American Journal of Pathology, Vol. 129, No. 2, November 1987
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RAPID COMMUNICATION

The Immunopathology of Sequential Tumor Biopsies in Patients Treated With Interleukin-2

Correlation of Response With T-Cell Infiltration and HLA-DR Expression

PAUL J. COHEN, MD, MICHAEL T. LOTZE, MD, JOHN R. ROBERTS, STEVEN A. ROSENBERG, MD, PhD, and ELAINE S. JAFFE, MD

From the Laboratory of Pathology and Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Sequential tumor biopsies from 9 patients with disseminated cancers were obtained before, during, and after treatment with interleukin-2 (IL-2) with or without the adoptive transfer of lymphokine-activated killer (LAK) cells. Infiltrating lymphoid and tumor cells were characterized in frozen sections by the use of monoclonal antibodies and the avidin-biotin complex (ABC) immunoperoxidase technique. Five patients had objective tumor regression (1 complete response of a follicular lymphoma, 4 partial responses of melanomas). Four patients (2 melanomas, 1 renal cell carcinoma, 1 breast carcinoma) were nonresponsive after treatment. After treatment, responsive tumors showed a pronounced infiltration of T cells, mainly Leu-2$^+$ (CD8, primarily cytotoxic/suppressor) cells. Macrophages, although increased, were fewer than the T cells, and Leu-7$^+$ or Leu-11$^+$ (NK and K) cells were virtually absent. In nonresponders, there was no significant increase in lymphoid cells after therapy, and no differences were noted between groups before therapy. In 4 of 5 responders, tumor cells were positive for HLA-DR before therapy; and in the remaining responder, the tumor became positive during treatment. Tumor cells in all biopsy specimens from nonresponders were DR$^-$ before and after the start of therapy. It is concluded that the expression of HLA-DR by tumor cells may play a role in the response to IL-2 with or without LAK and that marked infiltration by T cells accompanies, and possibly mediates, such a response. (Am J Pathol 1987, 129:208–216)

INTERLEUKIN-2 (IL-2)[1] is a 15,000-dalton glycoprotein, first described in 1976 as a factor produced by mitogen- or antigen-stimulated T cells, which mediated the expansion of T cells *in vitro*.[1] Originally called T-cell growth factor, subsequent investigations have supported a role for IL-2 as the second signal for T-cell mitogenesis.[2,3] In addition, the incubation of peripheral blood mononuclear cells in IL-2–containing media resulted in the appearance of cells capable of killing fresh autologous tumor cells, but not normal cells, in short-term chromium release assays.[4,5] These cells, designated lymphokine-activated killer (LAK) cells, are thought to arise from precursors that do not express mature B or T cell markers; the exact phenotype of the LAK effector cell remains elusive.

In animal tumor models, significant anti-tumor effects have been obtained with an adoptive immunotherapy approach with IL-2 and LAK cell infusions.[6-8] Clinical trials of high-dose IL-2 and the adoptive transfer of LAK cells in patients with advanced cancers unresponsive to conventional therapies have produced objective responses (>50% reduction in measurable tumor volume) in approximately 40% of patients to date.[9-11] Melanomas, renal cell carcinomas, and colorectal carcinomas have been the most responsive tumor types in patients treated thus

Accepted for publication August 31, 1987.
Address reprint requests to Elaine S. Jaffe, MD, Building 10, Room 2N202, Laboratory of Pathology, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892.

far. Significant anti-tumor responses also have been seen in patients with melanoma and renal cell carcinoma treated with IL-2 alone. To investigate the immunologic mechanisms of the tumor regression, as well as to evaluate possible predictors of a clinical response, we undertook a histopathologic and immunophenotypic analysis of sequential tumor biopsy specimens from patients with accessible metastatic deposits being treated with IL-2 or LAK/IL-2. We present evidence correlating clinical response with 1) the infiltration of tumors by T cells and macrophages and 2) the expression of HLA-DR on the tumor cell membrane.

