Photopheresis Therapy of Cutaneous T Cell Lymphoma

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Abstract

Systemically disseminated cutaneous T cell lymphoma has a very poor prognosis and is generally resistant to chemotherapy or radiotherapy. A method permitting the extracorporeal photoactivation of biologically inert 8-methoxypsoralen (8-MOP) by ultraviolet A energy to a form which covalently crosslinks DNA was tested in the management of this disease. Following oral administration of 8-MOP, a lymphocyte enriched blood fraction was exposed to 1–2 joules/cm² of ultraviolet A and then returned to the patient. The combination of ultraviolet A and 8-MOP led to an 88 ± 5% loss of viability of target lymphocytes, while 8-MOP alone was inactive. Twenty-seven of 37 patients with otherwise resistant cutaneous T cell lymphoma responded to treatment, with an average persistent 64% diminution in cutaneous involvement over a period of 22 ± 10 weeks. Responders included 8 of 10 individuals presenting with lymph node involvement, 24 of 29 with exfoliative erythroderma and 20 of 28 whose disease was resistant to chemotherapy. Undesirable side effects often encountered with standard chemotherapy, such as bone marrow suppression, gastrointestinal erosions and hair loss, were not seen. This study suggests that extracorporeal photochemotherapy is a promising treatment for widespread cutaneous T cell lymphoma.

Key words: cutaneous T cell lymphoma, photopheresis, photochemotherapy, leukapheresis

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The origins of photopheresis

Photopheresis is a new procedure (and new word) which is derived from extracorporeal photochemotherapy with leukapheresis. Apheresis is a term that refers to the procedure of removing the different elements of blood. Thus, plasmapheresis refers to the selective removal of plasma with return of erythrocytes and leukocytes. Leukapheresis refers to removal of buffy coat (lymphocytes, polymorphonuclear leukocytes, and monocytes) while returning most of the erythrocytes and plasma. In these procedures lost volume is often replaced with albumin solutions. In inadvertent depletion of erythrocytes, platelets, and granulocytes is still unavoidable. The ultimate goal of these manipulations is to modulate the immune system with minimal toxicity but traditional pheresis techniques do this in a non-specific manner. Photopheresis attempts to also provide a non-toxic immunomodulation but with a greater degree of specificity.

Leukapheresis is useful in the symptomatic management of many leukemic conditions. By reducing the leukocyte mass there is transient relief of microvascular sludging of leukocytes and mobilization of soft tissue deposits of leukemic cells. The role of leukapheresis is clearly palliative. This procedure has been applied to the management of the leukemic phase of cutaneous T cell lymphoma (CTCL).1,2 Typically, leukaphereses are begun at a twice or thrice weekly rate. Improvement occurs very quickly and benefits are temporary with recurrence of pretreatment signs and symptoms after leukapheresis is discontinued. Thus, adjunctive therapy is indicated to prolong the effects of apheresis in this condition.

Extracorporeal therapeutic maneuvers are attractive from at least two angles. Extracorporeal systems eliminate many of the checks and balances of the intact host which may counteract the effect desired. For example, as in the case with interleukin 2, some lymphokines have extremely short half lives when injected in the intact host.3 However, in the extracorporeal system, the same lymphokine may persist for days at levels stimulatory to exposed lymphocytes. Toxicity can also be minimized by utilizing therapies at levels otherwise toxic to the intact host with conventional delivery systems. By limiting cytotoxic therapy to the extracorporeal phase, the host immune responses are unimpeded and the patient spared considerable side effects.

An example of extracorporeal lymphocyte manipulation was introduced from work with murine autoimmune disease. In models of myelin induced allergic encephalitis it was found that T cells from sensitized animals could induce encephalitis when infused into naive syngeneic hosts. However, lethal damage to these cells conferred protection from the disease if the host was later challenged with viable sensitized T cells.4 These observations suggest that the new host recognized determinants on the damaged expanded clone of anti-myelin T cells which directed the encephalitic reaction. This work suggests that T cell clone specific (clonotypic) responses are indeed inducible. The
human equivalent of this murine model is the modality known as photopheresis. The treatment system involves lethally damaging a portion of a patient's lymphocyte compartment followed by reinfusion of these cells. The major difference is that the lethal damage in the murine system was extracorporeal treatment with mitomycin C and in the photopheresis system the damage is from extracorporeal irradiation of 8 methoxypsoralen containing lymphocytes with ultraviolet A light.

