

In: Plasmapheresis and Intravenous ...  
Editor: Rossana Allegro

ISBN: 978-1-63117-916-7  
© 2014 Nova Science Publishers, Inc.

#### *Chapter 4*

## **EFFECTS OF IVIG ON T CELL FUNCTIONS**

*Lauriane Padet<sup>1,2</sup> and Renée Bazin<sup>1,2,\*</sup>*

<sup>1</sup>Department of Research and Development,  
Héma-Québec, Québec, Canada

<sup>2</sup>Department of Biochemistry, Microbiology and  
Bioinformatics, Laval University, Québec, Canada

### **ABSTRACT**

Dysregulation of T cell functions results in the development and maintenance of autoimmune and systemic inflammatory disorders. In this context, CD4+ (helper) and CD8+ (cytotoxic) T cell activation leads to autoantibody production and self-reactive cytotoxicity respectively. IVIg is commonly used as a second line immunosuppressive therapy in patients suffering from these disorders. Over the past decades, clinical observations in IVIg-treated patients revealed significant modulations in T cell populations and functions (Th1/Th2 ratio, Treg numbers, cytokine expression, etc.). Similar observations on the immunomodulatory effects of IVIg on T cell populations (eg. activation and differentiation of various CD4+ subsets, reduction of CD8+ T cell cytotoxicity) were made using in vitro assays and animal models, suggesting that these experimental systems could be useful to decipher the mechanisms by which IVIg produces therapeutic effects in autoimmune and inflammatory disorders.

---

\* Corresponding author: Renée Bazin, PhD. Department of R and D, Héma-Québec, 1070 Avenue des Sciences-de-la-Vie, Québec (Qc), Canada, G1V 5C3. Phone: (418) 780-4362, Fax: (418) 780-2091. E-mail: renee.bazin@hema-quebec.qc.ca.

In this chapter, we will review the various effects of IVIg on T cells both *in vitro* and *in vivo*. We will also summarize the mechanisms proposed to explain these effects and discuss the reasons for the discrepancies between conclusions drawn from studies published during the last decades.

## INTRODUCTION

### IVIg and Autoimmunity

IVIg has been used for several decades to treat an increasing number of autoimmune and inflammatory disorders. Excellent review articles on the clinical use of IVIg have been recently published and therefore, this issue will not be discussed in this chapter [1-7]. We will rather focus on the effects of IVIg in autoimmune diseases with an emphasis on T cells on the following sections, using clinical observations obtained from IVIg-treated patients and experimental data derived from *in vitro* work or *in vivo* experimentations using animal models of disease aimed at understanding the mechanisms of IVIg action. Indeed, it has been reported more than 30 years ago that modulations in T cell populations and functions were induced in IVIg-treated patients suffering from autoimmune disorders. For example, Th0 and Th2 anti-inflammatory responses have been documented in IVIg-treated patients [8-10].

Other studies have shown that the ratio of CD4+ T cell subpopulations (Th1/Th2) is decreased following IVIg treatment [11-15], consistent with the induction of immunosuppressive effects. In addition, these clinical observations have stimulated considerable research efforts to better understand the mechanisms by which the infusion of high dose IVIg results in modulations in lymphocyte populations and therapeutic effects in a variety of autoimmune and inflammatory disorders.

### ROLE OF T CELLS IN AUTOIMMUNITY

T cells originate from lymphoid precursors in the bone marrow and migrate to the thymus where they differentiate. In the thymus, T cells reach a state in which they co-express the CD4 and CD8 molecules. These double positive cells are then first subjected to the process of positive selection, where a low affinity but sustained interaction between TCR and MHC molecules

results in cell survival. Following positive selection, the expression of one of the two co-receptors CD4 and CD8 is lost. The negative selection process also occurs in the thymus, where dendritic cells and macrophages present self MHC/peptide complexes to CD4<sup>+</sup> or CD8<sup>+</sup> T cells. This selection process is essential to remove autoreactive T cells. High affinity interactions with the self MHC/peptide complexes result in apoptosis. At the end of the positive and negative selection processes, a repertoire of mature naive T cells competent against non self-antigens but tolerant to self-antigens is generated [16-19].

Naive T cells require 3 signals for activation which are provided by antigen presenting cells (APC) in the lymphoid organs [20-24]. The first signal consists in the recognition of MHC/peptide complexes by the TCR. CD8<sup>+</sup> T cells interact specifically with MHC I/peptide complexes while CD4<sup>+</sup> T cells interact with MHC II/peptide complexes. TCR engagement leads to interleukin (IL)-2 production, an important survival factor for T cells. However, TCR engagement is not sufficient for clonal expansion and T cell differentiation. A second signal is needed and provided following interaction between CD28 expressed on T cells and CD80/CD86 costimulatory molecules expressed on mature APC with the contribution of interactions between other pairs of costimulatory molecules. Such costimulatory signaling amplifies IL-2 production and increases the expression of the alpha chain of IL-2 receptor (CD25). The high affinity binding of IL-2 to its receptor induces T cell proliferation. In the absence of costimulatory signals, T cells cannot be fully activated and undergo apoptosis or become anergic [25-27]. The third signal required for T cell activation and differentiation into effector cell is provided by cytokines secreted by APC which upon binding to receptors on T cells provide signals critical for the determination of the appropriate type of immune response [22, 28].

Even though the machinery responsible to establish T cell tolerance to self-antigens is highly efficient, tolerance can be broken. Several contributing factors have been identified and include genetic predispositions, microbial infections or other environmental factors [29-34]. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) [35], lymphoid protein tyrosine phosphatase non-receptor type 22 (PTPN22) [36], T cell immunoglobulin and mucin-domain containing (TIM) family [37], autoimmune regulator gene (AIRE) [38] are examples of genes that can contribute to the development of autoimmunity. Some MHC alleles can induce over-expression of self-peptides and increase the likelihood of developing autoimmune diseases [39, 40]. Ineffective presentation of self-antigens in the thymus can also lead to autoimmunity by allowing survival of autoreactive T cells.

Microbial infections can trigger autoimmunity by creating a strong pro-inflammatory environment that can trigger nonspecific polyclonal activation of autoreactive T cells [31]. In addition, pathogens can induce autoreactive T cell activation by molecular mimicry or by causing damage to cells or tissues, leading to enhanced presentation of self-antigens. Finally, other environmental factors such as diet, drugs and pollution have been proposed as contributors to the development of autoimmunity [41-43]. In addition to activated autoreactive T cells, pathogenic autoantibodies are major contributors to the development of autoimmune disorders such as immune thrombocytopenia, type I diabetes, rheumatoid arthritis and multiple sclerosis [44-47]. In fact, the production of such antibodies by autoreactive B cells is highly dependent on autoreactive CD4+ helper T cells. Cytotoxic auto-reactive CD8+ T cells also play a predominant role in several autoimmune disorders such as type I diabetes [48], multiple sclerosis [49, 50], chronic inflammatory demyelinating polyneuropathy [51], stiff-person syndrome [52] and polymyositis [53] by mediating organ or tissue destruction. Autoreactive CD8+ T cells may also participate in the etiology of immune thrombocytopenia [54]. Clearly, the different T cell subpopulations play a central role in the development of autoimmunity. Therefore, the clinical responses observed in many patients suffering from autoimmune disorders following IVIg administration have rapidly translated into the identification of T cells as potential direct or indirect targets of IVIg, given their major role in the pathophysiology of these diseases.

### **IS THE EFFECT OF IVIG ON T CELLS DIRECT OR INDIRECT?: THE SEARCH FOR AN ANSWER**

A number of studies aimed at elucidating the mechanisms by which IVIg can affect T cell functions have been reported in the literature.

These studies were done either in *ex vivo* experimental systems or using animal models of disease. A summary of these investigations is presented in the following paragraphs.

#### **Studies on Mitogen-Activated T Cells**

In the 90s, several research groups have focused on the effect of IVIg on the functions of activated T cells using mitogen stimulation to mimic T cell

activity during autoimmune disease. Phorbol esters such as phorbol myristate acetate (PMA) and phorbol dibutyrate (PDB) in combination with calcium ionophores (ionomycin) bypass surface TCR engagement and cross-linking requirements to directly activate intracellular signaling pathways leading to T cell activation [55, 56].

Using this experimental approach, opposing conclusions have been reached by different research groups. Some studies showed that IVIg decreased T cell activation upon peripheral blood mononuclear cell (PBMC) stimulation with PMA/ionomycin. More precisely, IVIg reduced the secretion of cytokines such as IL-2, IFN- $\gamma$ , TNF- $\beta$  and others, as well as T cell proliferation as evaluated by [ $^3\text{H}$ ]thymidine incorporation [57, 58].