Materials and Methods

Patient Population and Treatment

Detailed descriptions of patients and therapy have been previously published.[9-11] Briefly, all subjects had advanced disseminated tumors in whom prior therapy had failed, often including surgery, chemotherapy, and/or treatment with interferon. All patients were treated on an approved clinical protocol under a Federal Drug Administration (FDA)-approved IND (investigation of a new drug) for IL-2 with signed informed consent. Patients were typically treated with IL-2 at 30,000–300,000 U/kg three times a day for 1–3 weeks. For patients receiving LAK cells, daily 4-hour leukophereses were performed for 5 days with procurement of $1-3 \times 10^{10}$ mononuclear cells after a week of IL-2. These cells were cultured at $1-2 \times 10^6$ cells/ml in spinner flasks containing 1500 U/ml of IL-2. After 3–4 days of culture, the cells were administered intravenously over approximately 1 hour. During the period of cell infusions, IL-2 administration was reinstituted three times a day at the same dose. Other medications given with the IL-2 included acetaminophen (650 mg four times a day) and indomethacin (50 mg three times a day) to control fever and chills, ranitidine (300 mg every 12 hours) for prophylaxis of gastrointestinal bleeding, and frequent use of standard antiemetics. Patients with easily accessible sites of metastatic disease underwent biopsy before the initiation of therapy as well as during and after their treatment. Most patients studied had subcutaneous metastases of melanoma; others included 1 patient with generalized follicular lymphoma of the small cleaved cell type (Patient 1), 1 patient with cervical lymph node metastases from renal cell carcinoma (Patient 7), and 1 with locally recurrent breast carcinoma (Patient 6). The 9 patients presented here are all patients from whom to date at least two biopsy specimens had been obtained, one before and at least one during or after therapy. All tissue specimens were of subcutaneous lesions, with the exception of the three biopsy specimens from Patient 7 (cervical lymph nodes) and two from Patient 1 (cervical and axillary lymph nodes).

Tissue Processing and Reagents

Tissue was processed and examined without knowledge of a patient's clinical status. This was revealed to the pathologists only at the conclusion of this study. Fresh tissue was snap-frozen in OCT medium (Miles Laboratories, Naperville, Ill) in a dry ice/2-methyl butane bath and then stored in a liquid nitrogen freezer at −150 C for up to 10 months. Five-micron sections were made on a cryostat, mounted on gelatinized glass slides, and stained for membrane and cytoplasmic antigens with an avidin–biotin complex (ABC) immunoperoxidase technique as previously described.[12-14] The ABC kit was obtained from Vector Laboratories (Burlingame, Calif). The primary monoclonal antibodies used, their main immunoreactivities, and their sources are listed in Table 1. In addition, routine hematoxylin and eosin (H&E) stains were done on each biopsy specimen.

Table 1—Monoclonal Antibody Panel

Name	CD	Primary immunoreactivity	Source	Similar clones
Anti-Leu-1	CD5	T cells, pan-T reagent	Becton Dickinson	OKT1, T101
Anti-Leu-2	CD8	Class I reactive, primarily suppressor/cytotoxic T cells	Becton Dickinson	OKT8
Anti-Leu-3	CD4	Class II reactive, primarily helper/inducer T cells	Becton Dickinson	OKT4
Anti-Leu-4	CD3	T cells, pan-T reagent	Becton Dickinson	OKT3
Anti-Leu-7		Large granular lymphocytes, NK/K cells	Becton Dickinson	
Anti-Leu-11	CD16	Large granular lymphocytes, NK/K cells, neutrophils, LAK precursors	Becton Dickinson	
Anti-Leu-M3		Monocytes/macrophages	Becton Dickinson	
Anti-T11	CD2	Sheep E-rosette receptor, all T cells, NK cells, LAK precursors and effectors	Coulter	
Anti-B1	CD20	B cells in peripheral blood and lymph nodes	Coulter	
Anti-OKT9		Transferrin receptor	Ortho	
Anti-Tac	CD25	IL-2 receptor	Dr. T. Waldmann, NIH	
Anti-HLA-DR		Class II framework antigen B cells, monocytes/macrophages, activated T cells	Becton Dickinson	

Immunophenotypic Analysis

The histologic and immunophenotypic profiles of each of the tumor biopsies were analyzed according to the extent of the lymphoid infiltrate of the various cell types and by the expression of certain antigens on the tumor cells themselves. The lymphoid infiltrates were graded as follows: 0, no significant cellular infiltrate; +/−, rare positive cells; 1+, positive cells confined to the fibrovascular septae only; 2+, beginning infiltration into tumor nests; 3+, widespread but patchy infiltration into tumor nests; 4+, positive cells surrounding virtually every tumor cell. The level of expression of antigens (HLA-DR, Tac, OKT9, and surface immunoglobulin for Patient 1 with lymphoma) on tumor cells was graded as 0, not expressed, 1+, weak, 2+, moderate; or 3+, strong expression.

Results

Patients Treated and Responses

The characteristics of treated patients are presented in Table 2. Patient 1 achieved a complete remission. Patient 2 had clinically apparent shrinkage and disappearance of multiple subcutaneous nodules that were difficult to measure, primarily because of their small size, although other sites may not have responded, and brain metastases ultimately developed. Patients 3 through 5 sustained a partial response (>50% reduction in measurable tumor volume) in their subcutaneous metastases, in which all subcutaneous lesions behaved similarly. Liver metastases did not respond in Patient 5. Patients 6 through 9 did not respond. Four of five responses were in patients with melanoma. Three occurred with IL-2 alone, and two with IL-2/LAK therapy. Subsequent brain metastases developed in Patients 2 and 4, and they could not receive additional therapy. Patient 5 had progression of liver metastases and died several months after the conclusion of therapy. Patients 1 and 3 are alive 20 and 15 months after therapy, respectively.

