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Photopheresis: A New Therapeutic Concept

RICHARD L. EDELSON, M.D.

Professor and Chairman, Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut

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Photopheresis, the process by which peripheral blood is exposed in an extracorporeal flow system to photoactivated 8-methoxypsoralen (8-MOP), is a new treatment for disorders caused by aberrant T lymphocytes. It is now a standard therapy for advanced cutaneous T-cell lymphoma and shows promise in the treatment of two autoimmune disorders, pemphigus vulgaris and progressive systemic sclerosis (scleroderma). Additional diseases for which clinical trials are in progress include multiple sclerosis, organ transplant rejection, rheumatoid arthritis, and AIDS. The mechanism of action appears to involve a "vaccination" against the pathogenic T cells, in a clone-specific manner. Photoactivated 8-MOP initiates a cascade of immunologic events by forming covalent photoadducts with nuclear and cell surface-adherent DNA and possibly with other cellular molecules. For reasons not yet fully clarified, but probably related to enhanced cycling of the T-cell receptor for antigen, photopheresis increases the immunogenicity of the irradiated T cells so that their reinfusion induces a therapeutically significant immunologic reaction that targets unirradiated T cells of the pathogenic clone(s). The specificity of the induced immunologic reaction probably results from the extremely disproportionate expansion of the pathogenic clone(s), relative to the several million other clones of normal T cells.

This special issue of *The Yale Journal of Biology and Medicine* is devoted to an in-depth analysis of the clinical potential and scientific basis of photopheresis, an exciting new approach to the treatment of disorders, neoplastic or autoreactive, caused by aberrant T lymphocytes. Evidence has been rapidly accumulating, indicating that the human immune system, even when inundated by very large numbers of disease-provoking T cells, can be immunized against these cells by photopheresis in such a way that otherwise progressive disorders can be suppressed and even controlled. By bringing this body of work together for the first time under a single cover, we have attempted to tell the story in a cohesive manner, which will help stimulate others to contemplate, to investigate, and to implement the concept that clinicians may be able to harness the power of the immune system to control serious diseases.

Photopheresis, which is now approved by the Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma (mycosis fungoides, Sezary syndrome, and related presentations), is showing substantial promise in a variety of autoimmune disorders and is operative in over 40 centers in the United States and abroad (Table 1). Therefore, it is appropriate to review its status and to highlight several relevant practical issues. This article will serve as a clinical update and will summarize our growing knowledge as to how photopheresis works.

First, it would be helpful to discuss briefly the origin of the concept that diseases

Abbreviations: AMT: amino-methyl-trimethylpsoralen CTCL: cutaneous T-cell lymphoma EAE: experimental autoimmune encephalomyelitis 8-MOP: 8-methoxypsoralen FDA: Food and Drug Administration PET: photoinactivated effector T (cells) PHA: phytohemagglutinin TCR: T-cell receptor(s) UVA: ultraviolet A light

Address reprint requests to: Richard L. Edelson, M.D., Dept. of Dermatology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510

TABLE 1
Photopheresis Centers

U.S.A.

University of Pennsylvania
Philadelphia, Pennsylvania
University of California
San Francisco, California
Medical College of Wisconsin
Milwaukee, Wisconsin
Boston University
Boston, Massachusetts
University of Texas
Dallas, Texas
M.D. Anderson
Houston, Texas
Kenneth Norris Cancer Center
Los Angeles, California
University of Southern California
Los Angeles, California
Wishard Memorial
Indianapolis, Indiana

University of Iowa
Iowa City, Iowa
Washington Hospital Center
Washington, D.C.
University of Nebraska
Omaha, Nebraska
Columbia University
New York, New York
Georgetown University
Washington, D.C.
Cleveland Clinic
Cleveland, Ohio
Cedar Sinai Hospital
Miami, Florida
University of South Carolina
Charleston, South Carolina
Mt. Sinai Hospital, Miami
Miami, Florida
Civitan Blood Center
Gainesville, Florida
Oklahoma Blood Center
Oklahoma City, Oklahoma
Loyola University Hospital
Maywood, Illinois
University of Utah
Salt Lake City, Utah
Polyclinic Hospital
Harrisburg, Pennsylvania
University of Minnesota
Minneapolis, Minnesota

