Inhibition of Autoimmune Disease in a Murine Model of Systemic Lupus Erythematosus Induced by Exposure to Syngeneic Photoinactivated Lymphocytes

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MRL/l mice develop progressive, virulent autoimmune disease that has many of the features of systemic lupus erythematosus. Prophylactic treatment of MRL/l mice with syngeneic photoinactivated autoimmune splenocytes improves survival and inhibits the fulminant hyperproliferation of abnormal T cells and the production of high titer anti-DNA antibody invariably found in untreated mice. The proliferation of Thy 1+ splenic T cells was significantly decreased, and prolonged retention of the response to T-cell mitogen was found in treated mice. Treatment with unmodified cells induced a partial inhibition of disease features which did not prolong survival rates. These results suggest that phototherapy potentiates a normal immunoregulatory process which enables suppression of the development of abnormal cell populations in young MRL/l mice with relatively intact immune systems. J Invest Dermatol 94:52-57, 1990

The MRL/l mouse model of systemic lupus erythematosus provides an experimental system that permits exploration of the effect of T-cell directed therapeutic maneuvers on the course of autoimmune disease. MRL/l mice develop lymphoid hyperplasia, B-cell hyperactivity, autoantibodies, circulating immune complexes, depressed serum complement, thymic cortical atrophy [1,2], dermatitis [3,4], arthritis [5,6], and fatal immune complex glomerulonephritis. The genome of the MRL/l mouse contains the autosomal recessive mutation lpr. Homozygosity for this gene results in early onset of autoimmune disease with massive lymphadenopathy and splenomegaly due to a proliferation of phenotypically aberrant [7-9], functional inducer T cells [10]. Initiation of autoimmune disease in these mice correlates with the onset of T-cell hyperproliferation, suggesting that the primary defect in this model relates to a misregulation of inducer T cells resulting in polyclonal B-cell stimulation.

Therapies that ablate T cells can improve some of the parameters of autoimmune disease that are invariably manifested. In an age-related fashion, in untreated MRL/l mice [11,12], Neonatal thyroidectomy [13]; injection of monoclonal antibodies directed against the pan T-cell marker, Thy1 [11], or the inducer T-cell marker, L3T4 [14]; and total lymphoid irradiation [15] of MRL/l mice prevented the development of autoimmune disease, increased survival, and depressed T-cell proliferation.

Photochemotherapy [16] is a new treatment modality that may provide a clinically practical method to promote T-cell regulation through exposure of the recipient to autologous, photomodified lymphocytes using the drug 8-methoxypsoralen (8MOP) activated by ultraviolet A (UVA) light to form a DNA crosslinking agent [17]. Lymphocytes treated in this fashion fail to proliferate after stimulation by mitogen, antigen or alloantigen, but retain the capacity to serve as stimulator cells in mixed leukocyte culture. The photoinactivated cells die gradually in vitro over a 5-d interval [18]. Photochemotherapy has recently been demonstrated to be efficacious in the management of patients with the leukemic phase of the aggressive inducer T-cell malignancy, cutaneous T-cell lymphoma (CTCL) [16]. One mechanism by which photochemotherapy may achieve a beneficial effect in CTCL patients is through induction of an autoregulatory response in the recipient, putatively directed against abnormal T-cell idiotypes expressed on the reinfused lymphocytes.

If photochemotherapy invokes a host response that regulates abnormal inducer T-cell expansions, this approach could be studied in the MRL/l mouse model where a phenotypically aberrant, benign helper T-cell hyperproliferation promotes the development of autoimmune disease. To test whether reinfusion of photomodified lymphocytes can alter the course of a fulminant autoimmune process, recipient mice were exposed to syngeneic autoimmune splenocytes prior to the onset of overt disease when a relatively intact immune system was present. Treated mice were monitored by evaluation of reliable indicators of disease progression, including lymphoid organomegaly, survival, lymphocyte phenotype, and anti-DNA antibody production. Control groups were untreated or received injections of unmodified autoimmune cells or lysed phototreated cells to determine if the therapy was contingent on the presence of intact effector cells of the autoimmune process. The...
results obtained demonstrated a marked reduction in lymphoid hyperproliferation, prolonged survival, altered splenic lymphoid populations, and decreased production of anti-DNA antibodies in the recipients of phototreated autoimmune cells. Injection of unmodified cells alone was partially effective, while damaged cells had no effect, indicating that intact effector cells are required for induction of a response. The onset of autoimmune disease, as measured by these parameters, was significantly delayed in treated mice by preexposure to autoimmune effector cells.

