ABSTRACT

Patients with cancer often show impaired immune functions; however, the basis of this suppression is still not understood. In several experimental systems, human T-cells with receptors for Fc of immunoglobulin G may function as suppressors, and those with receptors for Fc of immunoglobulin M may function as helpers. Peripheral blood as well as tumor tissue infiltrates were examined for proportions and numbers of Tγ, Tδ, or la-positive T-cells. Forty-five untreated patients with solid tumors and 24 patients with lymphomas were studied. An increase in the percentage of peripheral blood Tγ cells (p < 0.001) and a decrease in Tδ cells (p < 0.0005) were recorded in all tumor patients when compared with 30 normal controls. Percentages and absolute numbers of peripheral blood la-positive T-cells were decreased (p < 0.001 and < 0.00001) in solid-tumor patients; by contrast, the proportion of peripheral blood la-positive T-cells was elevated (p < 0.005) in lymphoma subjects. Studies of cancer tissues from 46 untreated patients using immunofluorescence and mouse hybridoma antibody specific for T-cells showed that tumor lymphocytic infiltrates were composed mainly of T-cells. Double staining with fluorescein-conjugated specific anti-Tγ and anti-human la reagents detected relatively high proportions of Tγ and la-positive T-cells within solid-tumor lymphoid infiltrates. A comparison of peripheral blood and tumor lymphocytic T-cell profiles revealed that, in some patients, low proportions of la-positive T-cells in blood were paralleled by a high percentage of such cells in tumor lymphoid infiltrates.

INTRODUCTION

The host immune response to cancer continues to present a number of enigmatic and perplexing problems. A number of observations have documented the presence of blocking materials in the serum of animals or patients with various tumors. In some instances, evidence has been presented that blocking factors may actually be antigen-antibody complexes composed of host antibody and tumor antigens (2, 16, 17, 26, 40). Numerous studies of serum from large groups of tumor patients have established the presence of circulating immune complexes often in conjunction with progression of neoplastic disease (7, 15, 42, 43).

Much less is known about the host cell-mediated response to tumor. Depression of peripheral blood T-cell numbers and in vitro reactivity have repeatedly been observed in the face of disseminated cancer (13, 22, 31, 38). In addition, there is now a convincing body of data indicating that certain tumors are capable of inducing activation of various autologous suppressor cell mechanisms (9, 20, 53, 55). If, indeed, tumors are capable of activating endogenous suppressor cells, such a mechanism would appear to be uniquely suited for eventual tumor victory and the overcoming of natural host defense mechanisms. Several studies have appeared which have addressed the physiological importance and specificities of suppressor cell activation in various classes of animal or human cancers (20, 55), but more needs to be learned about how crucial such mechanisms are for eventual host survival.

The present study was directed at an analysis of peripheral blood and tissue T-cell subpopulations in a group of heterogeneous cancer patients. Recently, T-cells have been divided into several subpopulations on the basis of Fc receptors for IgG, IgM, IgA, and IgE (23, 27–29, 54). T-cells with IgG Fc receptors (Tγ) are believed to represent a possible suppressor population, whereas T-cells bearing Fc receptors for IgM have been shown to comprise T-cell help (29). However, recent studies comparing a number of cell surface markers using monoclonal hybridoma reagents have emphasized the possible heterogeneity of Tγ cells (37, 47). As yet, no distinct functional activities for Tα or Tγ cells have been defined.

In experimental mouse systems, the use of alloantisera directed against T-lymphocyte subsets has defined Ly antigens and cell surface marker profiles characteristic for T-helper and T-suppressor cells (3, 5, 6, 52). Unfortunately, at present no such alloantisera are as yet generally available, although several recently described mouse hybridoma reagents show considerable promise in this regard (35, 36).

