Editorial

Extracorporeal photophoresis in chronic cutaneous graft-versus-host disease

More than half the recipients of unmanipulated marrow grafts from histocompatible donors develop GVHD, and the increased use of donors who are HLA-matched but unrelated, or related but unmatched, indicates that GVHD will remain a significant clinical problem following allogeneic BMT.¹ Mortality due directly or indirectly to GVHD may be as high as 50%.² Any therapeutic approach which ameliorates GVHD deserves attention and recent data from two studies indicate that extracorporeal photophoresis (ECP) has an important role to play in chronic cutaneous GVHD.

Phototherapy has been used both experimentally and clinically to modify GVHD. Antigen-presenting cells (APC) and T lymphocytes are susceptible to photo-inactivation with UVB or with a combination of UVA and a photosensitising agent such as 8-methoxy psoralen (8-MOP).^{3,4} These effects have been exploited experimentally to facilitate organ transplantation⁵ and also to prevent the onset of GVHD in murine models.⁶ In particular, Ullrich⁷ treated donor mice with 8-MOP, and then irradiated spleen and marrow cells with UVA light. Only the recipients of non-irradiated cells developed GVHD. In clinical practice UVA phototherapy is either administered to the skin surface (psoralens + UVA = PUVA) or to leukocytes isolated from the patient (extracorporeal photophoresis or ECP). A limited number of clinical studies attest to the beneficial effect of PUVA in patients with drug-resistant GVHD affecting the skin or mucosal surfaces.^{8–12} PUVA significantly depletes the number and function of epidermal Langerhans cells,^{13–15} and affects cytokine production by epidermal keratinocytes.^{16–18} The treatment is non-invasive, simple to administer and relatively inexpensive. The main drawback is the long-term risk of non-melanoma skin cancer, such as squamous cell carcinoma, particularly in patients who may require long-term immunosuppressive therapy. In addition, PUVA has no significant benefit on the systemic manifestations of GVHD.

ECP was developed in the early 1980s and used initially for the treatment of Sezary's syndrome and other forms of cutaneous T cell lymphoma.¹⁹ Treatment takes place 2 h after the ingestion of 8-MOP. After centrifugation of whole blood, the plasma and leukocyte-rich fraction are passed through a clear plastic channel and irradiated with UVA light. Treatment takes 2–3 h and is usually undertaken on 2 successive days each month, although accelerated regimens have been used for patients with GVHD. The addition of a photosensitising agent (uvadex) directly into the leukocyte-rich fraction avoids the side-effects from psoralens, such as nausea, and provides a more consistent drug level. There is no direct carcinogenic impact on the skin, and the treatment does not produce immunosuppression.

Several reports of a therapeutic benefit from ECP in GVHD have appeared since 1994, mainly in small numbers of patients with chronic cutaneous GVHD, but no comparative data have been published.^{20–23}

In 1996, in collaboration with the Department of Haematology at the Hammersmith Hospital in London, we invited referrals of patients with chronic cutaneous GVHD in order to carry out a comparative study of PUVA and ECP. The initial objective was 4 months of PUVA twice-weekly, followed by ECP in those who did not respond. The two ECP centres were at the St John's Institute of Dermatology, St Thomas' Hospital, London, and the Department of Haematology, Rotherham. However, the entry criteria meant that only three patients received PUVA prior to ECP. Of the 11 patients treated, eight were receiving concomitant immunosuppression. One died from cyclosporin A toxicity shortly after starting ECP and was excluded from the analysis. The remainder received ECP every 2 weeks for 4 months, followed by treatment every 4 weeks for a further 3 months. Contemporaneously, a separate study was underway in Austria, using a similar ECP treatment protocol. The results of these two studies have now been published in abstract form and both show encouraging results. The UK study reported an overall improvement in skin scores of 50% after 6 months of treatment.²⁴ Clinical improvement was also noticed in patients with mucosal disease, but not in the systemic manifestations of GVHD, such as liver or lung function. In the Austrian study, skin changes improved in 11 out of 13 patients, and mucosal changes in 10 out of 12 patients.²⁵ In addition, liver function tests improved with alkaline phosphatase levels returning to normal in eight out of 12 patients. It is not clear how many of the Austrian patients were receiving immunosuppressive therapy during ECP, but all patients in both studies had already failed first- and second-line therapy, so the results are sufficiently impressive to warrant a larger randomised study. They also raise the question of how ECP is actually working.

