Shimane J. Med. Sci. Vol. 4, pp. 23—30, 1980

Effects of Cyclophosphamide on Lymph Nodes and Pyroninophilic Cells

(Cyclophosphamide effects/lymph nodes/pyroninophilic cells)

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(Received December 28, 1979)

Cyclophosphamide was given intraperitoneally to guinea pigs in a dose of 10 mg/day for a period of 5 days and the histological changes were studied on the lst, 5th and 10th days after the administration. Changes in number of pyroninophilic cells due to DNCB sensitization were also studied. On the lst day after discontinuation of Cyclophosphamide, the germinal centres, lymph follicles and paracortical areas of the lymph nodes all showed the same degree of cell component loss, but on the 10th day, only the germinal centres had been restored to their normal level, while the other sites showed a delay in recovery. The administration of Cyclophosphamide prior to DNCB sensitization resulted in a greater increase in pyroninophilic cells in the paracortical area of the regional lymph nodes than in the controls, between 7 to 13 days after sensitization. No increase was noted in the group that was given the drug after sensitization. Thus decrease in the lymph node component was accounted for by the B-cells which are susceptible to the effects of Cyclophosphamide, and the increase in pyroninophilic cells was attributed to this marked decrease in B-cells.

Lymph nodes play a major role in the development of contact sensitization in that there is swelling of regional lymph nodes (1) and the infliction of various types of damage to the lymph nodes inhibits the development of contact sensitization (2).

As Cyclophosphamide acts upon actively dividing cells, it is a substance being widely used as an anti-tumor preparation. There are reports that the administration of this drug prior to the development of sensitization enhances inflammation (3, 4). In this study, the effects of Cyclophosphamide on lymph nodes were observed, the changes in the number of pyroninophilic cells were monitored, and the relationship of the two discussed.

MATERIALS AND METHODS

White male guinea pigs weighing 250—300 g were used. Cyclophosphamide

in 100 mg vials was administered after dissolution in physiological saline.

Lymph node observations were made on 20 guinea pigs separated into groups of 4.

Group 1—Untreated

Group 2—Cyclophosphamide 10 mg/day was given i. p. for 5 days and lymph nodes were removed 1 day after discontinuation of administration

Group 3—Removed 5 days after discontinuation

Group 4—Removed after 10 days.

Two mesentery lymph nodes were removed, stained with H-E, PAS and Gomori's silver stains. The number of cells in the germinal centres, and the lymphocyte counts in the lymph follicles and paracortical areas were compared under a microscope. A special study of the paracortical area was made by comparing the findings derived from PAS and Gomori's silver stains.

Observation of the pyroninophilic cells in the paracortical area of the regional lymph nodes was made using 32 guinea pigs separated into groups of 3 to compare findings in a group given Cyclophosphamide prior to sensitization and a group given the drug immediately upon development of sensitization : Group 1 (12 animals) — untreated, Group 2 (12)—sensitized after 10 mg intraperitoneal administrations of Cyclophosphamide were commenced immediately after sensitization for a period of 5 days. To these animals 0.1 ml of DNCB acetone solution was applied bilaterally to the thighs. In Groups 1 and 2, the lymph nodes in the bilateral inguinal region in 2 animals each were removed prior to sensitization and 1, 4, 7, 10 and 13 days after sensitization, while in Group 3 removal was at 1, 4, 7 and 10 days after sensitization. The specimens were stained with Methylgreen-Pyronin and the pyroninophilic cells in the paracortical area were observed. The method used was to randomly select 100 sites in the paracortical area, to observe 100 cells and to count the number of pyroninophilic cells.

RESULTS

A slight variation in the number of lymphocytes was noted in the germinal centres, lymph follicles and paracortical area of the control group. After the discontinuation of Cyclophosphamide, a marked decrease in lymphocytes and the presence primarily of reticular cells were observed in the germinal centres on the lst day. On the 5th day, a slightly hyperplastic state was seen, and on the 10th day, the condition was much the same as that seen in the controls (Figs. 1—4).

Findings similar to those seen in the germinal centres were noted in the lymph follicles and paracortical area on the lst day, but by the 5th and 10th days the number of lymphocytes had gradually increased, however, the number was still less than that seen in the controls. In each animal, there was a slight variation in these findings (Figs. 1 —4).