Human la-like antigens were first described in association with B-cells and B-lymphoblastoid cell lines (24, 50). The human la antigens probably represent analogs of HLA-D determinants and may be closely related to immune response genes (14, 25, 41). La antigens have also been detected on granulocytic precursor cells as well as monocytes and macrophages (51). A small proportion of human T-cells has been shown to contain la-like antigens (1, 10). Most recently, la antigen has also been detected on a subpopulation of human Tγ cells (12, 21). In the early mouse studies, la-positive T-cells were linked to suppressor cell activity (33, 34), but subsequent in vitro studies in humans have suggested that la-positive T-cells may function as helpers (10, 30). Increases in proportions of la-positive T-cells have recently been demonstrated in certain human disease states and after immunization with tetanus toxoid (56). The present work has attempted to focus on peripheral blood and tissue T-cell subpopulation changes during the course of untreated cancer in the hope that more insight might thus be obtained into immune regulatory dysfunction during the course of human malignant disease.

MATERIALS AND METHODS

Peripheral blood was collected from 69 previously untreated...
patients with a wide variety of cancers. General categories and numbers of patients studied are shown in Table 1. Since irradiation or potent chemotherapeutic regimens are capable of affecting host lymphocyte subpopulations (38), no patient with any form of antecedent therapy was included in the study. Whenever possible, fresh tumor tissues were also collected and snap-frozen in liquid nitrogen, and 4-μm frozen sections were made to determine concurrent lymphocyte subpopulations within and adjacent to the tumors themselves. In all, a total of 11 patients were analyzed simultaneously for peripheral blood and tissue lymphocyte distribution. Tumor tissues alone without parallel peripheral blood were examined in an additional 35 patients.

Lymphocyte Cell Surface Marker Determinations. Lymphocytes from peripheral blood were obtained by Ficoll-Hypaque (4); subsequently, T-cells were isolated by neuraminidase erythrocyte-rosetting and centrifugation of rosettes through Ficoll gradients. After lysis of sheep cell rosettes with ammonium chloride, T-cells were assayed for proportions of Ty and Tθ subpopulations as described previously (28, 29, 48, 49). Total numbers of T-, Ty, and Tθ cells were determined by calculations from peripheral blood white blood cell differentials. A group of 30 normal controls was similarly studied to establish normal ranges and means.

Tissue Distribution of T-Lymphocytes. Previous studies from this laboratory have shown that many primary tumors contain substantial infiltrates of T-lymphocytes (19). One of the original objectives of the present work was to attempt to estimate the possible relative concentration of suppressor T-cells among tumor lymphocyte T-cell infiltrates. Since no unique T-cell alloantigen similar to mouse Ly antisera were available, several approaches were taken. T-cells within tissue were assayed by indirect immunofluorescence using serial 4-μm frozen sections and mouse hybridoma IgG with clear specificity for human T-cells. When double staining of tissues was attempted, in most instances rhodamine conjugates of goat anti-mouse IgG were used in parallel with mouse hybridoma anti-human T-cell reagents. These latter mouse anti-human T-cell antibodies were monoclonal products of 2 hybridomas developed and generously supplied by Dr. Shu Man Fu at Rockefeller University, New York, N. Y. The mouse hybridoma reagents showed strong membrane staining of 99 to 100% of specifically isolated human T-cells prepared by neuraminidase rosetting. No staining was noted with isolated normal human B-cells or several human B-cell lines (Raji, B-7, and B-35M). In addition, mouse hybridoma anti-human T-cell antibodies did not stain human monocytes, macrophages, or platelets. Mouse monoclonal IgG was used in dilutions (1:10 and 1:50) which avoided binding to tissue Fe receptors. In some instances, the hybridoma was also used in conjunction with the addition of aggregated human IgG (5 mg/ml) to saturate tissue Fe receptors. After no difference had been noted in tissue staining reactions with and without added aggregates in the hybridoma reagent, the remainder of tissue immunofluorescence studies were performed with suitable dilutions of the hybridoma anti-T-cell reagent alone. Ly-positive T-cells were identified by parallel staining using fluorescein-conjugated chicken or rabbit F(ab')2 fragments of antisera specific for human la antigens. Anti-human la reagents were prepared as described originally by Welsh and Turner (44) using a highly characterized rabbit anti-human la antisera kindly furnished by Dr. R. J. Winchester, Rockefeller University, New York, N. Y. Specificity and characteristics of these reagents have been described previously (11, 21, 30).