Photochemotherapy with psoralen and ultraviolet A light is known in the field of dermatology as PUVA. When 8 methoxypsoralen is activated by UVA light (320–400 nm) it becomes a crosslinking agent not unlike chemotherapeutic alkylating agents. Photopheresis involves photoactivation of psoralen in the leukapheresis sample which contains a portion of the lymphocyte compartment. A degree of selectivity is thus obtained by limiting light exposure to the desired compartment. Tissues not exposed to light are not affected. Another degree of selectivity is obtained by utilizing a crosslinking agent which would differentially target dividing cells. The first step of photopheresis (Fig. 1) involves performing a leukapheresis on a patient after they have ingested a dose of 8 methoxypsoralen. The leukapheresis yields a buffy coat sample which when diluted with saline and plasma is 750 ml in volume with a leukocyte count ranging from $5\times10^3$ to $10^3$ cells/mm$^3$. The hematocrit of these samples varies from 3–9%. Ultraviolet A light is then delivered to the buffy coat by pumping the leukocytes through

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Fig. 1 Photopheresis consists of several cycles which are carried out during a 3–4 hour period.
a thin clear plastic chamber close to the light source. Thus, psoralen is activated in lymphocytes as they pass through the irradiation chamber. After the irradiation with UVA is completed, the cells are returned via an intravenous line.

The clinical trial of photopheresis for cutaneous T cell lymphoma

The initial treatment protocol for CTCL consisted of performing two consecutive photopheresis treatments at four week intervals. Patients in the initial study of photopheresis for CTCL were eligible if they had erythroderma and/or circulating abnormal cells with biopsy confirmation of the diagnosis. Leukemics with lymphocyte counts >20,000/mm³ were excluded as were patients with cutaneous tumors and those with any visceral infiltration of lymphoma. No treatment other than emollients and 1% hydrocortisone were administered during the trial. A total of 37 patients were treated for a minimum of six months. Treatments were conducted in two consecutive daily sessions once monthly for six consecutive months. At that point patients were either continued on the same schedule if they were improving or they were accelerated to once weekly treatment sessions. When this regimen was followed it was found that 27 of 37 patients exhibited improvement. Of these, 29 were erythrodermic and 24 achieved

Fig. 2 Hands of an erythrodermic cutaneous T cell lymphoma patient prior to photopheresis.
marked clinical improvement (see Figs. 2 and 3). It is intriguing that onset of improvement is very gradual. At first there are temporary responses immediately following a two day cycle of therapy. Then, after 4–6 months there is gradually a permanent decrease in erythema, scaling, and pruritis.

The clinical response of the disease was consistent at the different centers that participated. The subset of CTCL which had the most dramatic remission rate was that of the erythrodermic stage with leukocyte counts under 15,000. The patient presented in Figures 2 and 3 was a member of this subset. The clearing typically occurs in a cephalocaudad progression. As the erythroderma cleared, the patients noticed a return of body hair and the capacity for eccrine sweating. Temperature intolerance and rigors tended to resolve with early signs of improvement. Discrete papules formed (or at least they were unmasked) in areas that were once erythrodermic. Histologically, these discrete papules often showed more diagnostic changes than did lesions existing prior to photopheresis.

Of 10 patients with proven lymph node involvement, 8 had marked improvement with photopheresis. In patients who have had lymph node enlargement it was observed that the clinical findings of adenopathy tend to revert during the initial stages of improvement often preceding skin improvement.

Fig. 3 Patient from Fig. 2 after 6 months of photopheresis.
Another subset of patients had discrete plaques in conjunction with circulating abnormal cells. In this population there were a total of 8 subjects. Over the course of the protocol, 3 of these patients exhibited improvement (Fig. 3). It is of interest that the circulating abnormal cell count rarely dropped during therapy for this group.

Several patients with tumor stage CTCL have been treated with photopheresis. While it was observed that tumor nodules would temporarily regress after therapy, no long term improvement was observed and these patients were subsequently treated with additional modalities in addition to photopheresis.

Fig. 4 Biopsy from a patient prior to photopheresis.
In patients who have achieved clinical remission, biopsies of previously involved skin have revealed surprising findings. As shown in Fig. 4, the typical pretreatment biopsy showed a heavy lichenoid infiltrate of the upper dermis with epidermotropic abnormal cells. In follow up biopsies, there is uniform reduction of epidermotropism and depletion of the dermal infiltrate. However there are still abnormal cells noted in perivascular regions as shown in Fig. 4. This finding suggests subsets within the tumor population exhibit differential sensitivities to photopheresis. The proposed concept of tumor progression by the evolution of increasingly aggressive subclones could be reversed to explain this finding. Presumably the more malignant subclones are preferentially eliminated by photopheresis, leaving more “benign” subclones still present in the perivascular regions. Alternatively, host anti-tumor immunity is sufficiently enhanced to keep all subclones in check, without completely eliminating them.