Pathologic Findings

There were striking histologic and immunophenotypic differences between the biopsy specimens obtained from Patients 1–5 and Patients 6–9. These are summarized in Table 3. Patient 1 had a follicular lymphoma, small cleaved type. The neoplastic nodules were composed predominantly of lymphoid cells staining for surface immunoglobulin, μ heavy chain,

Table 2—Patient Characteristics

Patient no.	Age	Sex	Diagnosis	Sites of disease	No. of IL-2 doses	Units IL-2 $\times 10^3$	No. of LAK doses	Total No. LAK cells $\times 10^{10}$	Dates of treatment	Dates of biopsy	Response status*
1	41	F	Follicular lymphoma	Liver, lymph nodes, bone marrow	18	1800	10	27.9	11/1/85 to 11/18/85	10/29/85 1/2/86	CR
2	30	M	Melanoma	Skin	27	2700	0	—	4/7/86 to 5/1/86	4/7/86 4/10/86 4/15/86 5/1/86	PR skin; brain metastases
3	51	M	Melanoma	Skin	21	2100	0	—	4/14/86 to 4/30/86	4/14/86 4/21/86 4/28/86 5/2/86	PR
4	50	M	Melanoma	Skin, liver, lung	20	2000	8	5.3	11/21/85 to 12/9/85	11/20/85 12/2/85 12/11/85	PR all sites
5	49	F	Melanoma	Liver, skin	29	3300	0	—	9/4/85 to 9/20/85	9/3/85 9/12/85 9/23/85	PR skin; NR liver
6	47	F	Breast carcinoma	Skin, liver	26	2600	5	11.03	4/7/86 to 4/22/86	4/7/86 4/24/86	NR
7	79	F	Renal cell carcinoma	Nodes, liver	16	1600	5	5.6	1/6/86 to 1/21/86	1/6/86 1/22/86 5/2/86	NR
8	40	F	Melanoma	Lung, skin	22	2200	4	7.9	1/13/86 to 1/27/86	12/3/85 1/29/86 2/20/86	NR
9	48	M	Melanoma	Lung, skin	18	1800	10	27.9	3/24/86 to 4/8/86	3/24/86 3/27/86 4/3/86	NR

*CR, complete response; PR, partial response, defined as >50% reduction in tumor volume; NR, no response.

Table 3—Selected Immunoreactivities of Lymphoid Infiltrates and Tumor Cells Before and During/After Therapy

Patient	Pan-T-cell markers Leu-1, Leu-4, T11 (CD5, CD3, CD2)		Monocyte marker Leu-M3		Helper: suppressor ratio	Class II antigen on tumor cells HLA-DR	
	Before therapy	During or after therapy	Before therapy	During or after therapy	Leu-3:Leu-2 (CD4:CD8) during therapy	Before therapy	During or after therapy
1	1+*	3+	ND	ND	2:3	3+	3+
2	2+	2+/3+/4+†	2+	2+/2+/3+	1:4	3+	3+/3+/3+
3	1+	2+/3+/3+	0	2+/3+/3+	1:2	2+	2+/2+/3+
4	1+	2+/3+	1+	2+/3+	1:3	3+	3+/3+
5	0	3+/4+	1+	3+/3+	1:2	0	3+/3+
6	0	0	0	0	—	0	0
7	1+	1+/1+	±	±/±	1:1	0	0/0
8	1+	1+/1+	2+	2+/2+	1:1	0	0/0
9	0	1+/1+	±	±/±	2:1	0	0/0

*For grading system, please see Materials and Methods section in the text.
†Each entry refers to a separate biopsy. Biopsies are listed in sequential order. Dates are shown in Table 2.

and κ light chain. Before therapy, the nodules were closely apposed, with little intervening lymph node parenchyma. A moderate infiltrate of T cells was present both in the interfollicular areas and, to a lesser extent, scattered within the nodules. After therapy with IL-2/LAK, the nodules were smaller, and a dense infiltrate of Leu-4^+ cells was present in between and within the follicles. Before therapy, the number of Leu-2^+cells was very small, and virtually all were outside the neoplastic nodules, as is usually the case in follicular lymphomas. In the posttherapy biopsy, markedly increased number of Leu-2^+ cells were seen within and around the nodules. These changes are shown in Figure 1. The lymphoma cells were strongly DR^+, as one would expect in a B-cell neoplasm.

After excision of this lymph node, which demonstrated residual disease, the patient's lymph nodes further regressed in size. At 3 months after completion of therapy, there was no palpable lymphadenopathy, and regression of all lymph nodes previously identified on CT scans was documented. A repeat bone marrow biopsy, which had been positive before therapy, was negative for lymphoma, and the peripheral blood was cleared of circulating monoclonal B cells. Before therapy, 50% of the circulating mononuclear cells were monoclonal B lymphocytes.