An antisera with specificity for human Ty cells was prepared in rabbits by immunization with pooled Ty cells isolated from 50 human cord blood samples. Human cord bloods were used as a source for human Ty cells in view of the demonstration of increased Ty suppressor activity in such samples (32). In brief, from batches of 2 to 3 human cord bloods, Ty cells were specifically isolated by ox cell EAg2 rosetting and centrifugation through Ficoll gradients. After lysis of adherent ox erythrocytes, Ty cells were frozen in the programmed cell freezer at −197° and stored in 10% fetal calf serum and dimethyl sulfoxide in liquid nitrogen. Simultaneously, non-Ty cells were similarly processed from the same human cord bloods. Rabbits were immunized i.v. with 2 to 5 × 10⁶ isolated cord Ty cells, and, after 3 to 4 weekly injections, antisera was harvested. After inactivation at 56° for 30 min, anti-Ty antisera were absorbed with T-null cells prepared from portions of freshly minced human thymus from children undergoing open heart surgery. Absorptions were also carried out with insolubilized fetal calf serum, ox erythrocytes, sheep erythrocytes, and finally the previously collected non-Ty cells from the same cord bloods used to prepare Ty cells. Absorbed anti-Ty antisera showed clear specificity for Ty cells with little or no reactivity for non-Ty, B-, or macrophage monocytes. These absorbed reagents also did not react with human L-cells (18, 50), null cells, or granulocytes. Because of recent evidence that the human T-cell subpopulation might indeed be heterogeneous, comparative studies using the mouse hybridoma reagent OKM1 (Ortho Pharmaceutical Corp., Raritan, N. J.) reactivity with monocyte-related antigens (37) were conducted. The OKM1 hybridoma was used in conjunction with rhodamine-conjugated goat F(ab')2 anti-mouse IgG in studies of isolated normal Ty cell populations.

The anti-Ty antisera did not block formation of Ty rosettes when T-cells were preincubated with F(ab')2 anti-Ty before EA T rosetting indicating that its specificity was not directed at Ty...
receptors themselves. In addition, treatment of normal lymphocytes or isolated T-cells with whole anti-Ty antisera plus complement failed to increase reactivity of treated cell populations to phytohemagglutinin, concanavalin A, or pokeweed mitogen (47). These findings indicated that a functional suppressor role for the T-cells identified by the hetero-anti-Ty reagent could not be identified. Representative studies of immunofluorescence specificity of absorbed F(ab)2 anti-Ty reagents as well as results of double staining with OKM1 to document Ty heterogeneity are shown in Table 2. Absorbed anti-Ty antisera studied as F(ab)2 fragments to avoid reactivity with tissue Fc receptors were used in indirect immunofluorescence with tissues using fluorescein isothiocyanate-conjugated goat F(ab)2 anti-rabbit F(ab)2 specifically isolated from insolubilized columns of rabbit F(ab)2. F(ab)2 fragments of anti-Ty antisera were prepared as described previously (46) to avoid inadvertent binding to Fc receptors on a variety of normal tissue or tumor cells. Double staining utilizing the monoclonal mouse anti-human T-cell antibody followed by a second layer of rhodamine-conjugated goat anti-mouse IgG could be accomplished using absorbed rabbit F(ab)2 anti-human Ty or rabbit F(ab)2 anti-human la antisera in conjunction with fluorescein-labeled goat anti-rabbit F(ab)2. Such techniques allowed direct identification of la-positive T-cells as well as Ty cells in frozen tissue sections. In some instances, rabbit F(ab)2 anti-human la reagents directly conjugated with fluorescein isothiocyanate were used in double staining experiments. A number of control experiments were done with fresh normal human lymph nodes and spleen to examine tissue reactivity of the immunofluorescence reagents used.

