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G Husby, … , R G Strickland, R C Williams Jr

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Research Article

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Tissue T and B Cell Infiltration of Primary and Metastatic Cancer

GUNNAR HUSBY, PETER M. HOAGLAND, ROBERT G. STRICKLAND, and RALPH C. WILLIAMS, JR.

From the Department of Medicine, Bernalillo County Medical Center, University of New Mexico, School of Medicine, Albuquerque, New Mexico 87108

ABSTRACT Immunofluorescent techniques were utilized to identify the types of infiltrating lymphocytes adjacent to human malignant tumors arising from a wide range of anatomic sites. 24 of 29 primary tumors and 5 of 8 metastatic lesions showed varying degrees of lymphocytic infiltration. T cells predominated in the infiltrates in primary tumors (mean 80%, range 50–100%) and this pattern was evident regardless of anatomic site or the presence or absence of metastatic spread. By contrast, B cells predominated at the margins of three of five tumor metastases. Mononuclear cells bearing the Fc receptor were not a prominent component of the infiltrates associated with either primary tumors or metastases, but tumor cell binding of fluoresceinated IgG aggregates was observed in 12 of 29 primary tumors. A significant reduction in peripheral blood T cell numbers occurred in a third of the patients studied. This decrease was not clearly related either to the extent of local tumor T cell infiltration or to the presence of disseminated disease. These preliminary findings provide a descriptive analysis of the local and systemic distributions of immunocompetent cells in cancer.

INTRODUCTION

One major area of incomplete understanding of tumor immunology relates to the precise mechanisms whereby host immune defenses are mobilized in response to various neoplasms. A large body of evidence has accumulated supporting the concept that the host immune response plays a critical role in whether or not the tumor or the invaded host prevails (1–5). There is good evidence for involvement of activated macrophages in host resistance to tumors (6–9). In addition, other mechanisms, such as antibody-mediated lymphocytotoxicity, appear to be potential effector systems for host destruction or abolition of tumor (10–12). However, perhaps the most evidence has accumulated implicating direct killing of tumor cells by activated lymphocytes (3, 4, 13–16). Such reactions appear to be closely analogous to graft versus host or host versus graft reactions, and it seems that T cells may be implicated as one of the prime cells types involved in this type of direct cell killing of tumor (2, 4). Indirect evidence also supports the importance of T cells in tumor immunity. Wybran and Fudenberg (17) found a significant reduction in peripheral blood lymphocytes forming "active" sheep red blood cell rosettes in patients with advanced or moderately extensive neoplasms, and subsequent studies appear to support the concept that as the tumor prevails over host defenses, proportions and numbers of T cells are diminished in the peripheral blood (18–21).

In the present study, we report our initial observations of the types of infiltrating cells adjacent to primary and metastatic malignancies together with parallel observations of the proportions and numbers of peripheral blood T and B lymphocytes in the patients studied. The results indicate that primary tumors from a broad range of anatomic sites are often infiltrated by cells, predominantly T lymphocytes. This observation was less frequent in metastatic lesions. The degree of tumor infiltration by T cells correlated well with the presence or absence of metastases; however, peripheral blood T lymphocytopenia in the patients studied here was not invariably related to disseminated malignancy or to the extent of tumor infiltration by T cells.

METHODS

Patients. 36 patients with malignant tumors, determined by surgical exploration and either biopsy or histologic examination of a resected specimen, formed the study population. The majority of these tumors were carcinomas of epithelial cell origin, arising from a wide variety of dif-

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TABLE I

Major Groups of Malignant Tumors Studied

Type	Number
Gastrointestinal	9
Colon and rectum	6
Stomach	2
Pancreas	1
Urogenital	8
Cervix	3
Bladder	2
Prostate	1
Ovary	1
Kidney	1
Breast	7
Skin	6
Miscellaneous	6
Lung	2
Carcinoma—undetermined primary	2
Thyroid	1
Neuroblastoma	1
Total tumors studied	36

ferent anatomic sites (Table I). The clinical details of the patients studied are shown in Table II. Of note was the fact that only 6 of the 36 patients had received treatment in the form of radiotherapy or chemotherapy before tumor tissue was obtained for diagnosis and the performance of further studies outlined below. In 28 patients, tissue from the primary tumor was available for study; in 7, metastatic lesions only were sampled, including two instances in which the site of the primary tumor was not established; in one patient both the primary tumor and a lymphoid metastasis were available for study (Table II). The evaluation of presence or absence of metastases was based on thorough clinical, laboratory, and radiological examinations, combined with histopathological examinations of preoperative biopsies and tissues specimens removed during surgical treatment. Results of reassessment of presence or absence of subsequent metastatic disease on the basis of clinical follow-up examinations at 4–6 mo are available in all subjects studied; however, final evidence for or against ultimate metastatic spread must await much longer follow-up.

Tissue studies. Fresh samples of resected tumor along with adjacent uninvolved tissue removed at surgery were kept in sterile, cold phosphate-buffered saline, pH 7.4, until snap-frozen in OCT compound (Ames Co., Div. of Miles Lab., Inc., Elkhart, Ind.). Frozen tissue sections, 4 μm thick, were cut on the cryostat and stained either by hematoxylin and eosin or in serial sections utilized for immunofluorescence studies after prior fixation in cold acetone, as previously described (22).

B cells and cells bearing Fc receptors. The IgG fraction of a rabbit anti-human $F(ab')_2$ was obtained by DEAE cellulose ion exchange chromatography. The purified IgG fraction was thereafter digested with pepsin (enzyme-protein ratio 1:50) at 37°C for 18 h, and the resulting $F(ab')_2$ fragments were labeled with fluorescein isothiocyanate and used to identify tissue cells with surface or cytoplasmic immunoglobulin (22). The $F(ab')_2$ fragment of the rabbit antibodies was employed to avoid reactions with the Fc receptors of tissue-fixed macrophages, polymorphonuclear leukocytes, or lymphocytes (23, 24). Cells with lymphocyte or plasma cell morphology that showed staining for immunoglobulins with $F(ab')_2$ fragments of rabbit anti-human $F(ab')_2$, either on the surface or in the cytoplasm, were regarded as belonging to the B cell line.

