Preface

Regulatory T cells (sometimes known as suppressor T cells) are a specialized subpopulation of T cells that act to suppress activation of the immune system and thereby maintain immune system homeostasis and tolerance to self-antigens. This book presents topical research in the study of regulatory T cells, including the role of regulatory T cells in the treatment of multiple sclerosis and hepatocellular carcinoma; genetic engineering of antigen-specific regulatory T cells; regulatory T cells in arthritis, allergic disorders and immunomodulation; and the roles of immunoregulatory cells and the mechanisms of immune response.

Chapter 1- Research in the past decades has clearly demonstrated that chronic inflammation and autoimmune mechanisms play critical roles in the pathogeneses of many diseases including cardiovascular diseases and atherosclerosis, which are the leading causes of morbidity and mortality worldwide. However, several important issues remain to be addressed including the mechanisms underlying the regulation of autoimmune inflammation. It has been recently reported that pro-inflammatory interleukin-17 (IL-17)-producing $CD4^+$ T helper cells (Th17 cells) interplay with immunosuppressive $CD4^+CD25^{high}Foxp3^+$ regulatory T cells (Tregs) in controlling inflammatory and autoimmune diseases. Therefore, in this chapter, we will analyze the progress in the field by focusing on the following topics: *1)* the new paradigm of multiple T helper cell subsets; *2)* IL-17 family cytokines and Th17 cells; *3)* Effects of Th17/IL-17 on various inflammation and autoimmune diseases; *4)* the interaction of Tregs and Th17 cells in regulating autoimmune diseases and vascular inflammation. Regulation of autoimmunity and inflammation lies in the interplays of the different T helper subsets, therefore, better understanding of these subsets' interactions with one another would greatly improve our approaches in developing therapy to combat inflammatory and autoimmune diseases.

Chapter 2- Regulatory T-cells (Tregs) are key regulators of the immune system, however their role in infectious diseases remains rather controversial. Chronic persistent infections in humans such as parasites, viruses and (myco)bacteria all can result in the induction of both $CD4^+$ and $CD8^+$ Tregs. In these chronic persistent infections, Tregs may dampen inflammation to limit tissue damage, but they may also inhibit ensuing effector immunity, thereby impairing pathogen clearance. All of these may depend on timing and location of Treg responses, early during infections Tregs may be essential balancing the trafficking of effector cells between the sites of immune induction and infection whereas in later stages Tregs may hamper pathogen clearance. A remarkably increased frequency of Tregs has been

observed at the site of infection, suggesting active local involvement. The vast majority of studies has focused on the role of (various subsets of) $CD4^+$ Treg cells. However, $CD8^+$ Treg cells play a prominent role as well, but surprisingly little is known about these cells, particularly in humans. The antigen specificity of such $CD8^+$ Tregs has been demonstrated for several pathogens and the genetic restriction of the T-cell responses can comprise classical as well as non-classical HLA class I family members. Here we review and discuss human $CD8^+$ Tregs and $CD8^+$ Treg subsets, with particular emphasis on their properties, effector mechanisms and their role in chronic, persistent infectious diseases.

Chapter 3- In the last years knowledge of regulatory T cells has expanded. They play a role in autoimmune disease but it is still unclear how they suppress auto-reactive T cells exactly. The possibilities to use regulatory T cells in the treatment of MS are discussed in this chapter. The focus will be on the function of HLA-G and $CD4^+CD25^+$ T cells expressing FoxP3 and CTLA-4.

Two types of Tregs with different ways of suppression are known. Adaptive Tregs are induced in the periphery and mainly secrete TGF-β and IL-10. These cytokines mediate suppression of T cells through induction of FoxP3. Natural Tregs are generated in the thymus and suppress T cells through HLA-G, CTLA-4 and FoxP3. HLA-G interrupts the signalling in APCs and T cells resulting in suppression. FoxP3 blocks the expression of IL-2 and consequently inhibits proliferation of T cells. The expression of FoxP3 is induced by TGF-β. TGF-β also induces $CD4^+CD25^-$ T cells to convert into $CD4^+CD25^+$ T cells which express FoxP3 and hence suppress T cell proliferation. The suppression mechanism of CTLA-4 is still unknown. It might be a positive feedback with TGF-β resulting in increased expression of FoxP3 or a block of co-stimulatory molecules on APCs causing reduced secretion of cytokines following reduced activation of T cells.

