STUDIES ON THE IMMUNOLOGICAL RESPONSE TO FOREIGN TUMOR TRANSPLANTS IN THE MOUSE

III. Changes in the Weight, and Content of Nucleic Acids and Protein, of Host Lymphoid Tissues*

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The events following the transplantation of tumor homografts have been followed in previous papers (1, 2). The timing was observed of graft breakdown, of the capacity of the draining lymph nodes to confer immunity on secondary hosts, and of the production of hemagglutinating antibody by the lymph nodes and spleen. It is the aim of the present work to relate these processes to changes in size of the host lymph nodes, spleen, and thymus. Further insight into them is given by analyses of the nucleic acids and protein nitrogen contents of the draining lymph nodes. Pentosenucleic acid (PNA) has frequently been linked with the process of protein synthesis (3, 4), and is therefore expected to increase during antibody production. The desoxypentosenucleic acid (DNA) content of a particular tissue gives an indication of the number of nuclei present, and an increase in the PNA/DNA ratio has therefore been widely interpreted as an increase in PNA per cell (5). This measure has been adopted in the present work. A further justification of its use is provided by the procedure of analysis used: the Schneider extraction gives a particularly accurate estimate of the ratio.

Changes in the nucleic acids and protein nitrogen contents of the popliteal lymph node of the rabbit have been investigated by Ehrich, Drabkin, and Forman (6), and by Harris and Harris (7). The PNA content of the lymph node cells was found to increase during antibody production. Homburger (8), and Savard and Homburger (9), found that the protein nitrogen of the lymph nodes increased after the implantation of tumors in mice. A susceptible but outbred strain of mice was used in this work, so that it was uncertain whether an immunological response was provoked. Kidd and Toolan (10–12)

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have reported histological changes in the lymph nodes of non-susceptible mice implanted with tumor.

Methods

The transplantable tumors, and most of the strains of mice used, have been described in an accompanying paper (1). The C57BL/6 strain carries the *b* allele at the histocompatibility-2 locus, and therefore is not susceptible to lymphosarcoma 6C3HED with which it was implanted. Implantation of the tumors was carried out by trocar subcutaneously in the flank.

At intervals after tumor implantation, the axillary, brachial, and inguinal lymph nodes draining the site of implantation were taken and pooled. The corresponding nodes from the opposite side (contralateral nodes), the spleen, and the thymus, were also taken. The lymph nodes were weighed and subjected to analysis in pools from four individual mice, but the spleens and thymuses were weighed individually. Tissue for analysis was treated by the method of Drasher (5). Two subsamples of 1 to 4 mg. dry weight each were weighed out for determination of nitrogen by the Markham method (13). Nucleic acids were separated from the remaining tissue by the method of Schneider (14). DNA was determined in the extract by Dische diphenylamine reaction (15), and PNA by the Mejbaum orcine method (16).

Four types of tumor-host combination were investigated. The reaction provoked by a sarcoma in a non-susceptible host strain was followed in the first series, by implantation of Sarcoma 1 in C57BR/a mice. The same combination of tumor and host was also used in the second series, with hosts which had sloughed off a graft of the tumor inoculated 32 days earlier, and which had therefore been immunized. In the third series the reaction to Sarcoma 1 was followed in susceptible hosts of the A strain, which is the strain of origin of the tumor. This series therefore constituted a control in which an immunological response to the strain-specific antigens of the tumor was not provoked. In the fourth series the response of non-susceptible hosts to a lymphoma was followed, by implantation of lymphosarcoma 6C3HED into C57BL/6 mice.

The full schedule of chemical analysis was carried out only with the draining lymph nodes in the first and second series. The wet weights alone of the remaining organs were measured. In the first, second, and third series, four spleens or thymuses, or four pools of lymph nodes, were taken at each interval. Each of the values given below therefore represent the means of four samples. In the fourth series, two organs or pools were taken at each interval.

Results and Discussion

Changes in the weights of the organs are shown in Fig. 1. The lymph nodes increased in weight after tumor implantation in all series, as did the spleen except in previously immunized hosts. On the other hand the thymus gave no significant response, except for a progressive reduction during the growth of the sarcoma in susceptible hosts.

