DEPRESSED CELL-MEDIATED IMMUNITY IN PATIENTS WITH PRIMARY INTRACRANIAL TUMORS

CHARACTERIZATION OF A HUMORAL IMMUNOSUPPRESSIVE FACTOR*

BY WILLIAM H. BROOKS,‡ MARTIN G. NETSKY, DAVID E. NORMANSELL, AND DAVID A. HORWITZ§

(From the Departments of Internal Medicine, Pathology, and Microbiology, University of Virginia School of Medicine, Charlottesville, Virginia 22903)

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Impaired cell-mediated immunity in certain patients with nonlymphoid cancer is indicated by depressed skin reactivity to common antigens (1, 2), delayed homograft rejection (3), decreased ability to become sensitized to dinitrofluorobenzene (4) or dinitrochlorobenzene (5, 6), and decreased in vitro lymphocyte responsiveness to the mitogen phytohemagglutinin (7, 8). It is not known whether depressed cell-mediated immunity precedes or follows the development of cancer.

Tumor-specific antigens and cell-mediated tumor immunity are noted in cases of human cancer. Blood lymphocytes from patients with some neoplasms inhibit tumor cell growth (9, 10) or undergo blast transformation when cultured with autochthonous tumor cells (11). In addition to the cell-mediated responses, the sera of these patients contain antibodies or antigen-antibody complexes (12) that specifically block lymphocyte-tumor cell interaction through a poorly understood phenomenon termed immunologic enhancement (13, 14).

In the present studies, patients with primary intracranial tumors were found to have depressed cell-mediated immunity. Unlike other patients with solid systemic tumors and impaired delayed hypersensitivity, our patients did not have clinical evidence of metastases, weight loss, or hematologic abnormalities. These patients, then, afforded an unusual opportunity to study host mechanisms responsible for defective cell-mediated immunity.

Although delayed hypersensitivity and other cell-mediated responses were depressed, we found evidence of lymphocyte sensitization to tumor antigens. To clarify these apparently paradoxical findings, we evaluated mononuclear leukocyte function with the one-way mixed lymphocyte reaction. Cellular reactivity in the absence of patient plasma was normal. Such plasma, however,

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‡ Present address: Division of Neurosurgery, University of Kentucky, Lexington, Ky. 40506.

§ Recipient of a fellowship award from the Arthritis Foundation. Reprint requests should be addressed to D.A.H.

strongly suppressed lymphocyte blast transformation. Immunosuppressive activity was found in IgG fractions isolated from patient sera.

Materials and Methods

23 patients with benign and malignant intracranial tumors were studied (Table I). 15 were men and 8 were women; ages ranged from 10 to 69 yr (median = 48). Patients were not receiving corticosteroids, cytotoxic drugs, or radiation therapy at the time they were studied. All venipunctures were performed before skin tests or blood transfusions. Healthy hospital employees served as controls. 10 were men and 10 were women; ages ranged from 24 to 63 years (median = 41).

Skin Testing for Delayed Hypersensitivity.—All subjects were skin tested by intradermal injections of 0.1 ml of the following antigens: intermediate strength purified protein deriva-

TABLE I

Clinical Data, Skin-Test Reactivity, and Leukocyte Response to Phytohemagglutinin of Patients with Intracranial Tumors

Clinical information						Skin tests*						PHA
Patient	Age	Sex	Diagnosis	HCT	WBC	SL	DNCB	PPD	SK-SD	CAND	TRICH	
	yr											*cpm*
Group I: Nonresponders to DNCB or skin-test antigens												
M.S.	69	F	Glioblastoma	40	10,500	3090	0	0	0	0	0	7,250
W.H.	52	M	Glioblastoma	40	11,500	1495	0	0	0	0	0	37,333
A.D.	52	F	Glioblastoma	39	10,500	1685	0	NT	NT	NT	NT	NT
W.K.	26	M	Astrocytoma	44	6,800	1297	0	0	4	0	0	12,243
N.T.	50	F	Hemangio-blastoma	45	7,400	1702	0	0	0	0	0	15,374
G.R.	10	M	Medullo-blastoma	45	8,100	1700	0	0	0	0	0	14,381
R.C.	14	M	Ependy-moma	48	7,500	1275	0	0	0	0	0	4,979
G.M.	52	F	Meningioma	40	9,500	2300	0	0	0	0	0	54,634
M.G.	48	F	Meningioma	42	7,500	1482	0	0	0	0	0	28,184
A.D.	26	M	Neuroma	47	11,100	2442	0	0	0	0	0	20,929
L.R.	65	F	Neuroma	41	7,500	2325	0	0	0	0	0	5,501
Group II: Nonresponders to DNCB with one positive skin test												
G.R.	54	M	Glioblastoma	45	9,600	3552	0	0	8	0	0	7,092
T.B.	61	M	Glioblastoma	41	11,500	2760	0	0	5	0	0	14,935
J.H.	27	M	Astrocytoma	42	10,500	1785	0	0	5	0	0	2,433
Group III: Responders to DNCB or two or more positive skin tests												
J.K.	42	M	Glioblastoma	40	8,500	1800	+	5	0	0	7	42,002
G.C.	39	M	Astrocytoma	41	5,400	972	+	4	10	0	0	16,301
S.D.	57	M	Astrocytoma	42	7,800	1568	0	0	22	12	0	14,909
Z.W.	54	F	Astrocytoma	47	10,500	1892	+	0	5	0	0	27,211
J.J.	12	F	Astrocytoma	39	10,500	2100	+	0	10	12	0	17,804
H.S.	25	M	Pinealoma	41	13,400	2268	+	0	8	6	8	73,282
C.F.	42	M	Pituitary	45	10,500	2450	+	0	12	0	0	28,570
R.C.	43	M	Pituitary	41	8,500	2125	NT	0	12	6	0	43,183
W.D.	48	M	Meningioma	48	9,500	1825	+	0	18	6	4	14,799