A similar study performed on purified T cells activated with PMA/ionomycin rather than PBMC also described the IVIg-mediated inhibition of IL-2 secretion, suggesting that IVIg exerts its anti-inflammatory effect by acting directly on T cells [59]. This inhibitory effect was not the consequence of cell death or repression of IL-2 mRNA expression [57, 59]. Therefore, a post-transcriptional regulation of IL-2 by IVIg was proposed to explain the inhibition of IL-2 secretion but the precise mechanisms by which IVIg affected T cell activation remained unclear [59].

Interestingly, another group reached an opposing conclusion following the demonstration of the absence of inhibitory effect of IVIg on T cells after PBMC or purified T cell stimulation with PDB/ionomycin [60].

The reason for this discrepancy is unclear since phorbol esters such as PMA and PDB produce similar effects on T cells. Another study provided new insights into the inhibitory mechanisms of IVIg by showing that IVIg inhibited T cell activation by PMA/ionomycin, but only when PBMC and not purified T cells were used [58].

These latter results suggested that accessory cells (B cells, monocytes) from PBMC were required for IVIg to exert its anti-inflammatory effect on T cells and support an indirect rather than direct effect of IVIg on T cells. It has also been suggested that IVIg modulated T cell functions by inducing soluble anti-inflammatory factors such as IL-1RA [61]. IL-1RA competes with IL-1 for the binding to the IL-1 receptor and thus deprives T cells of important activation signals [62].

In summary, although interesting data have emerged from studies using T cells activated with phorbol esters and ionomycin, the results obtained by various groups of investigators did not permit to clarify the exact mechanism by which IVIg affects T cell populations in IVIg-treated patients.

### **Studies on Lectin-Activated T Cells**

Lectins such as phytohemagglutinin (PHA), concanavalin A (ConA) and pokeweed mitogen (PWM) have also been used to study the effect of IVIg on T cell functions [63-72]. Lectins are sugar-binding proteins able to bind cell surface carbohydrates on T cells, leading to their aggregation and subsequent activation [73]. Several studies showed that IVIg inhibited T cell proliferation, CD25 expression and IFN- $\gamma$  secretion upon PBMC stimulation with PHA [64, 70, 72]. Similar results were obtained with purified T cells or T cell lines stimulated with PHA, suggesting that IVIg directly affected the functions of activated T cells [59, 69]. The inhibitory effect of IVIg on T cell was reported to be partially due to apoptosis induction [70], in contradiction with the results obtained with T cells activated with phorbol esters/ionomycin discussed above, in which T cell viability was not affected by IVIg [57, 59].

Overall, studies with T cells activated with phorbol esters/ionomycin and lectins did not permit to reach a consensus as to whether IVIg effects are a consequence of a direct or indirect action on these cells.

Recent data from our laboratory permitted to revisit the different conclusions from previous works using mitogen-activated T cells. Indeed, we showed that the putative inhibitory effect of IVIg on activated T cell functions was in fact the consequence of lectin neutralization by IVIg [74]. Lectins such as PHA are able to bind to immunoglobulins through the recognition of biantennary oligosaccharides found on the Fab region of some IgG [75-77].

Therefore, the experimental design used in the study of the effects of IVIg on lectin-stimulated T cells should have taken into account the possibility of mitogen neutralization, especially when IVIg is present at the same time as the lectin, which was the case in most of the above-mentioned studies. In our studies, we depleted IVIg preparations of PHA-reactive IgG and showed the absence of inhibitory effect of these depleted IVIg preparations when added together with PHA to T cell cultures. These results clearly demonstrated that the previously reported inhibitory effect of IVIg on lectin-activated T cells was the consequence of lectin neutralization rather than a direct effect of IVIg on T cells.

### **Studies on CD3/CD28-Activated T Cells**

In addition to studies in which T cells were activated with mitogens or lectins, a number of studies have been performed with T cells stimulated with

monoclonal anti-CD3 antibodies to induce T cell activation in a more physiologically relevant manner [78]. In these studies, anti-CD3 addition to PBMC cultures resulted in T cell proliferation. However, in the presence of IVIg, the functions of anti-CD3 activated T cells were reported to be significantly decreased [57, 58, 60]. Interestingly, it was shown that anti-CD3 activated T cells were affected by IVIg when accessory cells were present in the cultures. In contrast, addition of IVIg to purified T cells in the presence of anti-CD3 did not prevent T cell activation [58], suggesting that accessory cells (B cells and/or monocytes) were required for the inhibitory effect of IVIg. In fact, T cells need costimulatory signals provided by accessory cells (e.g., CD80) for optimal activation with anti-CD3 antibodies [79-82]. Binding of human IgG to Fc $\gamma$ R was shown to reduce the expression of CD80 on monocytes [83], suggesting that IVIg inhibited the anti-CD3-mediated T cell activation by decreasing costimulatory signals delivered by accessory cells to T cells [58].

In the light of the above observations on the role of accessory cells, the conclusions derived from studies reporting inhibitory effects of IVIg on activated purified T cells [59, 60] should be revisited. In fact, several studies showed that highly purified T cells cannot be fully activated with PMA/ionomycin or anti-CD3 in the absence of accessory cells [79, 84].

Therefore, one might consider that the above-mentioned studies with purified T cells were in fact done in the presence of a small amount of accessory cells, allowing T cell activation in the control conditions and IVIg-mediated inhibition in the experimental conditions. In fact, the early methods used to purify T cells included E-rosetting and nylon wool columns which do not yield a degree of purity as high as the more recent methods using positive selection with magnetic beads. Nevertheless, highly purified T cells can be activated in the absence of accessory cells using a combination of anti-CD3 and anti-CD28 [85]. Using this strategy, a group of investigators reported a partial but significant inhibition of highly purified T cell proliferation in the presence of IVIg [67], once again raising the possibility of a direct effect of IVIg on T cells. However, this possibility has been challenged recently in a study from our laboratory showing that IVIg can neutralize CD3/CD28 T cell expander beads, which are microbeads coated with anti-CD3 and anti-CD28 antibodies that are known to be very potent T cell activators. In fact, IVIg interfered with the binding of anti-CD3/CD28-coated microbeads to the T cells, thereby preventing activation [86]. Control experiments showed that prior incubation of purified T cells with IVIg did not affect the ability of CD3/CD28 beads to activate T cells and that IVIg did not affect the functions of T cells pre-activated with anti-CD3/CD28 beads [86].

In summary, the results derived from all the aforementioned *in vitro* studies must be interpreted with caution. A lesson can be learned from all these studies: experimental systems should be tightly controlled to rule out bystander effects or other possible interferences before making definitive conclusions on the mechanisms of action of IVIg on T cells.

## **Studies on CD4+ T Cell Subpopulations**

### *The Th1/Th2 Subsets*

CD4+ T cells can differentiate into various subpopulations of effector cells including T-helper 1 (Th1) and Th2 designed to eliminate different types of pathogens. Th1 and Th2 responses are elicited by professional APC and are characterized by their cytokine secretion profile [87-89]. The hallmark of a Th1 response is the production of interferon-gamma (IFN- $\gamma$ ) which activates the bactericidal activity of macrophages and induces the production of opsonizing and complement-fixing antibodies by B cells. The Th1 response is therefore important for the induction of cell-mediated immunity and plays a crucial role in the defence against intracellular pathogens [90]. On the other hand, the Th2 response is characterized by the secretion of IL-4, IL-5 and IL-13 and induces the production of neutralizing non-cytolytic antibodies by B cells, leading to humoral immunity. Th2 responses play a critical role in the defence against extracellular bacteria, parasites including helminths and toxins [91].

Early studies have associated the development of autoimmunity with a shift towards Th1 responses while a shift towards Th2 responses was believed to alleviate the disease [92, 93]. More recently this Th1/Th2 paradigm has been revisited since Th2 responses have also been implicated in autoimmune diseases, highlighting the complexity of immune response in autoimmunity [94].

Still, the Th1/Th2 concept continues to be accepted as part of the complex interactions observed during the development of autoimmune diseases. Therefore, restoration of the Th1/Th2 balance has been proposed as a therapeutic approach for the treatment of autoimmune disorders [95, 96].

Interestingly, several clinical studies have shown that IVIg modulated the Th1/Th2 ratio in favour of Th2 responses [8-10].