Current models of allo-reactivity in acute GVHD implicate TH1 lymphocytes in the afferent arm, and cytokine production by monocytes and macrophages in the efferent arm.²⁶ TH1 lymphocytes generate IL2 and interferon- γ ,²⁷ while monokines, such as IL1 and TNF α are thought to contribute to tissue damage.^{28,29} This model provides a rationale for many of the treatments currently used in GVHD, including thalidomide, which has anti-TNF α properties and the use of specific cytokine inhibitors.³⁰ In addition, TH2 cells have been used experimentally to prevent GVHD in a murine model.³¹

The pathogenesis of chronic GVHD is less well understood. Some investigators regard it as an extension of the allo-reactivity seen with acute GVHD, while others suggest that it may represent dysfunctional immune reconstitution with the production of auto-reactive clones. This is supported by the development of chronic GVHD following autologous BMT and the identification of specific class II donor anti-donor T cells.³⁰ Analysis of cytokine mRNA

expression in peripheral blood mononuclear cells shows up-regulation of interferon- γ with low levels of IL10 in chronic GVHD, again indicating a TH1-driven immune response.³² TNF α is also up-regulated, but only in extensive chronic GVHD, not in limited disease.³³

ECP can affect TH1/TH2 ratios,³⁴ so in the UK study we collaborated with the Department of Immunology at the Royal Free Hospital and examined lymphocyte function using a novel single cell cytokine assay, in which intracellular cytokines are fluorescently labelled and cells sorted using three-way FACS analysis. In particular, we measured IL2 and IL4 production by CD4 subsets, as an index of TH1/TH2 activity, and interferon production by CD8 subsets of an index of cytotoxic activity. Pre-treatment samples showed profound changes in the CD4 naive cell population, with very few cells and virtually no IL2 production.³⁵ In contrast, the memory cell subsets showed near normal IL2 production in the CD4 subsets, and increased interferon- γ production by the CD8 subset. Inhibition of these two cytokines was observed in patients on cyclosporin A therapy. By contrast, ECP did not alter cytokine activity, nor the TH1:TH2 ratio.³⁶

The mechanism of action of ECP in GVHD therefore remains an open question which requires further study. From a clinical standpoint any future study will need to be randomised and internationally based in order to recruit sufficient numbers. Because the immuno-modulatory effects of ECP may be obscured by immunosuppressive therapy, ECP should begin ideally before second-line therapy with cyclosporin A or azathioprine. One strategy would be to randomise patients with chronic GVHD to receive either steroids or ECP plus steroids. The two groups could then be compared in terms of clinical response, and/or the need for second-line therapy with cyclosporin A, etc. Long-term follow-up would also be crucial.

ECP is expensive, but its use in GVHD should not be difficult to justify if its therapeutic benefit can be reproduced in a randomised study.

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References

- Lazarus HM, Vogelsan GB, Rowe JM. Prevention and treatment of acute graft-versus-host disease: the old and the new. A report from The Eastern Cooperative Oncology (ECOG). *Bone Marrow Transplant* 1997; **19**: 577–600.
- Weisdorf D, Haake R, Blazar B *et al.* Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome. *Blood* 1990; **75**: 1024–1030.
- Warwick L, Morison MD, John A *et al.* Influence of PUVA and UVB radiation on delayed hypersensitivity in the guinea pig. *J Invest Dermatol* 1981; **76**: 484–488.
- Andreu G, Perrot JY, Pirenne F, Boccaccio C. The effect of ultraviolet B light on antigen-presenting cells: implications for transfusion-induced sensitization. *Semin Hematol* 1992; **29**: 122–131.
- Perez M, Edelson R, Laroche L *et al.* Inhibition of antiskin allograft immunity by infusions with syngeneic photoactivated effector lymphocytes. *J Invest Dermatol* 1989; **92**: 670–676.
- Deeg HJ. Modification of allointeractions after blood transfusion and marrow transplantation by ultraviolet light. *Semin Hematol* 1992; **29**: 95–101.
- Ullrich SE. Photoactivation of T-cell function with psoralen and UVA radiation suppresses the induction of experimental murine graft-versus-host disease across major histocompatibility barriers. *J Invest Dermatol* 1991; **96**: 303–308.
- Hymes SR, Morison WL, Farmer ER *et al.* Methoxsalen and ultraviolet A radiation in the treatment of chronic cutaneous graft-versus-host reaction. *J Am Acad Dermatol* 1985; **12**: 30–37.
- Eppinger T, Ehninger G, Steinfert M *et al.* 8-Methoxypsoralen and ultraviolet A therapy for cutaneous manifestations of graft-versus-host disease. *Transplantation* 1990; **50**: 807–811.
- Kapoor N, Pelligrini AE, Copelan E *et al.* Psoralen plus ultraviolet A (PUVA) in the treatment of chronic graft versus host disease: preliminary experience in standard treatment resistant patients. *Semin Hematol* 1992; **29**: 108–112.
- Aubin F, Brion A, Deconinck E *et al.* Phototherapy in the treatment of cutaneous graft-versus-host disease. *Transplantation* 1995; **59**: 151–155.
- Vogelsang GB, Wolff D, Altomonte V *et al.* Treatment of chronic graft-versus-host disease with ultraviolet irradiation and psoralen (PUVA). *Bone Marrow Transplant* 1996; **17**: 1061–1067.
- Friedmann PS. Disappearance of epidermal Langerhans cells during PUVA therapy. *Br J Dermatol* 1981; **105**: 219–221.
- Ree K. Reduction of Langerhans cells in human epidermis during PUVA therapy: a morphometric study. *J Invest Dermatol* 1982; **78**: 488–492.
- Ashworth J, Kahan MC, Breathnach SM. PUVA therapy decreases HLA-DR+ CD1a+ Langerhans cells and epidermal cell antigen-presenting capacity in human skin, but flow cytometrically-sorted residual HLA-DR+ CD1a+ Langerhans cells exhibit normal alloantigen-presenting function. *Br J Dermatol* 1989; **120**: 329–339.
- Okamoto H, Horio T, Maeda M. Alteration of lymphocyte functions by 8-methoxypsoralen and long-wave ultraviolet radiation. II. The effect of *in vivo* PUVA on IL-2 production. *J Invest Dermatol* 1987; **89**: 24–26.
- Aubin F, Kripke ML, Ullrich SE. Activation of keratinocytes with psoralen plus UVA radiation induces the release of soluble factors that suppress delayed and contact hypersensitivity. *J Invest Dermatol* 1991; **97**: 995–1000.
- Rivas JM, Ullrich SE. Systemic suppression of delayed-type hypersensitivity by supernatants from UV-irradiated keratinocytes. An essential role for keratinocytes derived IL-10. *J Immunol* 1992; **149**: 3865–3871.
- Edelson R, Berger C, Basparro F *et al.* Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. *New Engl J Med* 1987; **316**: 297–303.
- Owsianowski M, Gollnick H, Siegert W *et al.* Successful treatment of chronic graft-versus-host disease with extracorporeal photopheresis. *Bone Marrow Transplant* 1994; **14**: 845–848.
- Rossetti F, Zulian F, Dall'Amico R *et al.* Extracorporeal photochemotherapy as single therapy for extensive, cutaneous, chronic graft-versus-host disease. *Transplantation* 1995; **59**: 149–151.
- Gerber M, Gmeinhardt B, Volc-Platzer B *et al.* Complete remission of lichen-planus-like graft-versus-host disease