In the melanomas, before treatment, lymphoid cells were rare and confined to the fibrovascular septae, in between tumor cell nests (except for Patient 2, who had some permeation of lymphoid cells into tumor nests). These cells were virtually all T cells, because >90% stained with the pan-T-cell markers Leu-1, Leu-4, and T11. In the nonresponders, this infiltrate did not change significantly in either amount or distribution. In the responding patients (2–5), however, during and after therapy there was a marked increase in the number of lymphoid cells, which extended from the periphery into the tumor cell nests. In these patients, on H&E stains, the tumor cells could be seen to lose their polygonal shape, round up, and disaggregate as they were surrounded by lymphoid cells. The lymphoid cells remained >80–90% positive for pan-T markers. Moreover, there was a reversal of the predominance of Leu-3 (helper, CD4, Class II reactive) over Leu-2 (suppressor/cytotoxic, CD8, Class I reactive) cells usually found in normal tissues, including normal lymph nodes. (Ratios were not estimated on pretreatment biopsies because of the paucity of lymphoid cells in most instances.) Most of the infiltrating lymphoid cells were positive for HLA-DR and Tac after therapy; however, only rare lymphoid cells were HLA-DR^+ or Tac^+ before therapy. Illustrations of these findings are presented in Figure 2.

The infiltrating lymphocytes in the responding patients were apparently concentrated in tumor-bearing areas. In the early phases of this study, biopsy specimens were obtained after treatment with IL-2 from both normal-appearing skin and skin involved by an IL-2 induced rash. The normal skin showed no lymphoid infiltrate. Those areas of skin clinically involved by a rash showed only edema and a minimal perivascular lymphoid infiltrate.

The distribution of Leu-$M3^+$ cells (macrophages) paralleled that of the T cells, but these cells were present in fewer members (10–20% of mononuclear cells). Only very rare cells stained with Leu-7 or Leu-11 (large granular lymphocytes, natural killer [NK] cells) in any biopsy. Similarly, very few $B1^+$ (B) cells were noted in any biopsy (with the exception of the lymphoma, which was $B1^+$). Examination of the tumor cells with antibodies to Tac, T9, and HLA-DR suggested a correlation between the expression of HLA-DR with clinical response and histologic

