

Lymphocytosis in Rats Treated with Polyacids

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Summary

Inoculation of rats i.v. with dextran sulfate or polymethacrylic acid causes lymphocytosis. Electron microscopy of sections of cervical lymph nodes in control rats showed active passage of small lymphocytes through the high endothelial cells (HEC) of the post-capillary venules. In treated rats there was no indication of such passage: the HEC were free of small lymphocytes; and in about 25% of the venules apparently stationary lymphocytes, lacking microvilli, were found appressed to the luminal walls of the HEC.

Ormai and *Palkovits* (1) have pointed out that a variety of compounds will induce lymphocytosis in animals. Most of these compounds are high polymers, and some of them also have in common acid groupings along the polymer chain (2). Although it is not definitely known how such compounds bring about the increase of lymphocytes in the bloodstream, the work of *Morse* and colleagues (3) with pertussis vaccine and that of *Ormai* and *Palkovits* (1) with polymethacrylic acid agree in showing a decrease of those cells in the lymph nodes. In view of the many observations of the unique movement of the small lymphocytes through the high endothelial cells (HEC) of the postcapillary venules (PCV) of the lymph nodes (4, 5, 6), it was considered of interest to examine this region for any changes that could be detected upon polyacid-induced lymphocytosis.

Materials and Methods

Two polyacids were used: dextran sulfate (DS) was obtained from Pharmacia Fine Chemicals AB (approximate molecular weight of 500,000); polymethacrylic acid (PMAA) was purchased from Polysciences, Inc. Its molecular weight was determined viscometrically as 163,000

(7). Five pairs of sister rats (A0 and Sprague-Dawley (SD) strains) were used. Each sister of a pair of ether-anesthetized A0 rats was inoculated i.v. respectively with 1.7 mg of DS/100 gm body weight in Palbecco's DAB or with DAB alone, and SD rats were inoculated with phosphate-buffered saline (PBS), or with PMAA in PBS with an amount of PMAA equivalent to a concentration of 4 mg/100 gm of body weight. (The rats weighed 175-250 gm). White blood cells were counted before inoculation and three hours after. Then the animals were killed by exsanguination. The cervical lymph nodes of A0 rats were diced, fixed in 2.5% glutaraldehyde, washed, fixed in 1% osmic acid, and washed again - all in cacodylate buffer (pH 7.4). The same nodes of SD rats were prepared in the same way in veronal buffer (pH 7.3). After desiccation in acetone, the tissue dice were embedded in araldite (A0 rats) or Spurr's resin (8) (SD rats) and sectioned to pale gold-silver thickness. The sections were placed on copper grids and stained with uranyl nitrate and lead citrate.

Results

The average white cell count of the normal A0 rat was $7.15 \times 10^3/\mu\text{l}$ and for the SD rat $15.7 \times 10^3/\mu\text{l}$. Three hours after inoculation with DS the white cell count of the A0 rat had risen to $42 \times 10^3/\mu\text{l}$, and after inoculation with PMAA it had reached $37.2 \times 10^3/\mu\text{l}$ in the SD rat.

Sections of PCV in the lymph nodes of normal rats (Fig. 1, 2) always showed small lymphocytes in several HEC, indicative of an active traffic through those cells. The cytoplasm of the lymphocyte was clearly set off from that of the HEC by a distinct plasma membrane.

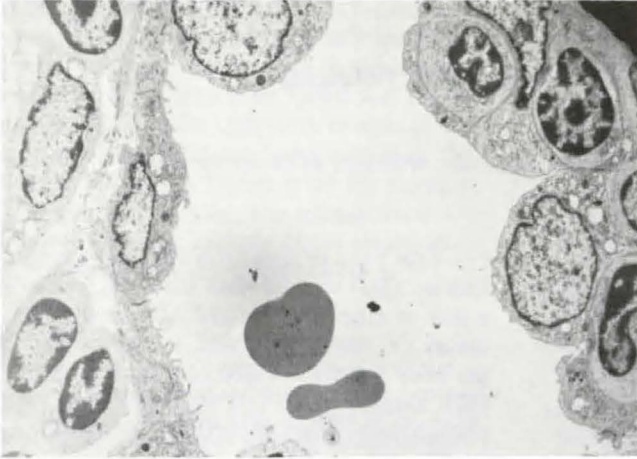


Fig. 1 Cross section of post-capillary venule of control SD rat. Lymphocytes are passing through high endothelial cells. X 2560.

Small microvilli were present on the surface of free lymphocytes (one shown in part at top of Fig. 1).

The PCV of the treated animals were unlike those of the control animals. No lymphocytes were found in the HEC of 12 PCV. In 4 PCV, near-spherical, apparently stationary, small lymphocytes (lacking microvilli) could be observed in the lumen, appressed to the walls of the HEC (Fig. 3, 4). On the whole, however, there was no indication of a traffic of lymphocytes through the HEC (Fig. 3-5). Careful examination of many sections did not disclose any visible cytological change in the membranes of the HEC of treated rats.

Discussion

Although *Gowans and Knight* (4) presented strong evidence that normal lymphocytes move from the PCV lumen through the HEC into the nodal tissue, other workers nevertheless have maintained that these cells circulate or could circulate in the reverse direction (6, 9, 10). This study adds the datum that, during polyacid-induced lymphocytosis, the circulation of the lymphocytes is interrupted and those cells do not pass through the HEC in any direction.

