Immunology, 1971, **21**, 1059.

The Growth of the Cell Population in the Draining Popliteal Lymph Node of Rats Injected with Rat Adrenal in Freund's Adjuvant

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(*Received* 24*th December* 1970)

Summary. The growth of the cell population and the rate of cell proliferation in the draining popliteal lymph node was studied in rats injected in the foot with adrenal tissue antigen-adjuvant emulsion and pertussis vaccine. The number of cells in the lymph node quadrupled during the first two days after injection prior to any significant increase in the rate of cell proliferation as measured by the number of cells incorporating ^{3}H-thymidine *in vitro*. After the second day proliferation of cells contributed to the growth of the lymph node's cell population. It is suggested that the early sudden growth of the cell population is brought about partly by an increase in the influx of lymphocytes from the blood into the lymph node and partly by a decrease in the efflux of lymphocytes with the efferent lymph from the lymph node.

INTRODUCTION

The events taking place in a lymph node after injection of an antigen into its draining area have been the subject of a number of studies (Turk and Stone, 1963; Taub, Krantz and Dresser, 1970; Dresser, Taub, and Krantz, 1970). The size of the node increases and the rate of cell proliferation within the node reaches a peak about 4 days after injection of antigens which evoke a delayed hypersensitivity response (Turk and Stone, 1963). In a recent report on the local effect of adjuvants injected into mice it was pointed out that the enlargement of the node preceeds the appearance of blast cells, suggesting that the enlargement is due to influx into the node of lymphocytes rather than to proliferation of cells within the node itself (Taub *et al.*, 1970). The same investigators subsequently confirmed this interpretation by showing that infused ^{51}Cr-labelled donor lymphocytes accumulated in a recently stimulated lymph node in numbers which were three to four times higher than in non-stimulated nodes (Dresser *et al.*, 1970).

In the study referred to above (Taub *et al.*, 1970) the lymph node enlargement was measured by increase in weight, and the rate of cell proliferation was judged by the number of blast cells found in sections of the lymph node. In another experimental

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model, rat allergic adrenalitis, we, too, observed a rapid increase in weight of the draining lymph node during the first days after injection of antigen (Werdelin and Witebsky, 1970). It was of interest to study in detail the quantitative aspects of this rapid, early enlargement of the draining lymph node. In the present report the number of cells in the growing node was counted in cell suspensions prepared therefrom and the rate of proliferation of these cells was measured by counts in autoradiographs of smears of the number of cells incorporating ^{3}H-thymidine.

MATERIALS AND METHODS

Female Lewis rats weighing 200–220 g were obtained from Simonsen Laboratories, Inc. (Gilroy, Calif.). Allergic adrenalitis was produced with a method slightly modified from that of Levine and Wenk (1968). Forty-two rats each received a single injection, in the right hind foot, of 0·06 ml of an emulsion of petroleum oil (Difco Laboratories, Detroit, Mich.) with homogenized rat adrenals containing per ml 250 mg rat adrenal, 2 mg *Mycobacterium tuberculosis* H37Ra and 2 mg *Mycobacterium butyricum*. Immediately after the foot pad injection 0·1 ml concentrated pertussis vaccine (corresponding to 180×10^9 micro-organisms per ml) was injected intracutaneously and subcutaneously in the dorsum of the same foot. The rats were killed in groups of six by an overdose of ether 2, 4, 6, 8, 10, 12 and 16 days after the injection. Twelve untreated rats served as controls. At autopsy the right popliteal lymph nodes from the six injected rats were removed (from the twelve normal rats both the right and left popliteal lymph nodes were taken). They were trimmed of fat and extracapsular connective tissue, weighed, pooled in chilled tissue culture medium 199 (Grand Island Biologicals, Grand Island, N.Y.), then cut into small pieces and gently strained through a No. 100 stainless steel mesh with 4 to 8 ml fresh medium 199. The volume of the resulting cell suspension was recorded, and a cell count was made for calculation of the total number of cells in the lymph nodes from the six rats. The crude suspension of cells was incubated with 0·5 mCi of ^{3}H-thymidine per ml cell suspension on a gently agitating water bath at 37° for 30 minutes. The cells were spun down at 100 ***g*** for 5 minutes and resuspended in fresh medium 199; after one additional wash the pellet was smeared on gelatin-coated slides. The smears were air-dried for 3 minutes, fixed with methanol and processed for autoradiographs with NTB-2 nuclear emulsion (Eastman Kodak, Rochester, N.Y.). Counts for ^{3}H-thymidine-labelled cells were made at $1000\times$ magnification on autoradiographs exposed for 28 or 30 days selected on the basis of technical quality. A well spread portion of the smear was selected and the total number of cells and of labelled cells present within the limits of an ocular grid delineating an area of 6400 μm^2 were counted and recorded. The immediately neighbouring field was counted next and so on until a total of 2000 cells had been screened.