* Mean diameter of induration after 48 hr, in millimeters.

Abbreviations used: HCT = hematocrit; WBC = white blood count; SL = small lymphocytes; DNCB = dinitrochlorobenzene; PPD = tuberculin; CAND = *Candida;* TRICH = trichophyton; PHA = phytohemagglutinin.

tive (PPD)[1]-tuberculin 5 units/0.1 ml (Dept. H. E. W., Tuberculosis Branch, Atlanta); *Candida* 1:10 (Hollister-Stier Laboratories, Yeadon, Pa.); trichophyton 1:10 (Hollister-Stier), and streptokinase 50 units-streptodornase 10 units ([SK-SD], Varidase; Lederle Laboratories, Pearl River, N. Y.). Induration was measured after 5, 24, and 48 hr to discriminate between immediate and delayed reactions.

Sensitization with dinitrochlorobenzene (DNCB) was performed by the method of Catalona et al. (6). DNCB was dissolved in acetone to make a stock solution of 2000 μg/0.1 ml. Solutions were refrigerated and replenished every 2 wk. At the time of sensitization, 2000 μg were placed on the volar aspect of the forearm and covered with a sterile gauze bandage. 50 and 100 μg were simultaneously applied to the other forearm to test for prior sensitization. Test sites were examined at 24 hr, 7 days, and 14 days. Subjects demonstrating either a spontaneous flare or a flare 24 or 48 hr after a subsequent challenge dose of 50 and 100 μg were designated responders. Subjects not responding after single or repeated application(s) were nonresponders.

Lymphocyte Tdr-^{3}H Incorporation in Response to Antigens and Phytohemagglutinin (PHA).—Washed blood leukocytes (2.5×10^6) were cultured in triplicate with antigens or PHA, using a modification of previously described techniques (15). The cells were suspended in 3 ml of minimal essential medium (MEM-S; Grand Island Biological Co., Grand Island, N. Y.) containing 20% heat-inactivated type AB human serum obtained from a single donor and were cultured in 12 × 75 mm plastic culture tubes (Falcon Plastics, Los Angeles, Calif.). Preservative-free PPD was obtained from Parke, Davis & Co., Detroit, Mich. All other antigens were dialyzed 48 hr before use to remove preservatives. 0.1 ml of each of the following was used for culture: phytohemagglutinin-M (PHA) (lot number 564590 Difco Laboratories, Inc., Detroit, Mich.) 1:10; SK-SD undiluted; trichophyton 1:150; *Candida* 1:100; and PPD 300 μg/ml. Cultures containing PHA were incubated for 4 days; all other cultures were incubated for 6 days at 37°C (5% CO_2). 2 hr before termination, 5 μCi of tritiated thymidine (Tdr-^{3}H) (New England Nuclear Corp., Boston, Mass.; specific activity 0.67 Ci/mmole) were added to each tube. Samples were then prepared for liquid scintillation counting according to the method of Horwitz, Stastny, and Ziff (15). A positive response to antigens was defined as at least a threefold increase of Tdr-^{3}H uptake compared with unstimulated cells. The cpm of PHA-stimulated leukocytes from controls were consistently 100 times that of unstimulated cells.

Mixed Lymphocyte-Tumor Cultures.—Primary intracranial tumors were obtained at craniotomy, minced with scalpel blades, and explanted into several 75-cm^2 plastic flasks (Falcon Plastics No. 3024). Nutrient mixture F-12 (Grand Island Biological Co.) supplemented with penicillin 100 units/ml, streptomycin 50 μg/ml, and 20% heat-inactivated fetal calf serum was used as the culture medium. When adequate growth was observed, usually in 14–21 days, cells were harvested with 0.025% trypsin-EDTA (Grand Island Biological Co.), washed twice with media (100 *g* for 10 min), and resuspended in a concentration of 4×10^5 cells/ml. Tumor cells were not treated with mitomycin C.