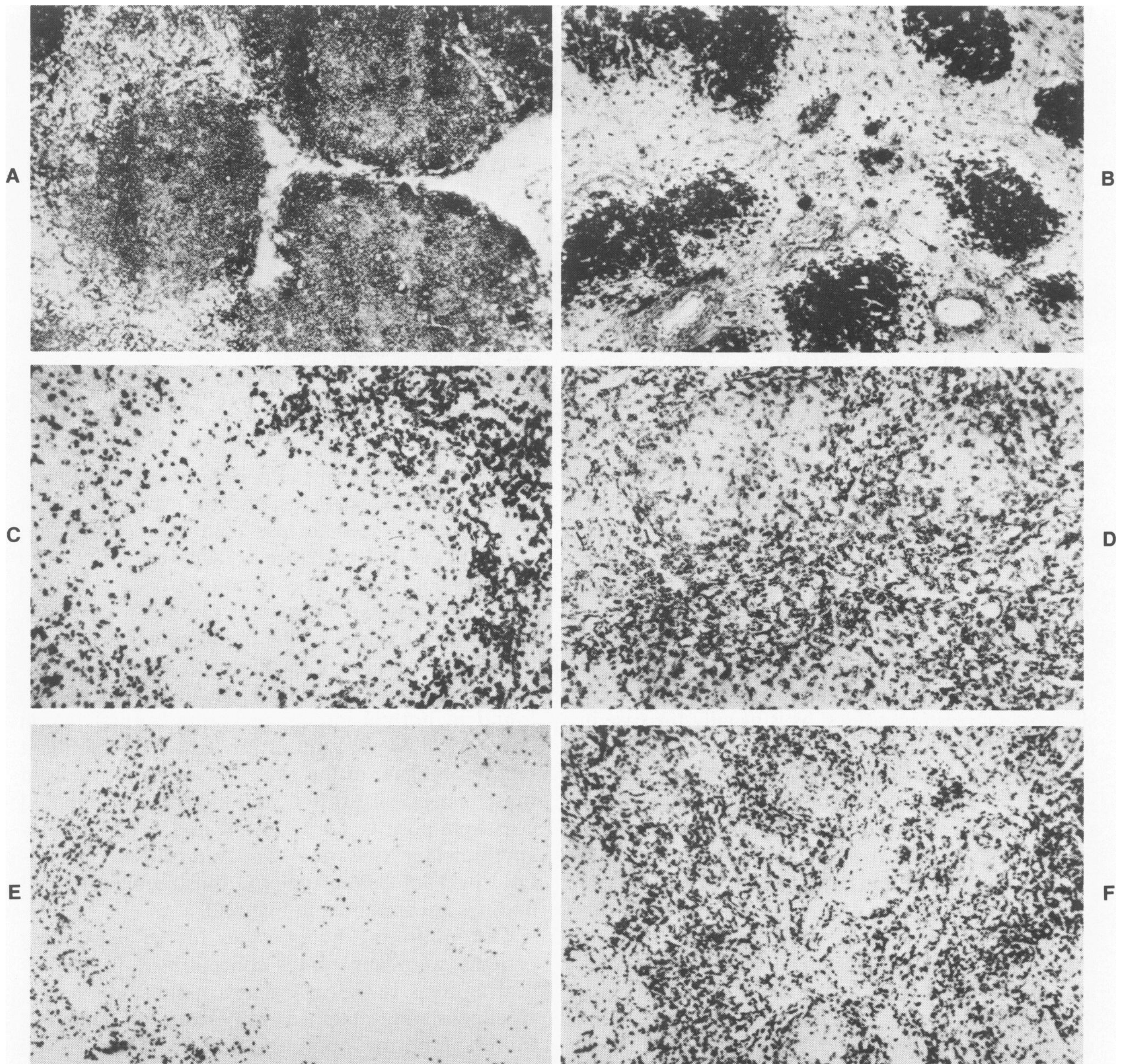

Figure 1—Biopsy specimens from Patient 1 with follicular lymphoma before (**A**, **C**, and **E**) and after (**B**, **D**, and **F**) treatment with IL-2/LAK. **A** and **B** are stained with anti-μ heavy chain, **C** and **D** with anti-Leu-4, and **E** and **F** with anti-Leu-2. The nodules of lymphoma, which show monoclonal staining for IgM, are markedly diminished in size in the posttherapy biopsy. The T-cell (Leu-4^+) infiltrate, mostly interfollicular before therapy, expands and extends into the lymphoma nodules after treatment. The Leu-2^+ cells constitute most of the expanded T-cell population. (Original magnification, ×160)

changes (Table 3). In Patients 1–4, HLA-DR was present on tumor cells before treatment. In Patient 5, HLA-DR appeared after the initiation of therapy and remained positive for at least 3 days after the cessation of treatment (the time of her last biopsy). In all 4 nonresponders, tumor cells were negative for HLA-DR before treatment and remained negative. In the pretherapy biopsies, the endothelium was HLA-DR^-, even when the tumor cells were positive. In biopsies taken during and immediately following therapy, the endothelium was HLA-DR^+, irrespective of response status or HLA-DR expression on tumor cells. There were insufficient specimens for evaluation of HLA-DR expression on the overlying epidermis. Photomicrographs illustrative of the findings are shown in Figure 3.

Tumor cells from all biopsies were positive for T9 (transferrin receptor). Tumor cells from biopsies in Patients 2, 3, 4, 7, and 8 were positive for Tac (IL-2 receptor). Because of this unexpected finding, samples from Patients 2, 3, 6, 7, 8, and 9 were also stained with 7G7, a monoclonal antibody that detects the

