Cellular Reactions Following Tumor Growth with Special Reference to Plasma-cellular Response

B. D. Baruah

(Department of Pathology and Cancer Research Centre, Assam Medical College, Dibrugarh, Assam, India)

SUMMARY

The local and remote cellular reactions of the host following tumor growth have been described. During the course of progressive growth of a transplanted (Rd/3 and Walker) and induced tumor in rats, the regional lymph nodes and spleen increased in weight, associated with the development of a characteristic plasma-cell response accompanied by diminution of lymphocytic tissue, while there was a slight local reaction and scanty plasma-cell accumulation around the tumor graft.

Induced tumor inhibition and regression were associated with an increased local host reaction around the tumor, consisting of an increased accumulation of plasma cells.

Following regression of the transplanted tumor, the animal became resistant to a second graft of the same tumor at a different site. Heat-killed tumor grafts which were no longer capable of stimulating this plasma-cell response failed to render the host resistant to subsequent inoculation of living tumor cells.

It was concluded that the plasma-cellular response is the morphological manifestation of an immune reaction, due to antigenic stimulation from these tumors, and that the plasma cells are involved in the production of antibodies. Judged by the plasma-cellular reaction, transplantation immunity to tumor and normal tissues conforms with the immune state generated by bacterial and other foreign protein antigens.

Reticulum cells contributed to the formation of plasma cells.

Growth and regression of a tumor tissue in experimental animals have been shown by various workers to be accompanied by changes in the organs of the host, especially in the lymph nodes and spleen and also locally around the tumor (8, 12, 15, 21, 25, 26, 28, 29, 31, 32, 34, 38, 39). Although there is a close association between the immunity to a transplantable tumor and presence of local cellular infiltrations of, e.g., lymphocytes and plasma cells, there has been a wide diversity of opinion concerning the specific cell or cells involved.

That the lymphocyte plays a part in tumor immunity was suggested because a large number of these cells were seen around any tumor graft, which were capable of eliciting immunity, and because there was a constant association between these cells and tumor destruction. Lymphocytic destruction of tumor homografts was first proposed by Wade (39). Da Fano (12) and Mottram and Russ (28) demonstrated experimentally the correlation between the presence of lymphocytes and the development of immunity to tumor homografts. Murphy (29) claimed that homografts are directly destroyed by the surrounding lymphocytes. Loeb (25) correlated the intensity of the lymphocytic reaction around the grafts with the genetic disparity between the graft and the host (individuality differentials). Apparently he did not ascribe to the lymphocyte any direct role in tumor destruction. Kidd and Toolan (24) emphasized the close association between the lymphocytes of the host and the cells of a degenerating tumor homograft, when inbred strains of mice were used. Weaver et al. (40), using diffusion chambers in mice, have shown that the lymphocytes are the cells responsible for the destruction of the tumor homograft. Moreover, the positive role of lymphocytes in tumor immunity received support from the investigations of Harris et al. (19), Dougherty...
et al. (14), and Kass (23), which claimed that the lymphocytes produce antibodies.

Recent work has shown that plasma cells are involved in the production of antibody against bacterial, viral, and other foreign protein antigens (4). Several workers have also observed plasma-cell development in the host, following homografts of tumor (15, 20, 21, 26, 31, 32) and normal tissue (36). Parsons (31, 32) first noted a widespread development of plasma cells in the lymph nodes of mice bearing transplanted generations of methylcholanthrene-induced tumors, Mal-Sarcoma 37. Similar observations have been reported by subsequent workers in man and in different animals (mice, rats, and chickens), with a variety of tumors (CSH mammary carcinoa, Walker carcinoa, benz-pyrene-induced tumor, chicken lymphoma) (7, 15, 20, 21, 26). They also found an association between local infiltration of plasma cells and the onset of tumor regression and destruction.

It is generally agreed that homografts of normal and tumor tissues are destroyed by a "specific immune" reaction, and several workers have been successful in demonstrating antibody production by such homografts. This problem has been approached here from a different angle, namely, by demonstrating plasma-cellular reaction in the tissues of the host, which may be considered as an indication of the immunologic changes taking place.

In the following experiments a detailed histological and cytological study was made in rats to observe the local and remote reactions of the host, following transplanted and induced tumors, during the stage of progressive growth and regression, with special reference to the plasma-cell equipment.