The regional lymph nodes have been shown to take part in the immunological response which brings about graft destruction in non-susceptible hosts (1, 2). The timing of their increase in weight is in excellent agreement with their immunological function. The increase took place 5 to 15 days after tumor implantation, that is to say at a time when the nodes can confer heightened graft resistance on secondary hosts, and are producing hemagglutinating antibody most rapidly. After implantation of tumor into previously immunized

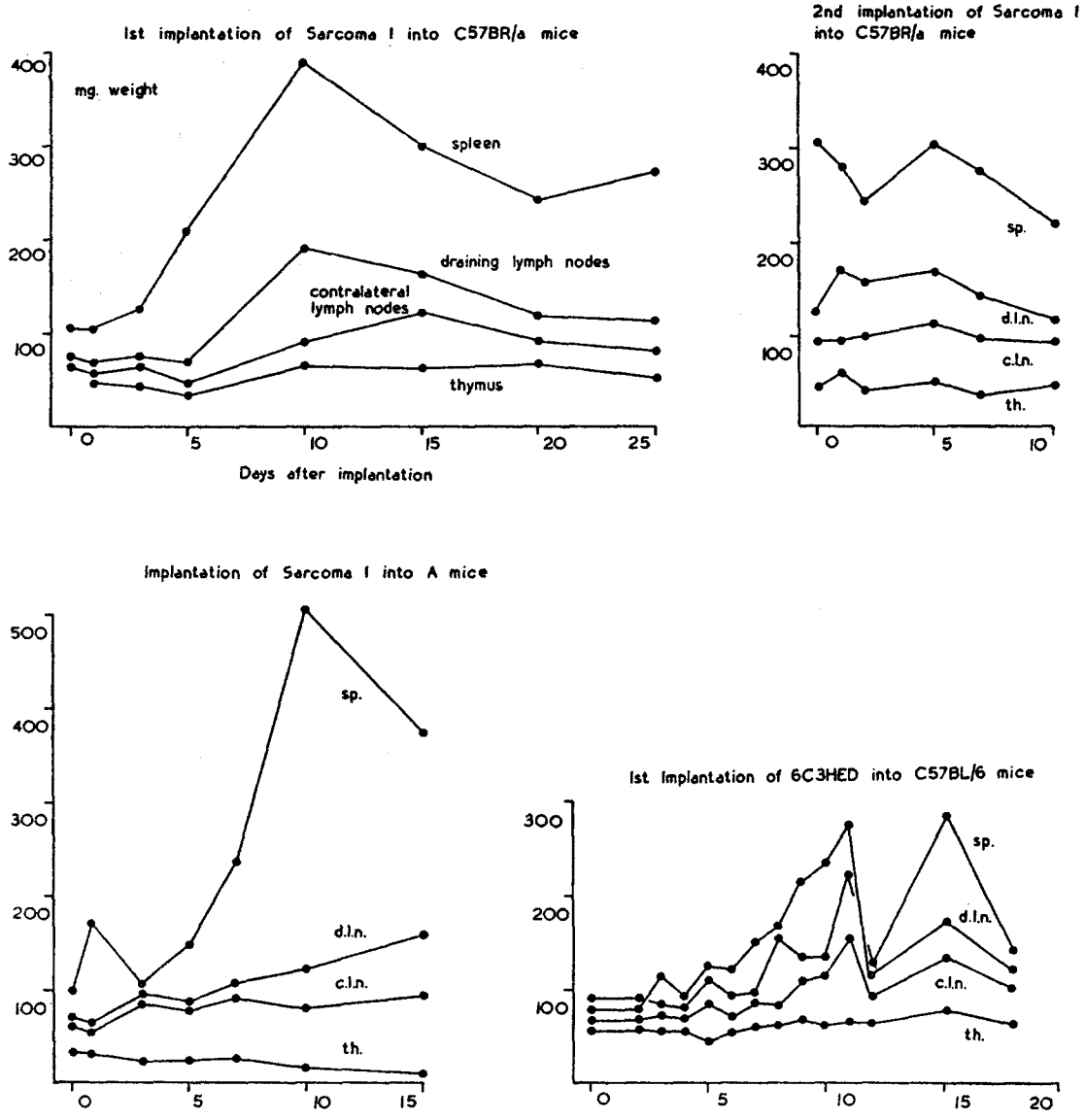

FIG. 1. Changes in the weight of host organs after tumor implantation.

hosts, the immunological response is more rapid, and a more rapid increase in the lymph node weight was accordingly observed. The lymph nodes draining the site of implantation are the only nodes which have been shown to participate significantly in the response; and as would follow from this fact, in both series of primary responses in non-susceptible hosts the increase was more marked in the draining nodes than in those from the opposite side. Some increase was also found in the contralateral nodes, possibly a sign in them of a weak immunological response.

The spleen has also been shown to participate in the immunological response (2), at least by the production of hemagglutinating antibody. It becomes active in this relation a few days later than the draining lymph nodes. The greatest increase in weight took place somewhat later in the spleen than in the draining lymph nodes during the response of non-susceptible hosts to the lymphosarcoma, though not to the sarcoma.

