

Early and Late Effects of Irradiation for Seminoma Testis on the Number of Blood Lymphocytes and their B and T Subpopulations

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Summary

High voltage irradiation of the iliac and paraaortic lymph nodes for seminoma testis induces a grave, acute blood lymphocytopenia. A major part of this effect is probably caused by irradiation of the blood. Indications are presented that B lymphocytes are more gravely affected than T lymphocytes. It is possible that the treatment leaves a relatively radioresistant subpopulation of lymphocytes, mostly consisting of T lymphocytes.

The total number of blood lymphocytes is normalized five to ten years after treatment. Six months after treatment the percentage of B lymphocytes is clearly higher than normal, remaining so for the first three years. The percentage of T lymphocytes is unchanged both during therapy and at all intervals thereafter. The results therefore indicate that the number of B lymphocytes is more rapidly normalized after radiotherapy than that of T lymphocytes, and that the post-irradiation lymphocytopenia is mainly a T lymphocytopenia. However, this shift in B/T lymphocyte ratio is not permanent, being completely or nearly normalized after ten years. This pattern of regeneration seems to be essentially the same in patients who have received additional treatment to the mediastinum, probably meaning that the thymus has only a marginal influence on the regeneration of T lymphocytes in the adult organism. It is possible that the rapid recovery of B lymphocytes relates to the ability of the bone marrow to extend into non-irradiated parts of the skeleton after radiation destruction.

During recent years several workers have reported that high-energy irradiation for various forms of cancer and for mb.Bechterew leads to blood lymphocytopenia (3, 6, 19). Conflicting results have been reported on the contribution of T and B lymphocytes to this (1, 2, 19), but present knowledge favours the view that B lymphocytes are most affected during the acute phase.

From our laboratories it has been reported (8) that patients irradiated for Hodgkin's disease up to 3 years ago have a significantly greater fraction of B lymphocytes in peripheral blood than healthy subjects and untreated patients. T-cells were not recorded, but the findings might indicate a more rapid and complete post-irradiation regeneration of B than T lymphocytes, causing a shift in blood lymphocyte subpopulations which might be permanent. This observation cannot be given general validity because the thymus had been included in the field and most of the patients had been splenectomized. In addition, patients with Hodgkin's disease may have an abnormal lymphocyte response to radiotherapy.

The present investigation was initiated for the following purposes:

1. To describe the acute changes in numbers of circulating lymphocytes in patients with solid tumours during radiotherapy in fields not including the thymus.
2. To clarify whether irradiation in patients with solid tumours causes the same chronic shift in lymphocyte subpopulations as in Hodgkin's disease.
3. To assess if such a shift is permanent and whether it occurs also in patients where the thymus has not been included in the radiation field.

Patients with seminoma testis were selected for the study because most of them are relatively young, they have a relatively good prognosis and can be observed for years, and the irradiated field is comparable to that given to patients with Hodgkin's disease.

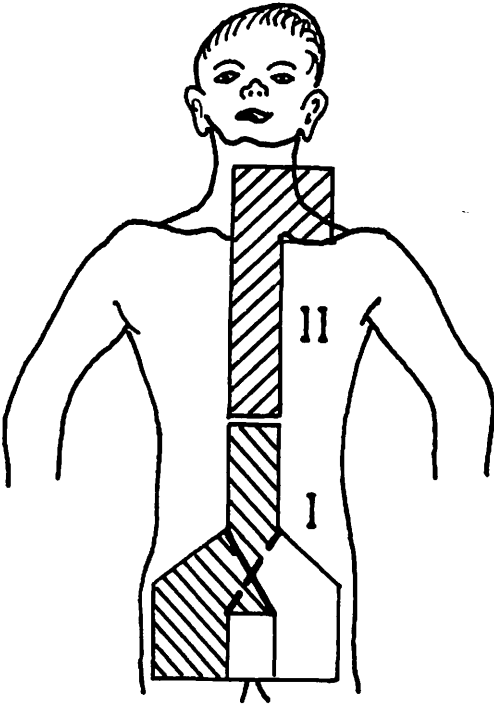


Fig. 1 Fields of irradiation. Field I, including a right or left iliacal part, was given to all patients (groups A, B and C). Field II was given only to group C patients.