Study of changes in the number of pyroninophilic cells in the paracortical area of the regional lymph nodes due to Cyclophosphamide injected prior to and immediately after sensitization, showed that in the controls, the mean numbers of cells were 1.7 prior to sensitization, 1.5 on the lst day after sensitization, 8.2 on the 4th day, 4.8 on the 7th day, 2.4 on the 10th day, and 2.0 on the 13th day, indicating that the peak was reached on the 4th day and that normal levels were reverted to by the 10th day. In contrast, in the group given Cyclophosphamide prior to sensitization, the mean numbers of cells were pre-sensitization 1.5 and 1.6 after sensitization on the lst day, 9.9 on the 4th day, indicating an increase over the controls, with a statistically significant difference, at the 1% level, after the 7th day. In the group injected immediately after sensitization, the mean numbers of cells were 0.8 on the lst day after sensitization, 1.0 on the 4th day, 2.2 on the 7th day, and 1.2 on the 10th day, that is, no increase. On the 4th and 7th days, there were significantly greater differences in decrease between the controls at the 1% level and also at the 5% level on the 10th day (Fig. 5).

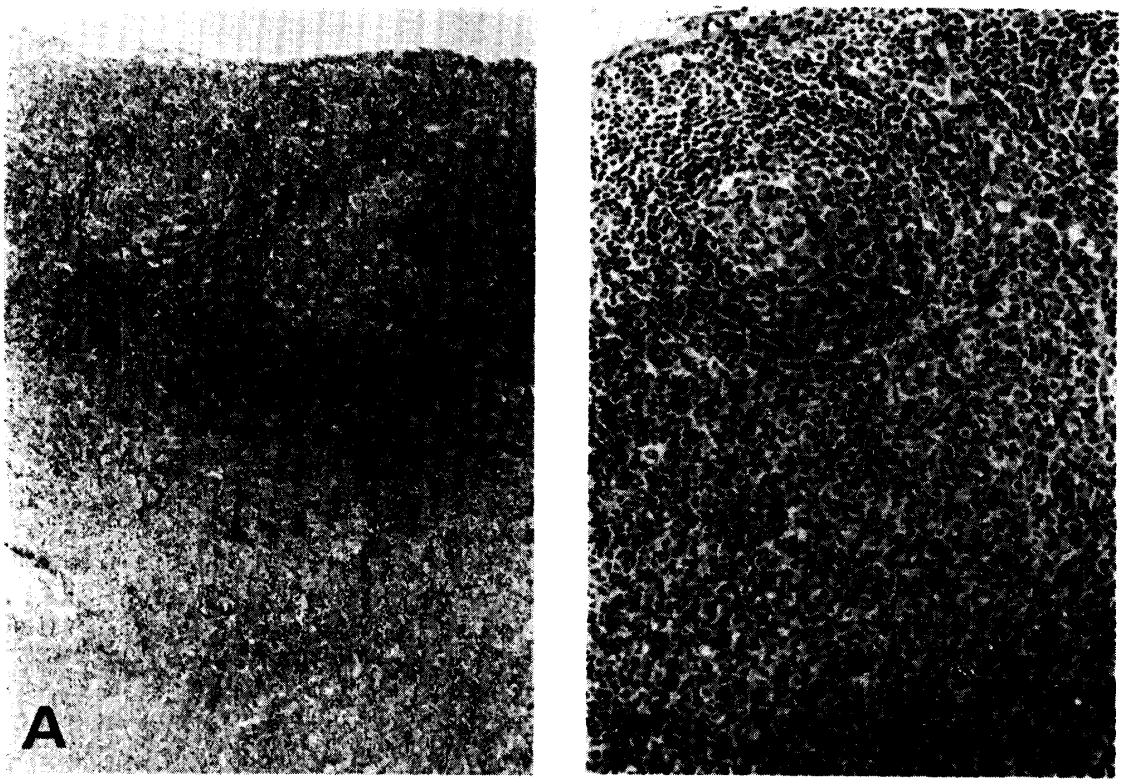

Fig. 1. Control group. Photos of the lymph follicles, germinal centres and paracortical area. A (×32) B (×80)