It seemed possible that studies of frozen tissue sections which examined cut tissues might show different patterns of immunofluorescence reactivity from those using living cells from whole blood. Accordingly, a number of control experiments used populations of T-rosetting cells, monocytes, B-cells, and L-cells isolated and prepared by cytocentrifugation. Staining of these cytocentrifuged preparations showed T-cell membrane but no intracellular staining with anti-T-cell hybridoma. Anti-Ty reagents likewise showed no evidence for reaction with intracellular or cytoplasmic antigens. Anti-la antibodies fragments F(ab)2 reacted predominantly with surface membrane determinants in B-cells and to a much fainter degree in monocytes and macrophages.

All immunofluorescence studies of both tissue and peripheral blood were performed using a Zeiss fluorescence microscope equipped with epifluorescence, a mercury HBO 200-watt lamp, and a BG12 primary filter. For fluorescein immunofluorescence, a 516-Å excitation filter and a 455- to 499-Å barrier filter were used. When the same fields were examined with rhodamine conjugates, a 560-Å excitation filter and a 590-Å barrier filter were used.

RESULTS

Peripheral blood from a total of 45 patients with various forms of solid tumors was studied. In addition, 6 patients with malignant lymphoma and 18 patients with Hodgkin's disease were also included in the study. Frozen tissue samples from a total of 46 different solid-tumor patients were also examined. In these latter instances, an attempt was made to prepare frozen sections from margins or sections of the tumor shown by concurrent hematoxylin and eosin staining to contain significant lymphocytic infiltrations (Fig. 1).

Peripheral Blood T-Cell Markers. Both proportions and absolute numbers of peripheral blood T-cells were markedly reduced in untreated patients with various solid tumors. The percentage and total number of Ty cells, however, were increased in tumor patients; in parallel, a marked diminution of T4 cells (both proportions and numbers) was observed in comparison to data accumulated concurrently in 30 normal controls. These findings are shown in Table 3. When specifically isolated T-cells were examined, it was also found that numbers and proportions of la-positive T-cells in peripheral blood of solid-tumor patients were also markedly reduced (Table 3). It seemed possible that reduction in T-cells, Ty cells, or the proportion of la-positive peripheral blood T-cells might conceivably be due to lymphocyte adsorption of plasma factors interfering with the various rosetting or immunofluorescence techniques. Accordingly, a number of control experiments were performed to examine this possibility. Incubation of normal mononuclear cells with serum or plasmas from cancer patients followed by washing in Hanks’ balanced salt solution prior to rosetting assays did not appreciably alter the results of subsequent Ty or T4 results. In addition, it seemed possible that the low proportion of peripheral blood la-positive T-cells in cancer patients might be due to the binding of host antibody to T-cell determinants or even la determinants themselves on this same cell population. However, this was not borne out by direct immunofluorescence examination of neuraminidase-rosetted T-cells using F(ab)2 anti-immunoglobulin reagents where less than 0.5% of the cells showed any surface immunoglobulin.

Results obtained in peripheral blood T-cell surface markers from patients with malignant lymphoma or Hodgkin’s disease produced slightly different results. In these patient groups, similar changes in total T-cells as well as T-cell subpopulations were noted; however, proportions of T-cells bearing la antigen were often markedly increased. These data are shown in Table 4. Great care was taken in these studies to exclude any artifacts involved in the mononuclear cell separations. Concurrent monitoring of T-cell preparations examined for la-like antigen showed no contamination of monocytes or macro-

<table>
<thead>
<tr>
<th>Normal donor</th>
<th>Whole monocyte-depleted lymphocytes</th>
<th>B-cells</th>
<th>T-cells</th>
<th>Ty</th>
<th>Non-Ty</th>
<th>Null or LH°-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17</td>
<td>3</td>
<td>14</td>
<td>66</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>14</td>
<td>2</td>
<td>11</td>
<td>59</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>53</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>50</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% of Ty°-cells staining with</th>
<th>% of Ty°-cells staining with</th>
<th>% of Ty°-cells staining with</th>
<th>% of Ty°-cells staining with</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKM1</td>
<td>both OKM1 and anti-Ty</td>
<td>anti-Ty</td>
<td>both OKM1 and anti-Ty</td>
</tr>
<tr>
<td>4 normal donors tested</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(different from A-D above)</td>
<td></td>
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</table>

A Mouse hybridoma anti-T-cell reacting with 99 to 100% normal peripheral blood erythrocyte-rosetting lymphocytes.
B Mean of 4 donors.