In autoimmune disorders, CD4+ T cells are activated following presentation of autoantigens by APC. Therefore, to study the mechanisms by which IVIg affects the CD4+ T cell response in a context relevant to autoimmunity,



we first used a mouse model of immunization using ovalbumin (OVA) as an antigen and evaluated the impact of IVIg infusion during the immunization period on the generation of OVA-specific T cells [97]. Using this strategy, we showed that IVIg significantly reduced the number of OVA-specific T cells produced in response of OVA immunization. We also showed that the *in vivo* activation of OVA-specific CD4<sup>+</sup> T cells from transgenic mice expressing a rearranged TCR transgene specific for the I-Ad-OVA<sub>323-339</sub> complex [98] was reduced in the presence of IVIg. These results thus confirmed the effect of IVIg on antigen-specific CD4<sup>+</sup> T cells, but did not permit to determine whether the effect of IVIg on these cells was direct or indirect. To address more directly this question, we used an *in vitro* OVA presentation assay and demonstrated that the presence of IVIg during the incubation of APC with OVA greatly affected the ability of APC to activate OVA-specific CD4<sup>+</sup> T cells. In contrast, OVA-loaded APC were able to efficiently activate OVA-specific CD4<sup>+</sup> T cells in the presence or absence of IVIg. These results thus suggested that the inhibitory effect of IVIg was dependent on APC and not directly on the CD4<sup>+</sup> T cells. Two mechanisms possibly involved in the inhibitory effect of IVIg on antigen presentation by APC were proposed. First, IVIg can compete with immune complexes of antigen for binding to FcγR, therefore reducing antigen internalization inside the APC. Second, IVIg can compete intracellularly with the internalized antigen for loading on MHC II molecules, thereby decreasing the amount of MHC II-peptide complexes presented on the surface of APC [99].

In summary, the above studies have revealed that IVIg does not directly affect the functions of CD4<sup>+</sup> T cells but acts mainly by decreasing the ability of APC to present antigens in an MHC II-restricted manner. In the context of an autoimmune disease, this effect of IVIg could result in a reduced presentation of self-antigens to autoreactive CD4<sup>+</sup> T cells and could thus contribute to the restore the Th1/Th2 ratio in IVIg-treated patients.

### ***The Th17 Subset***

Besides the classical Th1 and Th2 subsets, naive CD4<sup>+</sup> T cells can also differentiate into other subsets of effector cells such as Th9, Th17 and Th22 [100]. The Th17 subset recently attracted much interest owing to its involvement in the development of autoimmune and inflammatory disorders [101, 102]. Th17 cells are characterized by the secretion of IL-17A, IL-17F, IL-21 and IL-22 and by the expression of transcription factors distinct from Th1 and Th2. These pro-inflammatory cells play a major role in the immune response against extracellular bacteria and fungi [103-105].

In addition, memory Th17 cells are drivers of chronic inflammation and this property has been strongly associated with many human autoimmune and chronic inflammatory disorders, including psoriasis, Crohn's disease, rheumatoid arthritis, multiple sclerosis and uveitis [106-110].

The involvement of Th17 cells in autoimmune disorders has prompted an analysis of this cell population in IVIg-treated patients suffering from inflammatory myopathies. An imbalance ratio of Th1/Th17 was observed in these patients before IVIg treatment. In IVIg responders, the ratio of Th1/Th17 cells was lower than that observed in non-responders, suggesting a role for the Th17 mediated pathway in the anti-inflammatory response of IVIg [111]. The effect of IVIg on Th17 cells was also recently studied using a mouse model of EAE, an experimental model of multiple sclerosis [112].

In this model, the pathogenesis was improved by IVIg treatment and this effect was associated with a decreased number of Th17 cells in the draining lymph nodes and spleen. Based on their results in the mouse model of EAE, the authors proposed that the anti-inflammatory activity of IVIg could be mediated either by a direct interaction of IVIg with T cells or by modulating the functions of APCs. The same group of investigators also studied the effects of IVIg on Th17 cells in vitro, starting with CD4<sup>+</sup> T cells stimulated with monoclonal anti-CD3 and anti-CD28 antibodies in the presence of selected cytokines [113]. They showed that IVIg prevented the differentiation of CD4<sup>+</sup> T cells into Th17 cells [114] and proposed that this effect was related to the inhibition of phosphorylation of the signal transducer and activator of transcription 3 (Stat3), a transcription factor that plays a central role in the differentiation and stabilization of the Th17 lineage [115, 116]. It has been shown that CD3 ligation by monoclonal antibodies induces tyrosine and serine phosphorylation of Stat3 in human CD4<sup>+</sup> T cells [117]. Considering that anti-CD3/CD28 stimulation was used to trigger the activation of CD4<sup>+</sup> T cells prior to their differentiation into Th17 cells, a possible interference between IVIg and antibodies used for activation cannot be excluded to in part explain the inhibitory effect of IVIg on the differentiation of CD4<sup>+</sup> T cells, as previously discussed [74, 86].

Therefore, the elucidation of a direct or indirect effect of IVIg on Th17 cells will require further investigation in clinical studies with IVIg-treated patients, in animal models of diseases with a known Th17 component and also using in vitro systems with well-controlled experimental conditions.

### ***Regulatory T Cells***

Regulatory T cells (Tregs) have been originally described by Shimon Sakaguchi [118, 119] and were later characterized as CD4<sup>+</sup> CD25<sup>high</sup> Foxp3<sup>+</sup> T cells [120]. Most Tregs are formed during T cell maturation in the thymus, where some T cells specific for self-antigens are not eliminated through the process of negative selection but instead differentiate into natural Foxp3<sup>+</sup> Tregs. These cells were initially named natural Tregs or nTregs and represent about 5 % of the total CD4<sup>+</sup> T cells in circulation [121]. Recently, a panel of experts recommended that Tregs originating from the thymus should be named thymus-derived Tregs (tTregs) instead of nTregs [122].

tTregs are indispensable for the maintenance of homeostasis and peripheral tolerance and for the prevention of autoimmunity [123, 124]. In addition to tTregs, other Tregs have been described and arise from naive autoreactive T cells in the periphery under certain environmental conditions such as the composition of the cytokine milieu, the concentration of antigen and the nature of the antigen-presenting cells [125, 126]. The newly proposed nomenclature for these cells is peripherally-derived Treg (pTreg) instead of the previously used terms such as induced Tregs (iTregs) or adaptive Tregs (aTregs), and the word iTregs should now refer to in vitro-induced Tregs (iTregs) [122]. The importance of CD4<sup>+</sup> CD25<sup>high</sup> Foxp3<sup>+</sup> Tregs in the control of autoimmunity has been revealed following the identification of loss-of-function mutations in the Foxp3 gene leading to the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome [127-129].

Foxp3 is considered to be the master regulator of the Treg lineage. In the IPEX syndrome, the lack of Tregs is associated with a lethal auto-aggressive lymphoproliferation, highlighting the prime importance of Tregs for the prevention of autoimmunity.

Since that discovery, the development of a number of autoimmune diseases such as ITP [130, 131], SLE [132], Kawasaki disease [133], Guillain-Barré syndrome [134], type I diabetes [135, 136], multiple sclerosis [137, 138] has been associated with anomalies in Treg numbers or immunosuppressive functions.

Several clinical studies on IVIg-treated patients suffering from autoimmune disorders have revealed modulations in Treg populations.

For example, increased numbers of circulating Tregs have been reported in Kawasaki disease [133, 139], Guillain-Barré syndrome [134], eosinophilic granulomatosis [140] and autoimmune rheumatic disease [141] following IVIg infusion. The effect of IVIg on Tregs has also been studied in animal models of disease.

In the EAE mouse model, repeated injections of IVIg were shown to prevent the development of the disease. The protection was associated with increased numbers of tTregs in the blood, lymph nodes and spleen [142]. A further demonstration of the role of Tregs for the effects of IVIg in this model was the failure of IVIg to protect against EAE in mice that were depleted of Tregs. The effect of IVIg was also studied in a mouse model of herpes simplex virus-induced encephalitis, in which a single dose of IVIg was sufficient to increase the number of Tregs in peripheral lymphoid organs and protect mice against HSV infection.

The underlying mechanism for this effect was the induction by IVIg of a subset of IL-10-producing FoxP3<sup>-</sup> ICOS<sup>+</sup> CD4<sup>+</sup> T cells [143]. Another study revealed that IVIg ameliorated the OVA-induced airway hyperresponsiveness in mice by increasing pTreg numbers by a factor of 4 to 6 times [144].

In this latter model, the induction of OVA-specific pTregs was mediated by tolerogenic APC generated after IVIg exposure. This observation supports an indirect rather than direct effect of IVIg on this Treg population, in contrast with the observation done using the EAE model, in which a direct effect of IVIg on tTreg proliferation was reported [142].

In vitro studies have permitted to show a direct effect of IVIg on purified CD4<sup>+</sup> tTregs from healthy individuals, leading to their activation and increased expression of CD69, CD38, HLA-DR, IL-10, TGF- $\beta$  and FoxP3 [145, 146].

On the other hand, the generation of pTregs from naive T cells in the periphery seems to be rather an indirect consequence of the effect of IVIg on APC, leading to the generation of tolerogenic dendritic cells (DC) [144, 147, 148].