T_H17 cells also have an impact on MS. T_H17 cells are induced by IL-6 and TGF-β. These cytokines start the differentiation and activate the transcription factor ROR-γt that makes a T_H17 population expand. The presence of IL-6 inhibits the expression of FoxP3. Presence of TGF-β and absence of IL-6 induces FoxP3 which inhibits the expression of ROR-γt leading to the production of pro-inflammatory cytokines. Hence, FoxP3 and ROR-γt are of great influence and a disbalance between these factors will lead to a skewed balance in favour of T_H17 cells.

Consequently, increased levels of TGF-β will induce expression of FoxP3 and could normalise the situation.

The current treatment of MS is mainly symptomatic. Glatiramer acetate can promote activation of FoxP3, hence enhancing the function of Tregs. IFN-β up-regulates the expression of HLA-G. Unfortunately, these drugs do not cure MS, since glatiramer acetate and IFN-β are not able to skew the dysbalance back to normal.

Because TGF-β and HLA-G are able to induce the function of Tregs; the conclusion is that administration of TGF-β and HLA-G appears to be a future treatment of MS.

Chapter 4- The immune system requires a homeostatic equilibrium between the mechanisms that assure self-tolerance, those that control the capacity to mount life-long immunity to pathogenic microbes or toxins, and those that attenuate effector mechanisms from inducing immune pathology. A growing body of evidence suggests that an imbalance in this homeostasis plays a major role in the pathophysiology of autoimmune disease.

A great diversity of regulatory cells types are being progressively identified, such as tolerogenic DCs, $CD4^+CD25^+$ T_{Reg}, regulatory natural killer (rNK) and $CD8^+CD28^-$

suppressor T-lymphocytes, among others. Among the different types of immunoregulatory cells, $CD4^+CD25^+$ (IL-2 receptor alpha chain) regulatory T $CD4^+$ cells (T_{Reg}) play a pivotal role in the tolerance to self-antigens and tissue grafts, and in the suppression of autoimmune reactions. T_{Reg} exert modulatory functions upon the activity of self-aggressive T cells not deleted in the thymus and are one of the pillars for maintaining the immune system in homeostatic balance. Indeed, these cells modulate the intensity and quality of immune reactions through attenuation of the cytolytic activities of reactive immune cells. The outcome of different contributions of all these complex mechanisms under variable inflammatory conditions, the diversity of immune interactions between this constellation of cells conduct the evolution of the immune reaction. In this review, we summarize research characterizing the growing diversity of immunorregulatory cells subsets in the control of autoimmunity in rodents and humans. Here, we attempt to integrate the current experimental evidence on the major suppressive pathways of immunorregulation.

Chapter 5- Gastrointestinal mucosa is an important site for oral tolerance induction and allergic responses to exogenous innoxious antigens, such as food antigens. The failure or aberration of oral tolerance results in the development of allergic diseases, especially food allergy. Several factors, including mucosal barrier function, humoral factors, lymphocyte anergy, clonal deletion, and regulatory T cells, contribute to the development of oral tolerance. Accumulating clinical and experimental evidences have suggested that the decreased number and dysfunction of regulatory T cells are involved in the pathogenesis of food allergy and that the development of regulatory T cells is associated with outgrowing food allergy. Although the precise nature of regulatory T cells controlling oral tolerance to food Ags remains unknown, recent reports demonstrate that not only regulatory $CD4^+$ T cells but also subsets of $CD8^+$ T cells may function as Treg by inhibiting allergic responses. Increased understanding of the mechanisms of oral tolerance has made a paradigm shift of therapeutic strategies for food allergies from avoidance of foods to oral tolerance induction by augmentation of regulatory T cells. In this article, we review the roles of regulatory T cells in oral tolerance and the pathophysiology of food allergy, and discuss their therapeutic potential.