Aggregates of fluoresceinated IgG could not be utilized to detect tissue B cells, since this reagent also reacted with Fc receptors on polymorphonuclear granulocytes and macrophages (22–25). However, because a previous report by Tønder and Thunold (26) indicated that tumor cells carried Fc receptors, we used fluoresceinated IgG aggregates in an attempt to identify tumor cells bearing Fc receptors.

T cells. Indirect immunofluorescence was used to identify T cells in tissue sections (27, 28). Rabbit anti-human thymocyte antiserum was prepared as previously described (22), with isolated T cells obtained from thymuses of children undergoing cardiac surgery. Before use, the following absorptions of the rabbit antiserum were performed: eight serial absorptions each for 30–60 min at 37°C, with B cells (5×10^7 cells/ml) from patients with chronic lymphatic leukemia; three serial absorptions with rhesus-positive red cells (5×10^8/ml) from normal blood donors; two serial absorptions, for 30 min at 37°C, with polymorphonuclear leukocytes and monocytes (15×10^6 cells/ml) isolated during in vitro assays for chemotaxis (29); absorption with an equal volume of insolubilized (30) normal human serum, and finally absorption with an insolubilized homogenate of normal human liver tissue. The liver tissue used for absorption was obtained at autopsy from a subject killed in a traffic accident. Liver tissue frozen and thawed five times was minced with scissors and homogenized in an equal volume of phosphate-buffered saline at high speed for 5 min at 0–4°C, in a Sorvall Omnimixer (DuPont Instruments, Sorvall Operations, Newtown, Conn.). Liver homogenate was further disintegrated by sonication in a Bronson sonifier (Bronson Instruments, Inc., Stamford, Conn.) and subsequently centrifuged at 18,000 g for 20 min. The supernate (60 mg protein/ml) was insolublized with glutaraldehyde (30) and used for absorption of an equal volume of antiserum. In some experiments the sonicated liver homogenate was insolublized and used without centrifugation. Absorption with the liver preparations was used to avoid staining of endothelial cells and reticulin fibers. After incubation with anti-T cell antiserum, the sections were washed and subsequently stained with a fluoresceinated sheep antiserum to rabbit IgG. The absorbed anti-T cell antiserum appeared to show primary specificity for T cells in frozen sections of normal human spleen and lymph nodes (22). It did not stain germinal centers of lymph nodes nor plasma cells. Definite cell membrane staining was clearly evident in periarteriolar regions of human spleen as well as paracortical areas of normal human lymph nodes (22). The absorbed anti-T cell antiserum also stained an average of 65±10% of normal peripheral blood lymphocytes, while no staining of chronic lymphatic leukemia cells was observed. Staining with anti-T cell antiserum was completely abolished by absorption with homogenates or suspensions of human fetal thymocytes.

Since B cell identification depended entirely on detection of surface or cytoplasmic Ig with $F(ab')_2$ fragments of anti-immunoglobulins and T cells on indirect immunofluorescence, these techniques may have omitted enumeration of so-called null cells possessing Fc or complement receptors (31); thus it was recognized that values given for T and B cells identified by the above methods were estimates that had not allowed for null-cell detection.

The degree of lymphocytic infiltration in the tumor tissues studied was evaluated by calculating the sum of fluorescent

TABLE II

Clinical Details and Tissue Immunologic Findings in 36 Patients with Malignant Tumors

Patient	Age	Sex	Tumor site and type	Presence or absence of metastases	Prior treatment	Tissue examined	Degree of* lymphocytic infiltration	Tissue‡ lymphocyte proportions		Tumor cells binding aggregated IgG
								T cells	B cells	
1	71	F	Rectum adenocarcinoma	−	None	Primary tumor	Severe	80	20	−
2	51	F	Colon adenocarcinoma	−	None	Primary tumor	Severe	90	10	−
3	66	M	Colon adenocarcinoma	−	None	Primary tumor	Severe	80	20	+
4	46	F	Rectum adenocarcinoma	−	None	Primary tumor	Moderate	50	50	+
5	65	M	Colon adenocarbinoma	+	None	Primary tumor	Mild	80	20	+
6	57	M	Rectum adenocarcinoma	−	None	Primary tumor	Severe	90	10	−
7	72	M	Gastric adenocarcinoma	−	None	Primary tumor	Severe	70	30	+
8	84	M	Gastric adenocarcinoma	+	None	Lymph node metastasis	Severe	0	100	−
9	61	F	Pancreatic adenocarcinoma	+	None	Omental metastasis	None	—	—	−
10	29	F	Cervical squamous cell	−	None	Primary tumor	Moderate	95	5	+
11	29	F	Cervical squamous cell	−	None	Primary tumor	Moderate	80	20	+
12	63	F	Cervical squamous cell	+	None	Lymph node metastasis	None	—	—	−
13	79	M	Bladder transitional cell	−	Irradiation	Primary tumor	Mild	80	20	+
14	61	M	Bladder transitional cell	+	Irradiation	Liver metastasis	Mild	80	20	−
15	66	M	Prostate adenocarcinoma	+	None	Primary tumor	Mild	95	5	−
16	58	F	Ovarian serous cystadenocarcinoma	+	None	Primary tumor	Mild	60	40	−
17	59	M	Kidney hypernephroma	−	None	Primary tumor	Moderate	100	0	+
18	67	F	Breast adenocarcinoma	−	None	Primary tumor	Mild	50	50	−
19	26	F	Breast ductal cell carcinoma	+	None	Primary tumor	Mild	90	10	−
20	66	F	Breast ductal cell carcinoma	+	None	Primary tumor	None	—	—	−
21	48	F	Breast medullary carcinoma	−	None	Primary tumor	Severe	75	25	−
22	38	F	Breast ductal cell carcinoma	+	None	Primary tumor	Mild	90	10	+
23	50	F	Breast ductal cell carcinoma	+	None	Primary tumor	None	—	—	+
24	63	F	Breast adenocarcinoma	+	None	Primary tumor	Mild	80	20	−
						Lymph node metastasis	Moderate	15	85	−
25	66	M	Skin squamous cell carcinoma	−	None	Primary tumor	None	—	—	−
26	69	M	Skin squamous cell carcinoma	−	None	Primary tumor	Mild	80	20	−
27	56	M	Skin squamous cell carcinoma	+	None	Sternal metastasis	Mild	40	60	−
28	55	M	Skin squamous cell carcinoma	−	Irradiation	Primary tumor	None	—	—	−
29	70	M	Skin basal cell carcinoma	−	Irradiation	Primary tumor	Mild	100	0	−
30	56	M	Skin malignant melanoma	+	Chemotherapy	Primary tumor	None	—	—	+
31	61	M	Lung bronchogenic carcinoma	−	None	Primary tumor	Severe	90	10	−
32	55	M	Lung adenocarcinoma	+	None	Primary tumor	Moderate	65	35	+
33	60	M	Undetermined primary anaplastic carcinoma	+	None	Lymph node metastasis	None	—	—	−
34	67	F	Undetermined primary anaplastic carcinoma	+	None	Lymph node metastasis	Mild	90	10	−
35	62	F	Thyroid papillary carcinoma	−	None	Primary tumor	Severe	80	20	−
36	2	F	Neuroblastoma	+	Chemotherapy	Primary tumor	Mild	90	10	−