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Table 3
Lymphocyte cell surface markers in control subjects and patients with solid tumors

<table>
<thead>
<tr>
<th></th>
<th>WBC total</th>
<th>% of lymphocytes</th>
<th>No. of lymphocytes/cu mm</th>
<th>% of T-cells</th>
<th>No. of T-cells/cu mm</th>
<th>% of T(_Y) cells(^a)</th>
<th>No. of T(_Y) cells/cu mm(^b)</th>
<th>% of T(_B) cells(^b)</th>
<th>No. of T(_B) cells/cu mm(^b)</th>
<th>% of la-positive T-cells(^c)</th>
<th>No. of la-positive T-cells(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 30)</td>
<td>7583.3 ± 2206.3(^d)</td>
<td>44.4 ± 13.2</td>
<td>3177.1 ± 709.6</td>
<td>68.7 ± 12</td>
<td>2196.5 ± 697</td>
<td>8.2 ± 3.6</td>
<td>185.1 ± 86.1</td>
<td>39.6 ± 12.5</td>
<td>891.6 ± 462</td>
<td>3.6 ± 1.6</td>
<td>80.9 ± 50.1</td>
</tr>
<tr>
<td>Solid tumors (n = 45)</td>
<td>9869 ± 5456.3</td>
<td>23.7 ± 11.8</td>
<td>2056.5 ± 1243.7</td>
<td>51.9 ± 13</td>
<td>1050.5 ± 593.8</td>
<td>18.9 ± 15.7</td>
<td>210.8 ± 333.6</td>
<td>26.1 ± 16.9</td>
<td>258.7 ± 196.9</td>
<td>2.3 ± 2.2</td>
<td>18.1 ± 19.1</td>
</tr>
</tbody>
</table>

\(^a\) Comparison of percentage of T\(_Y\) cells between normal controls and solid-tumor patients (p < 0.001); when numbers of T\(_Y\) cells were compared in the 2 groups, however, no significant difference was noted (p < 0.05).

\(^b\) Comparison of % of T\(_B\) and absolute number of T\(_B\) cells between normal controls and solid-tumor patients (p < 0.0005 and < 0.00001, respectively).

\(^c\) Comparison of % of la-positive T-cells and number of these cells in normal controls and solid-tumor patients (p < 0.001 and < 0.00001, respectively).

\(^d\) Mean ± S.D.

Table 4
Lymphocyte cell surface markers in patients with Hodgkin's disease and non-Hodgkin's lymphomas

No significant difference for percentage and total numbers of T\(_Y\), T\(_B\), and la-positive T-cells was noted when Hodgkin's disease and non-Hodgkin's lymphomas were compared. However, in both of these groups, percentage of T\(_Y\), percentage of T\(_B\), numbers of T\(_B\), and percentage of la-positive T-cells were significantly different from normal controls (p < 0.00001, < 0.005, < 0.00001, and < 0.005, respectively).

<table>
<thead>
<tr>
<th></th>
<th>WBC total</th>
<th>% of lymphocytes</th>
<th>No. of lymphocytes/cu mm</th>
<th>% of T-cells</th>
<th>No. of T-cells/cu mm</th>
<th>% of T(_Y) cells(^a)</th>
<th>No. of T(_Y) cells/cu mm(^b)</th>
<th>% of T(_B) cells(^b)</th>
<th>No. of T(_B) cells/cu mm(^b)</th>
<th>% of la-positive T-cells(^c)</th>
<th>No. of la-positive T-cells/cu mm(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin's disease</td>
<td>7833.3 ± 4556.3</td>
<td>37.4 ± 12.2</td>
<td>2523.2 ± 648.4</td>
<td>50.5 ± 12.4</td>
<td>1251 ± 380.3</td>
<td>22.3 ± 13.6</td>
<td>270.5 ± 199.4</td>
<td>26.3 ± 13.5</td>
<td>327.4 ± 181.6</td>
<td>9.3 ± 8.9</td>
<td>112.2 ± 110.5</td>
</tr>
<tr>
<td>(18)(^a)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Non-Hodgkin's</td>
<td>11350 ± 7779.3</td>
<td>27.2 ± 10.6</td>
<td>3289.3 ± 3428.5</td>
<td>50.2 ± 15.7</td>
<td>1485.8 ± 1425</td>
<td>17.5 ± 7.7</td>
<td>317.5 ± 427.4</td>
<td>27.5 ± 24.5</td>
<td>487.8 ± 671.7</td>
<td>10.0 ± 7.6</td>
<td>115.8 ± 64.8</td>
</tr>
<tr>
<td>lymphomas (6)</td>
<td></td>
<td></td>
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\(^a\) Numbers in parentheses, number of patients studied.
phages using latex particle ingestion as well as peroxidase staining.

