STRUCTURAL AND FUNCTIONAL CHANGES IN LYMPHOID POPULATIONS DURING CARCINOGENESIS

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According to data in the literature [13] the surface of T- and B-lymphocytes at different stages of differentiation is characterized by differences in the quantitative and qualitative composition of marker antigens and receptors. Hence the importance of the study of individual lymphocyte populations differing with respect to these receptors, the presence or absence of which can give precise information on the stage of cell differentiation. From this point of view the receptor for the third component of complement, found in a subpopulation of mature B-lymphocytes and lost by plasma cells [6], is a suitable object.

The aim of this investigation was to study changes in the relative percentage of B-cells carrying the receptor for the third component of complement (LRC) in the thymus, lymph nodes, and peripheral blood of rats during the course of carcinogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats; a sarcoma of the thigh was induced by 7,12-dimethylbenz(a)anthracene (DMBA). At least 10 animals were used in each experiment. Lymphoid cells were isolated from the thymus, peripheral and regional lymph nodes, and also from peripheral blood on Ficoll–Verografin with a density of 1.077 g/cm^3 [10]. Lymphoid cells obtained from the same organs of normal rats were used as the control. The cell suspensions were equalized for number of cells, and their viability was tested with trypan blue. To detect LRC the method of indirect rosette formation was used. The number of cells was counted under the microscope, 300 karyocytes from each sample being examined. The results were expressed as percentages of the total number of cells examined. Na_3H-EDTA (0.01 M) in medium No. 199, which binds Ca^{++} and Mg^{++} ions [9], essential for reception by these cells, was used as the control, preventing macrophages and leukocytes from participating in this reaction. For morphological analysis of the suspensions films were made and stained by Pappenheim's method [3]. All data were subjected to statistical analysis by Student's t-test at the 0.05 level of significance.

EXPERIMENTAL RESULTS

The results of the study of relative percentages of LRC in organs of the immunocompetent system of rats during carcinogenesis showed (Table 1) that 1 month after injection of DMBA no significant changes were detectable in the relative percentage of LRC compared with control values in populations obtained from lymph nodes, thymus, and blood. Two months after injection of DMBA, among the lymph node and blood cells the relative percentage of LRC observed was lower. The addition of Na_3H-EDTA to the incubation medium had virtually no effect on the results obtained for thymus cells, evidence of the true accumulation of mature B-cells in the thymus.

With the appearance of palpable tumors after 3 months 2 weeks, an increase in the relative percentage of LRC was observed in suspensions obtained from lymph nodes, thymus, and blood. It was postulated on the basis of these results that the change in the relative per-

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TABLE 1. Relative Percentage of LRC in Suspensions Obtained from Lymph Nodes, Thymus, and Blood of Control and Experimental Animals during Chemically Induced Carcinogenesis (M ± m)

Source of lymphoid cells	Control	Periods of carcinogenesis				
		1 month	2 months	3 mos. 2 weeks	4 mos. 2 weeks	5 mos. 2 weeks
Lymph nodes	15,78±1,100	14,33±1,370	11,60±0,400	22,67±1,34	21,00±1,005	21,50±1,100
P			⩽0,01	⩽0,001	⩽0,001	⩽0,001
Thymus	1,05±0,195	1,33±0,100	3,00±0,412	3,67±0,406	3,00±0,332	3,40±0,400
P			⩽0,01	⩽0,001	⩽0,001	⩽0,001
Blood	5,93±1,410	6,33±0,766	2,67±0,330	12,47±1,76	13,33±1,83	12,80±1,500
P			⩽0,01	⩽0,001	⩽0,001	⩽0,001

Legend. Values of P given where differences from control are significant. Ten animals were used in each experiment.

centage of LRC in the central and peripheral organs of immunity reflected changes in their functional activity.

Since the functional activity of the lymphoid organs is also determined by the number of cells completing differentiation in them, it was interesting to compare these results on the number of the LRC with the total number of cells isolated from the thymus and lymph nodes of the experimental animals in the course of carcinogenesis.