Yale University
New Haven, Connecticut
University of Pittsburgh
Pittsburgh, Pennsylvania
Henry Ford Hospital
Detroit, Michigan
University of Michigan
Detroit, Michigan
Mayo Clinic
Rochester, Minnesota
Scripps Clinic
La Jolla, California
Mount Sinai Medical Center
New York, New York
Ochsner Foundation
New Orleans, Louisiana
Duke University
Durham, North Carolina
Vanderbilt University/VA Medical
Center
Nashville, Tennessee
Morristown Hospital
Morristown, New Jersey
New York University/VA Medical Center
New York, New York
Thomas Jefferson University
Philadelphia, Pennsylvania
Hahnemann University
Philadelphia, Pennsylvania
University of Cleveland
Cleveland, Ohio
Tulane University
New Orleans, Louisiana
University of Alabama
Birmingham, Alabama
Southwest Florida Blood Center
Tampa, Florida
Good Samaritan Hospital
Los Angeles, California
Harper Hospital
Detroit, Michigan
Northwestern University
Chicago, Illinois
Puget Sound Blood Center
Seattle, Washington
Grandview Hospital
Dayton, Ohio

Europe and Asia

University of Dusseldorf
Dusseldorf, West Germany
National Cancer Institute, Japan
10 participating centers
Aachen Hospital
Aachen, West Germany

University of Vienna
Vienna, Austria
Hospital Avicenne
Paris, France

caused by expanded populations of pathogenic T cells can be effectively treated by extracorporeal photochemotherapy. This procedure evolved from an awareness that current approaches to the treatment of disorders of the immune system are quite nonspecific and toxic. The normal human body selectively regulates the expansion and suppression of each of the several million distinct clones of T cells which form the core of the immune system, permitting the rapid mobilization of those T cells necessary for anti-microbial defenses but inhibiting the activity of the same cells when their efforts are no longer required. In comparison, our most commonly applied tools for treatment of disorders of the lymphocyte or immune system are extremely crude. Systemic corticosteroids, antimetabolites, and cyclosporine A do not discriminate between individual clones of pathogenic and benign T cells and lead to many documented side effects. Bioengineered molecules, such as monoclonal antibodies and cytokines, will certainly find niches in our therapeutic armamentarium but are nonspecific and affect many types of target cells. Therefore, none of these therapeutic agents approximate the efficiency with which the normal body deals with its own T cells.

MECHANISM UNDERLYING RESPONSE TO PHOTOPHERESIS

A far more desirable approach would be the development of a means of dealing directly with the clones of T cells which actually cause a disease, while sparing the innocent clones. Studies in experimental animals, largely conducted by Irun Cohen and his colleagues at the Weizmann Institute in Israel over the past decade, have suggested that this goal may well be attainable [1,2]. Those investigators found that physically altered pathogenic T cells can be presented to an animal in such a manner that, instead of causing disease, they actually vaccinate against the development of the illness. The experimental system which the investigators used most extensively is experimental autoimmune encephalomyelitis (EAE).

EAE can be induced in rats by immunizing them with basic myelin protein extracted from the central nervous system of guinea pigs and presented to the rat in complete Freund's adjuvant, a preparation which markedly enhances efficiency of immunization [3]. Virtually all of these rats develop an acute paralytic syndrome, which is frequently fatal and which is associated with a T-cell infiltration of the central nervous system. That the disease is actually caused by T cells which specifically react against basic myelin protein has been demonstrated by the extraction from affected rats of such T cells, clonal expansion of these cells *in vitro,* and intravenous introduction of these anti-basic myelin protein T cells into healthy rats. The recipients of these anti-basic myelin protein T cells then develop a florid EAE, even though they themselves were not immunized with basic myelin protein.