MATERIALS AND METHODS

Tuck male albino rats, weighing initially about 120–180 gm., were employed throughout. The animals were kept in cages under optimal conditions and given a diet of commercial rat cubes (No. 86, containing 20 per cent protein) and water ad libitum.

Tumor transplantation.—Rd/3 rat sarcoma (originally induced by 1,2,5,6-dibenzanthracene) and Walker carcinoa were used. Viable portions of a 10–12-day-old tumor were implanted subcutaneously into the right flank, with proper aseptic precautions. The tumors take in 100 per cent of these rats, becoming palpable within 6–8 days, grow steadily, and usually kill the animal between 17 and 30 days following implantation.

Induction of tumors.—1,2,5,6-Dibenzanthracene (DBA), 5 mg. in arachis oil, was injected into the subcutaneous tissue of the right side, twice in a week. Tumors appeared after 6–7 months in 80 per cent of the surviving rats and were very slow-growing.

Tumor inhibition and regression.—Tumor (Rd, 3 and Walker) inhibition and regression were induced by intraperitoneal injection of a small dose of DBA (1–2 mg), which has little or no effect on the body weight (18). DBA in the form of a suspension (1 per cent in 0.2 per cent Teepol) was injected intraperitoneally (2 mg/100 gm of body weight) on alternate days from the day of implantation of the tumor and continued until there was regression or definite inhibition. Each animal received a total dose of 14–47 mg. In the control group, rats were implanted with the tumor, and the same amount of 0.2 per cent Teepol was injected intraperitoneally on alternate days.

Twenty rats were used in each of these experiments. Animals were weighed, and the growth of the tumor was ascertained by palpation at semi-weekly intervals in two dimensions. Animals were killed with chloroform at different stages of tumor growth, inhibition, and regression. Tumors and different organs were dissected out and weighed to the nearest mg. Histological sections and smears were prepared and stained as described below.

Histological technic.—The tissues were fixed in 10 per cent formal saline and 95 per cent alcohol, embedded in paraffin in the usual way, and cut into sections about 6 μ thick.

Smear preparations.—These were made from the lymph node, spleen, and bone marrow. One-half of the lymph node or spleen was fixed for paraffin section, and the other half was scraped with a sharp scalpel. The scrapings were floated on normal rat serum and thoroughly mixed. A drop was transferred to a clean slide and then covered with a second slide, producing a uniform cell suspension. The slides were then pulled apart and dried. The opposing surfaces of both slides presented an even distribution of the material. They were then immediately fixed with methyl alcohol and stained. Plasma cells can be counted easily from these preparations, and a slight increase of these cells can be readily recognized.

Staining methods.—Sections and smears were stained with hematoxylin and eosin and Unna-Pappenheim (methyl green-pyronin). Unna-Pappenheim stain proved very satisfactory for the demonstration of plasma cells, since it permits better discrimination of different cell types. Moreover, this procedure can be considered as a differential stain for nucleic acid (10), methyl green being highly specific for "nuclear" deoxyribonucleic acid.
and pyronin being indicative of "cytoplasmic" ribonucleic acid.

Unna-Pappenheim stain (Gurr) in 1 per cent aqueous solution was used. Sections and smears were stained for 30–40 mins.

Estimation of the amount of plasma cells in different organs.—The amount of plasma cells was estimated in both smear preparations and histological sections.

a) Smear preparations: Figures for the relative percentage of plasma cells were obtained from counts of about 500 cells in the stained preparations. The cell counts obtained in this way are quite reliable and reproducible if the same technic is employed throughout.

b) Histological sections: Following a general study of the histological section, particular attention was paid to the plasma-cellular contents. A method of recording the quantity of plasma cells was attempted, based on subjective judgment, and the amount was expressed by means of 6 symbols (Paganaeus. 1948):

- 0 = No plasma cells or only isolated ones detectable.
- += Sparserness of plasma cells, in small groups.
- ++ = Moderate amount of plasma cells and larger groups of cells.
- +++ = Fair abundance of plasma cells.
- ++++ = Abundance of plasma cells.