* Determined by the sum of fluorescent cells as revealed by the anti-immunoglobulin and anti-T cell antisera.

‡ Lymphocytic cells showing no fluorescence with anti-immunoglobulin or anti-T cell antisera are not recorded.

cells, as revealed by the anti-F(ab')$_2$ and anti-T cell antisera. Each biopsy was thus classified as showing absent, mild, moderate, or severe lymphocytic infiltration. As a control, adjacent tissue sections were stained with hematoxylin and eosin and examined by light microscopy. Proportions of tissue T and B cells were estimated by counting the number of the fluorescent cells found in at least four neighboring sections stained with antisera to T cells and immunoglobulins, respectively. In the case of moderate or severe lymphocytic infiltrates, the relative proportions of T and B cells were estimated by counting 100 or more lymphocytic cells in several microscopic fields. When infiltrates were less extensive, fewer lymphocytes (15–100 cells) were available for counting. It was recognized, as noted above, that this method did not necessarily detect null cells or B cells bearing Fc or complement receptors.

Peripheral blood studies. Heparinized venous blood was obtained preoperatively from 30 of 36 patients in whom tumor tissue was subsequently examined. Lymphocyte-rich preparations were obtained by Ficoll-Hypaque density gradient separation. Lymphocyte preparations showed 95–98% viability by eosin Y or trypan blue dye staining. Lymphocyte recoveries by the Ficoll-Hypaque method averaged 85–88%. Monocyte contamination estimated by peroxidase

TABLE III

Summary of Tissue Immunologic Findings in 29 Primary Tumors Examined

Tumor group	Number studied	Degree of lymphocytic reaction: Moderate or severe	Degree of lymphocytic reaction: Mild or absent	Tissue lymphocytic proportions*: T cells	Tissue lymphocytic proportions*: B cells
Nonmetastatic					
Gastrointestinal	6	6	0	77 (50–90)	23 (10–50)
Urogenital	4	3	1	89 (80–100)	11 (0–20)
Breast	2	1	1	62 (50–75)	38 (25–50)
Skin	4	0	4	90 (80–100)	10 (0–20)
Miscellaneous	2	2	0	85 (80–90)	15 (10–20)
Total	18	12	6	86 (50–100)	14 (5–50)
Metastatic					
Gastrointestinal	1	0	1	80	20
Urogenital	2	0	2	77 (60–95)	23 (5–40)
Breast	5	0	5	87 (80–90)	13 (10–20)
Skin	1	0	1	—	—
Miscellaneous	2	1	1	77 (65–90)	23 (10–35)
Total	11	1	10	81 (60–95)	19 (5–40)

* Figures given are mean and range of T and B cell proportions in those tumors showing lymphoid infiltration.

(32) and esterase (33) stains averaged 12%. B cells in peripheral blood were identified by direct immunofluorescence with fluoresceinated-pepsin $F(ab')_2$ fragments of polyvalent rabbit IgG antibodies to human immunoglobulins (22). In addition, peripheral blood mononuclear cells bearing Fc receptors were identified with aggregates of fluoresceinated IgG by the method of Dickler and Kunkel (34). Total T cells in peripheral blood were identified by the sheep red cell rosette technique without addition of exogenous serum and after overnight incubation at 4°C in pH 7.4 phosphate-buffered saline, as previously described (35). In addition, active T cell rosettes were also determined by the method of Wybran and Fudenberg (17) in 21 patients and 14 control subjects. Proportions of B cells, aggregate-binding cells, and T cells (both total and active) were measured, and absolute numbers of these cells were obtained from parallel determinations of total lymphocyte counts.

RESULTS

Lymphocytic reactions at tumor margins. In most instances there was relatively good correlation between the estimations by immunofluorescence and light microscopy as far as the degree of lymphocytic infiltration was concerned. However, it must be pointed out that lymphocytic cells binding neither anti-T cell nor anti-immunoglobulin antisera (e.g., the so-called null cells) must certainly be present in such tissues, and such cells were not taken into consideration in the evaluation by immunofluorescence of degree of lymphocytic infiltration and the proportions of subpopulations of lymphocytic cells.