Cohen's group then made the startling observation that resistance to the subsequent development of EAE could be induced by first vaccinating potential recipients against the otherwise pathogenic anti-basic myelin protein T cells. This work was accomplished by initially treating the pathogenic cloned T cells in ways (glutaraldehyde, hydrostatic pressure) that made their cell membranes more rigid and more efficiently exposed cell surface antigens that distinguished the anti-basic myelin protein T cells from all other T cells. Their data indicated that the most likely T-cell surface structures leading to the observed effects were the T-cell receptors (TCR) for specific foreign antigen. T cells react with foreign antigens in a manner analogous to the way that antibodies bind to appropriate ligands. T cells accomplish this process via membrane receptors, unique to each clone of T cells, capable of binding appropriately presented foreign antigens. Each T-cell clone bears a unique receptor, composed of two

disulfide-linked protein chains with distinctive amino acid sequences [4]. It is the three-dimensional structure of these receptors that confers specific binding properties and permits each of the several million clones of T cells to operate independently.

One of the ways that the normal individual regulates the proliferation of individual clones of T cells is to single out the T cells on the basis of the T-cell receptor for antigen: antibodies and cellular responses are produced which selectively target the appropriate T-cell clone(s)'s T-cell receptor. Since the TCR of each clone has its own distinct amino acid sequence, it is also uniquely antigenic. It appeared that Cohen's team had discovered several ways to make the anti-basic myelin protein T-cell receptor sufficiently antigenic so that, when the treated cells were infused into rats, the animals developed such a strong clone-specific immunologic response that subsequently infused living pathogenic T cells of the same clone were either destroyed or inhibited. When these animals were appropriately prepared, it was not possible to induce in them florid EAE. Since it was still possible to induce in them other autoimmune disease by infusion of unrelated clones of T cells, it was clear that the effect was truly clone-specific.

Those studies were of critical importance and clearly demonstrated that it is possible to vaccinate against the activity of pathogenic clones of T cells. Three major hurdles existed, however, before it would be possible to consider extending these observations to the treatment of humans with diseases caused by expanded clones of malignant or autoreactive T cells. First, the methods employed by Cohen's group (glutaraldehyde, hydrostatic pressure) were not readily applicable to reinfusable human blood. Second, patients always present with disease, not prior to developing illness. Hence, it would ultimately need to be demonstrated that vaccination against T-cell activity could be effective even in the face of progressive disease, rather than prophylactically as in the rat system. Finally, in virtually all autoimmune diseases and even in most patients with cutaneous T-cell lymphoma, it is not possible accurately to identify or isolate the pathogenic clone(s). Therefore, the only plausible way to proceed was to deal with the intact patient's whole blood and hope that the pathogenic clone(s) would be sufficiently expanded compared to all other individual clones of normal T cells that *in vitro* cloning would not be required. As we will see, solutions to each of these challenges were forthcoming.

The impractical glutaraldehyde and hydrostatic pressure approaches were effectively replaced by use of ultraviolet A (UVA)-activated 8-methoxypsoralen (8-MOP). Several features distinguish psoralens from nearly all other clinically useful drugs [5,6]. First, in the absence of light they are extremely safe and, in fact, without known biologic function. Second, once activated by ultraviolet energy, they exhibit an exceptional ability to bind covalently to target molecules in a manner which severely interrupts cellular function. Third, the type of ultraviolet energy, ultraviolet A, which photoactivates psoralen compounds, is capable of passing through clear plate glass and certain plastics and is of lower energy than ultraviolet B, which causes sunburn and generally does not pass through those materials. Fourth, once photoactivated, the transiently energized psoralen remains active for microseconds (millionths of a second), an interval long enough to be chemically reactive and short enough so that, for practical purposes, once the light source is turned off, unbound psoralen reverts to its inert form. In essence, psoralens provide the clinician with drugs of high chemotherapeutic potency, but only where and when the psoralens come in contact with light of the appropriate wavelength. Compounds of this family, therefore, offer a completely directed form of chemotherapy.

In the early 1950s, a group of investigators led by Aaron B. Lerner and Thomas B. Fitzpatrick at the University of Michigan demonstrated that a concentrated oral form of 8-MOP had a high margin of safety: the ratio of the maximal safe concentration of the drug to that required for clinical effect [7]. Studies with this purified form of 8-MOP substantiated the earlier clinical findings of El Mofty in Egypt that its oral use in conjunction with exposure of the skin to sunlight improved many patients with vitiligo, by stimulating their residual melanocytes. In the early 1970s, John Voorhees and his colleagues, also at the University of Michigan, found that topically applied psoralen followed by sunlight improved some patients with psoriasis [8]. Psoriatic epidermal cells actually divide more rapidly than do cells of most cancers but are not malignant, since they do not invade other tissues and can be returned to normal behavior patterns by effective therapy. In the late 1970s, John Parrish and his colleagues at Harvard, including Madhu Pathak and Fitzpatrick, led a multi-institutional group which found that orally administered 8-MOP followed by total skin exposure to high-intensity UVA was largely effective in clearing extensive psoriatic lesions which had otherwise been quite resistant to other treatments [9].

