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Cutaneous T cell lymphoma and graft-versus-host disease: A comparison of *in vivo* effects of extracorporeal photochemotherapy on Foxp3+ regulatory T cells ☆

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Abstract Extracorporeal photochemotherapy (ECP) is a well established treatment for both cutaneous T cell lymphoma (CTCL) and graft-versus-host disease (GVHD). However, the general effector mechanism is not fully settled. Twenty-four patients with CTCL and 14 patients with GVHD were included to assess the relative numbers of regulatory T cells (Treg) and any change in the serum cytokine profile during 6 months of ECP therapy. The relative amount of Treg cells was twice as high in CTCL compared to GVHD and healthy controls. TGF-β was on average three times higher in GVHD than in CTCL. Both patient groups had a small but significant increase in TGF-β after treatment. Our results indicate a strengthened Treg function as a result of ECP. Elevated TGF-β may indicate high Treg activation in GVHD, whereas an increased number of Treg cells in CTCL could be interpreted as a response that is involved in down-regulating the lymphoma cells.

Introduction

Extracorporeal photochemotherapy (ECP), or photopheresis, was introduced by Edelson et al. in 1987 [1] as a treatment for cutaneous T cell lymphomas (CTCL). Encouraged by the promising results in CTCL, ECP has later been applied to a variety of T cell mediated diseases, such as graft-versus-host disease (GVHD) [2], preventing graft rejection in heart and renal transplantation [3–5], as well as in other autoimmune diseases [6,7]. The ECP procedure is based on leucapheresis, followed by extracorporeal treatment of the leucocytes with 8-methoxypsoralen (8-MOP) and illumination with ultraviolet light A (UVA) before being reinfused to the patient. The therapeutic effect of ECP is supposed to be explained by 8-MOP

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binding covalently to DNA in circulating lymphocytes when illuminated with UVA. This ultimately leads to cell proliferation arrest and induction of apoptosis in the treated cells [8,9].

GVHD is a condition that evolves when allogeneic immunocompetent cells are introduced into an immunoincompetent host, and was first described by Billingham in 1959 [10]. The main occurrence is after haematopoietic cell transplantation, but GVHD is also seen after solid organ transplantations and blood transfusions. The pathophysiology of GVHD is T cell recognition of target tissues as being foreign with a subsequent induction of an inflammatory and cytolytic response leading to injury. Regulatory T cells (Treg) have been reported to protect against chronic cutaneous GVHD [11]. Immunomodulation by phototherapeutic agents has been proven beneficial for some patients, especially with chronic GVHD in the skin. PUVA is most widely used, but success has also been reported with ECP [12,13]. The effect is thought to be mediated through triggering of certain tolerance mechanisms such as inhibition of pro-inflammatory cytokines, stimulation of Treg cells and production of anti-inflammatory cytokines [14–16].

CTCL represents a group of low-grade, non-Hodgkins' lymphomas, such as mycosis fungoides (MF) and Sezary syndrome (SS). Approximately one half of the patients with CTCL treated with ECP demonstrates a reduction in skin score by at least 50% within 12 months of treatment, and is categorized as responders [17]. A major problem with clinical ECP in CTCL is that we still do not know which kind of effect we aim at obtaining. Due to the findings of monoclonal T cells in peripheral blood, one explanation has been that ECP induces an anti-clonotypic cytolytic immunity directly against the lymphoma cells [18]. An essential issue in CTCL is whether ECP is inducing specific cytotoxicity against the lymphoma cells, or alternatively inducing specific or non-specific Treg cells controlling the CTCL proliferation. The success obtained with priming dendritic cells in cancer therapy could point to the first possibility [19], whereas the effect of ECP in GVHD, transplant rejection and autoimmunity favours the other view [2–7].