Chapter 6- The recognition and elimination of malignant cells by donor derived immune effector cells, the so-called graft-versus-leukemia effect (GVL), is key to the success of Allogeneic Hematopoietic Stem Cell Transplantation (AHSCT) in hematological malignancies. The ongoing challenge limiting the success of AHSCT is the maximization of this powerful GVL effect, while minimizing attack of the donor immune cells against the host normal tissue, the so-called graft-versus-host disease (GVHD). The role of CD4+CD25+FOXP3+ regulatory T cells (Treg) in the promotion of peripheral tolerance to self and thus the aversion of autoimmunity is now widely accepted. Furthermore, increasing evidence support their role in modulating alloresponses in the stem cell transplant setting, demonstrating that Tregs are critical players both in the engraftment of donor stem cells and the abrogation of allo-reactive responses that can result in GVHD. Importantly evidence from both murine models and human data suggests that Tregs dampen the alloresponse to host, thereby ameliorating the severity of GVHD without an apparent loss of useful GVL effect. This observation has sparked much research interest into both the underlying mechanisms by which Tregs mediate this effect and ways in which their function can be harnessed and translated into therapies to improve AHSCT outcomes. In this review we seek to summarize the existing data on the role of regulatory T cells in the stem cell transplant setting and

examine how their manipulation might be incorporated as a useful technique to reduce GVHD following allogeneic stem cell transplantation.

Chapter 7- Hepatocellular Carcinoma (HCC) is the fifth most common cancer worldwide and also the third most common cause of cancer-related death. HCC arises most frequently in males with cirrhosis, which is most often a consequence of chronic hepatitis infection (HBV and HCV) or alcohol abuse. To date, the only effective approaches for patients with HCC are resection or liver transplantation.

Immunological mechanisms are important in the surveillance of malignancy and control of tumor progression. Tumor-Infiltrating Lymphocytes (TILs) have been described in HCC, and extensive infiltration has been associated with reduced tumor recurrence following resection. However continued tumor-growth, despite the presence of a lymphocytic infiltration, including tumor-specific T cells within and surrounding tumors, suggests a failure of immune control. Although, many mechanisms have been proposed for this attenuated immune response, it becomes evident that direct suppression of effector cells, supported by regulatory T cells could play a pivotal role in the suppression of immune response to tumors. Initially described in context of immune disorders such as inflammatory autoimmune pathologies, regulatory T lymphocytes are characterized by their capacity to inhibit T helper response. To date, several regulatory T cells are described, however CD4+CD25+ regulatory T cells and Tr1 sub-populations remain best characterized.

Currently, there is no evidence for direct implication of CD4+CD25+ regulatory T cells in the malignancy and control of HCC progress. However, recent studies showed that both regulatory T cells subpopulations and particularly Tr1 have been implicated in the modulation of the immune response during HCV chronic infection, supporting HCC progress.

Chapter 8- A strong relationship exists between regulatory T (Treg) cells and the development and progression of cancer. Increasingly, evidence suggests that Treg cells can migrate into tumors and suppress effective antitumor responses in the tumor microenvironment. The critical question that needs to be addressed is the mechanism of action of these Treg cells *in vivo*. In this chapter, we consider the nature and characteristics of Treg cells in the tumor microenvironment, including dynamic processes of Treg recruitment, activation and induction during tumor pathogenesis and their potential mechanism for multiple suppressive effects. Enhancing our understanding of the underlying molecular mechanisms will offer an insight into how Treg cell function can be manipulated more effectively for antitumor therapy.