Patients and Methods

Three groups of patients were studied: *Group A* consisted of seventeen men studied before, during and immediately after their first course of radiotherapy. A total dose of 4000 rad was delivered to the iliacal and paraaortic lymph nodes by a 7 MeV linear accelerator in fractions of 200 rad a day five days a week (Fig. 1). The dose rate was about 70 rad/min. In all the patients orchietomy had been carried out 3-4 weeks before the start of irradiation. The secondary dose to the thymus was estimated to be about 4% of the field dose.

Group B included sixteen men who had been given similar treatment up to ten years ago. Some of these had received betatron irradiation at a somewhat lower dose rate, but with the same daily, total and secondary doses.

Group C included fourteen men who had been treated in the same way as group B, but who had received treatment to the mediastinum with the same doses six to eight weeks later.

Time of treatment for groups B and C is shown in Table 1.

All patients in Groups B and C were studied retrospectively. They were all clinically well and without signs of recurrence.

Table 1 Numbers of patients observed at each time interval after therapy

Time since therapy (years)	1/2	2	3	5	10
Group B	3	3	3	3	3
Group C	4	2	3	2	3
Total number forming mean values of fig. 2 and 3	7	5	6	5	6

Fasting blood samples were collected in the morning, before the patients got out of bed. Blood leukocytes were counted by an electronic white cell counter. Differential counts were performed on 400 cells. Lymphocytopenia was defined as < 1500 lymphocytes/mm³. Lymphocyte subpopulations were determined in suspensions of mononuclear cells separated from heparinized blood according to Boyum (4). Macrophages were not removed. The following tests were performed:

E-rosette forming cells: The spontaneous rosette test with sheep erythrocytes (SRBC) was used to detect T lymphocytes as described by Jondal et al. (14) and by Froland (10). Briefly, the mixture of SRBC and mononuclear cells was spun down and kept at 4°C overnight, and then the pellet was carefully resuspended by the use of a Pasteur pipette. No serum was present.

200 cells were counted in each preparation. It has been shown later that this modification of the test is not optimally sensitive and reproducible (13), but since the test has not been shown to favour any subpopulation of T lymphocytes, this modification will express the real percentage of T lymphocytes proportionately. Normal controls had 20-40% E-rosette forming cells.

EAC-rosette forming cells: This test was applied to detect cells with receptors for complement factor C'3 on their surface, as described in detail by *Jondal et al.* (14). Some of these cells are B lymphocytes, while others are monocytes-(15, 17). Briefly, SRBC were sensitized with rabbit-anti-SRBC and incubated with mouse complement (frozen-thawed mouse serum). The sensitized SRBC were mixed with the suspension of mononuclear cells and spun down, and after 14 minutes of incubations at room temperature the pellet was vigorously resuspended by a whirl-mixer. 200 cells were counted in each preparation. Normal controls had 20-40% EAC-rosette forming cells.

Ig-positive cells: This test was used to detect B lymphocytes. The mononuclear cells were stained in suspension with a fluorescein isothiocyanate labelled antiserum directed against the F(ab')₂ fragment of human IgG, as described in detail by *Froland and Natvig* (11). This test was carried out without knowledge of the rosette percentages. In normal controls the proportion of Ig-Positive cells was 4-20%.

Results

Group A: During treatment the average lymphocyte number fell from about 2200/mm³ to less than 500 (fig. 2). The mean percentage of Ig-positive dropped from 20 to 4, while the mean percentage of E-rosette forming cells remained constant or increased (Fig. 3). The percentage of EAC-rosette forming cells also tended to increase (fig. 3).

Groups B and C: The number of patients in these groups is too small for separate statistical analysis. Fig. 2 shows that patients studied six months after irradiation were still lymphocytopenic. A gradual increase of the total number of circulating lymphocytes was found with time after irradiation, and in patients studied five and ten years after the treatment, the total lymphocyte number was again normal.