Amongst the 29 primary tumors studied, 13 showed moderate or severe and 16 mild or absent lymphocytic infiltrations. Some differences in the degree of lymphocytic infiltration were apparent in comparing the different anatomical sites; however, the numbers in each subgroup are small (Table III). By contrast, only two of the eight metastatic lesions examined showed moderate or severe lymphocytic reactions. When the degree of lymphocytic infiltration of the primary tumor was contrasted in patients with or without metastases at the time of tissue removal, moderate or severe infiltration was present in only 1 of 11 with metastases, as compared to 12 of 18 patients without distant tumor spread (Table III). This difference is statistically significant ($P < 0.003$; Fisher's exact test). Of some interest was the finding that the lymphocytic reaction was mild or absent in all six tumors (five of which were primary) examined where the patient had received prior irradiation or chemotherapy.

Relative proportions of T and B cells in lymphocytic infiltrates. No lymphocytic reaction was present in eight of the tumor samples examined. These included five primary tumors and three metastatic lesions (Table II). Thus analysis of T and B cell proportions was possible in 29 tumor samples (24 primary and 5 metastases). In these 29 tumor samples showing lymphocytic infiltration, no substantial differences in the proportions of tissue T or B cells in relation to the degree of reaction or the anatomical site of the tumor were apparent. The major finding was the presence of a predominance of T cells in 24 of 29 tumor samples. The mean (± 1 SD) proportion of T cells in all tumors studied was $72 \pm 24\%$ whereas B cells constituted $25 \pm 24\%$. Repre-

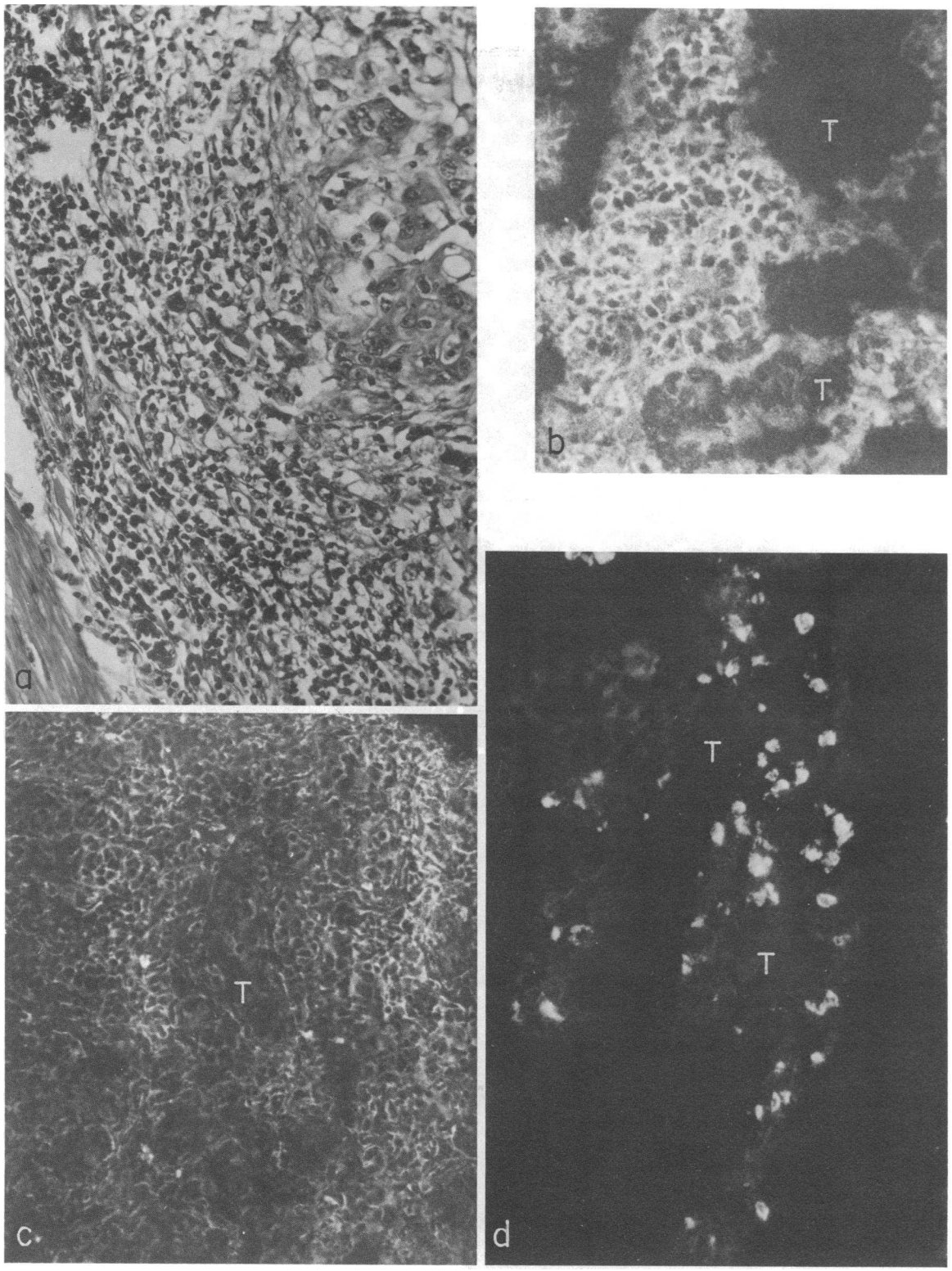

FIGURE 1 *a.* Tissue section of adenocarcinoma of the proximal colon stained with hematoxylin and eosin showing tumor cells surrounded by a heavy infiltrate of mononuclear cells (× 160). *b.* Section of the same tumor as in 1*a*, stained with anti-T cell antiserum, showing extensive T cell infiltrations between the malignant glands (T = tumor, × 160). *c.* Same section as in *b*, showing numerous T cells infiltrating the tumor (× 160). *d.* Section of the same tumor stained with anti-F(ab')$_2$, showing less extensive B cell infiltration (× 160).