Lymphocytes to be used as "responder" cells were obtained by concentrating leukocyte-rich plasma to 2–4 ml and layering 1 ml on 2.5 ml of 20% sodium Hypaque (Sterling-Winthrop Research Inst., Rensselear, N. Y.). After centrifuging at 2500 *g* for 10 min at 25°C, the supernatant was decanted and washed three times in MEM-S (150 *g* for 10 min). The cells were resuspended in a concentration of 6×10^5 mononuclear cells/ml. Differential counts revealed 80% lymphocytes (range 70–85%), 18% monocytes (range 10–26%), and 1–2% polymor-

[1] *Abbreviations used in this paper:* DNCB, dinitrochlorobenzene; MEM, minimal essential medium; MLC, mixed lymphocyte culture; MLTC, mixed lymphocyte-tumor culture; PHA, phytohemagglutinin; PPD, purified protein derivative; SK-SD, streptokinase-streptodornase; Tdr-^{3}H, tritiated thymidine.

phonuclear leukocytes. Cells stained by trypan blue were rare (<1%). Cultures containing 1 ml of stimulator cells and 1 ml of responder cells in 20% fetal calf serum were incubated for 6 days, then isotopically labeled and prepared for scintillation counting as before.

Suspensions of tumor cells were also placed on glass cover slips in Leighton culture tubes (H. W. Leighton Laboratories, Glen Ridge, N. J.), and were subsequently used for histologic study to determine contamination by fibroblasts. Slides stained with hematoxylin–eosin and phosphotungstic acid were reviewed by one of us, (M. G. N.). Contamination with fibroblasts was not considered significant. Control cultures were prepared with autologous fibroblasts from skin or dura, and with fetal brain. These tissues were explanted, cultured (using the previously described techniques), and used as the "stimulator" in mixed lymphocyte-tumor reactions (MLTC).

In some experiments, responder lymphocytes were irradiated with 2500 R (106 R/min) (Picker Vanguard (Picker X-ray Corp., Cleveland, Ohio), 280 kvp, cone size 15 × 20 cm) before MLTC. In other experiments, heat-inactivated autologous and normal human sera were substituted for fetal calf serum.

Mixed Lymphocyte Cultures.—Mixed lymphocyte cultures (MLC) were prepared by the method of Bach and Voynow (16). Responder lymphocytes were obtained as previously described. Stimulator cells were collected from sedimented venous blood, washed three times in MEM-S (150 *g* for 10 min), and incubated at 37°C for 25 min with 25 μg/ml of mitomycin C (lot number 7090 Nutritional Biochemicals Corp., Cleveland, Ohio). The cells were then washed three more times and adjusted to 4×10^5 leukocytes/ml. Each "one-way" culture contained 6×10^5 responder cells, 4×10^5 stimulator cells in 2.5 ml MEM-S containing 20% human plasma. Triplicate cultures were harvested at 6 days.

One person (W. H. B.) served as the source of stimulator cells in all experiments. As a positive control, responder cells from normal persons were included in each study with patient responder cells. To exclude a two-way response, PHA was added to mitomycin-treated W. H. B. stimulator cells. Uptake of Tdr-^{3}H was not increased in these cultures. In some experiments, the effect of patient plasma was compared with normal plasma on the reactivity of both patient and normal responder cells in the MLC. All plasmas were centrifuged at 1400 *g* for 20 min at 4°C to remove platelets.

Identification of Humoral Blocking Factors.—Serum from normal volunteers and from patients was dialyzed twice against 0.01 M potassium phosphate buffer, pH 8.0, and applied to a column of DEAE cellulose (Whatman DE52, H. Reeve Angel & Co., Inc., Clifton, N. J.) previously equilibrated with this buffer. Protein not adsorbed to the column under these conditions was concentrated to the original serum volume, dialyzed against phosphate-buffered saline, pH 7.3, and passed through a Millipore filter (0.45 μ) (Millipore Corp., Bedford, Mass.). These eluates contained only IgG, as judged by immunoelectrophoresis against polyvalent anti-whole human serum (Meloy, Springfield, Va.). Further analysis by quantitative immunodiffusion plates (Meloy) indicated that the eluates studied contained only IgG; no other immunoglobulin classes could be detected.

Cultures were prepared to evaluate the effect of patient IgG fractions on lymphocyte activation by PHA and in MLC. To study this effect in MLC, 0.1–5 mg/ml of IgG was added to cultures containing 20% normal plasma, W. H. B. stimulator cells, and normal responder cells. Similar concentrations of IgG were added to cultures containing normal leukocytes and PHA. Patient IgG was compared with equal concentrations of normal IgG.