See also Squier and Albert for additional criteria.

c) Connective tissue “capsule” of the tumor: Histological sections from different areas of the tumor were examined. The number of plasma cells and lymphocytes was counted in the connective tissue “capsule” around these grafts, where the infiltrating cells were the most dense. The two types of cells were for the most part mixed together, and counts were made from such areas, avoiding any special accumulation of either type. Two sections from each graft were studied, and in this way about 200 cells were counted in each section. The mean number per 1 sq. mm. was determined.

The gross plasma-cellular reaction was also expressed in arbitrary grades (0, ±, +, ++, ++++, ++++).

RESULTS

HISTOLOGICAL APPEARANCE AND AMOUNT OF PLASMA CELLS IN DIFFERENT ORGANS IN THE NORMAL RAT

The weights of the axillary and para-aortic (lumbar) lymph nodes in mg 100 gm body weight of normal rats varied from 7 to 10 mg. (av., 9 mg.) and 5 to 10 mg. (av., 8 mg.), respectively.

Histologically, the cortex appeared as a broad zone, almost surrounding and bordering the medulla, which was present as a narrow strip. The main cellular constituents were lymphocytes, and plasma cells were usually absent. The medullary cords contained lymphocytes, reticulum cells, and a varying number of plasma cells, which were almost entirely mature in type. Although the plasma-cellular content varied in different lymph nodes, the axillary and para-aortic lymph nodes contained only a few (0.26 per cent in smears and ± in sections). Therefore, these lymph nodes were considered to be suitable for any observation on the plasma-cellular reaction.

The spleen contained very few plasma cells (0.34 per cent in smears and ± in sections), mostly mature in type. They were present in the red pulp, lying mostly along the trabeculae. The Malpighian follicles did not contain any plasma cell.

The plasma cell has been classified on the basis of size of the cell, size and shape of the nucleus, and staining character of the nucleus and cytoplasm with Unna-Pappenheim stain.

Immature plasma cell.—It was a large, rounded, or oval cell, measuring about 10–14 μ in diameter. The nucleus was centrally placed. The chromatin content was diffusely dispersed within the nuclear membrane. Large, distinct, and usually round nucleoli were present which took on a slightly red color with pyronin. The cytoplasm was slightly vacuolated and conspicuously basophilic, forming a rather narrow border around the nucleus. With pyronin, it took on a slight to moderate red color. A perinuclear clear zone was usually absent.

With further maturation, the nucleus became slightly smaller, eccentrically placed with a condensation and thickening of the chromatin strands. The cytoplasm was increased, and the clear vacuole became evident in the cytoplasm adjacent to the nucleus. Mitosis was often seen in these cells.

Mature plasma cell.—It was a rounded or oval cell, sometimes polyhedral, of about 7–12 μ in diameter. The nucleus was more or less eccentrically placed, slightly smaller and richer in chromatin than in the immature type. The nuclear chromatin was clumped in an irregular pattern along the nuclear membrane, which often resembled a “cart-wheel” and stained deeply bluish-green. Nucleoli were usually not visible. The cytoplasm was abundant and distinctly basophilic, stained brilliant red with pyronin, was less vacuolated than in the immature type but with a characteristic “perinuclear clear area” adjacent to the nucleus. Tran-
tional forms between two types of plasma cells were frequently seen.

That the pyronin-stained material in the cytoplasm and the nuclei is ribonucleoprotein has been demonstrated by specific depolymerization by treatment with ribonuclease and hot trichloroacetic acid solution (5 per cent trichloroacetic acid for 15 minutes, at 90°C.) and subsequent staining with Unna-Pappenheim along with the controls. Ribonuclease treatment removed the entire red staining cellular material. In the latter, as the acid is a solvent for both types of nucleic acids, deoxyribonucleic acid was removed as well, and neither the green nor the red elements could be seen.

**Changes during the Progressive Growth of the Tumor**

The lymph nodes, especially the axillary and the para-aortic of the right side and the spleen, showed a considerable increase in weight along with the growth of the Rd/3 and Walker tumor (Table 1). A similar but less conspicuous increase in weight of these organs was also observed with the DBA-induced tumor. This increase in weight was quite unrelated to the incidence of metastases.

Some of these lymph nodes showed hemolymph node formation. Normal lymph nodes generally showed a bright to a darker yellowish color. In contrast, these affected lymph nodes showed a red or brownish-red coloration, varying in extent and degree. The amount of discoloration tended to be greater toward the later stages of tumor growth. In particular, the right axillary and para-aortic lymph nodes were mostly affected, and those with the Walker carcinoma showed a frequent and stronger response.