During that same period of time, Barbara Gilchrest and her co-workers at Harvard found that localized skin lesions of cutaneous T-cell lymphoma (CTCL) resolved in response to the same regimen [10]. CTCL is the most common adult malignancy of T lymphocytes and typically has an initial phase characterized by skin lesions containing large numbers of the malignant cells [11]. These clinical observations highlighted a series of laboratory studies, which had been proceeding in my laboratory (which was then at Columbia University), in Kenneth Kraemer's at the National Institutes of Health, and in Warwick Morrison's at Harvard, which all revealed that human T lymphocytes were quite sensitive to the combined effects of 8-MOP and UVA [12,13]. Furthermore, it had long been known that up to 25 percent of the blood supply passes through blood vessels of the skin and that UVA is capable of penetrating the full thickness of the skin, in attenuated amounts. Therefore, large numbers of circulating T cells must have been exposed to both UVA and 8-MOP during psoriasis treatments, and yet serious systemic sequelae to the immune system had not been reported. In fact, although approximately 2 percent of psoriatic patients treated repetitively with UVA and 8-MOP developed low-grade skin cancers, no increased numbers of patients with malignancies of white blood cells or increased susceptibility to infection were recognized among the thousands of subjects followed prospectively.

Paul Khavari from our group at Yale, working in close association with Cohen's group at the Weizmann Institute, found that exposure of the EAE-causing T-cell clones to 8-MOP/UVA was actually more effective than glutaraldehyde or hydrostatic pressure in leading to a beneficial vaccination effect in rats [14]. This finding was very exciting, because it suggested that extracorporeally routed human blood could be exposed to photoactivated 8-MOP and safely returned to the patient without introduction of significant systemic toxicity or generalized immunosuppression. Although there are clearly several ways to induce the vaccination against T cells, it was noteworthy that 8-MOP/UVA treatment of the cells was most effective and safest. Khavari also produced preliminary evidence that cell membranes isolated from 8-MOP/UVA-treated T cells could be used to vaccinate, indicating that the treatment had led to an effect in the T-cell membrane, in addition to the known effects of 8-MOP on nuclear DNA.

Carole Berger, Maritza Perez, and Liliane Laroche at Columbia and Yale Universi-

ties made several major observations in other experimental systems that were then developed to a greater degree and are discussed in detail elsewhere in this issue [15,16]. They found that the systemic lupus erythematosus-like disease which spontaneously develops in the inbred strain of MRL mice could be prevented or slowed by vaccinating young mice of that strain, prior to development of disease, with unfractionated 8-MOP/UVA-treated spleen lymphocytes from old diseased mice. These mice are clinically and serologically normal at birth but by young adulthood develop markedly elevated anti-native DNA antibodies, renal disease, splenomegaly, and massive lymphadenopathy. Berger and associates found that vaccination of young mice with 8-MOP/UVA-treated cells from diseased adults prevented development of the anti-DNA antibodies, greatly decreased splenomegaly and lymphadenopathy, and significantly prolonged survival times. Similarly, rejection of skin transplants from unrelated mice could be greatly impeded by vaccinating potential recipients with unfractionated 8-MOP/UVA-treated splenocytes from animals undergoing rejection of genetically identical skin grafts, and an immunologic response to a foreign antigen could be prevented by vaccination with unfractionated splenocytes from animals already manifesting the immunologic response against the same antigen. The power of this approach to control of the pathogenic clones was indicated by the capacity of this "T-cell vaccination" to occur in the absence of any other manipulation of the immune system. The extreme specificity of the approach was revealed by the clone-specific nature of the vaccination: the clones used in the photoinactivation and reinfusion steps induced immunity only against themselves and not against irrelevant T-cell clones.