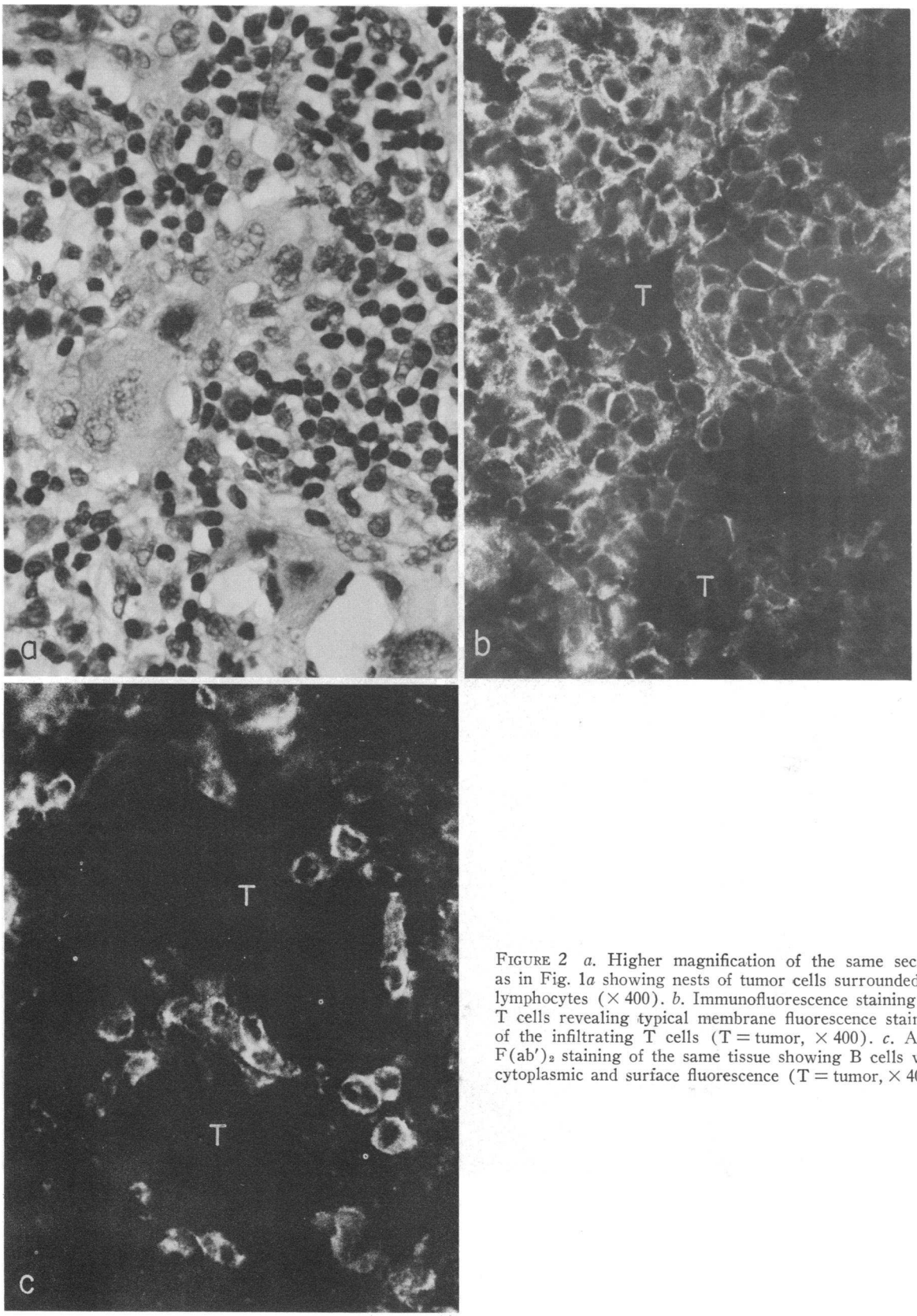

FIGURE 2 *a*. Higher magnification of the same section as in Fig. 1*a* showing nests of tumor cells surrounded by lymphocytes (× 400). *b*. Immunofluorescence staining for T cells revealing typical membrane fluorescence staining of the infiltrating T cells (T = tumor, × 400). *c*. Anti-$F(ab')_2$ staining of the same tissue showing B cells with cytoplasmic and surface fluorescence (T = tumor, × 400).