There was no evident difference between the total lymphocyte numbers of groups B and C, although the latter tended to have lower values.

The proportions of identifiable subpopulations of lymphocytes and monocytes six months to ten years after treatment are shown in fig. 3.

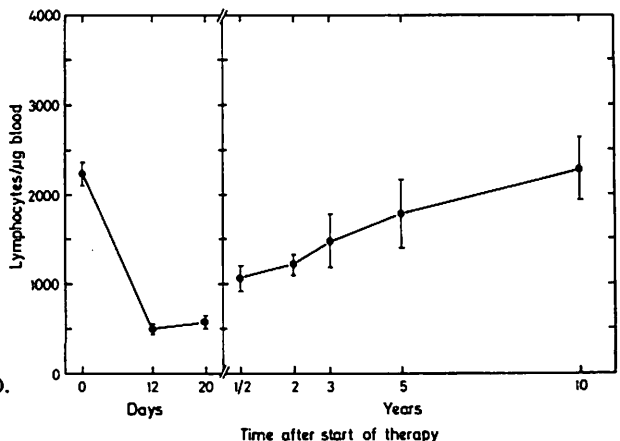


Fig. 2 Mean total lymphocyte counts at the different intervals after therapy \pm 1 SD. Left: Group A. Right: Mean of Group B and C

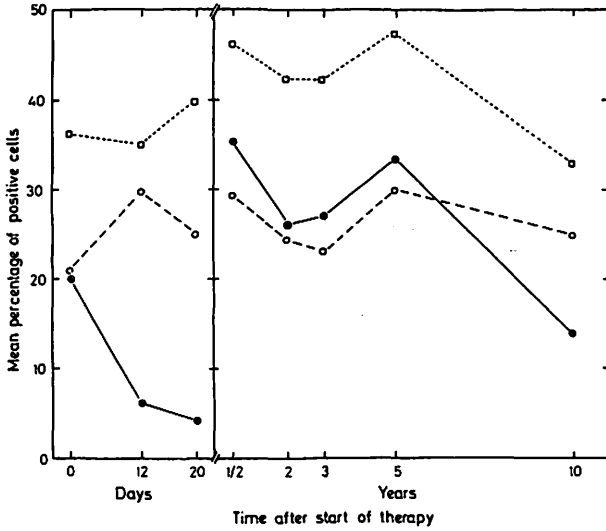


Fig. 3 Mean percentages of E-rosette forming lymphocytes (o - - o). Ig-positive lymphocytes (● — ●) and of mononuclear cells with receptors for mouse complement (EAC-rosette forming cells) (□ - - □). Left: group A patients. Right: Mean of groups B and C patients

The percentage of Ig-positive was higher than normal in patients studied six months to five years after treatment, but was normal at ten years. Group C tended to have somewhat higher values than group B, but the differences were small and not present at all intervals.

The percentage of E-rosette forming cells remained at a normal level at all intervals studied. No systematic difference was found between groups B and C.

The percentage of EAC-rosette forming cells was somewhat higher than in group A before treatment at all intervals, except at ten years. No systematic difference was found between groups B and C.

Discussion

The present investigation shows that irradiation of the iliac and paraaortic lymph nodes with cancericide doses induces a grave lymphocytopenia which is about maximal at an accumulated dose of 2400 rad (day 12). The Ig-positive cells are reduced relatively more than the E-rosette forming cells, while the percentage of EAC-rosette forming cells tends to increase. This is in accordance with the findings of *Blomgren et al.* (2) and parallel to those of *Stjernsvard et al.* (19), supporting the view that B lymphocytes are more radiosensitive *in vivo* than are T lymphocytes. The increase of the EAC percentage is probably due to a relative radioresistance of circulating monocytes.

The lymphocyte drop during irradiation of group A patients may have several causes: The irradiation of several lymph nodes and large bone marrow areas, a secondary effect on the thymus, and finally the exposure of the circulating blood.

