THE ROLE OF THE LYMPH NODES IN ACQUIRED ANTITUMOR IMMUNITY

COMMUNICATION I. CHANGES IN THE REGIONAL LYMPH NODES FOLLOWING HOMOTRANSPLANTATION OF THE BROWN-PEARCE TUMOR

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A considerable body of experimental data has accumulated in the field of immunology, indicating that the antibody is formed in certain cells of the lymphoid tissue [6,8,13]. These cells are considered to be young cellular elements of reticular origin, belonging to the lymphoid-plasmocyte series and known under the names: immature plasma cells [13], plasmoblasts and lymphoblasts [2,3], large lymph cells [19], etc. Immunomorphological changes are fundamental, lying at the root of the production of antibodies and their entrance into the blood circulation.

These data are of major interest in explaining the mechanism of acquired immunity to tumors, since there is no adequate basis for explaining it as a result of humoral antibodies [5,7]. A number of factors speak for a direct relationship between the lymph tissue and the immune antitumor processes; these became known as a result of a histological study of the cellular reaction about the periphery of a tumor [1,12,15], through experiments on the anti-blastic effect of lymph tissue in vitro [14,20], and by experiments on the passive transfer of antitumor immunity using cells from regional lymph nodes [18].

In connection with these data the question arose as to whether the process of the formation of antitumor immunity was reflected in morphological changes in the lymph tissue. Only isolated reports exist in the literature on an increase in weight of regional lymph nodes in animals with tumor homotransplants [9,10]; occasional references have been made to certain cellular changes in these nodes and in the spleen—lymphoid hyperplasia and plasma cell reaction [11,15,17]. We were not able to find any descriptions in the literature of the dynamics of the cell changes with development of antitumor immunity.

In our work we attempted to investigate the state of the lymph nodes during the growth and resorption of a tumor. For this purpose we used the schema: homotransplantation of the Brown-Pearce tumor to the helix of the ear in rabbits, a method worked out by us earlier [4]. This method is very suitable for resolving the proposed question, thanks to its following characteristics: with transplantation to the helix the development of the tumor attains a strict cyclic character; its active growth regularly alternates with resorption in comparatively uniform time intervals in the different animals. Resorption of the tumor node insures the development of effective immunity against the second transplantation of the same tumor. We could not detect an antitumor antibody in the blood of the immune rabbits [5]; thus, it was especially desirable to perform an immunomorphological investigation. Finally, the helix of the ear in the rabbit has well-developed regional lymph nodes.

In this report we present the results of an investigation of the lymph nodes at various stages in the growth and resorption of a tumor homotransplant.

METHOD

The investigation was carried out on rabbits of the chinchilla family, using both sexes. The Brown-Pearce tumor was transplanted to the rabbits by the method previously adopted [4]: a 0.28-0.3 ml 40% suspension of fresh tumor tissue was injected subcutaneously into the helix. In each case we made a quantitative appraisal of the tumor's developmental state, based on measurement of its area at the maximum cross section. Following transplantation, the rabbits were sacrificed at various stages of the tumor's development: in a growth period– on the 1st, 3rd and 6th days, and a resorption period–on the 10th, 15th and 34th days, which corresponded to the beginning, middle and end of tumor resorption (Table 1). As a control we sacrificed 8 normal rabbits, to whom no tumor was administered.

TABLE 1. Changes in the Weight of the Regional Lymph Nodes During the Development of the Brown-Pearce Tumor in the Helix of Rabbits (Mean Values)

Group of rabbits	Number of rabbits	Period of tumor development	Days following transplantation	Tumor measurement (in cm^2)			Total weight of the three nodes (in mg)			Weight of node No. 1 (in mg)		
				maximum	On the day animal was sacrificed	Percent decrease	Regional nodes	Contralateral nodes	Degree of increase	Regional node	Contralateral node	Degree of increase
First	8	Control rabbits					143	143	1.0	50	51	1.0
Second	4	Growth	1	-	-	-	229	219	1.0	89	72	1.2
Third	4	"	3	-	-	-	280	143	2.0	138	47	2.9
Fourth	6	"	6	-	1.45	-	333	119	2.8	163	39	4.2
Fifth	5	Resorption	10	2.35	2.11	10.2	470	231	2.1	-	-	-
Sixth	7	The same	15	2.35	1.35	42.5	670	308	2.1	205	64	3.4
Seventh	5	"	33	2.29	0.37	83.8	258	201	1.2	103	71	1.2