The weight of the adrenals was increased, and that of the thymus was decreased considerably, along with the growth of the tumor.

**Microscopic** — Considerable variations occurred in the development of plasma cells in the lymph nodes and spleen of these tumor-bearing animals, depending on the early or late development of the tumor, time of killing, and the size of the tumor.

**Smear preparations** — Although all the lymph nodes show increased number of plasma cells (immature and mature), the most striking increase has been observed in the axillary and para-aortic lymph nodes of the right side and the spleen (Table 1). They became much more numerous as the tumor progressed.

**Histological** —

Changes in the lymph nodes: As the tumor increased in size, the cortical lymphoid tissue became reduced, being replaced by the enlarged medullary tissue. The medullary cords were broad and crowded, with proliferated reticulum cells and plasma cells (immature and mature) (Figs. 1 and 2). Mitoses were frequent. Russell-body cells were also found (Fig. 4). The increased plasma cells were limited to the medullary cords only and usually appear in contact with the proliferated reticulum. No increase of plasma cells was seen in the cortical lymphoid tissue. Occasionally the medullary tissue was formed by a mass of coalescing cells with copious cytoplasm, many of them with eccentric nuclei. Free plasma cells were found in these areas, apparently in the process of detachment from the main mass. Eventually these cells became disunited, and free plasma cells were formed. All gradations from the reticulum cells to mature plasma cells could be seen.

Sinuses were dilated, and free histiocytes and a varying number of plasma cells were found. Macrophages, loaded with iron-containing materials, occurred, and mast cells were found to be increased. In the hemolymph nodes large numbers of erythrocytes were found in the sinuses, and many free macrophages showed erythrophagocytosis (Fig. 3).

There was a significantly constant relation between the amount of plasma-cell response and weight of these organs and of the tumor. No definite relation between the plasma-cell increase, hemolymph node formation, and metastasis was observed. Moreover, the lymph nodes which had secondary metastases showed a markedly diminished plasma-cell reaction. Ulceration and necrosis of the tumor did not appear to have any marked effect on the plasma-cellular reaction in these organs.

Changes in the spleen: In the spleen the red pulp became highly cellular and showed proliferation of the reticulum cells and increased number of immature and mature plasma cells, especially around the trabeculae (Fig. 5). Transitions from reticulum cells to mature plasma cells could be observed. The Malpighian follicles were not greatly affected. They were rather smaller in a few instances, and no increase of plasma cells was found.

A similar but less marked plasma-cell response in the draining lymph nodes and spleen was observed with DBA-induced tumors.

**Local reaction of the host around growing tumors** — The local reaction of the host around the tumor during its progressive growth was slight, and the cellular infiltration was scanty, consisting of lymphocytes, macrophages, plasma cells, and a few polymorphonuclear neutrophils, embedded in connective tissue. Table 2 shows the amount of infiltrating lymphocytes, and either plasma cells are absent or only a few are present. The tumor weight
## TABLE 1

**Weight of and Plasma-cellular Reaction in Different Organs of Rats during the Progressive Growth of Transplanted RD/3 Tumor**

<table>
<thead>
<tr>
<th>ANIMAL SERIES</th>
<th>WEIGHT (mg/100 gm. Body wt.)</th>
<th>TUMOR WEIGHT (gm.)</th>
<th>DAYS AFTER TUMOR TRANSPLANTATION</th>
<th>PERCENTAGE OF PLASMA CELLS IN SMEARS</th>
<th>PLASMA-CELLULAR REACTION IN HISTOLOGICAL SECTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Splenic</td>
<td>Rt. axillary lymph node</td>
<td>Lt. axillary lymph node</td>
<td>Rt. para-aortic lymph node</td>
<td>Thymus</td>
</tr>
<tr>
<td>1</td>
<td>354</td>
<td>14</td>
<td>11</td>
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</tr>
<tr>
<td>2</td>
<td>501</td>
<td>17</td>
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<td>9</td>
<td>170</td>
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<tr>
<td>3</td>
<td>652</td>
<td>21</td>
<td>24</td>
<td>8</td>
<td>98</td>
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<td>1024</td>
<td>27</td>
<td>34</td>
<td>7</td>
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</tr>
<tr>
<td>6</td>
<td>880</td>
<td>27</td>
<td>20</td>
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<td>84</td>
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<tr>
<td>7</td>
<td>854</td>
<td>16</td>
<td>12</td>
<td>7*</td>
<td>68</td>
</tr>
<tr>
<td>8</td>
<td>880</td>
<td>24</td>
<td>34</td>
<td>8</td>
<td>61</td>
</tr>
<tr>
<td>9</td>
<td>1265</td>
<td>17</td>
<td>16</td>
<td>6</td>
<td>72</td>
</tr>
<tr>
<td>Normal mean</td>
<td>314</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>165</td>
</tr>
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</table>

