The Regional Lymph Node in Mice Bearing Transplanted Syngeneic Lymphoma Cells (36454)

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(Introduced by L. Dmochowski)

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The events related to the induction of humoral and cell-mediated immunity in the regional lymph nodes draining the site of antigen injection have been extensively studied (1–5). The general consensus is that the regional lymph node plays a fundamental role in the immunological response. Surgical removal of this node may impair or even abolish the immune response of the host (6, 7). We describe a tumor–host system in which the lymph node draining the site of injection of the neoplastic cells displays minimal morphological changes even though the recipient is able to mount a relatively vigorous immune response.

Materials and Methods. C3Hf/Bu inbred mice kept under specific pathogen-free conditions were employed at about 12–14 weeks of age. A spontaneous lymphoma which arose in one of these mice constituted the test tumor cells. It was serially transplanted subcutaneously in the right thigh and it killed the animal in about 60 days. For the present studies 60 male animals were injected simultaneously with either 5×10^2 or 10^6 viable lymphoma cells after trypan blue exclusion. The first dose did not yield tumor takes and the second gave 100% take. Three animals per group were sacrificed at specified intervals. The regional, axillary and contralateral lymph nodes, and the spleen were the lymphopoietic organs studied. These were fixed in buffered formalin at pH 7.0. The sections were stained with hematoxylin–eosin and methyl green-pyronine. Serum was collected from each mouse and tested for circulating antibodies by the indirect immunofluorescent technique. Lymphoma cells were fixed in acetone for 10 min and incubated with the appropriate test and control sera for 30 min at room temperature. Following a double wash in buffer the cells were incubated with fluorescein labeled goat antimouse globulin serum for 30 min. The cells were then washed, counterstained with 0.006% Evans blue, mounted with buffered glycerol and examined under a Zeiss UV microscope. Specificity tests included testing of the positive sera on syngeneic tumor cells of mice with mammary carcinoma (Bittner virus positive and negative), fibrosarcoma and ovarian carcinoma, absorbing the positive sera with the above-mentioned cells and reexposing the lymphoma cells to the absorbed serum, and testing lymphoma cells with serum derived from syngeneic mice carrying the above-mentioned tumors.

Results. The morphological changes observed in the lymphoid organs were similar for both groups of mice during the first 2 weeks. The regional and systemic lymph nodes revealed very few changes compared to normal controls. These changes consisted of minute aggregates of few pyroninophilic cells in the subcortical region and mild reticulum cell hyperplasia in the cortical and medullary areas. After day 3 following the injection of the tumor cells, small abortive germinal centers were noted in the axillary nodes but not in the regional lymph nodes and very rarely so in the contralateral inguinal lymph node. The spleen, however, was the site of marked morphological changes indicative of immunological reaction. Twenty-four hours after tumor implantation, intense periarteriolar hyperplasia of pyroninophilic cells were noted in the small lymph follicles. The pyroninophilic cells became prominent during the fol-

lowing days and began to migrate into the marginal zone between red and white pulp. Germinal centers began to develop on day 5 accompanied by generalized hyperplasia of the lymphoid follicle and transformation of large pyroninophilic cells into mature plasma cells. After the second week the changes in those mice injected with 5×10^2 cells markedly subsided and 28 days following injection only occasional small germinal centers were still visible in the spleen. The mice injected with 10^6 cells continued to demonstrate violent changes in the spleen; during the third week the spleens were extremely hypertrophic with marked hyperplasia of lymph follicles, all of which contained prominent germinal centers and scattered aggregates of large pyroninophilic cells and mature plasmacytes. The marginal zone was sometimes effaced by these cells which also infiltrated the red pulp. The lymph nodes failed to reveal noteworthy changes. At the same time, the local tumor became grossly palpable. After week 3, groups of tumor cells appeared in the medullary part of the regional lymph node. These large, atypical pyroninophilic cells were easily distinguished from the local lymphocytes. The tumor grew very rapidly replacing the entire lymph node where only remnants of reticulum cells could be noted. Metastasis to spleen, systemic lymph nodes and liver followed. After week 4 the bone marrow and the gut became involved and the mice died about 60 days after tumor cell injections with generalized metastasis and extremely hypertrophic lymph nodes and spleen due to tumor replacement. These morphological changes were always accompanied by the appearance of circulating antibodies, presumably tumor specific. These antibodies were demonstrated after the second and fourth day following the injection of 10^6 and 5×10^2 cells, respectively. Serum from normal controls and from mice with other tumors never demonstrated a positive fluorescent reaction when tested with lymphoma tumor cells. The serum from mice with lymphoma were negative on mammary tumor cells, on fibrosarcoma cells and on ovarian carcinoma cells. Absorbing the positive sera with these cells did not remove the antibodies against the lymphoma cells.

Discussion. The morphological changes presently described are, by all evidence, related to the tumor cell injection. The mice are kept under specific pathogen-free conditions and their lymphoreticular tissues present small lymph follicles and only occasional pyroninophilic cells. No germinal center is ever noted. The circulating antibodies demonstrated by the indirect immunofluorescent technique are most likely tumor specific for lymphoma since the lymphoma cells arose in a syngeneic mouse and none of the controls presented these antibodies. Moreover, the positive sera did not react with other tumor cells. The morphological changes encountered in the present tumor–host system are at variance with previous reports concerning the role of the lymph nodes and spleen in the immune response. The regional lymph node never revealed changes which could be ascribed to a vigorous immune response. The other lymph nodes only revealed mild changes. The greatest alterations occurred in the spleen which revealed the classical histological picture of immune response. Several possibilities could account for these findings: (a) The tumor cells bypass the regional lymph node spreading via the blood circulation. Preliminary data utilizing tritium labeled lymphoma cells indicate, however, that the tumor cells do indeed accumulate in large numbers at the regional lymph node within 6 hr after injection. (b) The tumor cells do not stay a sufficiently long time in the lymph node to initiate an immune response. The same preliminary data mentioned above suggest that after 24 hr very few tumor cells may be present in the regional lymph node whereas they accumulate in the spleen and in a lesser number in other lymph nodes (c) Due to the syngeneic nature of the tumor cells and the immaturity of the lymphoid tissue an immune response cannot be mounted until the lymphoma cells reach an organ rich in immunocompetent cells, *i.e.*, the spleen, where recruitment of antigen-sensitive cells can be accomplished (8). (d) A variant of the above possibility could be a deficiency in the "trapping" of antigen-sensitive immunocytes (9) at the regional

and systemic lymph nodes. Since the immune system is rather underdeveloped, recirculating immunocompetent lymphocytes may be exiguous and the required "trapping" mechanisms necessary to begin an immune response may not function. These possibilities are further being explored in our laboratory. The present data suggest that the regional lymph node may not, in certain cases, be the first outpost of the immune surveillance mechanism in tumor cell rejection and that even its role in the immune response (7) may have been overestimated.

Summary. Mice injected with syngeneic lymphoma cells are able to mount an immunological reaction against the tumor cells. This results in marked morphological changes in the spleen and the appearance of circulating antibodies demonstrable by immunofluorescent techniques. The lymph nodes and particularly the regional node do not exhibit evidence of an immune response. This may indicate that in certain cases the draining nodes may not participate in the immune response of the host against tumor antigens.

The authors express their appreciation to Mrs. Shirley Fullen in helping prepare this manuscript.

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Received Jan. 6, 1972. P.S.E.B.M., 1972, Vol. 140.