By injecting India ink into the helix in preliminary experiments, it was established that the latter has three regional lymph nodes. The basic lymph flow was to a large node in the root of the helix, which we arbitrarily designated No. 1. In each rabbit we extracted three lymph nodes on the side of the tumor (regional), and three nodes on the opposite side (contralateral). After they were freed from the surrounding fat tissue, the nodes were subjected to careful examination and weighing.

Both the total weight of the three nodes and the individual weight of node No. 1 were noted, since the latter underwent the greatest increase in size.

For the morphological investigation we used prepared cytological samples, which were stained with azure-II-eosin. Node No. 1 was subjected to cytological investigation with a count of the basic cell forms, inasmuch as the changes in it were reflected the most clearly. For each node the cells were counted in 60 fields, 30 fields in each of two samples (objective ×90, ocular ×7, binocular eyepiece). For each of the cell forms in these two counts we took the average number for thirty fields, which corresponded to an average of 3500-4000 cells in total. Special attention was directed to changes in the content of reticular cells, lymphoblasts and plasmocytes; the corresponding data were subjected to statistical analysis. The lymphocytes and prolymphocytes, always composing more than 90% of the cells, were not counted.

RESULTS

The data from the macroscopic appraisal of the lymph nodes are presented in Table 1 and in Fig. 1. Development of the tumor in the helix occurred in the following manner: in all the rabbits the tumor survived the transplantation and showed a cyclic character of development. Hyperemia and edema were observed at the site of injection 24 hours after the inoculation. On the 3rd day the inflammatory signs decreased and it was possible to recognize the beginning of tumor growth. On the 6th day the tumor already showed large dimensions and looked like a large formation of oval form. The tumor nodule reached its maximum size on the 8th day in the majority of the animals. Then the tumor began to decrease in dimensions: by an average of 10.2% on the 10th day, and almost half by the 15th day. In a large part of the rabbits the resorption was finished after 3-4 weeks. Thus, when transplanted to the helix, the fate of the homotransplant from the Brown-Pearce tumor inoculate remarkably recalls the fate of a normal tissue transplant, both in the constancy of its developing regression and in the time intervals and suddenness of its onset [4].

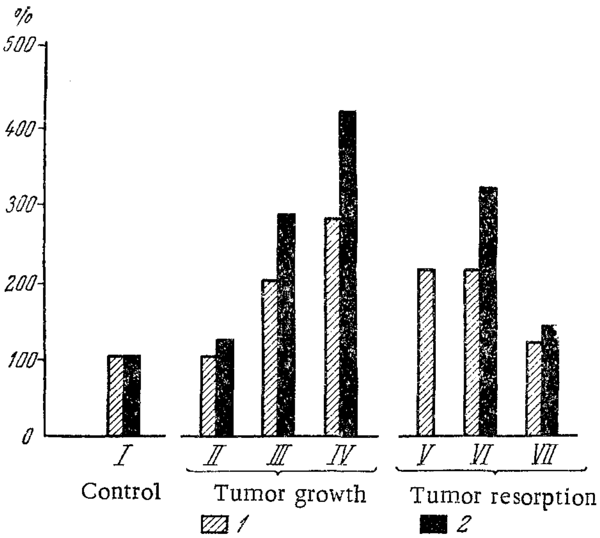

Fig. 1. Changes in the weight of the regional lymph nodes during the development of the Brown-Pearce tumor in the helix of rabbits (in percents of the weight of the contralateral nodes, the latter taken as 100%). I-VII: rabbit groups. 1) Total weight of the three nodes; 2) weight of node No. 1.