* Hemolymph node formation.

Each figure represents average of two rats.
and the presence of central necrosis (which always occurred toward the later stage of tumor growth) appeared to have no effect on the local plasma-cellular infiltration.

Peripheral blood showed absence of plasma cells at any stage.

Changes during Regression of the Tumor

All the tumor-bearing rats of the control group, receiving Teepol only, died within 16-20 days after transplantation of the tumor. In the DBA-treated rats, the effects produced on tumor growth were as follows:

a) The tumor growth occurred as usual, and, after attaining a certain size, it gradually commenced to regress and ultimately disappeared. In many cases, the animal was able to rid itself entirely of the tumor by a *shelling out* process.

b) Tumor growth was normal for a few days and then arrested and remained stationary.

c) Tumor growth was slow from the very beginning, and the tumor afterwards either regressed or remained stationary.

d) Growth of the tumor remained unaffected, with the tumor ultimately killing the animal in the usual course of time.

Forty-five per cent of the tumors showed definite regression, and 30 per cent showed marked inhibition of the tumor growth, in the case of the Rd/8 sarcoma, within a period varying from 14 to 35 days after transplantation. In the case of the Walker carcinoma, 25 per cent showed definite regression, and 40 per cent showed marked inhibition of the tumor growth, within 13-28 days after transplantation.

Macroscopic.—The weights of different organs and tumors following treatment with intraperitoneal injections of DBA are shown in Table 3. The spleen, right axillary and right para-aortic lymph nodes showed a considerable increase in weight. However, the general impression is that, in most animals with small regressing tumors, the weights of these organs were less than those in animals bearing growing tumors. The incidence of hemolymph node formation was frequent and more marked.

Microscopic.—The plasma-cellular reactions in different organs from these DBA-treated, tumor-bearing rats are shown in Table 3. They showed considerable increase of plasma-cellular content (immature and mature) in the red pulp of the spleen and the medulla of the right axillary and right para-aortic lymph nodes, depending on the tumor size. The animals having small regressing tumors showed a diminished plasma-cell content.

By the time all the tumor cells had disappeared, the plasma-cellular reaction in these organs became diminished and gradually returned to normal.

Local reaction of the host around the tumor during regression.—In both Rd/8 and Walker tumor showing regression and marked inhibition, there was an increased local reaction of the host around the tumor, consisting of an accumulation of a large number of plasma cells, without any appreciable alteration of the lymphocytic infiltration. Table 4 shows the number of lymphocytes and plasma cells around these tumors. It may be seen that the number of lymphocytes infiltrating did not differ much from those found in progressively growing tumors. This local plasma-cellular reaction was found to be much stronger in the tumor, which reached a larger size before its regression. The plasma-cell accumulation was more numerous about the nodules of viable tumor cells. Plasma cells also penetrated the tumor tissue (Fig. 6) and in a few instances appeared to have invaded the tumor, resulting in tumor cells degeneration and disappearance. When the tumor cells were no longer present, the local reaction subsided, and the plasma-cell accumulation diminished.

Effect of heat-killed tumor homograft on the plasma-cellular reaction.—To study the effect of dead and necrotic tissue on the plasma-cellular response, fresh pieces of growing tumor (Rd/8 and Walker) were treated in boiling saline solution for 5 minutes and grafted into the subcutaneous tissue of the right flank in a group of rats. All these tumor grafts showed regression after various intervals of time. Animals were killed during different stages of regression.