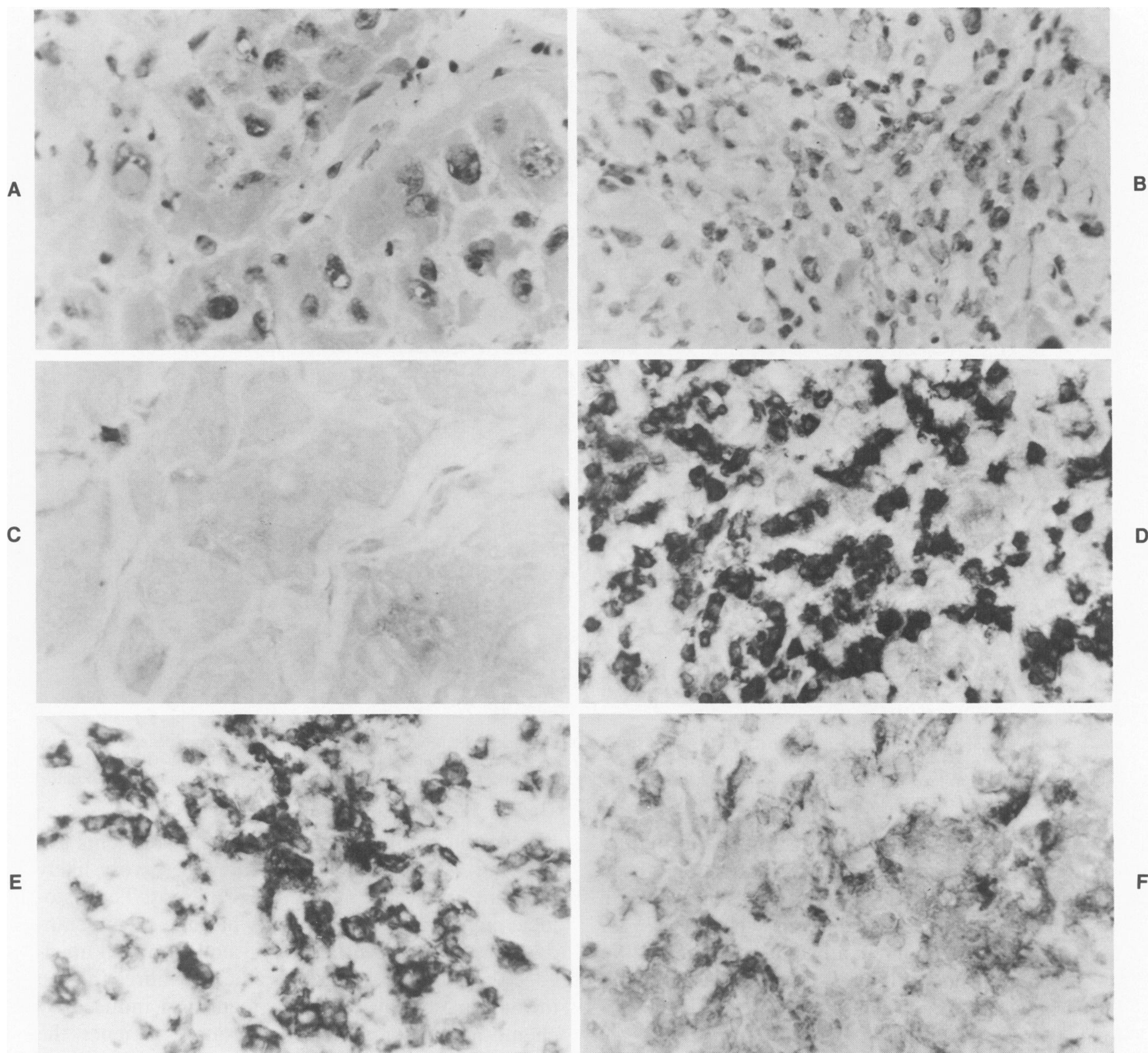

Figure 2—Biopsy specimens from Patient 3 with malignant melanoma before (**A** and **C**) and after (**B** and **D–G**) treatment with IL-2. H&E staining shows large polygonal melanoma cells before therapy, without a significant lymphoid infiltrate (**A**). Two days after completion of therapy with IL-2, on H&E (**B**), the tumor cells appear shrunken and rounded. Virtually every tumor cell is surrounded by mononuclear cells. Before therapy, Leu-4$^+$ cells were rare (**C**), but after treatment they became numerous (**D**). Leu-2$^+$ cells (**E**) were predominant over Leu-3$^+$ cells (**F**). (Original magnification, ×400)

IL-2 receptor in both the unoccupied and the occupied states. Tumor cells from biopsies in Patients 2, 7, and 8 showed positivity as well with 7G7. Staining for T9 and Tac did not change with therapy. Of interest was the observation that the Tac staining was cytoplasmic, in a perinuclear, Golgi-like pattern, not surface staining as in the infiltrating lymphocytes.

Discussion

This immunopathologic study identifies phenotypic correlates of clinical and pathologic tumor responsiveness of IL-2–mediated human tumor regression. In patients with an objective response, the tumors became densely infiltrated by large numbers of mature T lymphocytes, predominantly Leu-2 (CD8)-positive cytotoxic-suppressor cells. In responding patients with melanoma, individual tumor cells became less cohesive, smaller, and rounder, losing their usual polygonal shape. In the single follicular lymphoma treated, there was a marked diminution in the size of the neoplastic nodules. In nonresponders, neither a change in the appearance of the tumor cells