Among the solid-tumor cancer patients examined, an attempt to relate changes in peripheral blood T-cell subpopulation markers was made in conjunction with clinical profiles of individual patients at the time the assays were performed. In both solid-tumor patients and those with lymphatic neoplasms, a distinct relationship was apparent \((p < 0.05)\) between relative elevation of \(T_Y\) cells or \(Ia\)-positive T-cells and clear clinical evidence for metastatic disease at the time of study. A correlation \((p < 0.05)\) was also noted between total numbers of \(T_Y\) cells and those positive for \(Ia\) in the patients with lymphatic cancers. However, no distinct profile was apparent for either carcinoma of the breast or colon where sufficient numbers of subjects were studied to constitute distinct clinical groups. We also examined whether there appeared to be any relationship between proportions of \(Ia\)-positive T-cells in peripheral blood and the clinical extent of metastatic disease. No direct correlation was apparent.

Studies of Lymphocytes in Normal Control and Tumor Tissues. In 4-\(\mu\)m frozen sections from fresh normal human spleen and lymph nodes removed during various surgical procedures, direct staining for T-cells showed perifollicular and perivascular localization of collections of T-cells in the spleen. In addition, peripheral portions and subcapsular regions of normal lymph nodes stained predominantly for T-cells using the mouse hybridoma reagents. In normal human spleen, 5 to 12% (mean, 8.5%) of T-cells in perivascular locations were \(T_Y\) cells by double immunofluorescence, and a similar proportion (mean, 6%) was identified by double red and green immunofluorescence as \(Ia\)-positive T-cells. In 3 normal lymph nodes examined, only 2 to 5% of \(T_Y\) cells were noted in peripheral distribution; also, in similar subcapsular and peripheral follicular location, 3 to 8% (mean, 4%) \(Ia\)-positive T-cells were noted.

In general, T-cells predominated in interstitial tumor lymphoid infiltrates (Fig. 2). Direct comparison between hematoxylin- or eosin-stained frozen sections and adjacent serial 4-\(\mu\)m sections showed no more than 2 to 10% of null lymphocytes which could not be identified as T-cells, with the mouse monoclonal anti-human T-cell reagents or B-cells using fluorescein-labeled anti-human Ig or anti-la reagents.

Of great interest were the findings using double staining with rhodamine conjugates for T-cells in conjunction with fluorescein conjugates and F(ab')\(_2\) anti-\(T_Y\) reagents. Relatively high proportions of tissue \(T_Y\) cells were often noted. For instance, among the 20 tissues from carcinoma of the breast, an average of 12.4% of T-cells among lymphoid infiltrates were \(T_Y\) cells. In one patient with carcinoma of the colon, one with squamous cell carcinoma of the lung, and one with renal cell carcinoma, 26%, 23%, and 25%, respectively, of infiltrating T-cells were identified as \(T_Y\) by double rhodamine and fluorescein staining.