The results of this investigation show (Fig. 1) that 1 month after injection of DMBA the number of thymus cells was less than in the control, possibly on account of the immunodepressive effect of DMBA, resulting in death of some of the thymus cells. After 2 months, a sharp rise in the number of cells was observed in the thymus and lymph nodes. For instance, the number of cells obtained from the thymus at this time was almost 2.7 times greater than in the control ($P \leqslant 0.001$) and twice the number of cells present in the lymph nodes ($P \leqslant 0.001$), the number of which also was increased. Very possibly this marked increase in the number of thymocytes and also in the number of lymph node cells after 2 months of carcinogenesis was due to activation and subsequent proliferation of the cell populations present in these organs. After 2.5 months, it will be noted, the number of cells obtained from the thymus was considerably reduced (1×10^6-2×10^6/ml), whereas the number of cells in the lymph nodes was virtually unchanged. With the appearance of a tumor in the animal, different quantitative proportions were observed between the populations studied in the lymphoid organs. For instance, after 3 months 2 weeks fewer cells could be seen than during the latent period ($P \leqslant 0.001$) both in the thymus and in the lymph nodes; the figure for the thymus, moreover, was significantly below normal ($P \leqslant 0.01$).

The marked increase in the number of cells 2 months after injection of the carcinogen could thus indicate activation, accompanied by proliferation and maturation of the medullary precursors of T-cells. The simultaneous increase in the relative percentage of LRC in the thymus is an interesting fact in this connection. Reports have recently been published on the appearance of antibody-forming cells in the thymus of animals after sensitization by T-dependent and T-independent antigens [5]. If these data are compared with those of the present investigation, direct correlation can be postulated between the state of activation of the immunocompetent system, accompanied by an increase in the rate of migration of medullary precursors into the thymus and by the appearance of maturing B-cells, i.e., LRC, in it. The subsequent decrease in the number of cells in the thymus 2.5 months later could reflect the discharge of mature T-cells to the periphery. This reduction in the relative percentage of LRC in the lymph nodes at this time, the absence of plasma cells, and the increase in the total cell population could indicate proliferative processes and also a redistribution of the population in favor of the T-system, possibly due to specific retention of T-lymphocytes there [4]. Activation of the T-system, namely the appearance of mature T-cells at this time, has been demonstrated in the writers' laboratory by Maiskii et al. [1] in the macrophage migration inhibition and cytotoxic tests.

With the appearance of a tumor in the experimental animals, a reduction was observed in the number of cells present in their thymus. Meanwhile an increase in the relative percentage of LRC was observed in the lymph nodes, but as morphological analysis showed, virtually no plasma cells were found. According to the writers' earlier observations [2] ob-

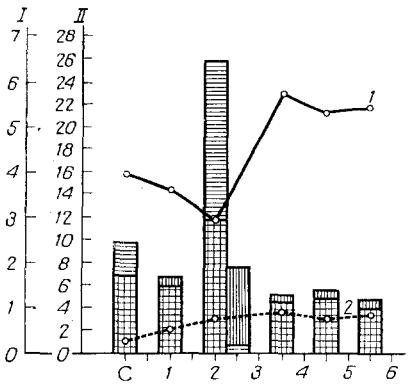

Fig. 1. Number of lymphoid cells and LRC in lymph nodes and thymus of rats at different stages of carcinogenesis. Abscissa, time after injection of DMBA (in months); ordinate: I) number of lymphoid cells (in relative units), II) number of LRC (in %). C) Control. Vertical shading: cells in lymph nodes, horizontal shading — cells in thymus; 1) percent of LRC in lymph nodes, 2) percent of LRC in thymus.

tained by the use of the negatively charged fluorescent probe 1-anilino-8-naphthalenesulfonate (ANS), a cell population characterized by a lower density of negative surface charges was identified at this same time in the thymus glands of the experimental animals. When these results are compared with those obtained by other workers relating to an increase in the density of negative surface charges in the course of maturation of T-lymphocytes [7], it can be postulated that the appearance of the cell population discovered in the present experiments in the thymus takes place through a disturbance of cell maturation processes. Communications describing suppressor T-cells during carcinogenesis, which stimulate tumor growth, are interesting in this connection [8, 11]. There is evidence that these cells are the least mature subpopulation and that they belong to the group of short-living T_1-cells. Their surface is characterized by a higher density of various receptors than that of mature T-cells [12]. On the basis of these data and the results of our preliminary experiments a lower density of negative charges can be postulated on the surface of suppressor T-cells on account of screening compared with other subpopulations of T-lymphocytes. These results thus suggest that through a disturbance of maturation processes in the thymus, intercellular relationships are modified in the peripheral organs of the immunocompetent system, and this is reflected by the accumulation of LRC and by delay of their maturation into antibody producers.

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