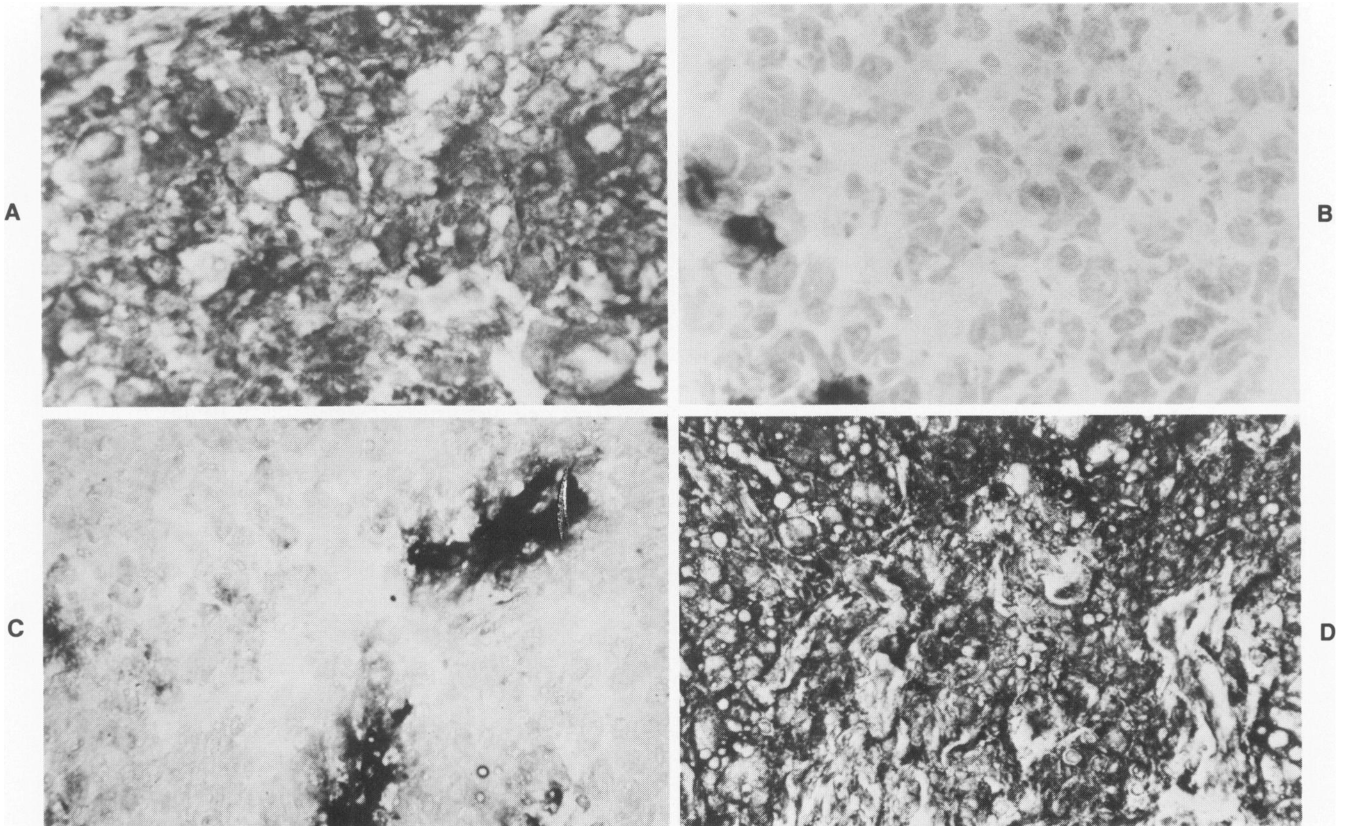

Figure 3—Biopsies from Patients 2 (**A**), 8 (**B**), and 5 (**C** and **D**) stained for HLA-DR. Tumor cells from Patient 2, who sustained a partial response, were strongly positive for HLA-DR before therapy. In Patient 8, who did not respond, the only cells staining before therapy were macrophages. Patient 5's tumor cells were negative before treatment (**C**), with staining only of macrophages. In a biopsy specimen obtained during therapy (**D**), tumor cells became strongly positive. Patient 5 had a partial response. (Original magnification, ×400)

nor the development of a significant inflammatory infiltrate was seen after therapy. Although it has been postulated that NK (Leu-11, Leu-7) cells play a role in host defense against tumor cells, few Leu-7$^+$ or Leu-11$^+$ cells were noted in any biopsy before or after therapy. Of particular interest is the suggested correlation between tumor regression clinically and histologic expression of HLA-DR on the tumor cells. All the responding tumors except one were HLA-DR$^+$ before therapy, and in 1 case a responding tumor became HLA-DR$^+$ after therapy. All tumors that were HLA-DR$^-$ before therapy and remained negative after therapy failed to show a response. However, 2 of the patients in whom skin lesions regressed apparently had no response of tumor in the brain and liver (Patients 2 and 5, respectively). HLA-DR expression in these latter sites in these 2 patients is unknown.

The histologic changes described here differ substantially from those reported in BCG-induced regression in cutaneous metastatic melanomas.[16] Two to 3 weeks after the injection of lesions, a marked granulomatous inflammatory infiltrate develops in the tumors, characterized by prominence of macrophages and giant cells. Thus, the cellular events underlying BCG's effect in melanoma are very likely different from those in IL-2/LAK therapy. The histologic picture also differs from what one sees in standard forms of cancer therapy. Although to our knowledge there are no studies specifically addressing the immunopathology of tumor regression induced by chemotherapy or radiation, in our experience the findings in such instances are coagulative necrosis of tumor cells, with prominence of polymorphs, histiocytes, and granulation tissue. A predominantly lymphocytic infiltrate is distinctly uncommon.