MATERIALS AND METHODS

Mice Because the onset of the disease process is slightly earlier in female MRL/l mice [2], male mice were selected for study to insure an adequate opportunity for therapeutic intervention. Young male MRL/MpJ-lpr/lpr mice were obtained from Jackson Laboratories (Bar Harbor, ME) at 4–6 weeks and used as recipients of photoinactivated autoimmune splenocytes or age-matched untreated controls. Donor male MRL/l mice were retired breeders received at 17–21 weeks. BALB/c mice were used as normal controls and were received from Jackson Labs at 6–7 weeks.

Cells Spleen cell suspensions were prepared under sterile conditions. Spleenocytes were liberated by macerating the spleens in a sterile petri dish with forceps. The cells were washed in Hanks buffered salt solution (HBSS, Gibco Laboratories, Grand Island, NY), and red blood cells were lysed with ammonium chloride (0.83%, in water) at 4°C for 10 min. The cells were washed and resuspended in RPMI 1640 (Gibco) containing 5% fetal calf serum (FCS) for culture or phosphate-buffered saline (PBS, Gibco) for treatment with 8-MOP-UVA. Lymph nodes were obtained from the retroperitoneal area and prepared in a similar fashion. Lymph node size was determined by measuring length and width with a ruler.

8 MOP-UVA Protocol The optimal concentration of 8-MOP (Elder, Bryan, OH) and dose of UVA for inhibition of lymphocyte proliferation was determined by development of a dose-response curve (data not presented). A drug level of 100 ng/ml and a light dose of 1 joule (J)/cm² of UVA energy were selected to photoinactivate the splenocytes.

The autoimmune status of the donor MRL/l mice was confirmed by demonstration of humoral anti-DNA antibody or the presence of overt lymphoid organomegaly. Splenocytes (20–50 × 10⁶/ml) were incubated with 100 ng/ml of 8-MOP in PBS at 32°C, for 20 min prior to irradiation with 1 J/cm² UVA. The light source consisted of a bank of four black lights (FL40, Sylvania GTE Products, Danvers, MA) emitting broad spectrum UVA energy (320–400 nm), filtered through a sheet of window glass to remove UVB emission. The light dose was calculated after measurement with a spectrophotometer (IL700A, International Light, Newburyport, MA). Previously performed controls included cells exposed to UVA alone or shielded with foil and placed on the irradiation surface [16]. These controls demonstrated that human lymphocytes as well as murine lymphocytes were viable at the time of reinfusion, but died gradually over a 5–10 interval in vitro and lost the capacity to respond to mitogens because of crosslinking of their DNA by photoaddition of 8-methoxypsoralen.

After irradiation, the cells were washed, resuspended in PBS at 20–50 × 10⁶ splenocytes/100 μl, and injected intravenously into the tail vein of recipient MRL/l mice. The viability of the phototreated cells was 90% or greater as determined by trypsin blue exclusion. Mice received their first injection at 6 weeks of age, a time point that is prior to the initiation of most of the features of the autoimmune process [19]. The mice were treated at 2-week intervals until conclusion of the experiment. In some protocols, groups of mice were treated in parallel with autoimmune splenocytes without 8-MOP-UVA inactivation or autoimmune cells that had been rapidly frozen and thawed to induce lysis. These mice received the equivalent number of whole or lyzed splenocytes as the 8-MOP-UVA experimental group.

Radioimmunoassay for Anti-Double Stranded DNA Antibodies A solid phase radioimmunoassay (RIA) was performed using calf thymus DNA (Sigma, Chemical Co., St. Louis, MO) at a concentration of 1 mg/ml in PBS. The DNA was sonicated for 30 min to obtain small fragments and aliquoted (100 μl/well) into the wells of a round bottom microtiter plate (Flow Laboratories, Rockville, MD). The wells were coated with the DNA solution by rotation on a plate shaker (Dynatech Laboratories, Alexandria, VA) for 1 h at 25°C. The plate was air-dried overnight in a laminar flow hood. The details of the RIA assay have been previously described [20]. Calf thymus DNA might contain some degree of single-stranded DNA which could result in some detection of autoantibody to both single- as well as double-stranded DNA. However, the final concentration of DNA in the wells was approximately 50 μg/ml and any single-stranded DNA contamination at this level would represent a minor component of the DNA present.