Finally, \(Ia\)-positive T-cells within tumor lymphoid infiltrates were also identified by double rhodamine (anti-T) and fluorescein (anti-\(Ia\)) immunofluorescence. No tumor cells examined in frozen tissue sections showed the clear-cut presence of human \(Ia\) antigen. Considerable variability in the proportion of \(Ia\)-positive T-cells was noted. In some cases, the percentage of \(Ia\)-positive T-cells closely approximated the percentage of \(T_Y\) cells, but, in others very high proportions of \(Ia\)-positive T-cells (50, 36, 73, 40, 83, 66, 45, 40, and 55%) were identified. In these 9 instances, 4 adenocarcinomas of the breast and 5 of the colon) there appeared to be distinct clusters of T-cells grouped together among lymphoid infiltrates which were \(Ia\) positive by fluorescein and clearly rhodamine positive for T-cells (Fig. 3). Comparison with proportions of \(Ia\)-positive T-cells simultaneously identified in peripheral blood often showed much lower values (1 to 5%), indicating marked relative tumor increments in these \(Ia\)-positive T-cell subpopulations. The double staining with the highly specific hybridoma mouse anti-T and the fluorescein-labeled rabbit F(ab')\(_2\) anti-human \(Ia\) reagent made clear definition possible in the same section. No confusion with B-cells, monocytes, or granulocytic precursors was possible since cells identified as T-cells were initially localized by their bright red membrane staining with the rhodamine conjugates and mouse anti-T monoclonal antibodies.

DISCUSSION

The studies reported here indicate that, besides a reduction in peripheral blood T-lymphocytes, patients with cancer showed significant increase in both proportions and numbers of the peripheral blood \(T_Y\) subpopulation; this was also accompanied by marked relative diminution in \(T_M\) cells. In addition, proportions and numbers of peripheral blood \(Ia\)-positive T-cells were decreased. These findings are of interest since, in several experimental systems, the \(T_Y\) population has been related to T-suppressor activity (29, 55). However, recent studies appear to indicate considerable heterogeneity within the \(T_Y\) cell subpopulation, both as to phenotype and possibly to function (37, 47). Recently, \(Ia\)-positive T-cells have been allocated primarily to the \(T_Y\) subpopulation in 2 independent studies (12, 21). Therefore, our finding of a relative increment in \(T_Y\) cells with a concomitant decrease in \(Ia\)-positive T-cells is of interest since it suggests the existence of at least 2 distinct subpopulations of \(T_Y\) cells varying independently in the case of cancer. Besides suppressor activity, \(T_Y\) cells may also be involved in cytotoxic or natural killer functions where Fc receptors may be necessary for biological activity (8). The increment in peripheral blood \(T_Y\) cells among tumor patients may reflect intrinsic activation of suppressor mechanisms or hypertrophy of potential cytotoxic T-cells capable of tumor cell elimination. Direct testing of these possibilities will now be necessary. In view of the known increment in circulating immune complexes associated with a number of primary human tumors (7, 15, 42, 43), it was surprising that peripheral blood \(T_Y\) cells were noted to be elevated among the untreated cancer patients studied. However, simultaneous determination of circulating immune complexes using the C1q binding method in many of the patients studied here did not reveal frequent elevations of complexes. Adherence of complexes to \(T_Y\) FC receptors could theoretically block some Fc receptors in vivo and thus mask true proportions of \(T_Y\) FC receptors assayed subsequently in vitro. That this was not in effect occurring was suggested by results in which isolated T-cells from cancer patients were incubated in medium at 37° for 12 hr to allow membrane turnover and were restudied for expression of Fc receptors. Such protocols did not show progressive increments in \(T_Y\) cell proportions in a number of patients studied.

Diminution in \(T_M\) cells (representing putative helper cells) is in keeping with decreased immune responsiveness noted previously in a wide variety of cancer subjects. The apparent relationship between demonstrable metastatic disease and pro-
portions of both Tγ and la-positive T-cells deserves further analysis. Direct isolation of these T-cell subpopulations from tumor tissues eventually may help to elucidate their functional importance.

