CELL COMPOSITION OF LYMPH NODES AND DNA CONTENT IN THEIR VARIOUS LYMPHOCYTE POPULATIONS IN MICE WITH TUMORS INDUCED BY MOLONEY MOUSE SARCOMA VIRUS

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The DNA content on populations rich in T- and B-cells and the numerical ratio between these cells in lymph nodes of intact mice and mice with tumors were investigated in experiments on BALB/c mice with a progressively growing sarcoma induced by Moloney virus. The fraction rich in T-lymphocytes characteristically had a higher DNA content than the fraction consisting mainly of B-lymphocytes. Development of the tumor led to a decrease in the relative number of macronucleolar lymphocytes and a simultaneous increase in the number of micronucleolar lymphocytes in the lymph nodes, especially the regional nodes.

KEY WORDS: macronucleolar and micronucleolar lymphocytes; DNA; Moloney sarcoma virus.

The study of the functional state of lymphocytes from lymph nodes located differently relative to a tumor and the ratio between the various population of lymphocytes can be used as immunological parameters of interaction between the host and a neoplasm developing in it. One of the essential functional indices of lymphocytes is determination of their DNA content.

The object of this investigation was to study the relative numbers of T- and B-lymphocytes in lymph nodes of BALB/c mice and the parallel determination of the DNA content in populations rich in T- and B-cells from intact animals and mice with tumors induced by Moloney sarcoma virus.

EXPERIMENTAL METHOD

A tumor was induced in BALB/c mice aged 10-14 days by the usual method described previously [1]. The regional (right inguinal) and remote (left axillary) lymph nodes relative to the tumor were taken from mice with a developing sarcoma for investigation. The corresponding lymph nodes from intact mice of the same age and sex served as the control. Cell suspensions were prepared from these lymph nodes, applied to slides, and stained with methylene blue by the method of Stockinger and Kellner [10] in order to study the ratio between different lymphocyte populations [2]. The original cell suspensions were separated into fractions rich in T- or B- lymphocytes by the cellular immunoadsorption method with certain modifications [3]. Mainly T-lymphocytes were present in the cell fraction which did not adhere to the sorbent, whereas most of the adherent cells were B-lymphocytes.

Cytological specimens of cells adherent and not adherent to the sorbent were stained by Feulgen's method. The DNA content in the nuclei of the lymphocytes was estimated from the optical density, measured on the MUF-5 instrument by consecutive cytophotometry, with scanning in three different directions; the mean optical density of each nucleus was then calculated and multiplied by its area. The results obtained for the DNA content in the lymphocyte nuclei were subjected to statistical analysis, allowing for instrumental error with a reliability of 95%, and were expressed in conventional units.

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TABLE 1. Cell Count in Lymph Nodes of BALB/c Mice during Development of Tumor Induced by Moloney Sarcoma Virus (in %, M ± m)

Lymph nodes	Group of animals	Lymphocytes		Blast forms and large lymphocytes	Other cells
		macronucleolar	micronucleolar		
Regional	Control	58,84±1,10	35,92±1,00	3,60±0,62	1,64±0,20
	Experimental	40,28±1,78	47,96±3,04	7,96±1,71	3,80±0,93
Distant	Control	59,50±1,21	34,64±1,62	4,32±0,47	1,52±0,27
	Experimental	50,20±1,87	40,15±4,08	7,60±1,27	2,05±0,54

TABLE 2. DNA Content (in conventional units) in Nuclei of Lymphocytes of Different Cell Fractions from Lymph Nodes of BALB/c Mice during Development of Tumor Induced by Moloney Sarcoma Virus (M± m)

Group of animals	Adherent fraction (B-cells)	Nonadherent fraction (P-cells)
Control*	6,84±0,061	8,06±0,067
Experimental:		
regional lymph nodes	7,23±0,083	8,49±0,068
remote lymph nodes	6,61±0,049	7,44± 0,052

*Data for both lymph notes.

EXPERIMENTAL RESULTS

Microscopic examination of the stained films from lymph nodes of the control and experimental mice revealed two main types of lymphocytes: macronucleolar, with one to three large, clearly distinguishable nucleoli and with a basophilic rim of cytoplasm, and micronucleolar, distinguished by the presence of numerous small, at times almost indistinguishable nucleoli and a weakly basophilic rim of cytoplasm. In addition, blast forms of lymphocytes and other cells, including plasma cells and nonlymphoid cells, were distinguished as separate groups. By the simultaneous use of immunological and cytological methods it was found that the macronucleolar lymphocytes belonged to the T-cell population and micronucleolar lymphocytes to the B-cell population [2]. The experiment shows that among cells from the lymph nodes of intact mice not adherent to the immunosorbent, i.e., mainly among the T-cell fraction, 82% of the cells when stained by the method of Stockinger and Kellner consisted of macronucleolar lymphocytes, whereas among the adherent cells 70.2% had the characteristic morphology of micronucleolar lymphocytes.

On the other hand, studies by Czechoslovak workers have shown that the presence of compact nucleoli and of nucleoli with a nucleome in lymphocytes is evidence that intensive DNA synthesis takes place in these cells [6, 9]. Subsequent investigations have shown that most "active" lymphocytes belong to the thymus-dependent population and that they are sensitive to the action of anti-θ-serum [4, 5]. Investigations by these workers and our own observation suggests that lymphocytes with large, compact nucleoli can be classed as T-cells.

The results of a study of the cell composition of lymph nodes of intact BALB/c mice and of mice with tumors induced by Moloney sarcoma virus are given in Table 1. They show that the cell composition of the lymph nodes of the intact mice was unconnected with their topography and was the same for both groups of lymph nodes studied.

In mice with a developing tumor the number of macronucleolar lymphocytes in the regional lymph nodes fell by 18% ($P < 0.05$), and in the remote lymph nodes by 9% ($P < 0.05$) compared with the control. Meanwhile, the relative number of micronucleolar lymphocytes increased in these lymph nodes, especially in the regional lymph nodes. Furthermore, transformation changes, expressed as an increase in the number of blast forms by 1.7-2.2 times, were observed in the lymph node cells.

The results of a study of the DNA content in the nuclei of lymphocytes from fractions rich in T- and B-cells are given in Table 2. They show that the cells of the nonadherent fractions, consisting mainly of T-lymphocytes, contained more DNA than cells adherent to the sorbent, most of which were B-lymphocytes ($P < 0.05$). This conclusion was valid both for the corresponding fractions obtained from the lymph nodes of intact mice and for the cell fractions of the experimental animals.

The differences thus found in the DNA content in the cell nuclei of the nonadherent and adherent fractions evidently reflect functional differences between the T- and B-cell populations of the lymph nodes.

Investigations with acridine orange have revealed differences in the nucleic acid content in lymphocyte populations. When lymphocytes are stained with this dye two types of cells giving strong and weak fluorescence can be distinguished [7]. Scott and Sneiby [8] showed that T-lymphocytes of human peripheral blood incorporate three times more ^{3}H-uridine than B-cells. The higher DNA content and greater incorporation of ^{3}H-uridine into T-cells than into B-lymphocytes can probably be explained by the diversity of function of this class of lymphocytes.

In animals with a developing tumor the DNA content is increased in both lymphocyte fractions obtained from the regional lymph nodes. This could indicate an increase in the functional activity of both group of cells in lymph nodes draining the zone of development of the neoplasm, as a result of antigenic stimulation transmitted to these lymph nodes.

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