Phenotypic Analysis Monoclonal antibody analysis was performed to determine the phenotype of splenocytes in treated mice and control animals. Spleen cell suspensions were prepared and resuspended in RPMI 1640 containing 0.3% bovine serum albumin (BSA). The anti-T-cell monoclonal antibodies used were anti-LYT 1.2, 1.3, 1.5, 2.4, 2.5, and 3.2 (Cedarlane Laboratories, Ontario, Canada). These antibodies were produced in mice, and use of a fluorescein-conjugated rabbit anti-mouse IgG or IgM secondary reagent required preliminary lysis of surface immunoglobulopositive B cells. To lyse the B cells, the splenocytes were incubated at 10 × 10⁶ cells/ml with a 1:20 dilution of the rabbit anti-mouse IgG or as a control nonspecific mouse ascites fluid in RPMI/3% BSA for 30 min at 4°C. The cells were resuspended in 2 ml of a 1:20 dilution of rabbit complement (Cedarlane Laboratories) and incubated at 37°C for 60 min. The cells were washed twice and resuspended in RPMI/3% BSA for assessment of viability by trypan blue exclusion. Non-viable cells were removed by flotation on Lympholyte-M (Cedarlane Laboratories) and centrifugation (500 × g, 20 min, 23°C). Indirect immunofluorescence analysis was performed as previously described [20].

Mitogen Assays Splenocytes were adjusted to 5 × 10⁶ cells/ml in RPMI 1640 containing 5% FCS. The cells were aliquoted at 100 μl/well in five replicate wells of a flat-bottom microtiter plate (Flow Laboratories). Background cultures received 100 μl of RPMI 1640/5% FCS. Stimulated cells received 100 μl of a 4-μg/ml solution of concanavalin A (Con A, Sigma). Splenocytes stimulated with lipopolysaccharide (LPS, Difco Laboratories, Detroit, MI) received 100 μl of a 100 μg/ml solution. Cultures were incubated for 3–4 days at 37°C under a 5% CO₂ atmosphere. The cultures were pulsed with 1 μCi/well of ³H-thymidine (Amersham, Arlington Heights, IL) and harvested 12–18 h later with a Mass II automated harvester (Whitaker M. A. Bioproducts, Walkersville, MD). The samples were counted in a liquid scintillation Beta counter (Amersham Corp.).

Statistics The weight, size, and cellularity of organs obtained from the groups of mice were analyzed for statistical significance by the standard Student t test. Survival data were analyzed using a z statistic to reject the null hypothesis at the α = 0.01 level of significance.

RESULTS

Reduced Lymphoid Organomegaly in Treated MRL/l Mice The weight and size of lymph nodes obtained from mice receiving biweekly injections of photoinactivated splenocytes from autoimmune donors (N = 29) was compared to organs obtained from age-matched littermates treated with unmodified autoimmune cells (N = 8) or untreated controls (N = 97, Fig 1). The weight and size of retroperitoneal lymph nodes obtained from mice treated with photoinactivated autoimmune splenocytes was significantly (p < 0.001) reduced in comparison with untreated controls at every age studied. A similar decrease in lymph node weight (p < 0.025) and size (p < 0.005) was demonstrated in young mice and...
maintained in the lymph nodes obtained from older mice (wt: p < 0.01; size: p < 0.001) treated with unmodified autoimmune cells.

Figure 2A demonstrates that spleens obtained from the recipients of photoactivated autoimmune splenocytes (N = 33) showed a significant (p < 0.001) reduction in weight and size when compared to controls (N = 121) at an early age (7-8 weeks). The spleens obtained from 19-34-week treated mice were increased in weight and size but remained reduced in comparison to the untreated age-matched controls (wt: p < 0.01, size: p < 0.025). MRL/l mice treated with unmodified autoimmune cells (N = 13) did not demonstrate a decrease in splenic weight at a young age. Older mice in this group had significantly smaller spleens when compared to controls (p < 0.001).

The cell yield obtained from the spleens of mice treated with photoactivated autoimmune splenocytes (N = 20) was significantly reduced in both young (p < 0.001) and old mice (p < 0.005) in comparison to controls (N = 100, Fig 2B). Mice receiving unmodified autoimmune cells (N = 9) also had reduced splenic cellularity (p < 0.01). In the group of 46 mice that received photoactivated autoimmune cells, only four mice (9%) were judged to be treatment failures based on early onset of lymphoid organomegaly. In contrast, 20% of the mice tested in the group receiving cells without inactivation were judged to be treatment failures.