The major significance of these observations was that *one could vaccinate against a particular T-cell activity without actually isolating or even identifying the clone(s) responsible for the activity.* The splenocyte populations in each of the above-described experimental systems contained markedly expanded clones of T cells capable of causing the lupus-like syndrome, mediating rejection of a particular skin graft, or reacting to a certain foreign antigen. It appeared that even though these expanded T-cell clones were admixed with many *smaller* irrelevant clones, the body was able selectively to react immunologically only against the expanded clones. That is, sufficient numbers of photoinactivated effector T or "PET" cells had been altered to lead to an immunologic reaction against them after their infusion, while the small numbers of T cells in each of the multitude of irrelevant clones came in "under the radar" of the immune system. That finding indicated that it should be possible to accomplish the same end by extracorporeally exposing human blood, containing expanded populations of disease-provoking T cells mixed with a multitude of much smaller clones of passenger normal T cells, to 8-MOP/UVA and then returning the photodamaged white blood cell mix to the patient.

CLINICAL TRIAL

The highly refined apparatus necessary to perform this extracorporeal procedure, known now as "photopheresis," was developed by engineers at Therakos, Inc., a subsidiary of Johnson and Johnson, Inc. The equipment combines an initial centrifugation step to enrich for lymphocytes with an elaborate ultraviolet exposure system, with all phases controlled by microprocessors and multiple monitors.

Several preliminary steps were required prior to beginning a clinical trial of photopheresis. First, we needed to determine the optimal conditions for the procedure. Because psoriasis patients readily tolerated blood levels of 50 to 200 nanograms of

8-MOP per milliliter of blood [9], we chose to administer the drug orally in exactly the same dosage as they received. Since we found that cell division by T cells could be almost completely blocked by 1 to 2 joules per square centimeter of UVA energy in the presence of 100 nanograms per milliliter of 8-MOP, we chose to expose the blood cells to this amount of energy. The weakness of the UVA energy, an advantage in minimizing undesirable side effects on other blood components, required an extremely thin blood film in the exposure system: in the currently used third-generation apparatus, blood is passed at a film thickness of only 1 millimeter between two high-intensity UVA energy sources, translating to a maximal distance between the targetted T cells and the light of only 0.5 millimeters. The total volume of patient blood outside the body during the treatment is approximately one unit or 500 milliliters, the same volume which an individual typically donates to a blood bank. To titrate the amount of UVA "seen" by the average T cell passing through the system to that which we had previously determined to be optimal for T-cell inactivation, Francis P. Gasparro and Regina Santella of our group produced monoclonal antibodies which recognized 8-MOP-DNA photoadducts in irradiated cells and permitted their quantification [17]. From predetermined dose-response curves, we were able to determine that 150 minutes of UVA exposure in the photopheresis apparatus were necessary to reach our goal of close to 2 joules per square centimeter of irradiation.

In order to justify using such an experimental modality, the first group of patients treated with this approach needed to fulfill certain requirements. They had to have an extremely debilitating and dangerous disease, unresponsive to standard therapy, caused by circulating T lymphocytes. The disease which we selected, the leukemic variant of cutaneous T-cell lymphoma (CTCL), is characterized by massive expansion of a malignant clone of helper T cells. Those malignant T cells migrate between the skin, which they infiltrate in such a manner that the total surface of that organ is red and swollen, and other body tissues via the blood and lymphatic vessels [11]. This phase of CTCL not only completely debilitates the victim but is associated with a median expected survival, when treated by standard means, of less than three years, with patients succumbing to opportunistic infections or destruction of vital organs by the malignant cells, as revealed by a recent multi-center prospective clinical study [18].

We began treating patients with a conservative regimen: photopheresis on two successive days at monthly intervals, a protocol initially intended merely to establish the toxicity of the new procedure. We realized that such a low frequency of treatments would permit irradiation of less than 10 percent of the total body burden of malignant cells, since so many of them were harbored in the skin and lymphoid tissues, and anticipated that, once the safety of the procedure was established, we would need to increase the frequency of treatments substantially. It therefore astounded us to see that four of the first five patients responded after only six to ten treatments and that eventually the severely involved skin of two of these patients cleared completely. This exceptional level of response, not noted with far more intensive cell removal by simple leukapheresis, indicated that, as the experimental studies in rodents suggested, the body's immunologic response to the reinfused UVA-irradiated T cells was leading to a profound clinical response.