Recently, De Groot et al. described the occurrence of short sequences of amino acid (Tregitopes) present within the structure of the IgG molecule able to induce the expansion of CD4<sup>+</sup> Tregs [149]. The corresponding peptides (also termed Tregitopes) were synthesized and shown to induce tTreg and pTreg (adaptive or antigen-specific Treg) activation and expansion following binding to MHC II molecules of APC, both in vitro and in vivo [149-151]. Since IVIg has been shown to be internalized inside APC [99, 152, 153], it can be speculated that Tregitopes derived from internalized IVIg are loaded onto MHC II molecules and presented by APC in IVIg-treated patients, therefore providing an additional explanation for the expansion and stimulation of Tregs following IVIg therapy [154, 155].

## Studies on CD8+ T Cells

Many subpopulations of cytotoxic CD8+ T cells have been defined based on their cytokine secretion profile. Type 1 CD8+ T cells (Tc1) secrete IFN- $\gamma$ , whereas Tc2 secrete IL-4 and IL-5. Tc17 secrete IL-17 whereas CD8+ Tregs act through the secretion of both TGF- $\beta$  and IL-10 [156, 157]. Altogether, CD8+ T cells play a major role in immunity against viruses and cancer. These cells acquire their cytotoxic function upon recognition by their TCR of MHC I-peptide complexes expressed on target cells. Cytotoxic CD8+ T cells rapidly destroy target cells following release of lytic granules (perforin, granzyme) or by expressing factors capable of signaling through death receptors expressed on the surface of target cells (FAS pathway) [158-160]. However, dysregulation in CD8+ T cells tolerance to self-antigens directly contributes to the initiation, progression and regulation of several autoimmune diseases such as type I diabetes, multiple sclerosis and others [161].

Data from clinical studies with IVIg-treated patients have shown a reduction in CD8+ T cell numbers in Crohn's disease [162] as well as a normalization of the CD8+ T cell repertoire in CIDP patients following high-dose IVIg infusion [163]. To better understand the effect of IVIg on CD8+ T cells observed in these clinical studies, we used a mouse model of OVA immunization similar to that described for the work on CD4+ T cells in Aubin et al. [164]. IVIg was administered at different intervals during the immunization of C57/BL6 mice with OVA. In these experiments, the number of CD8+ OVA-specific T cells was significantly lower in IVIg-treated animals, as evaluated using SIINFEKL-specific MHC I tetramers, in agreement with the clinical data. To decipher the mechanisms of IVIg effect on the CD8+ T cell response, an in vitro activation system using antigen cross-presentation was set up, in which APC cross-presented OVA peptides to OVA-specific CD8+ T cells (OT-I) purified from the spleen of mice expressing a rearranged TCR transgene specific for the H2-Kb-OVA<sub>257-264</sub> complex [165]. This assay permitted to show that IVIg inhibited the activation of OVA-specific CD8+ T cells when the antigen was added in the form of an immune complex (OVA-IC) [166]. The mechanism responsible for this inhibition was similar to that observed for the inhibition of the CD4+ T cell response, namely a competition between IVIg and OVA-IC for the binding to Fc $\gamma$ R expressed on APC, resulting in a decreased presentation of MHC I-peptide (SIINFEKL) complexes and consequently, a reduction in OVA-specific CD8+ T cell activation. Interestingly, an additional mechanism was revealed in experiments done with OVA-preloaded APC incubated with OVA-

specific CD8+ T cells in the presence or absence of IVIg. In the presence of IVIg, a significant reduction in OVA-specific CD8+ T cell activation and proliferation was observed, in contrast to the absence of IVIg effect on CD4+ T cells using a similar experimental approach [99].

Given that the expression of key costimulatory molecules on APC can be reduced following IVIg treatment [167], we speculate that this reduction may have a more drastic consequence on CD8+ compared to CD4+ T cell activation. Alternatively, IVIg may act by preventing the interaction between costimulatory molecules important for CD8+ but not CD4+ T cell activation.

In parallel, experiments addressing the cytotoxic activity of OVA-specific CD8+ T cells on target cells were performed both *in vivo* and *in vitro*. OVA immunization of C57/BL6 mice in the presence of IVIg resulted in a decreased number of perforin- and CD107a (a cell surface marker associated with degranulation)-expressing CD8+ T cells in the spleen [164].

In addition, OVA-specific CD8+ T cells activated following cross-presentation of OVA were less cytotoxic for their target cells (EL-4 decorated with SIINFEKL peptides) when IVIg was present during the incubation between activated CD8+ T cells and EL-4 cells.

This latter effect was shown to be related to TCR blockade by IVIg, thereby reducing the interaction between effector and target cells [164].

Altogether, the results from our studies on CD8+ T cells revealed that the effect of IVIg on their activation is, at least in part, the consequence of antigen cross-presentation impairment. As discussed above, interference of IVIg with the antigen presentation process also prevents CD4+ T cell activation, supporting the concept of an indirect rather than direct effect of IVIg on T cells. Yet, our work on CD8+ T cells also revealed a direct effect of IVIg on CD8+ T cell activation that was not observed on CD4+ T cells. Similarly, we showed that IVIg directly affects CD8+ T cell cytotoxicity, possibly as a consequence of TCR blockade. These latter results suggest that direct effects of IVIg on T cells synergize with the effects on antigen presentation to produce immunosuppressive effects in treated patients.

## CONCLUSION

Altogether, the studies using *ex-vivo* experimental systems or animal models of disease suggest that the inhibitory effect of IVIg on T cells is mostly the indirect consequence of an effect of IVIg on APC (decreased self-antigen presentation, generation of tolerogenic dendritic cells).

Nevertheless, several studies showed that IVIg selectively modulates the functions of T cell subpopulations (inhibition of Th17 differentiation, decrease cytotoxicity of activated CD8+ T cells, induction of Tregs with strong immunosuppressive potential). Overall, the studies done during the past decades on the effects of IVIg on T cells have revealed a diversity of mechanisms by which immunosuppression can occur. However, much work remains to be done to fully understand and control the effectiveness of IVIg therapy in a variety of autoimmune and inflammatory disorders.

### ACKNOWLEDGMENTS

The authors wish to thank Dr. Lionel Loubaki for helpful advices and careful reading of the manuscript.

### REFERENCES

- [1] Berger, M. Subcutaneous IgG in neurologic diseases. *Immunotherapy* 2014,6:71-83.
- [2] Cakmak, S. K., Cakmak, A., Gonul, M., A. Klc, Gul, U. Intravenous immunoglobulin therapy in dermatology: an update. *Inflamm. Allergy Drug Targets* 2013,12:132-146.
- [3] Gelfand, E. W. Intravenous immune globulin in autoimmune and inflammatory diseases. *N. Engl. J. Med.* 2012,367:2015-2025.
- [4] Imbach, P. 30 years of immunomodulation by intravenous immunoglobulin. *Immunotherapy* 2012,4:651-654.
- [5] Imbach, P. Treatment of immune thrombocytopenia with intravenous immunoglobulin and insights for other diseases. A historical review. *Swiss Med. Wkly.* 2012,142:w13593.
- [6] Navarro, R. P., Ballow, M., Fenrick, B., Pezalla, E. J. Considerations for the optimal use of immunoglobulin. *Am. J. Manag. Care* 2012,18:S67-78.
- [7] Winkelmann, A., Zettl, U. K. Use of intravenous immunoglobulin in the treatment of immune-mediated demyelinating diseases of the nervous system. *Curr. Pharm. Des.* 2012,18:4570-4582.
- [8] Graphou, O., Chioti, A., Pantazi, A., Tsukoura, C., Kontopoulou, V., Guorgiadou, E., et al. Effect of intravenous immunoglobulin treatment