An additional group of mice were recipients of photoinactivated autoimmune cells that had been lysed by freezing and thawing prior to injection (results not shown). These mice exhibited rapid onset of organomegaly (16-20 weeks) and died at an early age (60% mortality by 16 weeks of age).

Survival The survival of mice treated with injections of photoinactivated autoimmune splenocytes was compared with the survival of mice treated with unmodified cells or untreated controls (Table I). The group of mice that received unmodified cells from autoimmune donors demonstrated 50% mortality by 20 weeks of age. The 50% mortality level was reached in untreated control mice at 23 weeks of age. This rate of control mortality is similar to that reported in other studies [19]. In contrast, twice as many of the recipients of photoactivated autoimmune splenocytes were alive at 23 weeks of age. The increased survival demonstrated in the recipients of phototreated splenocytes was statistically significant in comparison to the controls (α = 0.01) and was maintained from 23-29 weeks, at which time all of the untreated control mice had died. The survival of the phototreated splenocyte group was significantly (α = 0.01) prolonged compared to the recipients of unmodified cells at 23 and 26 weeks.

Table I. Survival of MRL/l Mice Treated with Photoinactivated Autoimmune Splenocytes, Unmodified Cells or Untreated Controls

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>8 MOP-UVA cells (N = 22)</th>
<th>Unmodified cells (N = 20)</th>
<th>None (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>14</td>
<td>90</td>
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<td>17</td>
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<td>23</td>
<td>59*</td>
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<tr>
<td>26</td>
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<td>9</td>
<td>20</td>
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<tr>
<td>29</td>
<td>15*</td>
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</tr>
<tr>
<td>37</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Statistically significant increased survival at the α = 0.01 level in the 8 MOP-UVA cell recipient group compared to the unmodified cell recipients (23 and 26 weeks) and the non-treated group (23, 26, and 29 weeks).
of individual T
Effect stranded DNA was followed over time in MRL/l mice that received studied as an additional control to ascertain background levels of anibody level in mice that were recipients of photoinactivated auto-DNA antibody. The spleen of mice treated with photoinactivated autoimmune splenocytes was in the background range found in normal strains of mice.

Phenotype of Splenocytes from Mice Receiving Injections of Autoimmune Splenocytes Spleenocytes were obtained from mice receiving injections of photoinactivated autoimmune splenocytes at 13 to 26 weeks of age (Fig. 3). Spleenocytes from recipients of untreated autoimmune splenocytes and control mice were also studied. To adjust for the marked lymphoid depletion in the treated spleens, the results were expressed as the absolute number of antibody-reactive spleenocytes as determined by adjusting the number of recovered spleenocytes by the percentage of cells stained with each antibody. The spleens of mice treated with photoinactivated autoimmune splenocytes contained a non-significant decrease in B-lymphocytes bearing surface immunoglobulin in comparison with the recipients of unmodified cells or the untreated controls. The absolute number of Thyl.2 + T cells was significantly reduced (p < 0.001) in the photoinactivated cell treated group. The recipients of unmodified autoimmune splenocytes also had a profound reduction

in the absolute number of splenic T cells but not to the extent demonstrated in the spleens of mice treated with photomodified autoimmune cells (p < 0.001). The LYT1 + spleenocyte population was reduced in the spleens of mice receiving photomodified (p < 0.025) or untreated autoimmune splenocytes.

Response to LPS and Con A Splenocytes from mice that had received injections of photomodified or untreated autoimmune cells were studied at various time points to determine their capacity to respond to LPS and Con A in comparison to age-matched littermates. Because the treated mice had substantially fewer T cells than the controls, the results were adjusted to reflect the absolute number of B or T cells available for stimulation. In Fig 4A, the response of B cells to LPS stimulation from the three groups of mice is presented. The splenic B cells from the photoinactivated autoimmune cell treated mice were slightly less responsive to LPS stimulation than

Figure 3. Comparison of the absolute number of antibody positive cells obtained from the spleens of MRL/l mice treated with photoinactivated autoimmune spleenocytes (black bars), unmodified autoimmune cells (white bars), or untreated controls (striped bars). The percentage of positive cells was determined by indirect immunofluorescent staining and analyzed by cytofluorography. The total number of cells per spleen was multiplied by the percentage of reactive cells to obtain the absolute number of positive cells.