The adverse effects of photopheresis can be subdivided into immediate, subacute and chronic. To establish a large intravenous collection line (14 gauge), there is a need for reliable venous access. Ecchymoses and pain at the site are occasionally encountered. In the relatively rare patient with access problems there has been successful use of Hickman catheters, femoral catheters, and arteriovenous fistulae.

Overall there is no net removal of blood components. However, patients with borderline cardiac status may be unable to withstand removal of even one unit of plasma and buffy coat may require supplemental saline or albumin. For patients extremely sensitive to volume overload there is an additional problem. The buffy coat sample is diluted with saline so that a net gain of only 200–300 ml may be enough to precipitate failure especially after two consecutive days of therapy.

The long term toxicity can only begun to be addressed by looking at the longest duration of therapy. The initial patient treated was under therapy for nearly 3 years until therapy was stopped well into the patients remission. No untoward effects can be determined at this point in time, four years after his entering the protocol.

A few select patients had a febrile reaction within 8 hours of reinfusion of their treated cells. This temperature elevation is highest on the first day of the cycle and it decreases in intensity on the following day and on subsequent treatments. Multiple negative blood, urine, and sputum cultures have been obtained during these periods, indicating that sepsis was not the cause. Attempts to suppress the fever by premedicating with acetaminophen, antihistamines and meperidine have been only partially successful. It is noteworthy that all patients who exhibited this phenomenon attained remission. Now that photopheresis for CTCL is no longer purely experimental, it may be possible to suppress this febrile response by premedicating with systemic corticosteroids. However, if the mechanism of response involves the generation of an effective immunologic reaction to the reinfused cells, concomitant administration of a systemic immunosuppressive may impair the host response to treated cells and thus impair the photopheresis
While patients were undergoing therapy many studies were performed to detect deleterious effects of therapy on the immune system. Lymphocyte stimulation studies done repeatedly showed no suppression after months or years of therapy. Delayed hypersensitivity skin testing showed intact recall responses after patients had begun to clear. Indeed, many had to achieve clearing to facilitate skin testing. Leukocyte and lymphocyte counts have never been suppressed to low levels.

Fig. 5 Biopsy from the same patient as in Fig. 4 after 6 months of photopheresis.
Some patients have gradual onset of a hypoproliferative anemia during therapy. This is not due to loss of erythrocytes since there is no net withdrawal. The lack of a reticulocyte response to this anemia may reflect the suppression of erythropoiesis associated with "anemia of chronic disease" the chronic disease being CTCL. Alternatively, the photopheresis induced inflammatory response to CTCL may be the "chronic disease". Another possibility is that the treatment may in some way activate suppressor cells which can suppress erythropoiesis.7 Overall, the change in hematocrit before and after at least six months of photopheresis was less than 2%.

From many of the findings made to date one can predict a broad usefulness of this modality. The therapies which had been unsuccessfully employed in the responding CTCL patients included chlorambucil, cyclophosphamide, glucocorticoids, methotrexate, VP-16, interferon, nitrogen mustard, BCNU, deoxycoformycin, electron beam, cyclosporin A and CHOP chemotherapy. All have deleterious side effects yet all are used in a broad range of malignant disease. These observations suggest that other malignant diseases will respond like erythrodermic CTCL. The potential ability of photopheresis to reduce and supplant immunosuppressive therapy with immunomodulation makes it a welcome addition to the therapeutic armamentarium.

There are other examples of extracorporeal immunomodulation appearing now also. The use of lymphokine activated killer (LAK) cells for malignancy is a similar but technically much more impractical procedure. Interleukin 2 is known to stimulate proliferation by primed lymphocytes. When administered by itself it has no remarkable clinical impact on tumors. However, if lymphocytes from cancer patients are harvested and expanded in culture with interleukin 2 there are objective anti-tumor responses.3,8

**Photopheresis research**

The two areas of interest in photopheresis are the events occurring in the extracorporeal irradiation stage and in the host response to the irradiated sample. Furthermore, one can subdivide the effects of photopheresis into acute effects, which are temporally related to an individual treatment, and the long term effects which result from months of reinfusion of irradiated material.

During the irradiation phase there is very limited immediate killing of lymphocytes. However, the lymphocytes in the extracorporeal phase are severely damaged by photopheresis. When irradiated and nonirradiated controls are compared in lymphocyte stimulation assays (phytohemaglutinin) there is a profound loss of responsiveness. This suppression is extremely dependent on the hematocrit of the leukapheresis sample (Fig. 6). Lymphocytes respond to PHA by producing IL-2. When lymphocytes are studied for production of IL-2 in response to PHA, there is a marked deficiency of this in lymphocytes drawn from the photopheresis sample at the end of irradiation compared to controls drawn at the start of irradiation.9
Fig. 6 When PHA responsiveness is plotted against leukapheresis simple hematocrit it is apparent that the higher the hematocrit, the less suppression of responsiveness. This is felt to represent shielding of the lymphocytes by erythrocytes.