- on the Th1/Th2 balance in women with recurrent spontaneous abortions. *Am. J. Reprod. Immunol.* 2003,49:21-29.
- [9] Mouzaki, A., Theodoropoulou, M., Gianakopoulos, I., Vlaha, V., Kyrtsolis, M. C., Maniatis, A. Expression patterns of Th1 and Th2 cytokine genes in childhood idiopathic thrombocytopenic purpura (ITP) at presentation and their modulation by intravenous immunoglobulin G (IVIg) treatment: their role in prognosis. *Blood* 2002,100:1774-1779.
- [10] Yamada, H., Morikawa, M., Furuta, I., Kato, E. H., Shimada, S., Iwabuchi, K., et al. Intravenous immunoglobulin treatment in women with recurrent abortions: increased cytokine levels and reduced Th1/Th2 lymphocyte ratio in peripheral blood. *Am. J. Reprod. Immunol.* 2003,49: 84-89.
- [11] Bordet, J. C., Follea, G., Carosella, E., Dechavanne, M. Intravenous high-dose IgG therapy induced alterations of spleen lymphocyte IgM secretion and T cell subsets in patients with idiopathic thrombocytopenic purpura. *Thromb. Res.* 1987,47:165-174.
- [12] Dammacco, F., Iodice, G., Campobasso, N. Treatment of adult patients with idiopathic thrombocytopenic purpura with intravenous immunoglobulin: effects on circulating T cell subsets and PWM-induced antibody synthesis in vitro. *Br. J. Haematol.* 1986,62:125-135.
- [13] Kwak-Kim, J., Lee, S. K., Gilman-Sachs, A. Elevated Th1/Th2 cell ratios in a pregnant woman with a history of RSA, secondary Sjogren's syndrome and rheumatoid arthritis complicated with one fetal demise of twin pregnancy. *Am. J. Reprod. Immunol.* 2007,58:325-329.
- [14] Pogliani, E. M., Della Volpe, A., Casaroli, I., Maffe, P. F., Corneo, G. Lymphocyte subsets in patients with idiopathic thrombocytopenic purpura during high-dose gamma globulin therapy. *Allergol. Immunopathol. (Madr.)* 1991,19:113-116.
- [15] Tsubakio, T., Kurata, Y., Katagiri, S., Kanakura, Y., Tamaki, T., Kuyama, J., et al. Alteration of T cell subsets and immunoglobulin synthesis in vitro during high dose gamma-globulin therapy in patients with idiopathic thrombocytopenic purpura. *Clin. Exp. Immunol.* 1983,53: 697-702.
- [16] Klein, L., Hinterberger, M., Wirnsberger, G., Kyewski, B. Antigen presentation in the thymus for positive selection and central tolerance induction. *Nat. Rev. Immunol.* 2009,9:833-844.
- [17] Morris, G. P., Allen, P. M. How the TCR balances sensitivity and specificity for the recognition of self and pathogens. *Nat. Immunol.* 2012,13:121-128.



- 
- [18] Mouchess, M. L., Anderson, M. Central tolerance induction. *Curr. Top Microbiol. Immunol.* 2014,373:69-86.
- [19] Starr, T. K., Jameson, S. C., Hogquist, K. A. Positive and negative selection of T cells. *Annu. Rev. Immunol.* 2003,21:139-176.
- [20] Boyman, O., Sprent, J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat. Rev. Immunol.* 2012,12:180-190.
- [21] Brownlie, R. J., Zamoyska, R. T cell receptor signalling networks: branched, diversified and bounded. *Nat. Rev. Immunol.* 2013,13:257-269.
- [22] Curtsinger, J. M., Mescher, M. F. Inflammatory cytokines as a third signal for T cell activation. *Curr. Opin. Immunol.* 2010,22:333-340.
- [23] Hivroz, C., Chemin, K., Tourret, M., Bohineust, A. Crosstalk between T lymphocytes and dendritic cells. *Crit. Rev. Immunol.* 2012,32:139-155.
- [24] Leitner, J., Grabmeier-Pfistershammer, K., Steinberger, P. Receptors and ligands implicated in human T cell costimulatory processes. *Immunol. Lett.* 2010,128:89-97.
- [25] Appleman, L. J., Boussiotis, V. A. T cell anergy and costimulation. *Immunol. Rev.* 2003,192:161-180.
- [26] Baumann, S., Krueger, A., Kirchhoff, S., Krammer, P. H. Regulation of T cell apoptosis during the immune response. *Curr. Mol. Med.* 2002,2:257-272.
- [27] Saibil, S. D., Deenick, E. K., Ohashi, P. S. The sound of silence: modulating anergy in T lymphocytes. *Curr. Opin. Immunol.* 2007,19:658-664.
- [28] Gutcher, I., Becher, B. APC-derived cytokines and T cell polarization in autoimmune inflammation. *J. Clin. Invest.* 2007,117:1119-1127.
- [29] Atassi, M. Z., Casali, P. Molecular mechanisms of autoimmunity. *Autoimmunity* 2008,41:123-132.
- [30] Bogdanos, D. P., Smyk, D. S., Invernizzi, P., Rigopoulou, E. I., Blank, M., Sakkas, L., et al. Tracing environmental markers of autoimmunity: introducing the infectome. *Immunol. Res.* 2013,56:220-240.
- [31] Christen, U., von Herrath, M. G. Initiation of autoimmunity. *Curr. Opin. Immunol.* 2004,16:759-767.
- [32] Rose, A. M., Bell, L. C. Epistasis and immunity: the role of genetic interactions in autoimmune diseases. *Immunology* 2012,137:131-138.
- [33] Selmi, C., Lu, Q., Humble, M. C. Heritability versus the role of the environment in autoimmunity. *J. Autoimmun.* 2012,39:249-252.

- 
- [34] Tiniakou, E., Costenbader, K. H., Kriegel, M. A. Sex-specific environmental influences on the development of autoimmune diseases. *Clin. Immunol.* 2013,149:182-191.
- [35] Romo-Tena, J., Gomez-Martin, D., Alcocer-Varela, J. CTLA-4 and autoimmunity: new insights into the dual regulator of tolerance. *Autoimmun. Rev.* 2013,12:1171-1176.
- [36] Bottini, N., Peterson, E. J. Tyrosine Phosphatase PTPN22: Multifunctional Regulator of Immune Signaling, Development, and Disease. *Annu. Rev. Immunol.* 2013.
- [37] Li, Z., Ju, Z., Frieri, M. The T-cell immunoglobulin and mucin domain (Tim) gene family in asthma, allergy, and autoimmunity. *Allergy Asthma Proc.* 2013,34:e21-26.
- [38] Arstila, T. P., Jarva, H. Human APECED; a Sick Thymus Syndrome? *Front Immunol.* 2013,4:313.
- [39] Trowsdale, J., Knight, J. C. Major histocompatibility complex genomics and human disease. *Annu. Rev. Genomics Hum. Genet.* 2013,14:301-323.
- [40] Tsai, S., Santamaria, P. MHC Class II Polymorphisms, Autoreactive T-Cells, and Autoimmunity. *Front Immunol.* 2013,4:321.
- [41] Dedeoglu, F. Drug-induced autoimmunity. *Curr. Opin. Rheumatol.* 2009,21:547-551.
- [42] Farhat, S. C., Silva, C. A., Orione, M. A., Campos, L. M., Sallum, A. M., Braga, A. L. Air pollution in autoimmune rheumatic diseases: a review. *Autoimmun. Rev.* 2011,11:14-21.
- [43] Manzel, A., Muller, D. N., Hafler, D. A., Erdman, S. E., Linker, R. A., Kleinewietfeld, M. Role of "Western diet" in inflammatory autoimmune diseases. *Curr. Allergy Asthma Rep.* 2014,14:404.
- [44] Imboden, J. B. The immunopathogenesis of rheumatoid arthritis. *Annu. Rev. Pathol.* 2009,4:417-434.
- [45] La Torre, D. Immunobiology of beta-cell destruction. *Adv. Exp. Med. Biol.* 2012,771:194-218.
- [46] McMillan, R. The pathogenesis of chronic immune thrombocytopenic purpura. *Semin. Hematol.* 2007,44:S3-S11.
- [47] Reindl, M., Di Pauli, F., Rostasy, K., Berger, T. The spectrum of MOG autoantibody-associated demyelinating diseases. *Nat. Rev. Neurol.* 2013, 9:455-461.
- [48] Bulek, A. M., Cole, D. K., Skowera, A., Dolton, G., Gras, S., Madura, F., et al. Structural basis for the killing of human beta cells by CD8(+) T cells in type 1 diabetes. *Nat. Immunol.* 2012,13:283-289.