Figure 4. A: Comparison of the response to lipopolysaccharide of individual B lymphocytes obtained from the spleens of age-matched MRL/l mice treated with photoinactivated autoimmune spleenocytes (thin solid line with black squares), unmodified autoimmune cells (dotted line with stars), or untreated controls (thick solid line with crosses). The results were calculated by dividing the total cpm by the absolute number of Slg-positive B cells as determined by indirect immunofluorescence. For each experimental group a best fit trend line was drawn. B: Comparison of the response of individual T lymphocytes obtained from the spleens of age-matched MRL/l mice treated with photoinactivated autoimmune spleenocytes (thin solid line with black squares), unmodified autoimmune cells (dotted line with stars), or untreated controls (thick solid line with crosses) to concanavalin A. The results were calculated by dividing the total cpm by the absolute number of Thy-l-positive T cells as determined by indirect immunofluorescence. For each experimental group a best fit trend line was drawn.

Table II. Anti-ds DNA Antibody Levels in MRL/l Mice Treated with Photoinactivated Autoimmune Splenocytes, Untreated Controls, and Normal Non-Autoimmune Mice

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>8 MOP-UVA Tx cells (N = 5, X ± SD)</th>
<th>Untreated controls (N = 10, X ± SD)</th>
<th>Normal mice* (N = 10, X ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>901 ± 858</td>
<td>6355 ± 733</td>
<td>2353 ± 670</td>
</tr>
<tr>
<td>17</td>
<td>897 ± 924</td>
<td>15063 ± 4482</td>
<td>2353 ± 670</td>
</tr>
<tr>
<td>19</td>
<td>1971 ± 772</td>
<td>15063 ± 4482</td>
<td>2353 ± 670</td>
</tr>
</tbody>
</table>

* Normal sera were obtained from BALB/c or CBA/j mice.
controls. The recipients of unmodified autoimmune cells had an equivalent number of B cells when compared to controls (Fig 3), but these cells responded poorly to LPS in comparison to B cells obtained from control MRL/l and the recipients of photoactivated cells. Over time, the capacity to respond to LPS was lost in all three groups. In Fig 4B, the response to Con A of splenic T cells from all three groups of mice (adjusted to reflect the absolute number of T cells) is presented. Although the absolute number of T cells was significantly reduced in the group of mice receiving photoactivated splenocytes (Fig 3), the residual T cells responded better to Con A stimulation (p < 0.025) than T cells from the recipients of untreated autoimmune cells or controls. At older ages, the response to Con A was lost in all groups.

DISCUSSION

This study demonstrates that the fulminant progression of the autoimmune process in the MRL/l mouse may be ameliorated by exposing the animal at an early age to syngeneic lymphocytes from actively autoimmune mice. The inhibition of the disease process attained is comparable to the results achieved by other forms of anti-T cells therapy [11-15] but does not involve delivery of systemically toxic agents. These results indicate that treatment with autologous lymphocytes may provide an alternative form of clinically feasible immunoregulation that can prevent T-cell hyperproliferation and autoantibody formation.

Three groups of mice were evaluated in this study. The recipients of unmodified autoimmune cells represented a parallel treatment to the photoactivated group. These mice were exposed to the relevant T-cell idiootype on the autoimmune cells at an age when their immune system might retain the competence to respond to this abnormal expansion of autoreactive cells. Photoactivation was demonstrated to enhance the host response to injection of this effector T-cell population.

Analysis of both lymph nodes and spleens from mice treated with either photoactivated or unmodified autoimmune lymphocytes demonstrates that a significant reduction in lymphoid organomegaly can be achieved and maintained by this protocol. Lymphadenopathy has been found to be a reliable indicator of disease activity in the MRL/l mouse [19,21]. Mice treated with unmodified or photoactivated autoimmune splenocytes demonstrated a 50% reduction in lymphadenopathy. This decrease in lymph node size and weight was maintained beyond the normal life span of untreated littermates, in recipients of photoactivated cells. Recipients of unmodified cells did not demonstrate decreased splenomegaly until late in their treatment course, indicating that injection of unmodified cells may be relatively less effective than phototreated cells.

The survival results indicate that injection of unmodified autoimmune cells does not prolong survival and may in some animals result in earlier death. In contrast, only 9% of the recipients of photomodified autoimmune cells developed early disease, and the majority of these mice survived significantly longer than the untreated controls. The requirement for intact cells in this treatment regimen was indicated by the results of a group of mice that received lysed photoactivated autoimmune splenocytes. These mice died rapidly with manifestations of aggressive autoimmune disease.