Table 1

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<th>Pre-Culture</th>
<th>-UVA</th>
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<td>Helper T</td>
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<td>Suppressor T</td>
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<tr>
<td>Pan T</td>
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<td>95</td>
</tr>
<tr>
<td>B Cell</td>
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<td>H:S Ratio</td>
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When lymphocyte subsets are analyzed after 3 days of culture, it can be seen that even though the overall population has decreased (Fig. 8) there is no selectivity detected by subset analysis. Numbers are percentages.

When irradiated lymphocytes are then placed in tissue culture there is an accelerated decay that is proportional to the amount of light delivered (Fig. 7), with most lymphocytes dying over a several day period. Selectivity of killing was studied by examining lymphocyte subsets before and after in vitro culturing for 72 hours. The
Fig. 7 After irradiation of the lymphocytes there is a marked acceleration of death of the lymphocyte population.

irradiated cells still alive at this time had the same helper: suppressor ratio as did those not irradiated and controls (Table 1). Thus the psoralen and UVA is not selectively toxic to helper T cells in the photopheresis system. Thus, the acute effects of photopheresis on the irradiated specimen are generalized lymphocyte slow death and rapid impairment of T cell function.

The acute and chronic host responses to reinfusion of photopheresed material are less easily dissected in vivo. Lymphocyte surface markers can be utilized to demonstrate an immediate decrease in circulating T cells within 24 hours of a photopheresis treatment (Table 2). This may represent removal by the spleen, loss of markers by internalization, loss of markers by shedding, sequestration in the skin, or acute T cell toxicity. Over months, there is a small increase in the fraction of suppressor cells (Table 3). Since these findings are not seen in in vitro short term culture of treated cells it is apparent that the a host response has been invoked against cells not impacted
When lymphocyte subsets are analyzed in patients before and 24 hours after photopheresis, there is a marked decrease in T cell compartment markers. Numbers listed are percentages of peripheral blood lymphocytes.

**Table 2**

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**Table 3**

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<td>Suppressor T</td>
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<td>15</td>
</tr>
<tr>
<td>Pan T</td>
<td>69</td>
<td>68</td>
</tr>
</tbody>
</table>

When lymphocyte subsets are analyzed in patients before and after 6 months of photopheresis there is a slight decrease in the helper T cell portion and a slight increase in the suppressor T cell population. Numbers listed are percentages of peripheral blood lymphocytes.

It is known that the irradiated cells, when reinfused to the host, induce a beneficial response. There are multiple mechanisms by which this could take place, and in the final analysis the response is likely to be multifaceted. The alteration of surface antigens on abnormal cells may expose the host to new determinants. The irradiated blood fraction may contain generated lymphokines such as interferon, lymphotoxin, or possibly tumor necrosis factor, and this possibility needs to be extensively explored. The febrile response seen in some patients at 1–6 hours post reinfusion may be a sign of an important immediate host response, since all of the patients exhibiting this phenomenon cleared.

The killing of cells appears to be a crucial event. Although there appears to be no selectivity to the killing, it is noteworthy that those clones expanded in a disease process would be damaged to a greater degree than resting clones by several orders of magnitude. The killing of cells may also be an avenue of potentiating this form of therapy with adjunctive agents. Should the affected cells be more susceptible during various points of the cell cycle, adjunctive therapy to synchronize cells, arrest them in the appropriate point of the cell cycle, or to inhibit DNA repair could be synergistic with this therapy.

As suggested earlier, the long term induction of a clonotypic response may be
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of prime importance. This auto-immunization process might potentially be inhibited by concomitant cytotoxic or immunosuppressive therapy. Thus, as the study of photopheresis progresses, it will be necessary to clearly understand the mechanisms underlying the clinical responses.

Summary

Photopheresis is a relatively safe and technically simple modality for immunomodulation. The extracorporeal activation of a drug with UVA constitutes a novel form of drug delivery. The usefulness of this system in erythrodermic CTCL is well established. Immunomodulating therapy can thus be conducted outside the host where controlled conditions permit cellular manipulations not possible in the intact patient. Indeed, if one wished, as we did, to develop a system capable of stimulating an immunological response to damage, reinfused lymphocytes, the use of an extracorporeally photo-activated drug presents a distinctive special advantage. Because the only blood components immediately impacted by the photoactivated drug will be those in the extracorporeal system, mononuclear leukocytes localized to the reticuloendothelial system will be uninhibited by the drug. Therefore, unlike with classical chemotherapy which exposes the total body to the effects of the drugs, photopheresis leaves the reticuloendothelial system intact and capable of responding to the reinfused cells. This system is an excellent paradigm for future immunomodulating therapies of malignant diseases.

References