At rest, 2.5 l. of blood are entering and leaving radiation field I each minute (9). We have estimated that this field contains about 500 ml. of blood at rest. The blood in the field is then changed completely each twelfth second, and since the dose rate is 70 rad/min., the mean transit dose* is about 15 rad. During each therapeutical seance the whole blood volume will receive 20-25 rad, and when the total therapeutic dose has reached 2400 rad, the whole blood volume has received 240-300 rad. *In vitro* such a dose is lethal to a high proportion of the lymphocytes exposed to it as a single dose (21). In several animals (5, 7, 20) and in man (22), extracorporeal irradiation of the blood brings about a prompt exponential decline of the

*Transit dose: Dose received by any object passing through the radiation field at the speed of the blood

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number of blood lymphocytes at a transit dose of 15 rad or lower (18, 22). During extracorporeal blood irradiation the number of lymphocytes stabilizes at 20-30% of the number before treatment, as seems to happen also in the group A patients. We feel, therefore, that a major part of the lymphocytopenia in the group A patients can be explained by a direct effect on the circulating blood. Probably irradiation of lymphocytes circulating in the lymphatics also plays a part (5).

If it is right that the number of lymphocytes is quite stable during the last part of the treatment schedule, a selection of a relatively radio-resistant subpopulation of lymphocytes may be taking place. Our data indicate that this possible subpopulation consists mostly of T lymphocytes.

The retrospective study of irradiated patients shows that the lymphocytopenia lasts for years and indicates that blood B lymphocytes recover more rapidly than T lymphocytes. This is in accordance with our previous observations in patients with Hodgkin's disease (8) and are parallel to those induced by irradiation for mammary carcinoma (19). Our data indicate that the shift in B/T lymphocyte ratio induced by the radiotherapy is not permanent, but is normalized after five to ten years. Only marginal differences were found between the groups B and C, suggesting that irradiation of the thymus does not influence the main pattern of recovery. It must be emphasized that the response and influence of the thymus might be different when children are irradiated.

Evidence is available (12) that the T cell population can sustain itself for a long time *in vivo*, probably meaning that the net production of new T lymphocytes is quite low in the unirradiated adult organism. If no feed-back mechanisms exist which can increase the T lymphocyte production, this may be the main reason why T lymphocytes are so slowly reproduced.

Extensive radiotherapy is followed by bone marrow extension into non-irradiated parts of the skeleton (6). If the bone marrow is the bursa analogue in man (14), the organism can probably replace its destroyed capacity for B lymphocyte production rapidly after the present treatment schedule, leading to a relatively early recovery of the number of circulating B lymphocytes.

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In conclusion, irradiation for seminoma testis leads to a blood lymphocytopenia, which is relatively more pronounced for B than for T lymphocytes. Monocytes seem to be less reduced than T lymphocytes, causing a shift in B/T lymphocyte ratio, which is long-lasting, but not permanent, and which seems to be parallel to that described after irradiation for Hodgkin's disease. Additional irradiation including the thymus does not change the main pattern of regeneration.

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Lymphography in Childhood: Six Years Experience with 242 Cases

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Summary

From January, 1969 — December, 1974, 242 children less than 15 years of age underwent lymphography at the National Cancer Institute, Milan. Successful lymphatic cannulation was accomplished in 97% (440/463) of the sites where it was attempted. No major or permanent complications were encountered, although minor untoward effects might not have been recorded. In those children undergoing biopsy of opacified lymph nodes, lymphographic-histologic correlation was 98% (45/46).

Nonspecific reactive hyperplasia lymphographic patterns were encountered in 36% of all studies, confirming its high incidence in the pediatric age group.

This study has shown that lymphography in childhood can be as readily performed as in the adult and that its diagnostic accuracy is acceptable. As in adults, it is useful in treatment planning, evaluating results of therapy, and detecting recurrent tumor. The frequent occurrence of nonspecific reactive hyperplasia in the pediatric lymphogram should not be mistaken for evidence of tumor, particularly lymphoma.