Treatment with photoactivated autoimmune splenocytes not only decreased lymphoid hyperproliferation and increased survival but also prevented the induction of anti-DNA antibodies. The possibility that this inhibition of autoantibody production was related to a depletion of the abnormal T-cell population found in untreated MRL/l mice [22,23] was supported by phenotypic analysis of the splenic populations of treated mice. No significant difference in the absolute number of B cells was found in the spleens of treated mice in comparison to controls. The T-cell population was significantly reduced in the spleens of treated mice, confirming that this form of therapy is mainly T-cell directed and suggesting that inhibition of T-cell hyperproliferation can primarily or in conjunction with depletion of a relevant B-cell subset result in suppression of autoantibody production. The limited reduction in T cells found in the spleens of mice treated with unmodified autoimmune cells suggests that photoactivation significantly enhances the ability of syngeneic cell populations to inhibit the onset of autoimmune disease in these mice.

Mitogen assays of the T- and B-cell compartments of treated mice and controls demonstrated that B cells from treated mice responded to a slightly lesser degree than controls to LPS stimulation. A significant improvement in the observed T-cell hyporesponsiveness to the mitogen Con A [24,25] was found in the spleens of mice treated with photoactivated autoimmune splenocytes. This increased response was found in the residual T-cell population in treated spleens and may reflect retention of a normal T-cell subset. Alternatively, an autoregulatory T-cell population present in these spleens may have an increased capacity to respond to this mitogen.

These results demonstrate that a similar degree of resolution of the disease process as that achieved by total lymphoid irradiation, cyclosporin A, and monoclonal antibody therapy [11-15,26] can be achieved through the use of autoimmune splenocytes as an immunogen, rendering the recipient capable of inhibiting the fulminant proliferation of abnormal T-cells. Moreover, therapy with photoactivated autologous lymphocytes obviates the necessity for introducing cytotoxic agents and compromising normal immune status.

The role of psoralen and ultraviolet A light in enhancing the immunogenicity of the injected cells may be significant. Injection of cells alone was demonstrated to mediate a somewhat similar effect but mice treated in this fashion died earlier and did not have reduced splenomegaly until late in the treatment protocol. In addition, no normalization of the response to Con A, which may reflect the retention of normal lymphoid cells [24,25], was found in the spleens of mice treated with unmodified cells. Unmodified autoimmune cells were expected to mediate a similar effect to phototreated autoimmune cells if the treatment protocol mediated a response directed towards cells expressing an autoreactive T-cell idiootype. The addition of psoralen and UVA to the reinfused autoimmune cell population served to enhance the effectiveness of this treatment regimen. The partial effectiveness of the injection of unmodified autoimmune splenocytes also suggests that the inhibition of the T-cell hyperplasia in treated mice is not secondary to effects of unmetabolized drug but is dependent on the presence of the intact effector cells. Further support for this contention comes from the experiments using lysed cells treated with psoralen and UVA, where exacerbations of the disease process was noted.

The effect of bystander normal lymphocytes as well as dendritic cells and macrophages that inhabit the spleen has not been studied in these protocols, although their roles may be significant. Previous studies have demonstrated that myeloid cells are relatively resistant to the effects of 8-methoxypsoralen and ultraviolet A light [18]. Their contribution to phototherapy will be the focus of future investigations. In addition, the studies of Andersson et al demonstrated that it was possible to induce specific unresponsiveness to the effector cells mediating skin graft rejection by treating antigen-specific lymphocytes sensitized in vitro with Freund's complete adjuvant prior to injection into syngeneic recipients [27]. The requirement for adjuvant in their experiments may be overcome in our system by the reinfusion of phototreated bystander lymphocytes which gradually die and may release substantial amounts of lymphokines.

Although the underlying mechanism of this mode of therapy remains to be elucidated, the results indicate that this form of immunomodulation is comparable to other types of T-cell directed modalities. Studies performed by Cohen et al [28,29] have demonstrated that injection of inactivated T-cell clones capable of mediating autoimmune disease results in protection against the subsequent induction of the autoimmune process. In a similar fashion, reinfection of uncloned effector cells in young MRL/l mice might permit the development of an autoregulatory response mounted by a relatively intact immune system. Because treatment of autoimmune disease with autologous photomodified lymphocytes is clinically practical, further studies of this form of therapy may be of value in
the management of autoreactive processes as well as a spectrum of T-cell mediated dysfunctions.

REFERENCES