Investigation of the regional lymph nodes showed that during the period of tumor development they undergo profound changes, the nature of which is closely linked to the stage of the tumor process.

From Table 1 it can be seen that one day after the tumor transplantation the nodes on the experimental side did not differ from the controls in weight. In vivo palpation begins to detect an increase in node No. 1 on the 2nd day; on the 3rd day the nodes on the tumor side are already markedly increased, and their weight exceeds the weight of the nodes on the contralateral side by more than 2 times. Node No. 1 undergoes the greatest increase. Maximal hypertrophy of the nodes is attained on the 6th day following transplantation, the day before the tumor ceases to grow. The altered nodes markedly increase in volume, and appear succulent on sectioning. On the first days of the onset of tumor resorption the lymph nodes also begin to decrease, and by the end of resorption the weight of the regional nodes approaches the weight of the contralateral nodes. We were unable to detect any visible changes in the latter throughout the extent of the tumor process.

From this it would follow that the development of the Brown-Pearce tumor homotransplant in the helix of rabbits is accompanied by a markedly demonstrated enlargement of the regional lymph nodes. This enlargement develops from the beginning of tumor growth, and attains its maximum before the tumor stops growing and undergoes regression. As the tumor is resorbed the enlargement of the regional lymph nodes also gradually disappears.

As the microscopic investigation showed, the growth of the nodes is not related to metastasis of the tumor; only in one node were there observed individual tumor cells, undergoing degeneration.

Study of the prepared cytological samples showed that the basis for the hypertrophy lay in the profound changes in cell composition of the nodes (Table 2, Fig. 2).

In the normal rabbits the cytogram for the nodes of both sides was identical. The basic mass of cell elements consisted of lymphocytes and prolymphocytes. The number of lymphoblasts did not exceed 27 per 30 visual fields. There were a large number of reticular cells. The remaining cell elements (polyblasts, mature plasma cells, eosinophiles) were counted individually per 30 visual fields; as a rule, pseudoeosinophiles and macrophages were not encountered.

Even one day after transplantation of the tumor the cytogram for the node on the tumor side differed from the cytogram of the contralateral node. No changes of any kind were detected in the latter. In the experimental node we noted stimulation of the reticuloendothelium, with an increase in the number of reactive cells – polyblasts,

TABLE 2. Cell Changes in the Lymph Nodes During Development of the Brown-Pearce Tumor in the Helix of Rabbits

Group of rabbits	Number of rabbits	Period of tumor development	Day following transplantation	Lymph node	Cell forms: Reticulocytes	Polyblasts	Macrophages	Lymphoblasts	Plasmocytes	Eosinophiles	Pseudoeosinophiles	Mitoses
First	5	Control rabbits		R	111.8	3.6	0	19.1	4.1	6.0	0.2	1.0
				C	107.7	3.9	0.2	22.8	8.1	5.2	0.8	0.4
Second	3	Growth	1st	R	112.8	20.2	1.0	16.4	9.4	2.9	9.0	0
				C	127.2	4.2	0.3	16.7	3.0	1.7	1.5	0.5
Third	4	»	3rd	R	59.7	47.6	5.9	85.1	16.7	3.7	16.1	2.0
				C	122.3	17.5	0.3	20.8	4.2	1.0	2.8	0.2
Fourth	5	»	6th	R	55.5	26.1	4.8	134.4	15.1	0.7	2.7	1.8
				C	112.8	15.5	0.9	24.2	3.9	1.2	1.3	0.4
Fifth	3	Resorption	10th	R	61.2	13.2	2.3	144.5	18.8	2.5	1.3	6.2
				C	120.0	19.8	0.7	37.0	4.8	4.7	2.3	0.2
Sixth	5	The same	15th	R	69.0	10.6	2.4	115.7	16.5	1.8	1.8	2.1
				C	103.9	14.2	0.7	42.6	6.2	1.7	2.1	0.9
Seventh	4	» »	34th	R	105.9	4.7	2.5	49.5	2.5	2.0	1.0	0.6
				C	101.2	4.5	1.1	35.5	3.3	2.6	1.2	0.3

Note. In each column - the average number of cells per 30 visual fields (3500-4000 cells in total); R - regional node; C - contralateral node.

having a reticular derivation. Pseudoeosinophiles and single macrophages were noted.