### Table 3

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Plasma cells</th>
<th>Tumor wt. (g.)</th>
<th>Plasma-cellular reaction</th>
</tr>
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<tbody>
<tr>
<td>1080</td>
<td>58</td>
<td>1.6</td>
<td>+</td>
</tr>
<tr>
<td>960</td>
<td>28</td>
<td>10</td>
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</tr>
<tr>
<td>870</td>
<td>90</td>
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</tr>
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<td>42</td>
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<td>798</td>
<td>4</td>
<td>38</td>
<td>±</td>
</tr>
<tr>
<td>778</td>
<td>8</td>
<td>46</td>
<td>±</td>
</tr>
<tr>
<td>674</td>
<td>8</td>
<td>40</td>
<td>±</td>
</tr>
</tbody>
</table>

* Expressed in arbitrary grades.

Each figure represents the average of two animals.

In each case the tumor was growing at the time these data were collected.
## TABLE 3

**Effect of Intra-peritoneal Injection of DBA in Rats Bearing RD/3 Tumor on Tumor Growth and Plasma-cellular Reaction in Different Organs**

<table>
<thead>
<tr>
<th>ANIMAL SERIES</th>
<th>WEIGHT (mg/100 gm. BOD wt.)</th>
<th>DAYS AFTER TRANSPLANTATION</th>
<th>WT. OF TUMOR (om.)</th>
<th>CONDICTION OF TUMOR</th>
<th>PERCENTAGE OF PLASMA CELLS IN SMEARS</th>
<th>PLASMA-CELLULAR REACTION IN HISTOLOGICAL SECTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spleen</td>
<td>Rt. axillary lymph node</td>
<td>Lt. axillary lymph node</td>
<td>Rt. para-aortic lymph node</td>
<td>Lt. para-aortic lymph node</td>
<td>Spleen</td>
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<td>382</td>
<td>105†</td>
<td>81†</td>
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<td>10</td>
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</tr>
<tr>
<td>3</td>
<td>960</td>
<td>23</td>
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<td>9</td>
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<td>130†</td>
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<td>6</td>
<td>464</td>
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<td>10†</td>
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<td>11</td>
<td>33</td>
</tr>
</tbody>
</table>

* R = regressing; S = stationary; G = growing.
† Hemolymph node formation.
The weights of the right axillary and right para-aortic lymph nodes and of the spleen were not greatly affected. Microscopically, the tumors were completely necrotic. The local host reaction around the tumor was very slight, and the cellular reaction was scanty, consisting of fibroblasts and infiltration of polymorphonuclear neutrophils, lymphocytes, and macrophages. Plasma cells were few in number or entirely lacking. The right axillary and para-aortic lymph nodes and the spleen did not show any plasma-cell increase.

After regression of the tumors induced by DBA, or of heat-killed tumor graft, the animals were tested for susceptibility to the same viable tumor by subcutaneous inoculation into the opposite side. The animals in which regression of tumors (Rd/3 and Walker tumor) had occurred were found to be resistant to the inoculation of the same tumor and survived, whereas the animals which showed regression of heat-killed tumor grafts were susceptible to the tumor growth and died in the usual course of time.

**DISCUSSION**

These results show that the host reacts to a progressively growing transplanted and induced tumor by developing a characteristic plasma-cellular reaction, mostly in the lymph nodes which drain the site of inoculation of the tumor and the spleen. Inhibition and regression of induced tumor are regularly associated with an increased host reaction around the tumor, with accumulation of a large number of plasma cells, without affecting the local lymphocytic infiltration. This local plasma-cell accumulation remained high as long as any viable graft tissue persisted. When the tumor cells were no longer present, the local reaction subsided, and the plasma-cell accumulation diminished.

Following induced regression of the transplanted tumor, the animal became resistant to a subsequent challenge with the same tumor at a different site. Presence of living tumor cells was essential for this plasma-cellular response. Heat-killed tumor graft, which was no longer capable of stimulating this response, failed to render the host resistant to a subsequent inoculation of living tumor cells. Such a phenomenon is strongly suggestive of the presence of a circulating antibody.

