population were reported. Whereas Tregs have been shown to be reduced in the periphery, high prevalence of Treg was noted in the inflamed joints [90]. In vitro, it has been shown that Treg that were capable of suppressing the proliferation of activated cells on one hand, were not capable of controlling the secretion of TNF alpha on the other hand [91]. These findings suggest that Tregs in RA might migrate and accumulate in the joints but would not be efficient enough to control inflammation. Interestingly, the suppression potency was recovered in patients that responded clinically to treatments with monoclonal anti-TNF alpha antibodies [91].

Similar functional deficiency was described in sarcoidosis which is an inflammatory disease characterized by the presence of granuloma in diseased organs. TNF alpha also plays a major role in the pathophysiology of the disease. An accumulation of Tregs has been observed in diseased organs but, in opposite to RA, an expansion of Treg was also observed in the periphery [92]. Treg function has been shown to be impaired in two other immune diseases which are multiple sclerosis [93] and psoriasis [94]. In both cases, Treg isolated from patients were shown to be incapable of completely suppressing the proliferation of effector cells in vitro.

These numerous examples of animal models and human diseases strongly argue for the essential role of Tregs in the prevention of aberrant immune responses. Therefore, it is becoming more and more evident that the manipulation of Treg cells would be a promising approach to treat auto-immune diseases.

Conclusion

Since the seminal articles by Nishizuka et al. and by Gershon et al., it has been for long debated whether suppressor/regulatory T cells did actually even exist. Today, this question is no longer accurate and the subject of regulatory T cells is the object of a constantly growing interest in immunologists and clinicians. However, even if significant progresses have been made since the definition of CD4+C25+ T cells in

1995 and of foxP3 seven years later, the most important questions about Tregs are still unanswered: we still do not know what are the best surface markers for Tregs in rodents and in humans and more importantly, we still do not know the exact mechanisms of suppression. There is no doubt that the history of Tregs will make a new big leap ahead when the answers of this crucial question come.

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