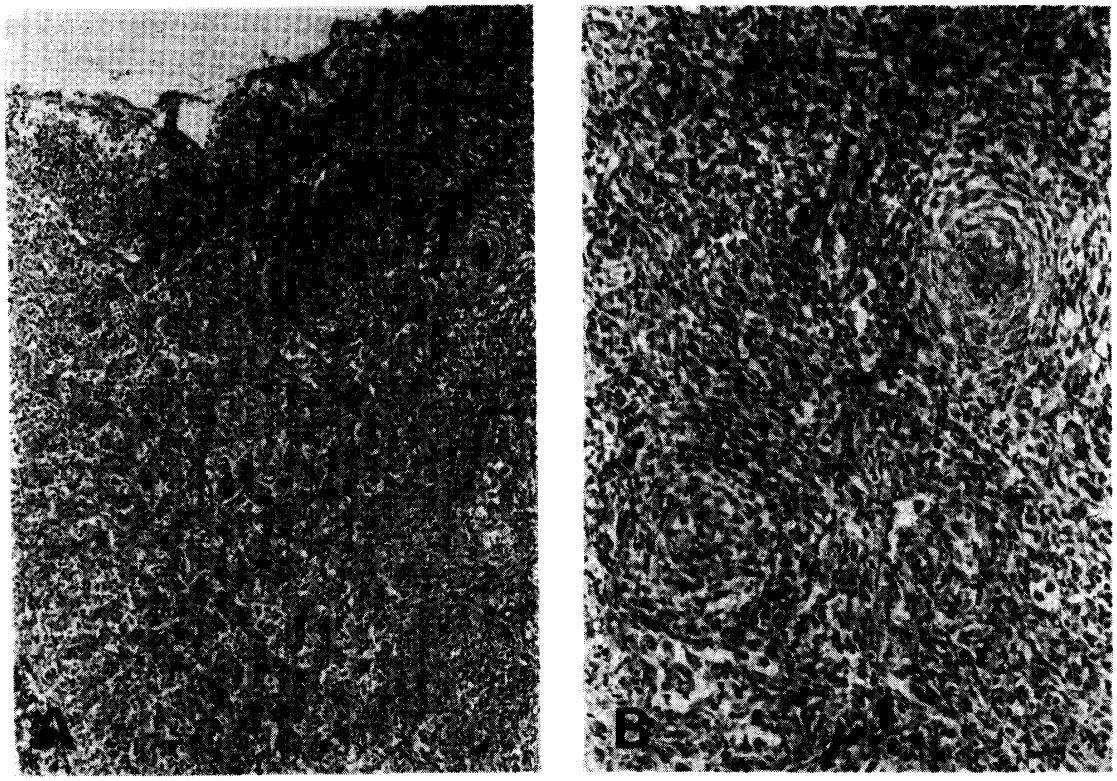

Fig. 2. Lymph node findings—1 day after discontinuation of Cyclophosphamide i. p., 10 mg/daily ×5. A generalized marked decrease in lymphocytes can be seen while reticular cells are evident in the germinal centres. A (×32) B (×80)