Parsons (30-32), from a series of investigations on mice, considered the plasma cells, which developed in tumor-bearing mice, to be potentially malignant and a transitional form, illustrating a change of normal lymphoid tissue into tumor cells. She further observed that a sarcoma resen-

**TABLE 4**

<table>
<thead>
<tr>
<th>MEAN COUNTS PER 1 SQ. MM. AREA</th>
<th>TUMOR WEIGHT (G.M.)</th>
<th>PLASMA-CELLULAR REACTION AROUND THE TUMOR*</th>
<th>CONDITION OF THE TUMOR</th>
<th>DEGREE OF TUMOR INHIBITION†</th>
<th>TOTAL AMOUNT OF DBA RECEIVED (ML)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphocytes</strong></td>
<td><strong>Plasma cells</strong></td>
<td></td>
<td></td>
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<tr>
<td>746</td>
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<td>regressing</td>
<td>++++</td>
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<tr>
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<td>23</td>
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<tr>
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<td>1210</td>
<td>940</td>
<td>6.5</td>
<td>++</td>
<td>&quot;</td>
<td>+</td>
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</table>

* Expressed in arbitrary grades.
† Degree of tumor inhibition:

- 0 = no inhibition.
- + = slight inhibition.
- ++ = moderate inhibition.
- +++ = marked inhibition.
- ++++ = definite regression.
bling that of the original tumor could be produced in normal mice by inoculating them with lymph nodes rich in such plasma cells. In a later work, Parsons et al. (33) noted a similar plasma-cell increase in normal mice, following injection with pentose-nucleotides, and attempted to explain that these and similar substances elaborated during the metabolic process of a developing sarcoma might be responsible for the widespread development of plasma cells. Apparently Parsons did not connect the plasma-cellular reaction with the possibility of antibody formation. These views are no longer tenable in the light of the current concept of plasma-cell function. Moreover in our own experiments, regional lymph nodes from tumor-bearing rats (rich in plasma cells), when grafted intraperitoneally into a normal susceptible host, did not give rise to any tumor (3).

These findings suggest that the increased plasma-cell production is the morphological manifestation of a systemic immune reaction to circulating antigen(s), which leave the graft and reach the lymph nodes and spleen, where they excite this reaction like any other antigens (3, 4, 6, 36, 37), and that plasma cells are involved in the production of antibodies. Other elements do not appear to have the same significance. It has been reported that the weight and P32 uptake of the lymph nodes in tumor-bearing mice are markedly increased, suggesting the release of antigenic protein from the tumor tissue (1). Recent study on the nucleic acid content also supports the participation of the draining lymph nodes in the formation of antibody against the transplanted tumor. Protein nitrogen and ribonucleic acid in these organs have been found to be increased, suggesting synthesis of protein (2, 22, 35). Such enlarged lymph nodes have also been shown to take part in the immunological response conferring passive tumor transplantation immunity (adoptive immunity) in secondary hosts (3, 27). By the Schultz-Dale technic, it has been demonstrated that the presence of antibody in the host is the basic mechanism involved in this passive tumor transplantation immunity (5). Further, the growth of the tumor cells is inhibited by treating them in vitro with an excess of splenic and lymph node cells (rich in plasma cells) from tumor-bearing animals (3). By use of a modified paper chromatographic technic, it has been shown that Rd/3 tumor phospholipide fraction gives a strong fixation with the extract from lymph nodes and spleen draining this growing tumor in rats. The only histologically demonstrable change in these organs which takes part in the immunological phe-

omenon is the increased accumulation of plasma cells. Attempts to identify the antigenic substance in the tumor have so far been inconclusive. Darcy (13), from his studies on normal tissue homografts, believed that the local plasma-cell accumulation is the effect of an immune reaction and not causally connected with the graft destruction, and he suggested a resorptive function of these plasma cells to explain his findings. Our results show that there exists a positive relationship between local plasma-cell infiltration and tumor inhibition and regression, and the essential difference between the local reaction initiated by a growing and a regressing tumor consists in a marked accumulation of plasma cells around the latter graft. The appearance of plasma cells in large numbers around regressing tumors indicates an active reaction on the part of the host against the transplant and plays an important part in homograft destruction.