Chapter 9- Adaptive immune responses are based on the cooperative harmony between $CD4^+$ and $CD8^+$ T cells. This principle applies not only to typical effector T cells but also to regulatory T cells. Studies of $CD4^+$ regulatory T cells have greatly advanced after the discovery of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg), whereas progress in understanding the dynamics of $CD8^+$ regulatory T cells has been much slower. Although several $CD8^+$ regulatory T cells with different markers have been reported, these cells have not been studied as extensively as $CD4^+$ Treg. Among $CD8^+$ regulatory T cells, the $CD8^+CD122^+$ regulatory T cells in mice and their human counterparts, $CD8^+CXCR3^+$ cells, appear to be the most promising for further study. Following the initial discovery of $CD8^+CD122^+$ regulatory T cells, the same research group has clarified part of the molecular mechanism of these regulatory T cells and determined the important roles of these cells in certain autoimmune diseases by using experimental mouse models. In this review, we summarize the advantages and disadvantages of each $CD8^+$ regulatory T cell population in comparison with $CD4^+$ Treg

and other $CD8^+$ regulatory T cells. Furthermore, we present experimental results showing the synergistic effect of $CD8^+CD122^+$ regulatory T cells and $CD4^+$ Treg and suggest that both $CD4^+$ and $CD8^+$ regulatory T cells be used for the treatment of immune diseases.

Chapter 10- Regulatory T cells (Treg) are crucial for suppression of autoimmune responses and employ a number of mechanisms for controlling the immune response. Treg are abundant at the site of inflammation in arthritis yet fail to control the pathological process. Recently the characterisation of surface markers which help define Treg in inflammation have aided investigations into why this failure of immune regulation occurs and has increased our understanding of Treg mechanisms of action. These investigations are complex due to overlap of Treg markers with those found on activated cells. There is increasing evidence that Treg in arthritis have defects in suppressive function. Here we discuss defects in Treg from patients with inflammatory arthritis, with the focus on adult rheumatoid arthritis (RA), and childhood arthritis (juvenile idiopathic arthritis JIA). We review evidence for how these Treg defects might be affected by treatment and possible novel therapeutic strategies.

Chapter 11- Regulatory T cells (Tregs) play a key role in the maintenance of immune homeostasis *in vivo*. The unique characteristics of Tregs endowed by foxp3, inability to produce IL-2 while constitutively expressing IL-2Rα (CD25), make themselves absolutely dependent on the responding cells for IL-2. When IL-2 is produced and secreted by the responder cells, it would bind preferentially to IL-2Rs on nearby Tregs, as the density of IL-2R on Tregs is higher than that on the responding cells. Therefore, Tregs start to proliferate earlier than the responding cells, and suppress the latter, resulting in an elegant loop of negative feedback. Furthermore, Tregs suppress the responder cells by lowering the production of IL-2 as well as by inducing earlier shedding of IL-2Rα from the responding cells. Meanwhile, the fate of secreted IL-2 and the mode of suppression are different according to the concentration of IL-2; if the stimulating signal is weak, a small amount of IL-2 is secreted from the responder cells and will bind preferentially to the IL-2Rs on Tregs. Therefore, Tregs will proliferate earlier and suppress the responder cells. If the stimulating signal is strong and the initially secreted IL-2 is enough, it would also bind to the IL-2Rs on the responder cells. In this case, the responder cells proliferate initially, but their late expansion is suppressed by Tregs. Either way, in the steady state, Tregs always win the responder cells in the competition for IL-2, hence the state of tolerance. However, in infection or inflammatory state, the suppressive activities of Tregs are suppressed (contra-suppression) by certain molecules secreted from the innate immune cells (i.e., dendritic cells), such as IL-6 or IL-21, resulting in the proliferation of the responder cells, hence immune response is elicited. In conclusion, regulation of IL-2 and IL-2Rα is an important mechanism of immune homeostasis maintained by Tregs.

Chapter 12- Foxp3 expression is required for the development and function of T regulatory cells. The last years intensive research focused on elucidating the transcriptional mechanisms behind Foxp3 expression have revealed a gene strictly regulated by epigenetic mechanisms, where multiple enhancer elements coordinates the expression of Foxp3. In this short communication article we will summarize the recent advances in studies of Foxp3 expression control.