A multi-institutional study was organized, with advice from the Food and Drug Administration, to expand the numbers of patients [19]. At the medical centers of Columbia University, University of Pennsylvania, University of California in San

Francisco, University of Pittsburgh, Yale University, University of Vienna, and University of Dusseldorf, a total of 37 patients with CTCL unresponsive to standard therapy were treated and 27 responded, including nine with complete or nearly complete clearing of the skin infiltration. Responders included 20 of 26 subjects presenting with lymph node involvement. This level of clinical response in a disorder otherwise known to be extremely resistant to conventional chemotherapy was noteworthy. Several additional features of the study were also remarkable. As compared to standard chemotherapy, the systemic side effects were minimal and in no instance prevented continuation of the treatments. Approximately 10 percent of the patients had a fever following the reinfusion of the damaged cells, but this febrile response completely resolved within 24 hours. Other problems, associated with leukapheresis (increased susceptibility to infection and depletion of other blood elements) or with standard chemotherapy of CTCL (bone marrow suppression, hair loss, intestinal bleeding from erosions, severe nausea), were not experienced. Nevertheless, the follow-up period has been too short to permit recognition of long-term side effects, and it remains possible that idiosyncratic adverse reactions will be recognized as larger groups of patients are treated now that the procedure has received approval from the FDA for management of advanced CTCL. Elsewhere in this issue, Peter Heald has updated the now extensive experience at the Yale–New Haven Medical Center with photopheresis treatment of CTCL patients.

RECOMMENDATIONS FOR USE OF PHOTOPHERESIS IN CTCL

Since that clinical trial was completed four years ago, additional clinical experience with those and other CTCL patients led to the following conclusions and recommendations.

Cutaneous T-Cell Lymphoma (CTCL) Patient Selection

Our longest clinical experience has been with the erythrodermic variant of CTCL, since 27 patients in the original clinical trial were in this category. The best responders were individuals who were immunocompetent, as described below, so that they were apparently able to mount an immunologic response to the reinfused photodamaged pathogenic lymphocytes. Patients who were not immunocompetent sometimes responded significantly, if they were treated frequently (up to twice weekly). This response by the immunoincompetent patients results from simple physical destruction of the malignant cells in the absence of an immunologic reaction against them; this type of response tends to be relatively short-lived, and the frequent treatments necessary to maintain it are impractical. Therefore, we recommend limitation of the therapy to the immunocompetent group.

More recently, we and others have begun to include patients with cutaneous tumors or extensive plaques in the absence of identifiable visceral tumors. It seems quite likely that such patients have hematogenous spread, given the known high recurrence rate of skin lesions following clearing induced by total body electron beam radiotherapy. Although the initial results have been variable, several of these patients appear to be maintaining remission following total body electron beam irradiation coupled with monthly photopheresis.

In this group of patients with tumors or extensive plaques, we are attempting to use photopheresis to maintain post-beam remissions. This group, in fact, now constitutes the largest new population whom we are treating. We have chosen to use photopheresis

for these patients, since the chemotherapeutic alternatives have limited efficacy and major attendant side effects. Prospective analysis of the results obtained in this population of patients by the cumulative photopheresis treatment group will determine whether the optimism generated by our preliminary observations in extensive plaque and tumor patients (treated by beam plus photopheresis) can be sustained.

Patients with plaque stage disease involving less than 15 percent of the body surface and lacking identifiable abnormal circulating cells are treated by us with either electron beam, PUVA, or nitrogen mustard, since those therapies can be rather effective in controlling the disease. Patients with plaque stage CTCL involving more than 15 percent of the body, with or without recognizable abnormal circulating T cells, as well as patients with more limited skin involvement but with readily demonstrable blood involvement, are treated by us with electron beam followed by photopheresis. We reiterate that, while we are encouraged by the preliminary results, it is too early to assess the efficacy of this combination of modalities formally.