It is possible to believe that a barrier to lymphocyte circulation in treated rats might be an altered membrane of the HEC, especially

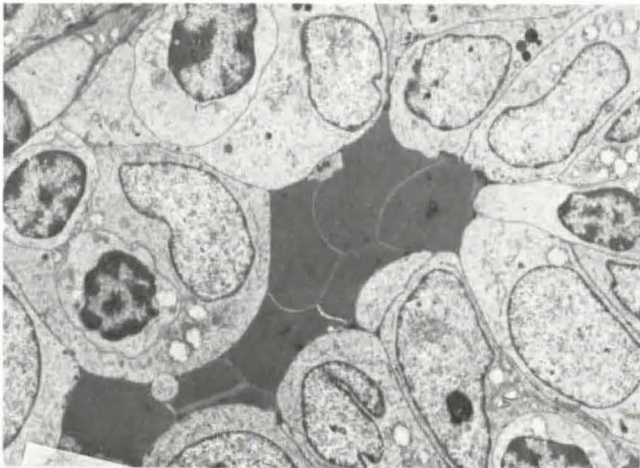


Fig. 2 As in Fig. 1 Lumen of venule crowded with red blood cells. X 2560.

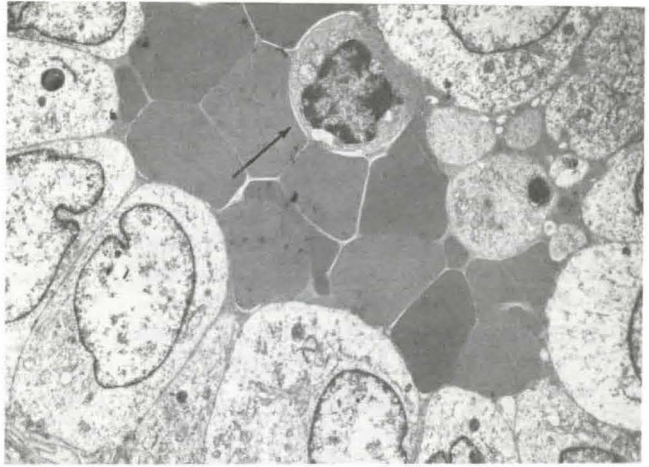


Fig. 3 Cross section of post-capillary venule of SD rat treated with polymethacrylic acid. Arrow points to apparently stationary lymphocyte amid red blood cells. X 2560.

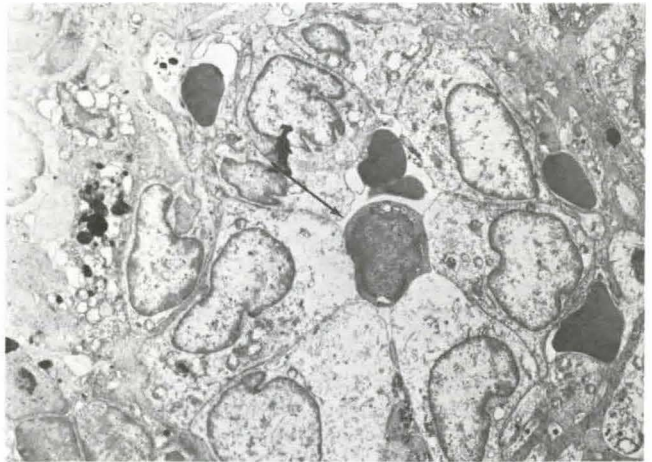


Fig. 4 As in Fig. 3, with arrow pointing to apparently stationary lymphocyte. X 2560.

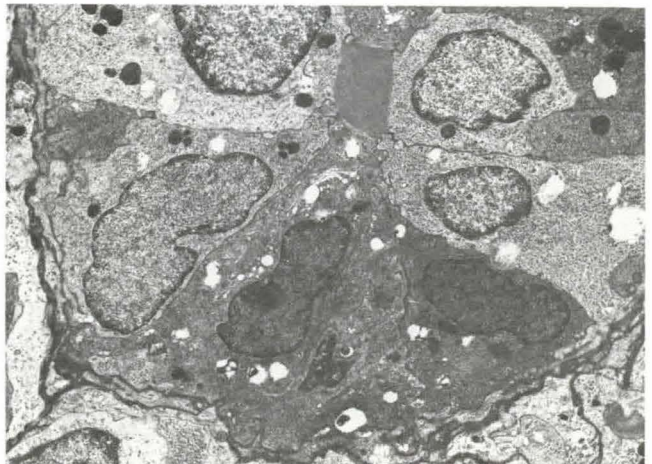


Fig. 5 Cross section of post-capillary venule of A0 rat treated with dextran sulfat. X 3760.

if one agrees with *Gowans* (11) that the role of the HEC in lymphocyte passage is macrophagelike. However, *Schoefl* (5) found that small lymphocytes in Peyer's patches move between the endothelial cells rather than within them. Moreover, pertussis-treated lymphocytes (3, 12) and trypsin-treated lymphocytes (13) also accumulate in the bloodstream, an indication that lymphocytosis can be attributed to an altered lymphocyte surface. The fact that microvilli are not evident in the stationary lymphocytes again suggests that the surface of the lymphocyte has been modified by the polyacids.

Acknowledgements

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