Circulating Lymphocyte Populations in Hodgkin’s Disease After Mantle and Paraaortic Irradiation

By Marshall R. Posner, Ellis Reinherz, Heather Lane, Peter Mauch, Samuel Hellman, and Stuart F. Schlossman

The effect of mantle and paraaortic radiation on peripheral blood lymphocytes was studied in 11 previously untreated patients with early stage Hodgkin’s disease using a series of monoclonal antibodies defining immunoregulatory lymphoid cells. Immediately following the completion of radiotherapy, there was a significant reduction in the number of lymphocytes and the percent of circulating T cells. This acute decrease in T cells was due to a marked diminution in the number of inducer T cells, while the fraction of suppressor T cells remained constant. These changes persisted for up to 12 mo and were accompanied by a later increase in the fraction of circulating B lymphocytes and cells bearing surface Ia. It thus appears that mantle and paraaortic radiotherapy causes a relatively selective reduction in the inducer T-cell population. The implications of a change in the ratio of inducer to suppressor T cells is discussed.

UNTREATED PATIENTS with Hodgkin’s disease have multiple defects in cell-mediated immunity as measured by both in vitro and in vivo tests of T-cell function that worsen with increasing stage of disease.1-3 Following treatment with radiotherapy, these defects become more pronounced and are associated with an increased incidence of viral infections and a decreased ability to respond to vaccination with bacterial antigens.4,5 The etiology of clinical immunosuppression following radiotherapy remains unexplained, although a decrease in circulating T cells, accompanied by a B lymphocytosis and a depression in T-cell function lasting several years has been reported following total nodal irradiation (TNI).6

Recently, a series of monoclonal antibodies have been developed that have been used to describe with precision subpopulations of human lymphoid cells with specific immunoregulatory functions.7-11 Abnormalities in the ratio of inducer to suppressor subpopulations or in the state of activation of these subsets in circulating T cells have been described in patients with autoimmune diseases,12 graft-versus-host disease,13 and immunodeficiency states.14 It is possible, on the basis of these studies, to predict that selective alterations in immunoregulatory subpopulations of T cells may be responsible for the observed immunosuppression following radiotherapy in patients with Hodgkin’s disease. Moreover, recent therapeutic use of total lymphoid irradiation (TLI) as an immunosuppressive agent in rheumatoid arthritis15,16 emphasizes the importance of defining the effects of radiation on immunoregulatory cells of which Hodgkin’s disease may provide a model.

In a previous study we utilized this series of monoclonal antibodies as a probe to analyze lymphoid subsets in patients with untreated Hodgkin’s disease.17 It was found that patients with early stage disease, unassociated with B symptoms, had normal numbers of circulating T lymphocytes and normal percentages of circulating inducer and suppressor subpopulations.

In contrast, patients with B symptoms were found to have decreased numbers of circulating lymphocytes and a decreased percentage of circulating T cells, although they maintained a normal ratio of inducer to suppressor cells. Both symptomatic and asymptomatic patients lacked evidence of activation of peripheral blood T cells as manifested by the appearance of increased numbers of Ia-positive cells. It appears, therefore, that patients with early stage Hodgkin’s disease can provide a unique model to study the immunosuppressive effects of radiotherapy on lymphoid subsets. In the present study we report the effect of radiotherapy on circulating lymphoid populations within the first 4 and 12 mo after irradiation in early stage Hodgkin’s disease. In the results reported, it will be shown that radiotherapy to mantle and paraaortic areas resulted in significant changes in the number of circulating T cells and the ratio of inducer to suppressor cells.

MATERIALS AND METHODS

Eleven previously untreated patients were studied. The histologic diagnosis of Hodgkin’s disease was confirmed, and the subtypes were identified according to the Rye modification of the Lukes and Butler classification. Patients were staged according to the Ann Arbor Clinical Staging Classification. All patients underwent staging laparotomy and splenectomy as part of their evaluation. Patients received 3600–4000 rad to mantle and 3600–4000 rad to a paraaortic-splenic pedicle field, each over 4 wk, with a 2-wk interruption between fields. The clinical characteristics of these patients are summarized in Table 1.
After informed consent, venous blood was obtained in heparinized syringes prior to and after radiation therapy in each patient. Peripheral blood mononuclear cells were separated by sedimentation over Ficoll-Hypaque, frozen under liquid nitrogen, and thawed for analysis.