Patient Evaluation

In addition to obtaining baseline abdominal CAT scans (to identify retroperitoneal nodes, splenic or hepatic involvement, and so on), chest X-rays (to identify hilar adenopathy or diffuse parenchymal infiltration), and routine blood profiles, cardiograms, and urinalyses, two specialized determinations of immunocompetence have been particularly helpful. Blood T-cell subsets should be analyzed using commercially available (Ortho, Becton-Dickinson, Coulter) monoclonal antibodies to determine both absolute and relative numbers of phenotypically abnormal cells. An indication of probable immunocompetence can be obtained from an estimation of the residual phenotypically normal helper/inducer and suppressor/cytotoxic T-cell subsets. For example, if the majority of the OKT3-positive T cells lack either OKT1 or OKT4 positivity, it can be assumed that few residual normal helper T cells persist and that the individual may be immunocompromised. Reactivity with the monoclonal antibody BE2, a marker of the malignant state, should be performed with a cytofluorograph. BE2 antigen is expressed in low quantity by many malignant T cells, and appropriate adjustment of the gains, as shown by Peter Heald at Yale, on the cytofluorograph will be required to recognize its presence. If this BE2 determination is accurately performed, the level of positive cells can be assessed longitudinally to help monitor response to therapy. It is noteworthy that Heald and Berger have shown that BE2 is actually an activation antigen, aberrantly expressed by CTCL cells. A phytohemagglutinin (PHA) stimulation assay of peripheral blood lymphocytes appears to be sufficient to distinguish many of the immunocompetent patients from the immunoincompetent ones. It is our recommendation to preclude those individuals with a greater than 50 percent diminution of PHA responsiveness from the treatment program.

Adjunctive Therapies

As indicated above, we routinely treat cutaneous tumors with radiotherapy and then introduce photopheresis. We have also been impressed with long-term responses of several patients who have received radiotherapy to markedly enlarged peripheral lymph nodes which were present before photopheresis or which developed during the course of treatment. It is our impression that simultaneous daily administration of immunosuppressive medications, particularly corticosteroids, should be avoided because of the preclusion of immunologic response to reinfused cells. Instead, we have

been quite impressed with efficacy of weekly oral methotrexate given as a single dose, ranging from 15 to 25 mg. Several partial responders to photopheresis improved greatly with addition of the methotrexate over two or more months.

Frequency of Treatment

During the clinical trial and in our subsequent experience, we found that some patients were particularly resistant to the effects of photopheresis. Some of these patients may benefit from increasing the frequency of treatment to every two weeks. In these individuals, when the response is maximal, treatment frequency may be decreased to every four or five weeks. Some patients who are less immunocompetent may then experience renewed disease progression with return to lower treatment frequency. The transient improvement which they experience while being treated more frequently is probably a reflection of physical destruction of the malignant cells in the absence of an immunologic response against them.

Length of Treatment Course

Whereas a number of erythrodermic CTCL patients have responded quite impressively within three months, most require significantly longer treatment periods. The majority of patients during the clinical trial required six months to demonstrate a 25 percent improvement. In addition, the majority of patients reach a maximal plateau of response, if less than complete, in six to eighteen months.

Weaning Off Photopheresis

With the exception of the rare individual who responds completely and can be maintained in remission completely off photopheresis, most patients require a gradual diminution of treatment frequency to determine the minimal acceptable intensity of the treatment program. We tend to stretch the intervals between treatments progressively to six weeks, to eight weeks, and occasionally to longer periods, while closely monitoring the clinical course. Because of the limited total experience with this approach, it is too early to make definitive recommendations.

Survival Data

The information on the survival of the 27 erythrodermic CTCL patients from the clinical trial is encouraging. The majority of the patients (67 percent) are still alive, with a median survival time from the diagnosis of erythrodermic CTCL of 43.5 months. This time span is in excess of the anticipated 30 months reported by the National Mycosis Fungoides Study Group in the *Annals of Internal Medicine*. Since most of the patients are still alive, the data are certain to become even more impressive with time.

While several of the complete responses have been remarkable and some have been maintained off therapy for more than four years, it is not surprising that the established malignancy in other patients can be difficult or impossible to eradicate by an immunologic reaction against the malignant cells. Yet one must be encouraged by the apparent capacity of continually administered photopheresis to slow the progression of the disease in a majority of the incomplete responders.