On the third day following transplantation the changes in the node on the tumor side were more profoundly developed. The decrease in the number of typical reticular cells was striking. However, the other cell forms of reticular derivation were increased in number –polyblasts and macrophages, associated with a well-defined erythrophagia. A small increase was detectable in the number of mature plasmocytes. The most characteristic feature of the microscopic picture was the appearance of new cell forms, having the following traits: primarily large cells of round or oval form, sometimes having pseudopods. A large round nucleus occupied a central or slightly eccentric position, and contained a fine network structure which stained weakly. The cytoplasm was markedly basophilic and often vacuolated. Mitotic figures were occasionally seen in these cells.

The described cells are apparently of reticular derivation, since transitional forms can be found between them and the typical reticular cells. The morphological characteristics justify placing the described cells in the category of young cells of the hemocytoblast-lymphoblast type. For brevity in designation we unite them into one group, the lymphoblasts.

On the 6th day following transplantation of the tumor the changes in the cell composition of the regional node reached full development. Most characteristic was a further increase in the number of cells of the lymphoblast type. Proliferation of these cells occurred throughout the entire node. In the cortical layer these cells sometimes suggested plasmoblasts (Fig. 3), but we refrained from using this name since from these cells we trace the gamma transitional forms to the typical prolymphocytes, and not to plasmocytes. At this time there is the well defined appearance of reticuloendothelial stimulation and polyblastic reaction in the contralateral node.

In the process of tumor resorption the neogenesis of lymphoblast-like cells continues, but its intensity decreases; the cells of the earlier developmental intervals become smaller, while larger prolymphocytes appear. The number of plasma cells does not increase in comparison with the previous periods. The number of normal reticular cells goes up. A small lymphoblastic reaction can be detected in the contralateral node.

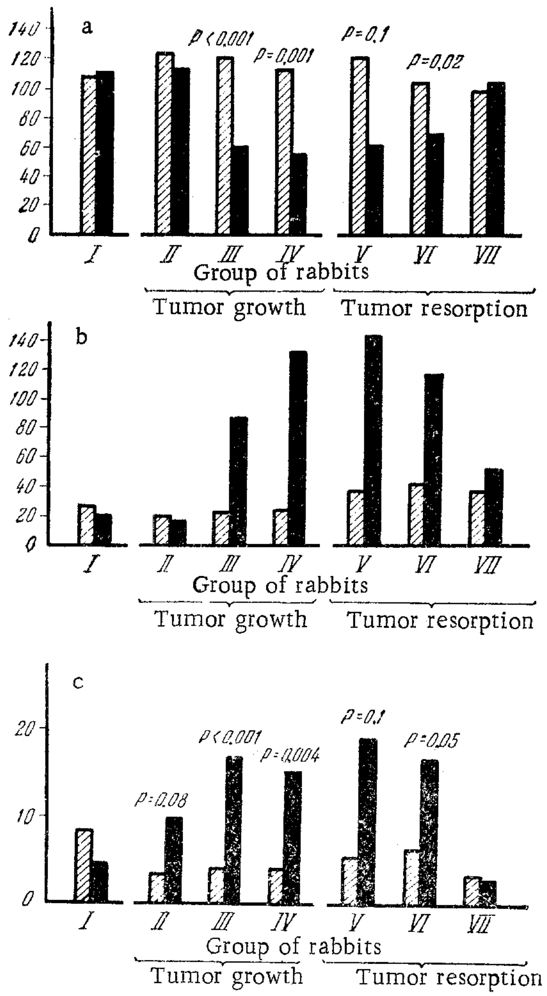

Fig. 2. Dynamics of the cell forms in the lymph nodes during the development of the Brown-Pearce tumor in the helix of rabbits. a) Reticular cells; b) lymphoblasts; c) mature plasma cells; black bar–regional node; cross-hatched bar–contralateral node. On the ordinate axis–number of cells per 30 visual fields (a,b,c).