Monoclonal antibodies with well-defined specificities were used. Anti-T3, anti-T4, and anti-T8 react with 100% of peripheral blood T cells (T\(^3\)), the inducer (T\(^4\)) and the suppressor (T\(^8\)) subsets, respectively. \(^6\) Anti-Ia reacts with the bimolecular glycoprotein complex representing the common framework of Ia in man. \(^9\) Mol and B1 react with circulating peripheral blood monocytes and B cells, respectively. \(^10\), 11

After thawing, cells were prepared for cytofluorographic analysis as previously described. \(^12\) In brief, after suspension of 10\(^6\) cells in SMEM with 5% pooled AB serum, the cells were pelleted, treated with 0.1 ml of monoclonal antibody, and incubated at 4°C for 30 min. Cells were washed three times and treated with 0.1 ml of fluorescein-conjugated goat anti-mouse immunoglobulins. The cells were incubated for 30 min at 4°C and washed an additional 3 times. Analysis was performed on a cytofluorograph (Ortho Diagnostic Systems, Westwood, Mass.). The intensity of fluorescence per cell was recorded on a pulse-height analyzer and a histogram was generated. Background was obtained by substituting 0.1 ml of a nonreactive monoclonal antibody, and the percent of cells showing fluorescence above this background with each monoclonal antibody was calculated using 10,000 cells/sample.

RESULTS

As shown in Table 2, pre- and postsplenectomy samples from patients did not differ significantly in either the number of circulating lymphocytes, the T4:T8 ratio, or in the percentages of the lymphoid subsets represented, confirming previously reported results. \(^13\) In the initial 4 mo following radiotherapy, there was a significant decrease in the number of circulating lymphocytes and an accompanying decline in the absolute number and percent of T3\(^+\) cells, mostly accounted for in the inducer (T4\(^+\)) population. The absolute number and percent of T8\(^+\) cells did not change significantly during this period, while the T4:T8 ratio declined significantly. The fraction of Ia-positive cells rose significantly. There was also an increase in the percentage of circulating Mol\(^+\) cells and B cells after radiotherapy that was not significant.

In patient samples examined between 5 and 12 mo after radiotherapy, there was a further decline in the number of circulating lymphocytes, but this was not significantly different from the immediate postirradiation period. The significant decrease in the fraction of T3\(^+\) and T4\(^+\) lymphocytes persisted, and although the fraction of T8\(^+\) (suppressor) cells declined, this was not significantly different from the preirradiation samples. There was, however, a significant decrease in the absolute number of T8\(^+\) cells compared to both preirradiation periods accompanying the further decrease in lymphocytes. The T4:T8 ratio was again significantly different then pre- or postsplenectomy values. The fraction of circulating Ia\(^+\) and Mol\(^+\) cells also remained elevated. The percentage of circulating B cells increased significantly compared to preirradiation samples and also when compared to the early postirradiation period, although there was no significant increase in the absolute numbers of circulating B cells.

DISCUSSION

We have utilized monoclonal antibodies to study the effects of radiotherapy to mantle and paraaortic areas on lymphoid populations in patients with early stage Hodgkin's disease. A significant reduction in the number of lymphocytes and the percent of circulating T cells occurred acutely following irradiation. Within the T-cell population there was a relatively selective diminution in the inducer subset that persisted for more

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Status</th>
<th>Patients</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range)</td>
<td>32 (12-63)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td>1 IA, 6 IA, 3 IIA</td>
<td></td>
</tr>
</tbody>
</table>

NS, nodular sclerosing; LP, lymphocyte predominant; LD, lymphocyte depleted; MC, mixed cellularity.

### Table 2. Results of Subset Analysis Over the Course of Treatment

<table>
<thead>
<tr>
<th>Status</th>
<th>Number</th>
<th>Lymphocytes/cu mm</th>
<th>T3</th>
<th>T4</th>
<th>T8</th>
<th>Ia</th>
<th>Mol</th>
<th>B1</th>
<th>T4:T8 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postsplenectomy</td>
<td>9</td>
<td>1,643 ± 162</td>
<td>53 ± 3</td>
<td>30 ± 3</td>
<td>25 ± 4</td>
<td>11 ± 1</td>
<td>15 ± 5</td>
<td>4 ± 1</td>
<td>2.08 ± .88</td>
</tr>
<tr>
<td>Postirradiation</td>
<td>5</td>
<td>2,253 ± 541</td>
<td>50 ± 5</td>
<td>32 ± 5</td>
<td>19 ± 2</td>
<td>12 ± 2</td>
<td>12 ± 4</td>
<td>6 ± 2</td>
<td>1.58 ± .30</td>
</tr>
<tr>
<td>(1-4 mo)</td>
<td>9</td>
<td>1,124 ± 198†</td>
<td>29 ± 7†</td>
<td>11 ± 2†</td>
<td>25 ± 5</td>
<td>25 ± 2†</td>
<td>22 ± 3</td>
<td>8 ± 1</td>
<td>.45 ± .10†</td>
</tr>
<tr>
<td>Postsplenectomy</td>
<td>5</td>
<td>762 ± 110†</td>
<td>25 ± 6†</td>
<td>14 ± 4†</td>
<td>16 ± 6</td>
<td>28 ± 1†</td>
<td>19 ± 5</td>
<td>17 ± 2†</td>
<td>1.06 ± .19†</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SEM. Subset results are expressed as percent positive.
†Significantly different from presplenectomy and postsplenectomy; p < 0.05 (Mann-Whitney-Wilcoxon test).
‡Significantly different from all other groups; p < 0.05.
than 5 months. In contrast, the fraction of T cells in the suppressor subpopulation was not significantly different from pretherapy levels. The reduction in T cells was accompanied by a later significant increase in the fraction, but not the absolute number, of B cells and an associated increase in Ia+ cells.