A comparison can be made between the responses of susceptible and non-susceptible hosts to the sarcoma. The lymph nodes in susceptible hosts increased in weight less rapidly than in non-susceptible hosts, and up to the 5th day after implantation the same held true of the spleen. The later stages of growth of the tumor in susceptible hosts are not comparable with its growth in non-susceptible hosts, since a far greater size is attained, and ulceration frequently occurs. The initial increases in the size of the lymph nodes and spleen in the third series suggests a response to the antigens of material contaminating the tumor, in susceptible and also presumably in non-susceptible hosts.

The results of the chemical analyses in series one and two are shown in Table I. The protein content of the draining lymph nodes increased as their weight increased, as shown by the nitrogen analyses. There are no indications of changes in the proportion of protein.

Considerable changes took place in the PNA and DNA content of the nodes after tumor implantation. The changes in the PNA/DNA ratio are shown graphically in Fig. 2. The ratio shows an initial lag during the primary response, then increases to a maximum value at 15 days after implantation, and then declines. During the secondary response there is a shorter lag, and the ratio attains a greater value. These features are in complete agreement with the observations on the immunological function of the nodes, when the ratio increases during antibody production. The conclusion can be drawn that during the reaction to foreign tumor grafts, as well as during the response to classical antigens (6, 7), the PNA/DNA ratio is an *excellent* indicator of antibody production.

SUMMARY

A study was made of variation in weight of the host lymph nodes, spleen, and thymus, after implantation of transplantable tumors in susceptible and

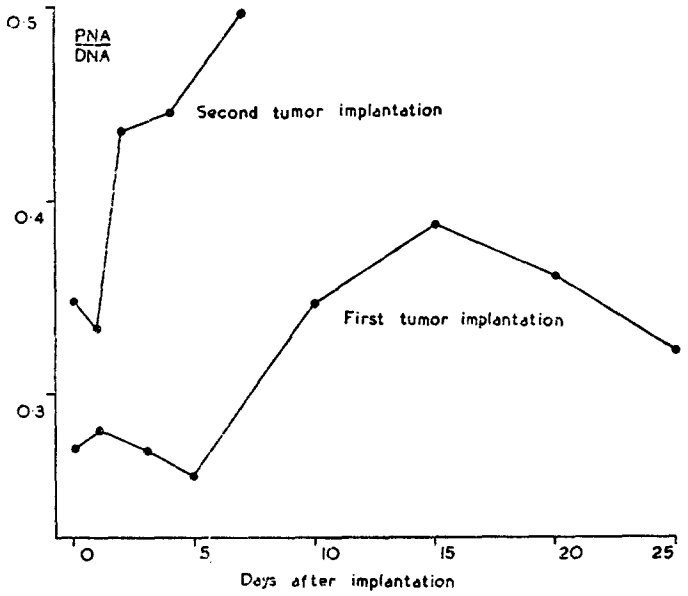

FIG. 2. Changes in the PNA/DNA ratio of the pooled axillary, brachial, and inguinal lymph nodes draining the site of implantation of Sarcoma 1 in non-susceptible (C57BR/a) mice.

TABLE I

Nucleic Acids and Protein Nitrogen Contents of the Pooled Axillary, Brachial, and Inguinal Lymph nodes Draining the Site of Implantation of Sarcoma 1 in Non-Susceptible (C57BR/a) Mice

Days after implantation	Pooled nodes, per four mice				PNA/DNA
	Mean mg. dry weight	Mean μg. PNA content	Mean μg. DNA content	Mean μg. N content	
Series 1: First Implantation of Tumor					
0	8.3	276	1022	990	0.270
1	7.5	293	1047	966	0.280
3	8.3	354	1318	1055	0.268
5	8.9	322	1257	1044	0.256
10	23.2	798	2299	2772	0.347
15	21.5	689	1780	2963	0.387
20	14.8	375	1041	1857	0.360
25	15.4	509	1582	1949	0.322
Series 2: Second Implantation of Tumor					
0	14.0	521	1491	1746	0.349
1	19.3	569	1714	2615	0.332
2	18.7	734	1681	2445	0.437
4	23.4	800	1800	2579	0.445
7	18.9	714	1440	2204	0.496

non-susceptible hosts. The lymph nodes and spleens of non-susceptible hosts increased in weight during the period when the organs were participating in the immunological response, though an increase also took place in susceptible hosts. Variations in protein nitrogen and pentose- and desoxypentosenucleic acid of the draining lymph nodes of non-susceptible mice were also studied. The protein nitrogen content increased with the weight of the nodes. Increase in the PNA/DNA ratio occurred while the lymph node cells were engaged in production of antibody. Increase in the PNA/DNA ratio was interpreted as an increase in PNA per cell, and therefore of the rate of protein synthesis.

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