Treg cells constitute a subset of T cells with immunosuppressive properties, and identifying discriminatory cell-surface markers for the characterization and isolation of Treg cells has been a crucial goal for some time. Treg cells were first identified as T helper cells (CD4+) that strongly expressed CD25 (IL2-Rα), both in mice and humans [20,21]. In humans, however, it has been shown that activated T cells generally up-regulate CD25 expression [22], thus decreasing the purity of Treg cell isolation by this marker and limiting its utility when studying Treg cells. It is known that Treg cells express several other characteristic molecules in addition to being CD25high, such as glucocorticoid-induced tumour necrosis factor receptor (GITR), cytotoxic T-lymphocyte antigen 4 (CTLA4) and L-selectin (CD62L). The above mentioned cell-surface or intracellular molecules are, unfortunately, also expressed in activated T cells, thus making an exclusive isolation of viable Treg cells a substantial problem. At present the transcription factor Forkhead box p3 (Foxp3) is considered the most specific marker for Treg cells. This protein is, however, intracellular and not available for isolation of living cells. The search for Treg specific cell-surface markers continues, and several candidates have recently been assessed [23]. The importance of Foxp3 was demonstrated by showing that Foxp3-mutant mice have a Treg cell-deficiency and develop a severe lymphoproliferative autoimmune syndrome [24]. Similarly, humans lacking Foxp3 suffer from an aggressive autoimmune syndrome, known as immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, a rare recessive disorder that results in early death [25]. When first discovered in mice, Foxp3 was thought to exclusively identify Treg cells also in humans, but recent evidence supports that Foxp3 indeed can be induced upon T cell activation, and that these Foxp3+ T effector cells are without immunosuppressive properties. Hence, low expression of specific cell-surface markers should also be exploited to assess and identify Treg cells, for instance, that they have low expression of CD127 (known as IL7-Rα), whereas activated, effector T cells are CD127high [26,27]. Treg cells are thought to be capable of inhibiting immune responses against a variety of antigens, including the ones expressed by malignant cells [28]. In preventing autoimmune disease, the presence of Treg cells is naturally thought to be beneficial, whereas in the context of CTCL high numbers of these regulatory cells have mainly been supposed to inhibit the surveillance and clearance of tumour cells. Indeed, investigators have suggested that there is an association between a high tumour burden or tumour progression and an increase in Treg cells [29]. CD103, an αEβ7 integrin that mediates T cell retention in the epithelial compartment, has been shown to be an excellent marker for identifying *in vivo*-activated (induced) Treg cells [30,31]. These CD103+CD4+Foxp3+ Treg cells have been shown to be more potent suppressors in autoimmune arthritis [30] and in reversing chronic GVHD than primary CD4+CD25high Treg cells [32]. CD39 is a member of the ectonucleoside triphosphate diphosphohydrolase family, and is expressed on the surface of various cells. Its function is to degrade ATP, which acts as a "danger signal" that activates the immune system, to AMP. In combination with CD73, another ectonucleotidase, it results in the production of adenosine, that exhibit inhibitory and antiproliferative effects. Recently, it has been shown that CD39 is confined to a subset of the Treg cells, thought to represent regulatory cells with effector/memory-like properties. In the same study, the expression of CD39 among the Treg cells was also reflected in the capacity to degrade ATP, as well as in suppressive capacity [33]. CD45RA is a surface marker of primary (naïve) T cells in contrast to secondary (antigen-stimulated) T cells.

This study was performed to determine the relative numbers of Treg cells, characterized by the phenotype CD3+CD4+Foxp3+CD127−, prior to ECP and after 6 months of ECP treatment in patients with CTCL or GVHD. Further characterization was done to analyze the expression of CTLA4, CD25, CD45RA, CD103 and CD39 among the Foxp3+ cells. In addition we studied whether there was a measurable change towards a tolerogenic profile, by assessing the serum concentrations of IL-4, IL-6, IL-10, IL-17, IFN-γ and TGF-β, before and after 6 months of ECP treatment.

Materials and methods

Study populations

Freeze-stored serum and peripheral blood mononuclear cells (PBMC) from a total of 58 individuals were evaluated. The