From this extensive experience with CTCL, we may now have important leads as to how to apply these principles to the treatment of a wide spectrum of diseases caused by lymphocytes: from lymphocytic leukemias to autoimmune disorders, such as rheuma-

toid arthritis and lupus, and even potentially to organ rejection. Current clinical trials include treatment of patients with rapidly progressive systemic sclerosis, rheumatoid arthritis, multiple sclerosis, chronic lymphocytic leukemia, pemphigus vulgaris, and rejection of transplanted organs. The majority of the treatment centers are in departments of dermatology, even though several of the diseases in the clinical trials are non-dermatologic. In general, dermatology groups are fully managing those individuals with major cutaneous components to their disorder, while merely supervising the photopheresis procedure in management of non-dermatologic patients. This latter situation is analogous to that of the radiotherapist's relationship to the referring oncologist. Clearly, however, the jurisdiction over the procedure will vary from center to center, particularly as those in other medical specialties become increasingly aware of photopheresis.

FUTURE PHOTOACTIVATABLE DRUGS

But is 8-MOP, a naturally occurring plant product, the ideal chemical agent for photopheresis? Whereas it has the advantage over some other naturally occurring psoralens, such as angelicin, of being able to form bifunctional adducts and therefore DNA cross-links, its absorption from the intestinal tract is highly variable and unpredictable. For example, in the same patient, under apparently identical conditions, Gasparro and associates have shown that blood levels may vary by more than 200 percent on different days. Clearly then, a simple advance will be the development of a solution of known concentration which could then be administered directly into the flow system of photopheresis. Blood levels, currently obtained after oral administration of 8-MOP, are in the range of 100 nanograms per milliliter or 500 micrograms per total five-liter blood volume of the average adult. If to 500 ml of extracorporeal blood only 50 micrograms of 8-MOP were added, one would obtain a more predictable 100-nanogram concentration per milliliter in UVA-irradiated blood, which would quickly be diluted to only one-tenth that level on return of the blood to the patient. One could actually add ten times that amount to the blood and potentially shorten the irradiation period greatly.

A second simple forward step would be to replace 8-MOP with its synthetic analogue, amino-methyl-trimethylpsoralen (AMT) [20], which has far greater water solubility as well as affinity for DNA, leading to, as shown by Gasparro, Berger, and colleagues, much greater activity per molecule. More efficient formation of monoadducts with DNA and then conversion of the monoadducts to bifunctional adducts or DNA cross-linkers is also possible by re-engineering of the lights, since Gasparro and associates have shown that the DNA cross-link occurs most efficiently at 340 nM of UVA [21]. In short, it should be possible in the near future to refine the procedure substantially.

It may also become desirable to deliver the photoactivatable drugs more selectively to appropriate target cells in extracorporeally routed blood. We have obtained laboratory evidence that this process could be accomplished in several ways. For example, by placing monoclonal antibodies on the surface of lipid vesicles, liposomes, which contain the photoactive molecule pyrene, Yemul and colleagues [22] have been able to cause these vesicles to bind selectively to target cells against which the monoclonal antibodies are directed. Subsequently delivered UVA leads to destruction of only the cells to which the liposomes had bound. Whereas liposomes delivered intravenously are rapidly filtered out of the bloodstream by the liver and spleen, they

might be more effectively used in the extracorporeal system of photopheresis where they would be directly mixed with the target white blood cells.

CONCLUSIONS

Photopheresis is an exciting new therapeutic approach to the management of diseases caused by abnormal circulating lymphocytes. Whereas it is already approved by the FDA for management of extensive cutaneous T-cell lymphoma, its applicability in the treatment of a broad spectrum of autoimmune diseases will be determined from the results of additional clinical trials. Elsewhere in this issue, preliminary clinical or research findings are presented, suggesting that photopheresis may have a place in the treatment of such diversified immunologic disorders as scleroderma, rheumatoid arthritis, multiple sclerosis, pemphigus vulgaris, and heart transplant rejection. The mechanism of action appears to be, at least in large part, an autovaccination against pathogenic clone(s) of T cells. The exciting possibility, therefore, exists that the importance of this new therapeutic modality may substantially increase in the next decade.

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