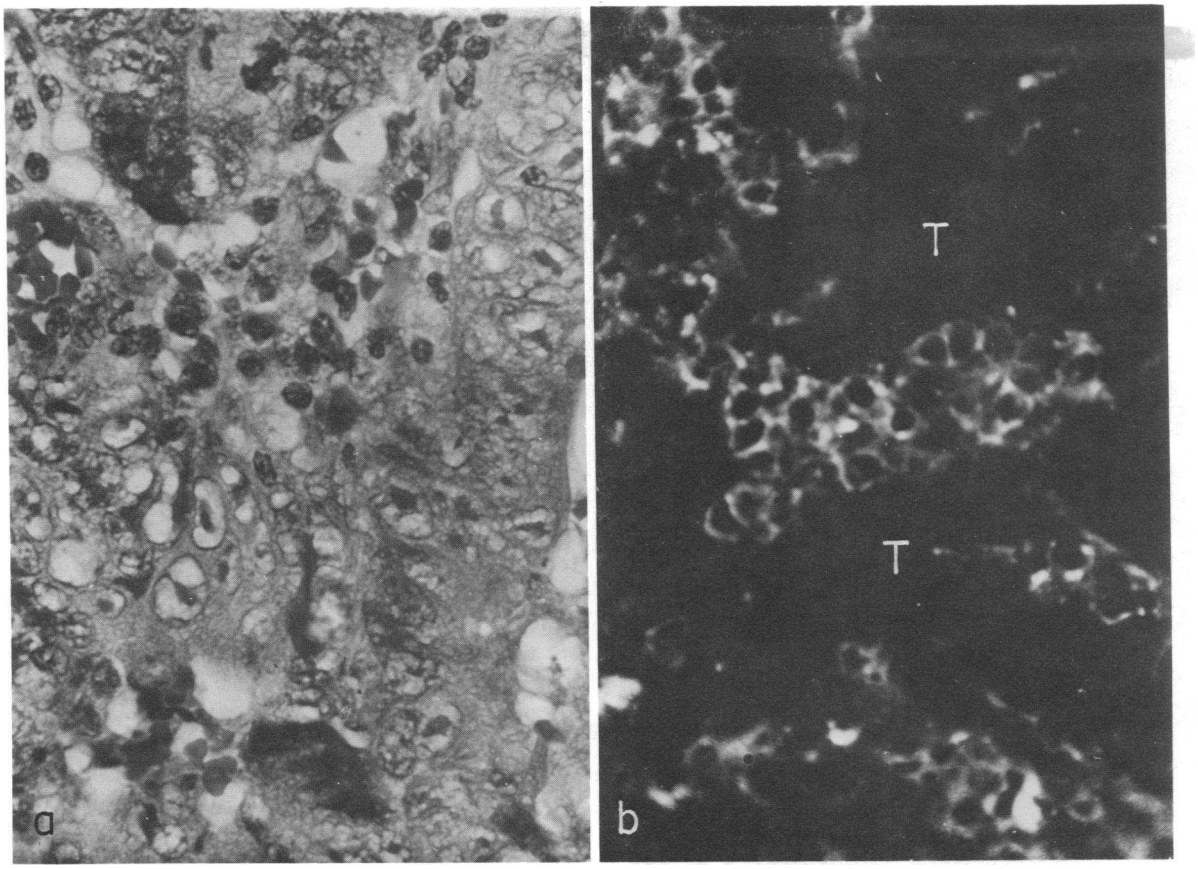

FIGURE 3 *a.* Adenocarcinoma of sigmoid colon stained with hematoxylin and eosin. Moderate lymphocytic infiltration of the tumor is seen (× 400). *b.* Immunofluorescence surface staining of T cells from the same tumor (× 400).

sentative examples of T and B cell infiltrates of tumor are shown in Figs. 1, 2, and 3. Cells binding IgG aggregates were not identified as a major component of the infiltrate in any of the tumors studied; however, occasional mononuclear cells bearing Fc receptors reacting with fluorescein labeled IgG aggregates were noted. These never constituted more than 5–10% of tissue infiltrates. T cell proportions in primary tumors ranged from 50 to 100% and predominated in 22 of 24 tumors studied (Table III), whereas in metastatic lesions T cell proportions ranged from 0 to 80% and predominated in only two of five studied (liver metastases, patient 14; lymph node metastases, patient 34). B cells in primary tumor lymphoid infiltrates constituted 5–50% of infiltrating cells and did not predominate in any sample examined (Table III), whereas B cells were the major infiltrating lymphocyte in three of the five metastases. Of particular interest was patient 24, Table II, where the primary tumor showed 80% T cells and 20% B cells, but a lymph node metastatic lesion contained only 14% T cells. Also of note were similar findings in patient 8, Table II, where lymph node metastases from gastric carcinoma showed no T cells, but 100% cells bearing surface Ig and apparently B cells. A similar predominance of B cells in metastatic lesions was recorded in patient 27. The staining pattern of the B cells in these patients was atypical, in that the infiltrating lymphocytes appeared to be covered with cell surface Ig and to be present in an unusual conglomerate mass (Fig. 4). Since B cell predominance adjacent to metastatic implants was noted in lymph nodes, it is difficult to estimate how much impetus for this was provided by the local environment of the lymph node itself. One sternal metastasis, however, also showed B cell predominance (patient 27).

Aggregated IgG binding in tumor tissue. In Table II, it can be seen that 12 of 37 tumor samples examined showed definite binding of fluorescein-labeled IgG aggregates. Examples of these reactions are presented in Fig. 5. Particulate lumpy surface binding of fluorescent aggregates could usually be defined on tumor cell membranes by comparisons with serial H and E-stained sections and the use of perioxidase and nonspecific esterase for mononuclear cell identification (32, 33). In all in-

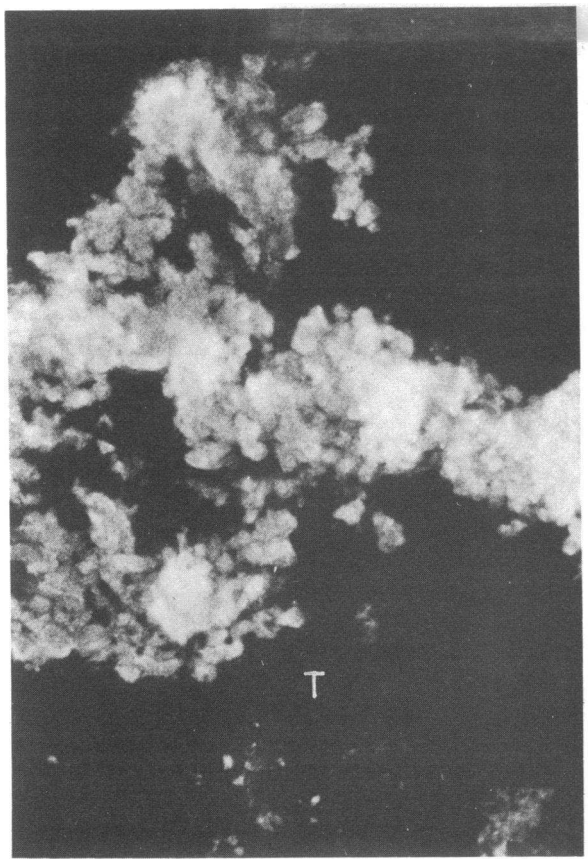

FIGURE 4 Lymph node metastasis from a poorly differentiated adenocarcinoma of stomach stained with anti-F(ab')$_2$, showing an extensive infiltration with conglomerate Ig-bearing cells. The staining pattern clearly differs from that seen in Figs. 1*d* and 2*c* (× 160).

stances the aggregate binding to tumor cell membranes was noted with primary tumors and not metastatic lesions. It was observed in all major anatomic tumor categories studied.