- 
- [49] Deckert, M., Sanchez-Ruiz, M., Brunn, A., Schluter, D. Role of CD8 T-cell-mediated autoimmune diseases of the central nervous system. *Crit. Rev. Immunol.* 2010,30:311-326.
- [50] Friese, M. A., Fugger, L. Pathogenic CD8(+) T cells in multiple sclerosis. *Ann. Neurol.* 2009,66:132-141.
- [51] Schneider-Hohendorf, T., Schwab, N., Uceyler, N., Gobel, K., Sommer, C., Wiendl, H. CD8+ T-cell immunity in chronic inflammatory demyelinating polyradiculoneuropathy. *Neurology* 2012,78:402-408.
- [52] Poh, M. Q., Simon, N. G., Buckland, M. E., Salisbury, E., Watson, S. Evidence of T-cell mediated neuronal injury in stiff-person syndrome with anti-amphiphysin antibodies. *J. Neurol. Sci.* 2013.
- [53] Takahashi, A., Takeda, I., Kanno, T., Nakahara, T., Odajima, H., Kasukawa, R. CD8-positive T cell-induced liver damage was found in a patient with polymyositis. *Intern. Med.* 2006,45:1059-1063.
- [54] Kashiwagi, H., Tomiyama, Y. Pathophysiology and management of primary immune thrombocytopenia. *Int. J. Hematol.* 2013,98:24-33.
- [55] Chopra, R. K., Nagel, J. E., Chrest, F. J., Boto, W. M., Pyle, R. S., Dorsey, B., et al. Regulation of interleukin 2 and interleukin 2 receptor gene expression in human T cells: I. Effect of Ca<sup>2+</sup>-ionophore on phorbol myristate acetate co-stimulated cells. *Clin. Exp. Immunol.* 1987, 69: 433-440.
- [56] Zlotnik, A., Daine, B. Activation of IL 1-dependent and IL 1-independent T cell lines by calcium ionophore and phorbol ester. *J. Immunol.* 1986,136:1033-1037.
- [57] Andersson, U. G., Bjork, L., Skansen-Saphir, U., Andersson, J. P. Down-regulation of cytokine production and interleukin-2 receptor expression by pooled human IgG. *Immunology* 1993,79:211-216.
- [58] Skansen-Saphir, U., Andersson, J., Bjork, L., Ekberg, C., Fehniger, T. E., Henter, J. I., et al. Down-regulation of lymphokine synthesis by intravenous gammaglobulin is dependent upon accessory cells. *Scand. J. Immunol.* 1998,47:229-235.
- [59] Modiano, J. F., Amran, D., Lack, G., Bradley, K., Ball, C., Domenico, J., et al. Posttranscriptional regulation of T-cell IL-2 production by human pooled immunoglobulin. *Clin. Immunol. Immunopathol.* 1997,83: 77-85.
- [60] Amran, D., Renz, H., Lack, G., Bradley, K., Gelfand, E. W. Suppression of cytokine-dependent human T-cell proliferation by intravenous immunoglobulin. *Clin. Immunol. Immunopathol.* 1994,73:180-186.

- [61] Arend, W. P., Leung, D. Y. IgG induction of IL-1 receptor antagonist production by human monocytes. *Immunol. Rev.* 1994,139:71-78.
- [62] Arend, W. P., Welgus, H. G., Thompson, R. C., Eisenberg, S. P. Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. *J. Clin. Invest.* 1990,85:1694-1697.
- [63] Heidt, S., Roelen, D. L., Eijnsink, C., Eikmans, M., Claas, F. H., Mulder, A. Intravenous immunoglobulin preparations have no direct effect on B cell proliferation and immunoglobulin production. *Clin. Exp. Immunol.* 2009,158:99-105.
- [64] Kawada, K., Terasaki, P. I. Evidence for immunosuppression by high-dose gammaglobulin. *Exp. Hematol.* 1987,15:133-136.
- [65] Klaesson, S., Ringden, O., Markling, L., Remberger, M., Lundkvist, I. Immune modulatory effects of immunoglobulins on cell-mediated immune responses in vitro. *Scand. J. Immunol.* 1993,38:477-484.
- [66] Kondo, N., Ozawa, T., Mushiake, K., Motoyoshi, F., Kameyama, T., Kasahara, K., et al. Suppression of immunoglobulin production of lymphocytes by intravenous immunoglobulin. *J. Clin. Immunol.* 1991, 11:152-158.
- [67] MacMillan, H. F., Lee, T., Issekutz, A. C. Intravenous immunoglobulin G-mediated inhibition of T-cell proliferation reflects an endogenous mechanism by which IgG modulates T-cell activation. *Clin. Immunol.* 2009,132:222-233.
- [68] Stohl, W. Modulation of the immune response by immunoglobulin for intravenous use. I. Inhibition of pokeweed mitogen-induced B cell differentiation. *Clin. Exp. Immunol.* 1985,62:200-207.
- [69] Tawfik, D. S., Cowan, K. R., Walsh, A. M., Hamilton, W. S., Goldman, F. D. Exogenous immunoglobulin downregulates T-cell receptor signaling and cytokine production. *Pediatr. Allergy Immunol.* 2012,23: 88-95.
- [70] Tha-In, T., Metselaar, H. J., Tilanus, H. W., Boor, P. P., Mancham, S., Kuipers, E. J., et al. Superior immunomodulatory effects of intravenous immunoglobulins on human T-cells and dendritic cells: comparison to calcineurin inhibitors. *Transplantation* 2006,81:1725-1734.
- [71] Toyoda, M., Zhang, X., Petrosian, A., Galera, O. A., Wang, S. J., Jordan, S. C. Modulation of immunoglobulin production and cytokine mRNA expression in peripheral blood mononuclear cells by intravenous immunoglobulin. *J. Clin. Immunol.* 1994,14:178-189.

- 
- [72] Van Schaik, I. N., Lundkvist, I., Vermeulen, M., Brand, A. Polyvalent immunoglobulin for intravenous use interferes with cell proliferation in vitro. *J. Clin. Immunol.* 1992,12:325-334.
- [73] Kay, J. E. Mechanisms of T lymphocyte activation. *Immunol. Lett.* 1991, 29:51-54.
- [74] Padet, L., St-Amour, I., Aubin, E., Bazin, R. Neutralization of mitogenic lectins by intravenous immunoglobulin (IVIg) prevents T cell activation: does IVIg really have a direct effect on T cells? *Clin. Exp. Immunol.* 2011,166:352-360.
- [75] Green, E. D., Baenziger, J. U. Oligosaccharide specificities of Phaseolus vulgaris leucoagglutinating and erythroagglutinating phytohemagglutinins. Interactions with N-glycanase-released oligosaccharides. *J. Biol. Chem.* 1987,262:12018-12029.
- [76] Holland, M., Yagi, H., Takahashi, N., Kato, K., Savage, C. O., Goodall, D. M., et al. Differential glycosylation of polyclonal IgG, IgG-Fc and IgG-Fab isolated from the sera of patients with ANCA-associated systemic vasculitis. *Biochim. Biophys. Acta* 2006,1760:669-677.
- [77] Jefferis, R. Glycosylation of recombinant antibody therapeutics. *Biotechnol. Prog.* 2005,21:11-16.
- [78] Dixon, J. F., Law, J. L., Favero, J. J. Activation of human T lymphocytes by crosslinking of anti-CD3 monoclonal antibodies. *J. Leukoc. Biol.* 1989,46:214-220.
- [79] Davis, L., Lipsky, P. E. Signals involved in T cell activation. II. Distinct roles of intact accessory cells, phorbol esters, and interleukin 1 in activation and cell cycle progression of resting T lymphocytes. *J. Immunol.* 1986,136:3588-3596.
- [80] Umetsu, D. T., Katzen, D., Chatila, T., Miller, R., Jabara, H. H., Maher, M., et al. Requirements for activation of human peripheral blood T cells by mouse monoclonal antibodies to CD3. *Clin. Immunol. Immunopathol.* 1987,43:48-64.
- [81] Verwilghen, J., Baroja, M. L., Van Vaeck, F., Van Damme, J., Ceuppens, J. L. Differences in the stimulating capacity of immobilized anti-CD3 monoclonal antibodies: variable dependence on interleukin-1 as a helper signal for T-cell activation. *Immunology* 1991,72:269-276.
- [82] Williams, J. M., Deloria, D., Hansen, J. A., Dinarello, C. A., Loertscher, R., Shapiro, H. M., et al. The events of primary T cell activation can be staged by use of Sepharose-bound anti-T3 (64.1) monoclonal antibody and purified interleukin 1. *J. Immunol.* 1985,135:2249-2255.