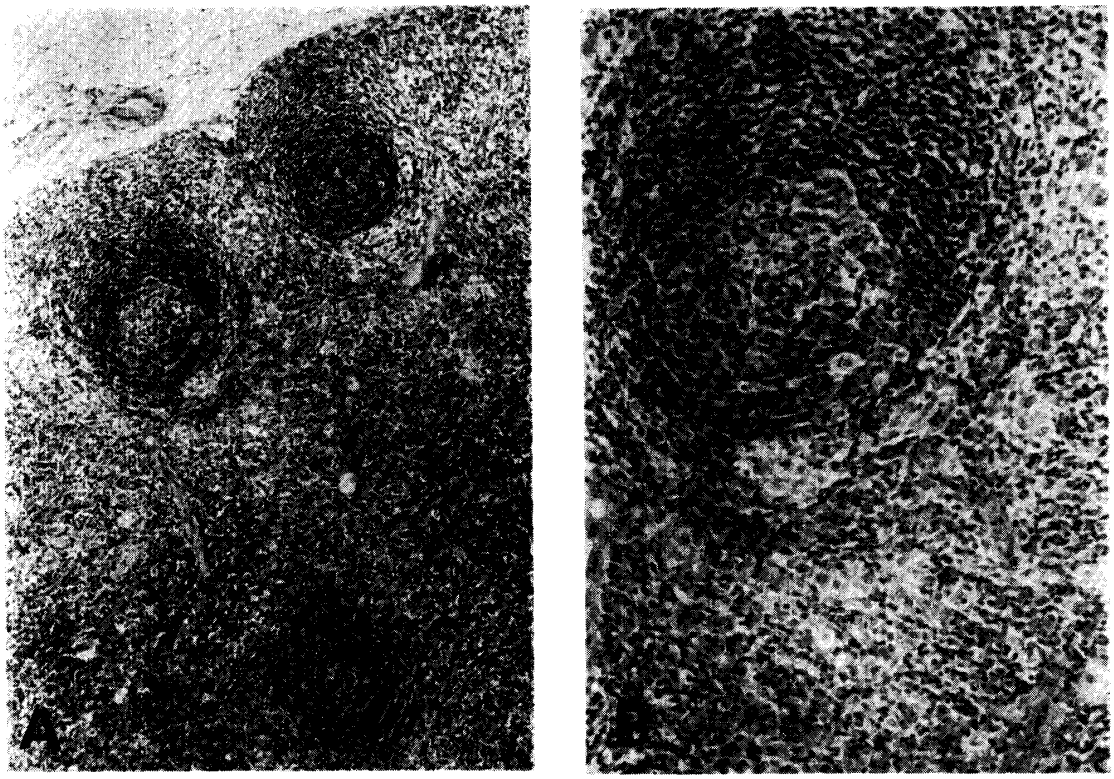

Fig. 3. Lymph node findings—5 days after discontinuation of Cyclophosphamide i. p., 10 mg/daily ×5. The germinal centres present a rather hyperplastic pattern, but in general the numbers of lymphocytes are less than in the controls. A (×32) B (×80)

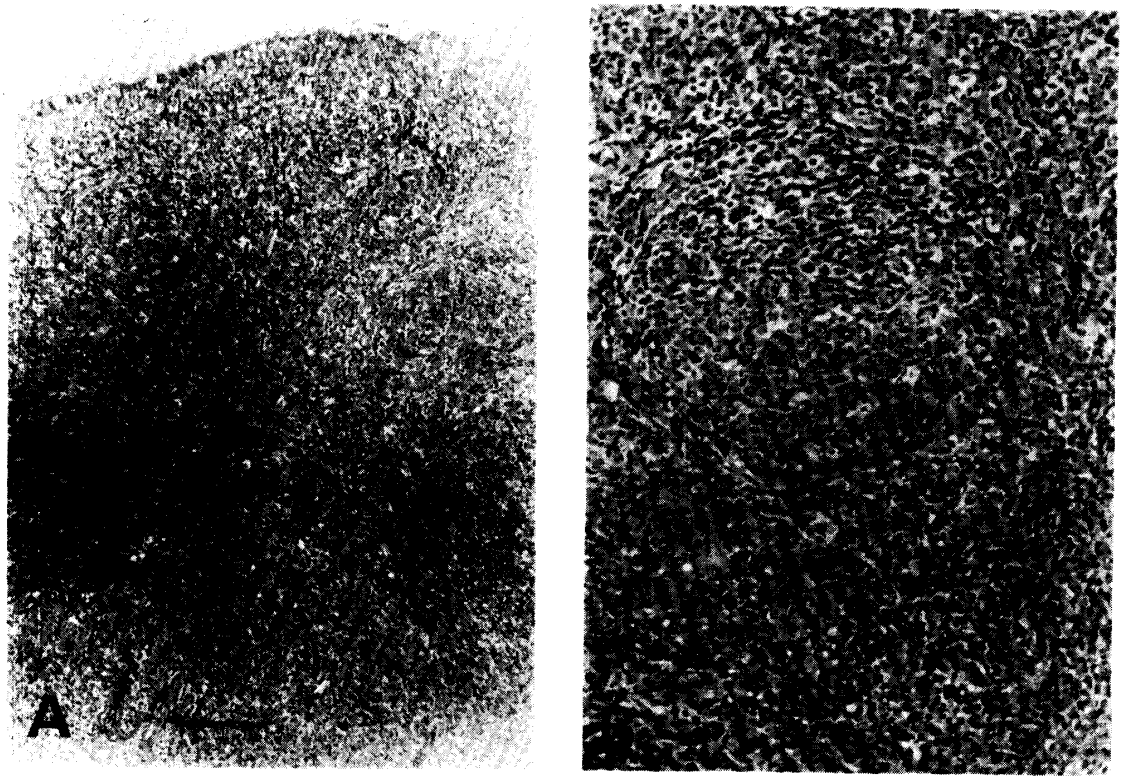

Fig. 4. Lymph node findings—10 days after discontinuation of Cyclophosphamide i. p., 10 mg/daily ×5. The number of lymphocytes is slightly less than the controls, but the number in the germinal centres is approximately the same. A (×32) B (×80)