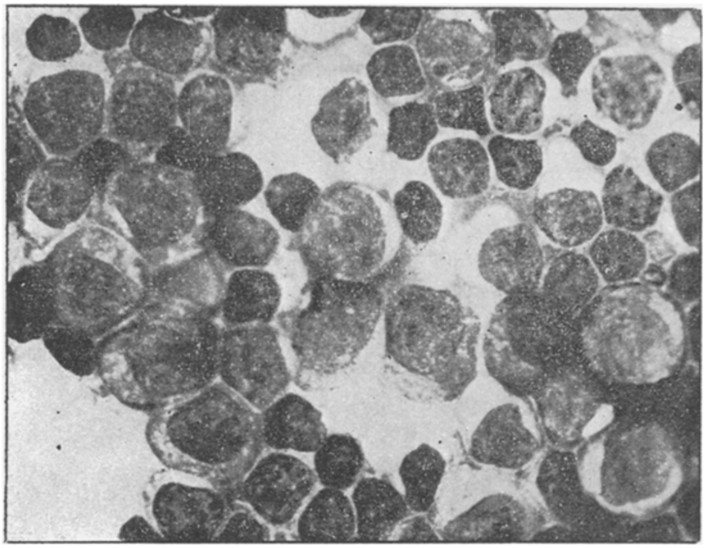

Fig. 3. Cellular reaction in the regional lymph node at the period of its maximal hypertrophy. The large lymphoblast-like cells are numerous. Magnification ×800.

As the tumor resorption approaches the end, all the cellular shifts gradually decrease and the cytogram of the node increasingly approximates the normal.

Thus, the investigation showed that the basis for hypertrophy of the regional lymph node, developing in response to tumor transplantation, lies in the intense proliferation of cellular elements of reticular derivation, belonging, by their cytological characteristics, to the young cell category of the lymphoblast type. This cellular reaction primarily develops in the regional nodes, but it is also noted, to a weak degree, in other lymph nodes. Complete development of the cellular reaction is attained on the 6th day following transplantation of the tumor, i. e., before the tumor begins resorption. During the process of tumor resorption the cell changes gradually disappear.

A comparison of our results with literature data [2,3,13,19] shows that the character of the changes observed, the time intervals of their onset, their dynamics and the site of development are very similar to the cell changes which characterize the process of immunogenesis associated with the action of foreign body antigens. It is important to emphasize that we did not observe a transition of the basic mass of young cell forms to mature plasma cells.

It is of interest to juxtapose that fact with our data showing that during regression of the Brown-Pearce tumor in the helix we could not demonstrate antibody in the blood of the rabbits [5]. The literature records that the appearance of adult plasmocytes is characteristic for the process of hyperimmunization, and observes a parallel relationship between the degree of their formation and the rise in antibody titer in the blood [2,13].

The results of the investigation show that the process of development of antitumor immunity, associated with temporary survival and subsequent resorption of the tumor homotransplant, is vividly reflected by morphological shifts in the regional lymph nodes which are characteristic for the processes of immunogenesis. A deeper study of the immunological nature of these shifts will serve as a project for subsequent experiments.

SUMMARY

The author studied the state of the lymph nodes in dynamics during the growth and resolution of the Brown-Pearce tumor transplant in the helix of rabbits. From the very first days of the tumor growth the regional lymph nodes are subject to considerable hypertrophy, reaching its greatest development prior to the resorption of the tumor. At the basis of hypertrophy there is an intensive formation of cellular elements of reticular origin and those related to the young cells of the lymphoblast type. Cellular reaction develops mostly in the regional lymph nodes and reaches its complete development on the 6th day after the inoculation of the tumor. Cellular shifts are reduced in the process of tumor regression and disappear with its completion.

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