RESULTS

The results of one experiment are seen in Fig. 1. The number of cells in the node increased from 4×10^6 to 18×10^6 during the first 2 days after injection of antigen, and from 18×10^6 to 105×10^6 during the next 2 days. The increase in numbers during the first 4 days correlated well with the increase in the weights of the nodes (from an average of 4 mg in the untreated rats to 16 mg on day 2 and to 58 mg on day 4). After the fourth

day the rate of increase in the number of cells in the node was more modest; a maximum of 180×10^6 cells was reached by the tenth day. Subsequently a slight drop was recorded. The percentage of ^{3}H-thymidine-incorporating cells in nodes from untreated animals was 0·7. Two days after injection of antigen it was 1·0. By day 4 it jumped to 5·2 per cent; from then on it gradually declined to a subnormal level by day 16.

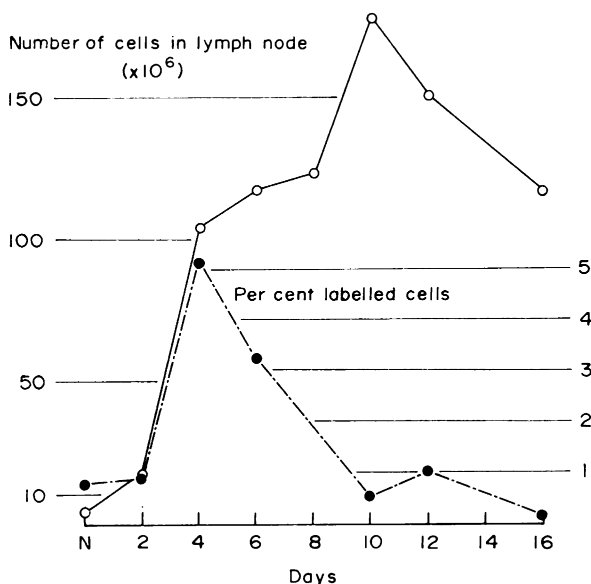

FIG. 1. Open circles, number of cells (mean of six nodes) and solid circles, percentage of ^{3}H-thymidine-incorporating cells in the draining popliteal lymph node.

In another experiment of essentially identical design, the number of cells in the draining popliteal lymph node and the rate of cell proliferation as measured by percentage of ^{3}H-thymidine-incorporating cells showed the same fluctuations as reported above.

DISCUSSION

Previous studies in our laboratory revealed that adrenal-specific inflammation resulted from injection into rats of rat adrenal homogenate in Freund's complete adjuvant with simultaneous inoculation of pertussis vaccine (Werdelin and Witebsky, 1970). The same antigen in incomplete adjuvant gave no adrenalitis, whereas adrenal homogenate with complete adjuvant alone or with incomplete adjuvant accompanied by pertussis vaccine gave rise to diminished adrenalitis. No adrenalitis followed injection of other rat tissue homogenates in Freund's complete adjuvant, or injection of the adjuvant emulsion alone. Using the optimal combination of adrenal homogenate, Freund's complete adjuvant, and pertussis vaccine, a considerable accumulation of lymphocytes was demonstrated in the draining lymph nodes during the first 2 days after injection.