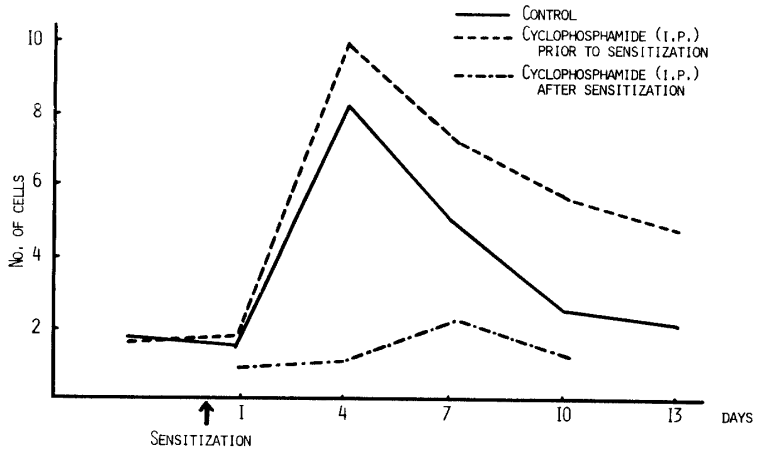

Fig. 5. Changes in the number of pyroninophilic cells in the paracortical area of the regional lymph nodes.

DISCUSSION

Assuming that there would be some difference in the effects of Cyclophosphamide, depending on the site within the lymph nodes, the three sites of germinal centres, lymph follicles and paracortical area were observed. A similar degree of damage was noted in all sites on the lst day after discontinuation of injection. In a similar study Turk and Poulter (5), found that the paracortical area in particular is not affected, but that this site is markedly affected when neonatal thymectomy is performed in mice, and in guinea pigs when injected with Cyclophosphamide after being treated with anti-lymphatic serum. On the 5th day, increases in cells were noted only in the germinal centres, but by the 10th day the number had reverted to normal levels. However, recovery in the lymph follicles and paracortical area was incomplete. This is consistent with the finding that the recovery of peripheral lymphocytes was slightly delayed in comparison to that of the granulocytes (6). Further, Revell who performed histological studies on the long term effects of Cyclophosphamide treatment in guinea pigs also reported a decrease in lymphocytes while the reticular cells remained unchanged. He pointed out that there was a marked decrease, particularly in B-cells (7). Todome reported similar findings (8). The reports of Fanci *et al.* (9) and Katz *et al.* (10) also confirm that the B-cells are affected by Cyclophosphamide.

In contact sensitization, a large number of pyroninophilic cells appeared in the paracortical area of the regional lymph nodes and divided into small lymphocytes within several days. These cells are said to possess the so-called "cell antibody". A study was made of the effects on these cells. In the control group, the number reached its peak on the 4th day after sensitization and on the 10th day decreased to about the pre-sensitization level (11). This finding is consistent with findings of Turk who used oxazolone application in which the number increased on the 3rd, 4th and 5th days, but decreased markedly on the 9th day (11). Also, Oort and Turk made determinations using autoradiography and noted many cells with an increased ^{3}H-thymidine uptake in the paracortical area, and in which peak levels were seen on the 4th day (12). Many other studies have been conducted such as staining of specimens with methyl green and determining the lymph node weight and DNA-P and RNA-P values as reported by Fujita (1), changes in pyroninophilic cell number at the lst and 2nd sensitization, as presented by Wiest and Jung (13) and in-vitro confirmation studies by Okamoto (14). Our study was undertaken because there are apparently no reports on such effects seen with Cyclophosphamide administration. The group injected with Cyclophosphamide prior to sensitization showed an increase in cell number over the controls on the 7th, 10th and 13th days after sensitization, and such indicates that there are more cells with antibody and that these cells remain present for a comparatively long period of time.

Study of the effects of Cyclophosphamide on lymph nodes and pyronino-

philic cells showed that the paracortical area of the lymph nodes was prominent in T-cells which had sustained damage similar to that of the other sites. Nevertheless, the pyroninophilic cells were increased by pre-treatment with Cyclophosphamide, assumedly because the B-cells were more specifically affected. The histological findings indicate a decrease in B-cells, and according to Todome's report, there is an increase in T-cells within the lymph nodes, although there is a slight variation depending upon the amount of Cyclophosphamide injected. Thus, there may be some interacting mechanism between the two types of cells (12). Turk stated that the suppressor B-cells sustain damage and as a result the effects on T-cell are no longer inhibited (15). Further, the finding that the pyroninophilic cells did not increase in the animals given injections after sensitization is attributed to the fact that Cyclophosphamide inflicts damage on cells which are undergoing division, and thus the pyroninophilic cells, as in the case of B-cells, are also damaged.

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