- 
- [83] Barcy, S., Wettendorff, M., Leo, O., Urbain, J., Kruger, M., Ceuppens, J. L., et al. FcR cross-linking on monocytes results in impaired T cell stimulatory capacity. *Int. Immunol.* 1995,7:179-189.
- [84] Schwartz, R. H. A cell culture model for T lymphocyte clonal anergy. *Science* 1990,248:1349-1356.
- [85] Trickett, A., Kwan, Y. L. T cell stimulation and expansion using anti-CD3/CD28 beads. *J. Immunol. Methods* 2003,275:251-255.
- [86] Padet, L., Bazin, R. IVIg prevents the in vitro activation of T cells by neutralizing the T cell activators. *Immunol. Lett.* 2013,150:54-60.
- [87] Luckheeram, R. V., Zhou, R., Verma, A. D., Xia, B. CD4(+)T cells: differentiation and functions. *Clin. Dev. Immunol.* 2012,2012:925135.
- [88] Yamane, H., Paul, W. E. Early signaling events that underlie fate decisions of naive CD4(+) T cells toward distinct T-helper cell subsets. *Immunol. Rev.* 2013,252:12-23.
- [89] Mosmann, T. R., Cherwinski, H., Bond, M. W., Giedlin, M. A., Coffman, R. L. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 1986,136:2348-2357.
- [90] Cope, A., Le Friec, G., Cardone, J., Kemper, C. The Th1 life cycle: molecular control of IFN-gamma to IL-10 switching. *Trends Immunol.* 2011,32:278-286.
- [91] Li, Z., Zhang, Y., Sun, B. Current understanding of Th2 cell differentiation and function. *Protein Cell* 2011,2:604-611.
- [92] Lafaille, J. J. The role of helper T cell subsets in autoimmune diseases. *Cytokine Growth Factor Rev.* 1998,9:139-151.
- [93] Liblau, R. S., Singer, S. M., McDevitt, H. O. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol. Today* 1995,16:34-38.
- [94] Crane, I. J., Forrester, J. V. Th1 and Th2 lymphocytes in autoimmune disease. *Crit. Rev. Immunol.* 2005,25:75-102.
- [95] Adorini, L., Guery, J. C., Trembleau, S. Manipulation of the Th1/Th2 cell balance: an approach to treat human autoimmune diseases? *Autoimmunity* 1996,23:53-68.
- [96] Kidd, P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern. Med. Rev.* 2003,8:223-246.
- [97] Aubin, E., Lemieux, R., Bazin, R. Indirect inhibition of in vivo and in vitro T-cell responses by intravenous immunoglobulins due to impaired antigen presentation. *Blood* 2010,115:1727-1734.

- 
- [98] Shimonkevitz, R., Colon, S., Kappler, J. W., Marrack, P., Grey, H. M. Antigen recognition by H-2-restricted T cells. II. A tryptic ovalbumin peptide that substitutes for processed antigen. *J. Immunol.* 1984,133:2067-2074.
- [99] Aubin, E., Proulx, D. P., Trepanier, P., Lemieux, R., Bazin, R. Prevention of T cell activation by interference of internalized intravenous immunoglobulin (IVIg) with MHC II-dependent native antigen presentation. *Clin. Immunol.* 2011,141:273-283.
- [100] Liu, Z., Fan, H., Jiang, S. CD4(+) T-cell subsets in transplantation. *Immunol. Rev.* 2013,252:183-191.
- [101] McGeachy, M. J. Th17 memory cells: live long and proliferate. *J. Leukoc. Biol.* 2013,94:921-926.
- [102] Kurebayashi, Y., Nagai, S., Ikejiri, A., Koyasu, S. Recent advances in understanding the molecular mechanisms of the development and function of Th17 cells. *Genes Cells* 2013,18:247-265.
- [103] Kaufmann, S. H., Kuchroo, V. K. Th17 cells. *Microbes Infect.* 2009,11:579-583.
- [104] Korn, T., Bettelli, E., Oukka, M., Kuchroo, V. K. IL-17 and Th17 Cells. *Annu. Rev. Immunol.* 2009,27:485-517.
- [105] Miossec, P., Korn, T., Kuchroo, V. K. Interleukin-17 and type 17 helper T cells. *N. Engl. J. Med.* 2009,361:888-898.
- [106] Annunziato, F., Cosmi, L., Liotta, F., Maggi, E., Romagnani, S. Type 17 T helper cells-origins, features and possible roles in rheumatic disease. *Nat. Rev. Rheumatol.* 2009,5:325-331.
- [107] Larsen, J. M., Bonefeld, C. M., Poulsen, S. S., Geisler, C., Skov, L. IL-23 and T(H)17-mediated inflammation in human allergic contact dermatitis. *J. Allergy Clin. Immunol.* 2009,123:486-492.
- [108] Louten, J., Boniface, K., de Waal Malefyt, R. Development and function of TH17 cells in health and disease. *J. Allergy Clin. Immunol.* 2009,123:1004-1011.
- [109] Maddur, M. S., Miossec, P., Kaveri, S. V., Bayry, J. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. *Am. J. Pathol.* 2012,181:8-18.
- [110] Van Beelen, A. J., Teunissen, M. B., Kapsenberg, M. L., de Jong, E. C. Interleukin-17 in inflammatory skin disorders. *Curr. Opin. Allergy Clin. Immunol.* 2007,7:374-381.
- [111] Tournadre, A., Porcherot, M., Cherin, P., Marie, I., Hachulla, E., Miossec, P. Th1 and Th17 balance in inflammatory myopathies: inter-

- action with dendritic cells and possible link with response to high-dose immunoglobulins. *Cytokine* 2009,46:297-301.
- [112] Othy, S., Hegde, P., Topcu, S., Sharma, M., Maddur, M. S., Lacroix-Desmazes, S., et al. Intravenous gammaglobulin inhibits encephalitogenic potential of pathogenic T cells and interferes with their trafficking to the central nervous system, implicating sphingosine-1 phosphate receptor 1-mammalian target of rapamycin axis. *J. Immunol.* 2013,190:4535-4541.
- [113] Maddur, M. S., Vani, J., Hegde, P., Lacroix-Desmazes, S., Kaveri, S. V., Bayry, J. Inhibition of differentiation, amplification, and function of human TH17 cells by intravenous immunoglobulin. *J. Allergy Clin. Immunol.* 2011,127:823-830 e821-827.
- [114] Maddur, M. S., Kaveri, S. V., Bayry, J. Comparison of different IVIg preparations on IL-17 production by human Th17 cells. *Autoimmun. Rev.* 2011,10:809-810.
- [115] Harris, T. J., Grosso, J. F., Yen, H. R., Xin, H., Kortylewski, M., Albesiano, E., et al. Cutting edge: An in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. *J. Immunol.* 2007,179:4313-4317.
- [116] Ma, C. S., Chew, G. Y., Simpson, N., Priyadarshi, A., Wong, M., Grimbacher, B., et al. Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. *J. Exp. Med.* 2008,205:1551-1557.
- [117] Gerwien, J., Nielsen, M., Labuda, T., Nissen, M. H., Svejgaard, A., Geisler, C., et al. Cutting edge: TCR stimulation by antibody and bacterial superantigen induces Stat3 activation in human T cells. *J. Immunol.* 1999,163:1742-1745.
- [118] Sakaguchi, S., Fukuma, K., Kuribayashi, K., Masuda, T. 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.* 1985,161:72-87.
- [119] Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., Toda, M. 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.* 1995, 155:1151-1164.
- [120] Hori, S., Nomura, T., Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003,299:1057-1061.



- [121] Allan, S. E., Alstad, A. N., Merindol, N., Crellin, N. K., Amendola, M., Bacchetta, R., et al. Generation of potent and stable human CD4<sup>+</sup> T regulatory cells by activation-independent expression of FOXP3. *Mol. Ther.* 2008,16:194-202.
- [122] Abbas, A. K., Benoist, C., Bluestone, J. A., Campbell, D. J., Ghosh, S., Hori, S., et al. Regulatory T cells: recommendations to simplify the nomenclature. *Nat. Immunol.* 2013,14:307-308.
- [123] Ohkura, N., Kitagawa, Y., Sakaguchi, S. Development and maintenance of regulatory T cells. *Immunity* 2013,38:414-423.
- [124] Wan, Y. Y. Regulatory T cells: immune suppression and beyond. *Cell Mol. Immunol.* 2010,7:204-210.
- [125] Bluestone, J. A., Abbas, A. K. Natural versus adaptive regulatory T cells. *Nat. Rev. Immunol.* 2003,3:253-257.
- [126] Lohr, J., Knoechel, B., Abbas, A. K. Regulatory T cells in the periphery. *Immunol. Rev.* 2006,212:149-162.
- [127] Bennett, C. L., Christie, J., Ramsdell, F., Brunkow, M. E., Ferguson, P. J., Whitesell, L., et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* 2001,27:20-21.
- [128] Gambineri, E., Torgerson, T. R., Ochs, H. D. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr. Opin. Rheumatol.* 2003, 15:430-435.
- [129] Wildin, R. S., Ramsdell, F., Peake, J., Faravelli, F., Casanova, J. L., Buist, N., et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* 2001,27:18-20.
- [130] Liu, B., Zhao, H., Poon, M. C., Han, Z., Gu, D., Xu, M., et al. Abnormality of CD4(+)CD25(+) regulatory T cells in idiopathic thrombocytopenic purpura. *Eur. J. Haematol.* 2007,78:139-143.
- [131] Sakakura, M., Wada, H., Tawara, I., Nobori, T., Sugiyama, T., Sagawa, N., et al. Reduced Cd4+Cd25<sup>+</sup> T cells in patients with idiopathic thrombocytopenic purpura. *Thromb. Res.* 2007,120:187-193.
- [132] Liu, M. F., Wang, C. R., Fung, L. L., Wu, C. R. Decreased CD4+CD25<sup>+</sup> T cells in peripheral blood of patients with systemic lupus erythematosus. *Scand. J. Immunol.* 2004,59:198-202.