It has been shown that cortisone, which abrogates the resistance to homologous and heterologous tumor grafts, inhibits this plasma-cell response in these organs. However, once the immune reaction is established, cortisone fails to interfere with it, in spite of damage to the lymphocytes (3). Cortisone also increases the incidence of lymph node metastases. In this connection, our finding of diminished plasma-cell response in the lymph nodes with secondary metastases may be relevant. This shows that cortisone strongly depresses antibody formation to antigens of the transplanted tumor tissue by inhibiting plasma-cell development. These findings further show a constant relation between the plasma-cellular reaction and tumor resistance, which cannot be explained in terms of lymphocytes alone.

Antigenic properties capable of immunization have been shown with induced tumors in rats and mice, whereas spontaneous tumors failed to immunize by similar methods in mice (17, 41). These results are consistent with the finding that the regional lymph nodes and spleen from mice bearing spontaneous mammary carcinoma do not show any increased plasma-cell response (3).

For a long time cancer investigators noticed a striking resemblance of tumor immunity in animals to immunity elicited by most bacteria and foreign proteins. Various attempts have been made to interpret tumor resistance in terms of antibodies from the study of serological response. An effort has been made here to find indirect evidence of antibody development from the study of histological changes in the host, following tumor homografts. The conclusion can be drawn from this study that, during reaction to bacterial and other foreign protein antigens, tumor, and normal

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1 R. Wilson, personal communication, 1957.
tissue grafts, the plasma cellular response may be considered as an indication of the immunological changes taking place. Judged by the plasma cellular reaction, transplantation immunity to tumor and normal tissues conforms in the main outline with the immune state generated by bacterial and other foreign protein antigens.

A large number of these lymph nodes showed hemolymph node formation. Wohllwill and Jetter (42) in their study on irradiated dogs noticed a close association between hemolymph node and plasma-cell development. Our findings do not support such a relationship in tumor-bearing rats.

Our observations also give no evidence in favor of lymphocytic origin of plasma cells. On the contrary, lymphocytes are reduced in number in the lymph nodes during the height of plasma-cell development. Further, in the lymph nodes, lymphocytes are located chiefly in the cortex, whereas plasma cells occur in the medullary cords. In the spleen, lymphocytes develop largely in the Malpighian follicles, whereas plasma cells are found in the red pulp. The thymus contains lymphocytes and no plasma cells. These observations tend to refute the lymphocytic origin of plasma cells. The reticulum-cell hyperplasia in the lymph node and spleen undoubtedly contributes to the formation of plasma cells. In a few instances plasma cells are seen in direct contact with the proliferating reticulum cells, without any relation to the diminishing lymphocytic tissue. All gradations from reticulum cells and mature plasma cells can be observed.

ACKNOWLEDGMENTS

This work was carried out in the Department of Pathology and Cancer Research Unit, University of Sheffield, England. I wish to express my thanks to Prof. H. N. Green for his keen interest and valuable criticism throughout this work. My thanks are due to Miss K. de Paula Hanika (Mrs. Lunts) for her technical assistance.

REFERENCES

12. Da Pan, C. A Cytological Analysis of the Reaction in Animals Resistant to Implanted Carcinoma. 8th Scientific Report, Imperial Cancer Research Fund, pp. 57-78, 1912.


Fig. 1.—Right axillary lymph node of a rat bearing a 12-day-old growing Rd/3 tumor, showing reduction of the cortex, increased medullary tissue, and dilatation of the sinuses. Unna Pappenheim, ×63.

Fig. 2.—Same under higher magnification, showing large number of plasma cells in the medullary cord. U. Pap., ×800.

Fig. 3.—Right para-aortic lymph node of a rat bearing a 14-day-old Walker carcinoma, showing hemolymph node formation. Note the dilated sinuses filled up with erythrocytes and erythrophagocytosis. Medullary cord is filled up with plasma cells. Hematoxylin and eosin, ×400.

Fig. 4.—Right axillary lymph node of a rat bearing a 14-day-old Walker carcinoma. Medullary cord showing increased number of plasma cells and Russell body cells. H. & E., ×380.

Fig. 5.—Spleen from a 16-day-old Rd/3 tumor-bearing rat, showing large number of plasma cells in the red pulp. U. Pap., ×580.

Fig. 6.—Rd/3 tumor during regression, induced by DBA. Note the large number of cells penetrating the tumor tissue. Most of these cells are plasma cells. U. Pap., ×580.
Cellular Reactions Following Tumor Growth with Special Reference to Plasma-cellular Response

B. D. Baruah


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