The attainment of a complete response in Patient 1 with follicular lymphoma is intriguing, especially because she had massive disease that had failed to respond to repeated chemotherapeutic treatments. Follicular lymphomas are low-grade tumors. Many patients have a history of lymph nodes that wax and wane in size over many years. Several lines of evidence have caused investigators to postulate that host immunity may have an important role in the clinical course of these lymphomas.[17] Spontaneous regressions have been documented, sometimes after infections, and immunomodulators such as α-interferon

have shown efficacy in low-grade, but not high-grade, lymphomas.[18,19] Moreover, it has been known for some time that although follicular lymphomas are monoclonal B cell proliferations, T cells account for up to 50% of the cells present in an involved lymph node.[15,20] The infiltrating T cells are present mainly in between nodules but also are seen within the follicles, intimately associated with the monoclonal B cells. The single best predictor of a response to anti-idiotype treatment is the amount of normal T-cell infiltration of the lymphoma, and it is postulated that the mechanism of action of anti-idiotype therapy involves immunoregulatory circuits.[20,21] It has recently been shown that IL-2 has a synergistic effect when combined with anti-idiotype antibody treatment in a murine lymphoma model.[22] In addition, in one study, some patients with follicular lymphoma who histologically progressed to a diffuse pattern, which is usually associated with a more aggressive course, continued to pursue an indolent course. These patients were identified as having marked reactive T-cell infiltration of their lymph nodes.[23] Thus, it appears that host T cells may represent a significant immune response, regulating to some extent the proliferation of the tumor cells. Our histologic findings are entirely consistent with stimulation of the lymphoma patient's T cells as the mechanism of remission.

A most provocative finding of this study is the correlation between HLA-DR expression and clinical response (5 of 5 responders, 0 of 4 nonresponders). HLA-DR antigens are Class II histocompatibility molecules analogous to the Ia antigens in mice and have a critical role in cell–cell interactions of the immune system.[24] In particular, the activation of $CD4^+$ T lymphocytes is thought to be dependent on the recognition of Class II molecules in conjunction with foreign antigen on the surface of accessory cells. HLA-DR molecules normally are found mainly on lymphoid and endothelial cells, but recent reports have described variable expression on normal epithelial cells.[25] HLA-DR is not present on normal skin melanocytes, nor in benign nevi,[26] but has repeatedly been shown to be present on a substantial proportion of melanomas, both in patient biopsies and in cell lines.[26,27] Furthermore, in cell lines negative for HLA-DR, HLA-DR synthesis and expression can be induced by γ-interferon.[28–30] Gamma-interferon levels are transiently elevated in patients undergoing IL-2 treatment.[31] This possibly accounts for the induction of HLA-DR in the tumor of Patient 5. In *in vitro* experiments investigating the capacity of melanoma cell lines to stimulate autologous T cells, it was found that HLA-DR expression on the tumor cells was necessary but not sufficient for blastogenesis.[32,33] It is hypothesized that the melanoma cells may directly interact with immune cells because of the presence of both Ia and tumor-associated antigens on the tumor cells' surfaces.

An unexpected finding was immunoreactivity with ant-Tac in tumor cells from 4 of 6 malignant melanomas and a renal cell carcinoma. Of the 5 cases positive for anti-Tac, 4 were studied with 7G7, an antibody that detects a separate epitope on the IL-2 receptor. Three of the 4 were also positive with 7G7. The significance of this immunoreactivity is unclear. However, it is thought that one of the two chains of the IL-2 receptor is expressed constitutively in a variety of cells, and that the membrane expression of this chain is lost when the fourth exon of the gene is deleted (D. Nelson, National Cancer Institute, personal communication), suggesting a possible mechanism for the accumulation of cytoplasmic immunoreactive material seen in this study.

The putative LAK effector cell may show heterogeneous phenotypic characteristics and is thought to be typically positive for Leu-11 (CD 16), negative for T3 (CD 3), usually negative for Leu-1 (CD 5), and variably positive for T11 (CD 2) and T8 (CD 8).[34,35] In our study, cells that were $T3^+$, $T11^+$, and $Leu\text{-}1^+$ were equal in number and distribution and were clearly distinct from the very rare $Leu\text{-}11^+$ cells. Therefore, we believe our results are most consistent with activation of the T-cell arm of the immune system.

We cannot at this time distinguish whether this lymphoid infiltrate actually results in tumor cell damage or is the consequence of cell damage mediated by other mechanisms. Moreover, the prominence of macrophages in tumor cell nests associated with cell necrosis could be a primary or a secondary phenomenon as well; ie, the $Leu\text{-}M3^+$ cells could be important in mediating tumor cell necrosis or might be present in response to cell necrosis. However, the apparent requirement for a response of HLA-DR expression on tumor cells suggests specific recognition of tumor cell antigens by cells that, when activated after the administration of IL-2, induce the local accumulation of cells with tumor cytolytic function. It will be useful to determine whether other tumors treated by IL-2/LAK, especially renal cell carcinomas, commonly express HLA-DR antigens in patients who respond. The usefulness of HLA-DR expression as a predictive test will be further evaluated.

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