The studies of tissue distribution of lymphocytes within various tumor tissues were of considerable interest. Peripheral blood increments in Tγ cells as identified by ox cell EA rosetting were often reflected by similar increments in Tγ tumor cellular infiltrates. Since the rabbit anti-Tγ antiserum identified an average of only a little over one-half of the Tγ cells by immunofluorescence, it seems likely that, in many instances, estimates of tumor localized Tγ cells were low. The studies of apparent cell surface antigen heterogeneity within Tγ cells using the OKM1 hybridoma reacting with a monocyte-related antigen were also of considerable interest. In view of the fact that our own studies have not identified suppressor activity in Tγ cells reacting with the anti-Tγ heteroantisem (47), it seems possible that the original suppressor cell activity ascribed to Tγ cells may relate to Tγ cells which are OKM1 positive or to some other minor Tγ subtraction. Certainly, further work is indicated in this area, particularly in cancer patients where Tγ cells have been noted to be elevated. In parallel, the identification of very high proportions of la-positive T-cells often noted in clusters within certain breast and colon carcinomas was of great interest. The occurrence of clusters of la-positive T-cells grouped together within the interstices of tumors suggests a similar or concerted function. Whether such cells represent groups of helper or suppressor T-cells now remains to be determined. The recent report by Yu et al. (56) indicating a rise in proportion of peripheral blood la-positive T-cells after immunization suggests that such cells may represent an activated T-cell subpopulation. A puzzling finding was that, in the 9 cases where clusters of la-positive T-lymphocytes were noted in tumor interstices, parallel identification of Tγ cells was often much lower. At present, we have no good explanation for these findings except that the immunofluorescent localization of Tγ cells was considerably weaker in intensity than that with the anti-la reagents. In addition, as noted above, Tγ cell heterogeneity may well have been a factor (37, 47). The increments in la-positive T-cells in tumor tissues were opposite to the findings in peripheral blood of many of these same patients where la-positive T-cells were diminished. These findings suggest that some sort of distinct immunological function, possibly help or suppression, might be concentrated within tumor tissues and therefore be noticeably diminished within circulating peripheral blood mononuclear lymphocytes. No noticeable relationship between clusters of tumor-localized la-positive T-cells and infiltrating macrophages identified in serial hematoxylin- and eosin-stained sections was apparent. A direct answer to this important question must now await the development of reliable techniques for isolation and functional assessment of these various lymphocyte populations from fresh tumor tissues. Similar assessment of the distribution of T-cells and mononuclear cells in a variety of tissues expressing human la-like antigens has recently been described by Seymour et al. (39).

Great caution, of course, must be used in any study such as the current analysis where single determinations of either blood lymphocyte phenotypic markers or patterns of tissue-infiltrating T-cells are performed, particularly with respect to the ongoing course of the neoplastic process. The one positive aspect in the current report was the fact that all patients were studied before initiation of any therapy. However, single observations in such a situation are not as useful as, for instance, serial studies among carefully followed patients.

Finally, the present study provides useful information regarding specific lymphocyte subpopulations studied in parallel in human peripheral blood and tumor tissues. It was of interest that the major mononuclear cell type identified by the techniques used here were T-cells. Their dominance within tumor lymphocytic infiltrates should provide an additional impetus for more refined functional studies of this important aspect of human host response to primary tumor.

REFERENCES

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Fig. 1. Typical sections of tumor tissues studied for types of lymphocytes involved as mononuclear infiltrates. A, carcinoma of breast with heavy interstitial lymphocytic infiltrate; B, frozen section of adenocarcinoma of lung with nodular lymphoid interstitial infiltrates. H & E, × 200.
Fig. 2. Carcinoma of breast having lymphocytic infiltrate stained with monoclonal hybridoma mouse anti-human T-cell reagent. A majority of the lymphocytes in this case were identified as T-cells. A, ×250; B, membrane staining of T-cells in tumor infiltrate, ×400.
Fig. 3. Frozen section of carcinoma of breast where heavy T-lymphocyte infiltrate was identified with mouse hybridoma anti-T-cell antibody and rhodamine label. Double staining for la-positive T-cells in this case showed clusters of such cells within portions of the tumor. A, clusters of la-positive T-cells, x 250; B, x 540.
T-Lymphocyte Subpopulations in Peripheral Blood and Tissues of Cancer Patients


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