The major finding in this study was that the number of cells in the draining lymph node increased four-fold in the virtual absence of increased cell proliferation during the first 2 days after injection of antigen. For the discussion of the mechanisms involved in the

growth of the draining lymph node's cell population it is convenient to regard the size of that population as the result of a number of balancing factors. Of particular relevance here are influx of cells into the node, proliferation of cells within the node, death of cells within the node, and efflux of cells from the node. In the steady state the number of cells added by influx plus proliferation equals the number of cells lost by efflux and by death. The present results clearly show that the rate of cell proliferation was not increased in the first 2 days after antigen and the balance therefore was upset by a dominance of influx over efflux.

The influx of cells into the node may occur by two different routes. The lymphocytes may arrive with the blood and enter the lymph node through the endothelial cells of the postcapillary venules in the paracortical zone (Gowans and Knight, 1964; Marchesi and Gowans, 1963). The presence in the circulation of antigen-reactive lymphocytes capable of undergoing mitotic division after exposure to sheep red blood cells has previously been demonstrated in rats (Rabin and Rose, 1970). It has been estimated that in the steady state the total population of blood lymphocytes in the mouse (approximately 25×10^6 cells) return to the lymph nodes every 5 or 6 hours (Gesner and Gowans, 1952; Morse and Riester, 1967). The number of cells entering the lymph nodes from the blood in the rat would, assuming a similar high rate of recirculation in that species, be sufficient to explain the sudden increase in cell population observed in the present experiment, especially when the influx into stimulated nodes is increased as indicated by the data of Griscelli, Vassalli and McCluskey (1969) and by Dresser *et al.* (1970). The mechanisms behind the increased influx of lymphocytes into stimulated lymph nodes is not known. Some studies have shown an increase in the height of the endothelium of the postcapillary venules of stimulated lymphoid tissue (discussed by Smith and Henon, 1959) and suggested that the permeability for lymphocytes is increased. The other route by which lymphocytes may enter lymph nodes is via the afferent lymphatics. Data obtained from sheep stimulated by application of skin homografts indicate that the number of cells in the afferent lymph is very low and that most of these are macrophages (Hall, 1967).

Lymphocytes leave the node with the efferent lymph, but may, at least in theory, also leave by direct entry into the blood (Yoffey, 1967). Whether the latter pathway really may be taken by long-lived recirculating cells is not known, but studies in rats have shown that at most very few of the newly-formed cells from the draining popliteal lymph node of rats leave by 'direct entry' unless the thoracic duct is obstructed (Werdelin, Wick and McCluskey, unpublished observations). At least two observations indicate that there may be a decrease in the output of cells from a lymph node shortly after injection of antigen into its area of drainage. The medullary sinuses become plugged with lymphocytes, the plugs extending out into the cortex for some distance (WHO Report, 1969) as if there was a sudden stoppage of the pathways of lymphatic drainage. Others have shown that the number of cells issuing through the thoracic duct in mice decreases sharply soon after intravenous injection of pertussis vaccine (Morse and Barron, 1970) which indicates that the output of cells from the nodes draining into the duct is decreased.

The accumulation of lymphocytes in the lymph node resulting from a combination of increased influx and decreased efflux is desirable from a teleological point of view. A large number of lymphocytes are brought in this way into the area into which the antigen is drained and detained there for some time. This allows contact between antigen and reactive cells leading to blast transformation and the subsequent generation of clones of cells capable of maintaining the immune response.

ACKNOWLEDGMENT

This work was supported by United States Public Health Service Research Grant CA-02357 and National Institute of Allergy and Infectious Disease Grant 2 RO1 AI09115. Dr Ole Werdelin is a Dr Henry C. and Bertha H. Buswell Research Fellow.

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