Peripheral blood findings. Studies of peripheral blood T and B cell markers were obtained in 26 of 28 patients in whom the primary tumor was studied for infiltrating cells and four of eight patients in whom metastatic tissue lesions were examined. The findings were compared to similar data in 80 control subjects free of cancer studied concurrently. T cell numbers below 1 SD from the normal mean were recorded in 10 of the 30 patients studied. Mean total T cell proportions and numbers in the patients with cancer were significantly reduced when compared to corresponding values in normal controls (Table IV, top half). No difference from control values was apparent, however, in peripheral blood B cell proportions or numbers, as identified by the presence of Fc receptors or by fluorescein-labeled pepsin-digested anti-F(ab')$_2$. Proportions and numbers of active T cell rosettes did not differ significantly from control values among the limited number of patients studied. When patients with metastases were compared to those without tumor dissemination, the reduction in mean proportions and numbers of total T cells was most apparent in the patients with metastatic disease (Table IV, bottom half). 6 of 14 patients with metastases showed total T cell number below 1 SD from the normal mean compared to 4 of 16 without metastases. Again, no differences in B cell proportions or numbers were apparent between these two groups.

Finally, an attempt was made to correlate the relative proportions of T cells infiltrating the tumors studied and the corresponding peripheral blood T cell distributions. No clear correlation between the degree of lymphocytic infiltration or extent of tissue T cell reaction and peripheral blood T cell proportions or numbers was observed among the small numbers of patients studied (Fig. 6).

TABLE IV

Peripheral Blood Lymphocyte Cell Surface Markers in Patients with Cancer Compared to Normal Controls

	Total peripheral blood lymphocytes	Total T cells	T cells	Total Fc receptor cells	Fc receptor cells	Total cells with surface Ig‡	Cells with surface Ig‡
		per mm^3	%	*per mm*^{3}	%	*per mm*^{3}	%
Cancer patients (30)	2,203±860*	1,344±783	57±19	180±87	8±6	254±213	10±7
Normal controls (80)	2,466±94	1,614±609	67±11	211±166	6±2	335±184	12±5
P value	0.10	0.05	0.0005	0.25	0.20	0.20	0.25
Cancer patients with metastases (14)	2,084±638	1,053±460	53±17	182±160	9±7	192±94	10±6
Cancer patients without metastases (16)	2,308±1,064	1,587±916	61±21	190±135	7±5	292±284	10±7
P value	0.25	0.05	0.15	0.475	0.25	0.15	0.45

* Numbers refer to means±1 SD.

‡ Determined by fluoresceinated pepsin F(ab')$_2$ fragments of rabbit IgG antibodies to human F(ab')$_2$.

DISCUSSION

The present report constitutes our initial attempts to apply techniques recently developed in this laboratory (22) to determine the types of lymphocytic cells infiltrating primary and metastatic tumors. The most striking and uniform pattern to emerge was the finding of predominant T cell infiltrations of neoplastic tissues showing mild, moderate, or extensive lymphoid cell collections. The present study appears to be the first to directly demonstrate T cells infiltrating human tumors. Other workers have indirectly suggested this possibility, based on their observations of a high number of infiltrating lymphocytes that do not react with classical B cell markers (36). Only three exceptions to this pattern were recorded where B cells formed the major infiltrating lymphocyte. These observations were all made in metastatic lesions, as distinct from primary tumors. Of great interest was the reciprocal pattern of primary tumor T cell infiltrate and metastatic lesion pattern, principally of B cell infiltrations, recorded in one patient with untreated metastatic carcinoma of the breast. As previously noted and as shown in Fig. 4, the staining pattern of these B cells was atypical, and an alternative interpretation could be that these are lymphoid cells covered by absorbed antibody. Attempts to elute such tissue sections with buffers at low pH or with chaotropic reagents have thus far been unsuccessful.

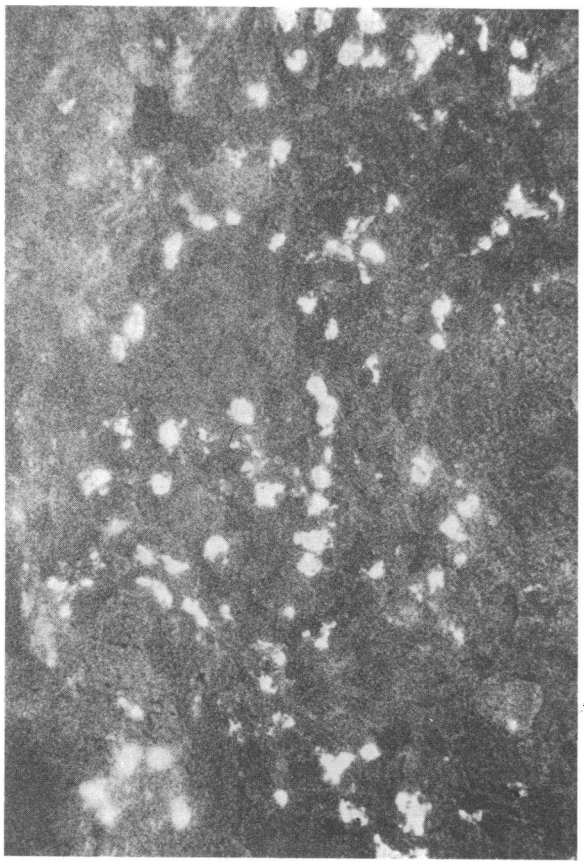

FIGURE 5 Section from carcinoma of colon showing binding of aggregated IgG to malignant cells (× 160).