- (GVHD) with extracorporeal photochemotherapy (ECP). *Bone Marrow Transplant* 1997; **19**: 517–519.
- 23 Balda B-R, Konstantinow A, Starz H *et al.* Extracorporeal photochemotherapy as an effective treatment modality in chronic graft-versus-host disease. *J Eur Acad Dermatol Venereol* 1996; **7**: 155–162.
- 24 Child FJ, Ratnaval R, Samson D *et al.* An open study to compare PUVA and extracorporeal photopheresis (ECP) in the treatment of chronic cutaneous graft versus host disease. *Br J Dermatol* 1988; **139** (Suppl. 1): 27–28.
- 25 Rabitsch W, Reiter E, Keil F *et al.* Extracorporeal photopheresis (ECP) in extensive chronic graft-versus-host disease (GVHD). *J Am Soc Hematol* 1997; **90** (Suppl. 1): Abstr. 1676.
- 26 Ferrara JLM. Paradigm shift for graft-versus-host disease. *Bone Marrow Transplant* 1994; **14**: 183–184.
- 27 Antin JH, Ferrara JLM. Cytokine dysregulation and acute graft-versus-host disease. *Blood* 1992; **80**: 2964–2968.
- 28 Nestel FP, Price KS, Seemayer TA, Lapp WS. Macrophage priming and lipopolysaccharide-triggered release of tumor necrosis factor alpha during graft-versus-host disease. *J Exp Med* 1992; **175**: 405–413.
- 29 Abhyankar S, Gilliland DG, Ferrara JLM. Interleukin-1 is a critical effector molecule during cytokine dysregulation in graft-versus-host disease to minor histocompatibility antigens. *Transplantation* 1993; **56**: 1518–1523.
- 30 Lazarus HM, Rowe JM. New and experimental therapies for treating graft-versus-host disease. *Blood Rev* 1995; **9**: 117–133.
- 31 Fowler DH, Kurasawa K, Husebekk A *et al.* Cells of the Th2 cytokine phenotype prevent LPS-induced lethality during murine graft-versus-host reaction. *J Immunol* 1994; **152**: 1004–1011.
- 32 Korholz D, Kunst D, Hempel L *et al.* Decreased interleukin 10 and increased interferon- γ production in patients with chronic graft-versus-host disease after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1997; **19**: 691–695.
- 33 Tanaka J, Imamura M, Kasai M *et al.* The important balance between cytokines derived from type 1 and type 2 helper T cells in the control of graft-versus-host disease. *Bone Marrow Transplant* 1997; **19**: 571–576.
- 34 Di Renzo M, Rubegri P, D'Aloe G *et al.* Extracorporeal photopheresis restores TH1/TH2 imbalance in patients with mycosis fungoides: early stage. *Immunology* 1997; **92**: 99–103.
- 35 Salooja N, Tilling R, Russell-Jones R, Janossy G. Absolute cytokine production by peripheral blood lymphocytes in patients with chronic cutaneous graft-versus-host disease (cGVHD). *Bone Marrow Transplant* 1998; **21** (Suppl. 1): Abstr. 423.
- 36 Darvay A, Tilling R, Janossy G, Russell-Jones R. The effect of extracorporeal photopheresis (ECP) on TH1/TH2 cytokine production in patients with chronic cutaneous graft versus host disease (ccGVHD). *Br J Dermatol* 1998; **139** (Suppl. 1): 44.