- [133] Furuno, K., Yuge, T., Kusuhara, K., Takada, H., Nishio, H., Khajoe, V., et al. CD25+CD4+ regulatory T cells in patients with Kawasaki disease. *J. Pediatr.* 2004,145:385-390.
- [134] Chi, L. J., Wang, H. B., Zhang, Y., Wang, W. Z. Abnormality of circulating CD4(+)CD25(+) regulatory T cell in patients with Guillain-Barre syndrome. *J. Neuroimmunol.* 2007,192:206-214.
- [135] Brusko, T. M., Wasserfall, C. H., Clare-Salzler, M. J., Schatz, D. A., Atkinson, M. A. Functional defects and the influence of age on the frequency of CD4+ CD25+ T-cells in type 1 diabetes. *Diabetes* 2005, 54: 1407-1414.
- [136] Lindley, S., Dayan, C. M., Bishop, A., Roep, B. O., Peakman, M., Tree, T. I. Defective suppressor function in CD4(+)CD25(+) T-cells from patients with type 1 diabetes. *Diabetes* 2005,54:92-99.
- [137] Haas, J., Hug, A., Viehover, A., Fritzsching, B., Falk, C. S., Filser, A., et al. Reduced suppressive effect of CD4+CD25high regulatory T cells on the T cell immune response against myelin oligodendrocyte glycoprotein in patients with multiple sclerosis. *Eur. J. Immunol.* 2005,35:3343-3352.
- [138] Viglietta, V., Baecher-Allan, C., Weiner, H. L., Hafler, D. A. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* 2004,199:971-979.
- [139] Olivito, B., Taddio, A., Simonini, G., Massai, C., Ciullini, S., Gambineri, E., et al. Defective FOXP3 expression in patients with acute Kawasaki disease and restoration by intravenous immunoglobulin therapy. *Clin. Exp. Rheumatol.* 2010,28:93-97.
- [140] Tsurikisawa, N., Saito, H., Oshikata, C., Tsuburai, T., Akiyama, K. High-dose intravenous immunoglobulin treatment increases regulatory T cells in patients with eosinophilic granulomatosis with polyangiitis. *J. Rheumatol.* 2012,39:1019-1025.
- [141] Bayry, J., Mouthon, L., Kaveri, S. V. Intravenous immunoglobulin expands regulatory T cells in autoimmune rheumatic disease. *J. Rheumatol.* 2012,39:450-451.
- [142] Ephrem, A., Chamat, S., Miquel, C., Fisson, S., Mouthon, L., Caligiuri, G., et al. Expansion of CD4+CD25+ regulatory T cells by intravenous immunoglobulin: a critical factor in controlling experimental autoimmune encephalomyelitis. *Blood* 2008,111:715-722.
- [143] Ramakrishna, C., Newo, A. N., Shen, Y. W., Cantin, E. Passively administered pooled human immunoglobulins exert IL-10 dependent anti-inflammatory effects that protect against fatal HSV encephalitis. *PLoS Pathog.* 2011,7:e1002071.

- [144] Massoud, A. H., Guay, J., Shalaby, K. H., Bjur, E., Ablona, A., Chan, D., et al. Intravenous immunoglobulin attenuates airway inflammation through induction of forkhead box protein 3-positive regulatory T cells. *J. Allergy Clin. Immunol.* 2012,129:1656-1665 e1653.
- [145] Kessel, A., Ammuri, H., Peri, R., Pavlotzky, E. R., Blank, M., Shoenfeld, Y., et al. Intravenous immunoglobulin therapy affects T regulatory cells by increasing their suppressive function. *J. Immunol.* 2007,179:5571-5575.
- [146] Tha-In, T., Metselaar, H. J., Bushell, A. R., Kwekkeboom, J., Wood, K. J. Intravenous immunoglobulins promote skin allograft acceptance by triggering functional activation of CD4+Foxp3+ T cells. *Transplantation* 2010,89:1446-1455.
- [147] Massoud, A. H., Yona, M., Xue, D., Chouiali, F., Alturaihi, H., Ablona, A., et al. Dendritic cell immunoreceptor: A novel receptor for intravenous immunoglobulin mediates induction of regulatory T cells. *J. Allergy Clin. Immunol.* 2013.
- [148] Trinath, J., Hegde, P., Sharma, M., Maddur, M. S., Rabin, M., Vallat, J. M., et al. Intravenous immunoglobulin expands regulatory T cells via induction of cyclooxygenase-2-dependent prostaglandin E2 in human dendritic cells. *Blood* 2013,122:1419-1427.
- [149] De Groot, A. S., Moise, L., McMurry, J. A., Wambre, E., Van Overtvelt, L., Moingeon, P., et al. Activation of natural regulatory T cells by IgG Fc-derived peptide "Tregitopes". *Blood* 2008,112:3303-3311.
- [150] Cousens, L. P., Su, Y., McClaine, E., Li, X., Terry, F., Smith, R., et al. Application of IgG-derived natural Treg epitopes (IgG Tregitopes) to antigen-specific tolerance induction in a murine model of type 1 diabetes. *J. Diabetes Res.* 2013,2013:621693.
- [151] Van der Marel, S., Majowicz, A., Kwkkers, K., van Logtenstein, R., te Velde, A. A., De Groot, A. S., et al. Adeno-associated virus mediated delivery of Tregitope 167 ameliorates experimental colitis. *World J. Gastroenterol.* 2012,18:4288-4299.
- [152] Paquin Proulx, D., Aubin, E., Lemieux, R., Bazin, R. Inhibition of B cell-mediated antigen presentation by intravenous immunoglobulins (IVIg). *Clin. Immunol.* 2010,135:422-429.
- [153] Proulx, D. P., Aubin, E., Lemieux, R., Bazin, R. Spontaneous internalization of IVIg in activated B cells. *Immunol. Lett.* 2009,124:18-26.
- [154] Cousens, L. P., Tassone, R., Mazer, B. D., Ramachandiran, V., Scott, D. W., De Groot, A. S. Tregitope update: mechanism of action parallels IVIg. *Autoimmun. Rev.* 2013,12:436-443.

- [155] Elyaman, W., Khoury, S. J., Scott, D. W., De Groot, A. S. Potential application of tregitopes as immunomodulating agents in multiple sclerosis. *Neurol. Res. Int.* 2011,2011:256460.
- [156] Yen, H. R., Harris, T. J., Wada, S., Grosso, J. F., Getnet, D., Goldberg, M. V., et al. Tc17 CD8 T cells: functional plasticity and subset diversity. *J. Immunol.* 2009,183:7161-7168.
- [157] Wang, Y. M., Alexander, S. I. CD8 regulatory T cells: what's old is now new. *Immunol. Cell Biol.* 2009,87:192-193.
- [158] Anikeeva, N., Sykulev, Y. Mechanisms controlling granule-mediated cytolytic activity of cytotoxic T lymphocytes. *Immunol. Res.* 2011,51:183-194.
- [159] Lieberman, J. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. *Nat. Rev. Immunol.* 2003,3:361-370.
- [160] Mosmann, T. R., Li, L., Sad, S. Functions of CD8 T-cell subsets secreting different cytokine patterns. *Semin. Immunol.* 1997,9:87-92.
- [161] Walter, U., Santamaria, P. CD8+ T cells in autoimmunity. *Curr. Opin. Immunol.* 2005,17:624-631.
- [162] Korber, J., Kottgen, E., Renz, H. A case of Crohn's disease with increased CD8 T-cell activation and remission during therapy with intravenous immunoglobulins. *Scand. J. Gastroenterol.* 1998,33:1113-1117.
- [163] Mausberg, A. K., Dorok, M., Stettner, M., Muller, M., Hartung, H. P., Dehmel, T., et al. Recovery of the T-cell repertoire in CIDP by IV immunoglobulins. *Neurology* 2013,80:296-303.
- [164] Trepanier, P., Chabot, D., Bazin, R. Intravenous immunoglobulin modulates the expansion and cytotoxicity of CD8(+) T cells. *Immunology* 2014,141:233-241.
- [165] Hogquist, K. A., Jameson, S. C., Heath, W. R., Howard, J. L., Bevan, M. J., Carbone, F. R. T cell receptor antagonist peptides induce positive selection. *Cell* 1994,76:17-27.
- [166] Trepanier, P., Bazin, R. Intravenous immunoglobulin (IVIg) inhibits CD8 cytotoxic T-cell activation. *Blood* 2012,120:2769-2770.
- [167] Misra, N., Bayary, J., Dasgupta, S., Ephrem, A., Huyen, J. P., Delignat, S., et al. Intravenous immunoglobulin and dendritic cells. *Clin. Rev. Allergy Immunol.* 2005,29:201-205.