Chapter 13- Regulatory T cells (Tregs) potently suppress a variety of innate and adoptive immune responses mediated by T effector cells (Teff). However, because of their low number (comprising only minor subset (4-10%) of the total CD4 T cell population) and their multiple specificities, large numbers of expanded antigen-specific Tregs are required in order to

suppress an ongoing inflammatory response. This scarcity of Tregs greatly limits their clinical use against autoimmune inflammatory conditions. To overcome the scarcity of antigen-specific Tregs, several attempts have been made to redirect the specificity of Tregs to a pre-defined target antigen.

In this chapter, we review three approaches to endow Tregs with the required novel specificity; all rely on the ability to genetically engineer the recognition of Treg by their transduction with any receptor-gene of interest. The first approach utilizes a chimeric receptor whose specificity is comprised of a class-II-peptide complex; this receptor redirects the genetically modified Tregs against pathogenic antigen-specific effector T cells, resulting in suppression of an experimental model of multiple sclerosis, experimental allergic encephalomyelitis (EAE). The second approach makes use of TCR α and β chain gene transduction to generate MHC-1:peptide specific Tregs that were shown to suppress arthritis and rejection of a non-HLA-matched transplant. The third approach is based on an antibody-based chimeric receptor, which we have used to generate murine Tregs that suppress TNBS colitis in a murine model. In this review, we describe the activity of each of these modified Tregs, compare their reactivity to unmodified nTreg, and discuss their potential as well as obstacles towards future clinical application.

Chapter 14- The immune system protects the host from infection and exogenous stimuli using innate and acquired immune systems and is critical to maintaining health. These stimuli lead to type 1 helper T cell (Th1) and/or Th2 type cytokine secretion according to the character of the exogenous or endogenous stimuli. In addition, FoxP3$^+$(forkhead box P3$^+$) CD4$^+$CD25$^+$regulatory T cells: natural regulatory T cell (nTreg), IL-10 producing regulatory T cells (Tr1), and Th17 cells play a pivotal role in this immune balance triggering immune suppression and stimulation. Altogether they form a four-way balance.

In allergic diseases including atopic dermatitis (AD) and nasal allergy, it is understood that the cytokine profile comprises Th2 dominant, and this milieu gives rise to phenotypical abnormality and pathologic conditions including cellular infiltration and overproduction of IgE.

Chapter 15- Regulatory T cells (Tregs) are an important part of the complex adaptive system of the body that is able to respond to environmental challenges. Tregs maintain peripheral tolerance and play an important role in inflammatory reaction control. Several subsets of Tregs have been described. Naturally occurring CD4$^+$CD25$^+$ Tregs are recognized as a major subset of immune cells maintaining immune self-tolerance in the periphery. Inhibitory potential of these cells against self-reacting T cells is linked to the thymic origin of Tregs. Another subtype of Tregs that is inducible. Such Tregs are generated in the periphery and exert their suppressive potential substantially in the form of anti-inflammatory activity. The latter plays an important role in cooperation of three principal anti-inflammatory mechanisms, which have been developed in the course of evolution: macrophages possessing the suppressive activity (most ancient), Tregs, and stress hormones (phylogenetically youngest). Normally, all three mechanisms of inflammation control are well equalized. However the balance may be disturbed by age due to repeated episodes of stress and HPA axis activation. Consequently, stress hormone release coupled with antigen overload results in Treg accumulation. In time HPA axis activation replaces by its depletion that is manifested by both basal cortisol level decrease and the reduction of stress-induced cortisol response. So, cortisol being in low concentration in the blood is not able any more to control inflammation

and Tregs become a principal mechanism of anti-inflammatory machinary. Superfluous Treg accumulation results in development functional somatic syndromes, such as chronic fatigue syndrome, and in some patients, by reason of anticancer immunity suppression, to tumor growth. On the other hand, in childhood the lack of adequate antigen loading may lead to delay of Treg maturation. Allergy and asthma manifestation may be a consequence of such Treg insufficiency. Thus, both excess and deficiency in Treg number may be at the bottom of morbid conditions. The advances in modern pharmacology allow devising the methods of Treg level control.