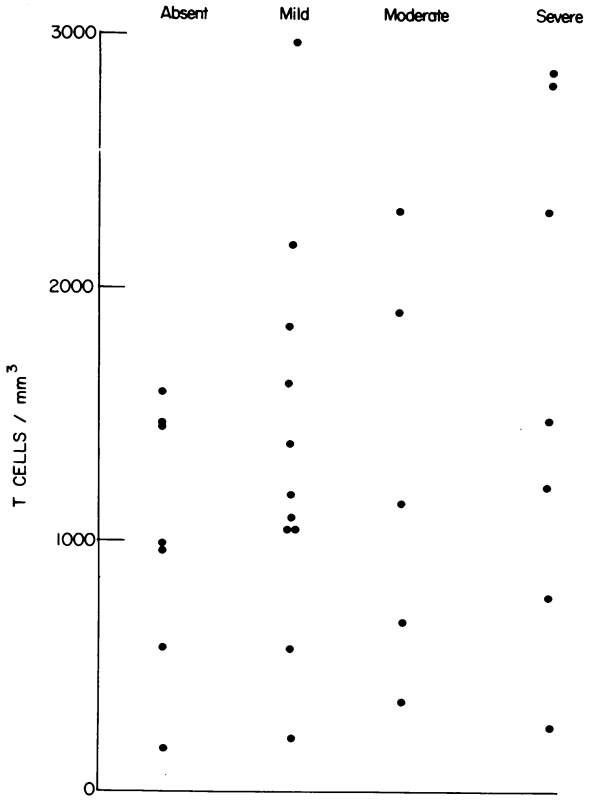

FIGURE 6 Relationship between peripheral blood T cell numbers (cells/mm³) and degree of lymphocytic reaction at tumor margins in 30 patients with malignant tumors.

It was recognized that utilizing F(ab')₂ fragments of anti-immunoglobulin antibodies as B cell markers and indirect anti-T cell immunofluorescence as a T cell marker meant that some lymphoid cells bearing Fc or complement receptors and cells classified by Brier et al. (31) as null cells were not enumerated. Thus the proportions of B cells estimated by the present methods may be relatively low. More work will be needed to define the possible contribution of null cells to such infiltrates.

A further finding with respect to the cell types infiltrating tumors was the relative paucity of cells bearing the Fc receptor and detectable by the use of fluoresceinated IgG aggregates. While in vitro assays using circulating lymphocytes from patients with cancer have suggested that such cells play an important role in tumor-cell killing (37–39), the present findings imply that such a mechanism may not be of major importance within the local environment of a tumor cell mass. T

cells constituted the main type of infiltrating lymphocyte in a wide variety of human tumors from differing anatomic sites. Also of interest were the findings of a significant inverse relationship between the degree of lymphocytic (T cell) tumor infiltrate and relative frequency of clinical metastatic disease. It was also clear that in six patients who had received prior radiation or chemotherapy, tumor lymphocytic infiltrates were mild or absent in all instances. Previous studies by Stjernswärd et al. (18) revealed a reduction in peripheral blood T cells, as measured by the sheep red cell rosette technique, in a significant proportion of patients undergoing prior radiation therapy for carcinoma of the breast.

The patterns of lymphocytic reaction observed in the present study suggest that T cells may be of considerable importance in the generation or completion of the local host immune response to various tumors. However, since the total number of patients studied is small and long-term follow-up studies have not yet been completed, no firm conclusions are yet justified. Nevertheless a number of studies have been already reported relating lymphoid patterns locally and in draining regional lymph nodes to survival and clinical course, both in human tumors and in experimental animals (40–44).

Since T cell infiltrates predominate in primary tumors and many such lymphoid collections appear to be extensive, it is difficult to understand why many such tumors eventually prevail. The host response appears intact, as demonstrated by such features as the juxtaposition of T cells at the tumor margins, but nevertheless, the tumor often overcomes the host defenses. Recent work has indicated that effector T cell function may depend on intracellular levels of cyclic GMP or AMP (45, 46). Cyclic GMP increase leads to increase in effector T cell function and cyclic AMP increment to diminished cellular activity (47, 48). If some mechanism were available by which tumors could induce elevation of intracellular cyclic AMP in infiltrating T cells, then cell-mediated immunity against the tumor might be suppressed. Plescia et al. have recently postulated that tumors may elaborate or generate prostaglandins which could divert, short-circuit, or defuse defending T cells (49). It is conceivable that this could occur by elevation of their intracellular or membrane-associated cyclic AMP (50, 51). Currently, studies are in progress to examine these questions directly in tissue sections of the various tumors studied, using direct immunofluorescent identification and methods recently described (52).

No direct predictive patterns or profiles were noted when peripheral blood T and B cell quantitation was compared with similar analyses of tissue T and B cell distributions. Peripheral blood T cell numbers, as determined by sheep red cell rosette technique, were substantially reduced in a third of the patients studied but no striking correlation was apparent between decreased circulating T cells and either the presence of disseminated malignancy or the degree of local lymphocytic infiltration. However, considerably more data are needed before such patterns of relationships may emerge. Indeed, recent reports of nonimmunoglobulin serum factors inhibiting E rosette formation (53) indicate the need for caution in interpreting peripheral blood data.

Our observation of tumor cell binding of fluorescent IgG aggregates confirmed previous reports by Tønder and Thunold (26). It was of note that in all instances where tumor cell binding of aggregates was demonstrated, primary tumors and not metastases were involved. The presence of cell membrane Fc receptors did not appear to confer any survival advantage on the tumor, as has been hypothesized previously (54), since only 4 of the 12 tumors showing receptors for aggregated IgG were associated with concurrent metastatic disease. It is not clear, however, that attempts to detect Fc receptors in tissue sections of tumors are completely accurate, since some receptors might already be saturated by endogenous complexes or aggregated γ-globulins and therefore not be available to react with additional aggregates applied to the tissues. Indeed, if tumor cells possessed higher ratios of Fc receptors per unit of surface membrane than normal host cells, the tumor could achieve a survival advantage over normal host cells by virtue of a greater tumor cell membrane saturation with serum-enhancing or blocking factors.

In conclusion, the present study emphasizes the central paradox of tumor immunology. Examination of the environment in the immediate vicinity of neoplastic tissue has clearly indicated that lymphoid cells are not prevented from infiltrating the tumor in large numbers. Nevertheless the tumor often prevails over the host. Thus, examination of local factors in and around such neoplastic tissues may eventually explain how an apparently effective immune response is subverted.

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