Previous studies of T cell function in patients with Hodgkin's disease following radiotherapy have shown defects in the ability of T cells to respond in vitro to T cell receptor (TCR)/CD3+ complexes and TCR/CD3+ antibodies. Moreover, clinical evidence of immunosuppression as manifested by an increased incidence of viral infections and decreased antibody response to bacterial vaccines has been reported in patients following radiotherapy.6 8 This is not surprising, given the decrease in the T4+ or CD4+ population observed in these patients and the central role, as described in previous studies, of this subset in the immune response. It has been shown that the T4+ population is required for the production of immunoglobulins in the pokeweed mitogen driven system and for the development of cell-mediated lymphocytotoxicity and concanavalin-A-induced suppression in which the T8+ population is the final effector.7 8

Major alterations in the T4+ and T8+ populations and changes in the normal ratio of these subsets have been reported in autoimmune and immunodeficiency diseases.1,2,4,9 In the former, decreased suppression or an increased inducer population, and in the latter, increased suppression or loss of an inducer population, have been observed. Resolution of these changes occurs with resolution of the disease.1,2 This indicates that changes in immunoregulatory subpopulations that occur either spontaneously or as a result of therapy may have a significant impact on these diseases.

The etiology of radiotherapy-induced alteration in lymphoid subsets observed in the present study may be explained by one of several different mechanisms. First, the T4+ and T8+ populations appear to be distributed differently throughout the body. T4+ cells predominate in lymph nodes and the peripheral circulation, while T8+ cells appear predominant in the gut epithelium and the bone marrow.20 21 Both TLI and mantle and paraaortic radiation are directed primarily at the thymus and lymph nodes. The profound effects seen on inducer cells, therefore, may be attributable to effects of radiotherapy on the microenvironment occupied by T4+ cells. Differences in turnover of these subsets might also explain the differential effects of radiotherapy.

Alternatively, radiotherapy may activate a population of suppressor cells. In this regard, animal studies have demonstrated that TLI in NZB/NZW mice results in virtual elimination of autoimmune disease, and in BALB/c mice induces tolerance to foreign proteins as well as prevents the occurrence of graft-versus-host disease in bone marrow transplants.22 23 Moreover, studies of patients undergoing bone marrow transplantation have demonstrated that during immunologic recovery, a marked reversal of the normal T4+/T8+ ratio occurs early in the course of bone marrow recovery and is associated with evidence of activation of T cells as manifested by the presence of increased numbers of Ia+ T cells in the circulation of these patients.13,24-26

Recently, on the basis of the results of animal studies and the apparent lack of marked toxicity of TLI in patients with Hodgkin's disease, TLI has been used as therapy for patients with severe rheumatoid arthritis unresponsive to other forms of treatment.15,16 A reduction of symptoms occurred in these patients, accompanied by a marked reduction in circulating inducer T cells and a reversal of the normal ratio of inducer to suppressor cells. Even though these patients have dissimilar aberrations of immunoregulatory cells, the present study demonstrates that radiotherapy reduces the number of circulating T cells and reverses the normal ratio of inducer to suppressor cells in patients with Hodgkin's disease. Moreover, this depression in the numbers of inducer T cells occurs after patients receive only mantle and paraaortic-splenic pedicle irradiation rather than more extensive total lymphoid irradiation.

The present study demonstrates that radiation therapy to mantle and paraaortic-splenic pedicle areas in patients with Hodgkin's disease results in a significant, prolonged alteration in T-cell populations. These findings support the notion that a significant alteration in the ratio of inducer to suppressor cells contributes to the immunosuppression seen in these patients after radiotherapy.

REFERENCES


Circulating lymphocyte populations in Hodgkin’s disease after mantle and paraaortic irradiation

MR Posner, E Reinherz, H Lane, P Mauch, S Hellman and SF Schlossman