RESULTS

Comparison of Delayed and In Vitro Hypersensitivity of Tumor Patients and Controls.—All control subjects tested could be sensitized to DNCB (Table II), and only one of this group required secondary challenge to demonstrate a

positive response. In contrast, only 7 of 22 patients with benign and malignant tumors could be sensitized to DNCB. Reapplication was necessary in all but one of the patient responders.

When control subjects were skin tested with PPD, SK-SD, *Candida*, and trichophyton, all responded to at least two antigens. Patients were significantly less responsive to at least three of the four skin-test antigens (Table II). They were divided into three groups on the basis of the findings (Table I). Group I (11 patients) and group II (3 patients) were unresponsive to DNCB.

TABLE II

Comparison of Delayed Hypersensitivity and Lymphocyte Proliferative Reactivity of Patients and Controls

	No. of responders		*P* value*
	Normals	Patients	
Delayed hypersensitivity‡			
Sensitization to DNCB	8/8	7/22	0.001
Response to common antigens			
PPD	8/15	1/22	0.001
SK-SD	14/15	11/22	0.006
CAND	6/15	5/22	>0.1
TRICH	7/15	1/22	0.004
Lymphocyte proliferative reactivity			
PHA response§	16/16	17/22	>0.1
Response to common antigens‖			
PPD	11/16	6/23	0.01
SK-SD	14/15	10/23	0.002
CAND	5/15	2/23	>0.05
TRICH	2/16	0/23	>0.1

* Fisher exact probability test.

‡ Greater than 5 mm induration at 48 hr.

§ Greater than 100-fold increase in Tdr-^{3}H uptake over unstimulated values.

‖ Greater than threefold increase in Tdr-^{3}H uptake over unstimulated values.

Group I was anergic to the four skin-test antigens, and group II patients had one positive skin test. Group III included seven patients who were sensitized to DNCB and two others with two positive skin tests. As in previous studies, SK-SD yielded the greatest number of positive responders and the strongest reactions observed (17). Nonetheless, the intensity of the 48-hr reaction in group III patients (mean 12 mm, range 5–22 mm) was significantly less than in the controls (mean 19 mm, range 8–60 mm) ($P < 0.01$).[2]

Delayed hypersensitivity in the patients was impaired, although their physical condition was good. They did not have symptoms suggesting general debilitation, nor had they lost weight. There was no anemia, granulocytopenia, or lymphopenia (Table I).

[2] Analysis of variance.

The lymphocyte proliferative response to common antigens, as estimated by measuring the incorporation of Tdr-^{3}H, paralleled the skin-test results. Significantly fewer patients responded to PPD and SK-SD when patients and controls were compared (Table II). A significant difference in the blastogenic response to PHA was not observed. Five patients in groups I and II, however, had depressed PHA response (Tables I and II).

Lymphocyte Tdr-^{3}H Incorporation in Response to Autochthonous Tumor Cells.—Increased incorporation of Tdr-^{3}H was observed in patient lymphocytes when they were cultured with autochthonous tumor cells in media supplemented with fetal calf serum. In 11 of 13 patients, DNA synthesis in the mixed lymphocyte-tumor cell culture was at least twofold greater than the sum of lymphocyte and tumor cells cultured separately. The lymphocyte reactivity of group I patients was similar to that of group III patients (Table III).

In some cultures, lymphocytes irradiated with 2500 R were cultured with tumor cells. Tdr-^{3}H uptake was reduced to background levels. These results excluded the possibility that tumor cells were stimulated to proliferate in the presence of autologous lymphocytes.

Increased Tdr-^{3}H uptake was not observed when lymphocytes were cultured with fetal brain or autologous fibroblasts. These results indicate spe-

TABLE III

Lymphocyte Recognition of Tumor Antigens on Autochthonous Cells by Mixed Lymphocyte-Tumor Culture (MLTC)

Patient	Skin test: Group	Skin test: Diagnosis	BKG*	Tumor cells‡	MLTC	R.I.§
				cpm		
M.S.	I	Glioblastoma	537	81	2649	4.1
W.H.	I	Glioblastoma	165	50	3348	15.5
N.T.	I	Hemangioblastoma	138	76	449	2.1
R.C.	I	Ependymoma	131	93	1076	4.8
G.M.	I	Meningioma	202	313	1376	2.6
M.G.	I	Meningioma	105	134	479	2.9
A.D.	I	Neuroma	152	72	1229	5.5
T.B.	II	Glioblastoma	366	151	815	1.5
J.H.	II	Astrocytoma	283	895	5006	4.3
J.K.	III	Glioblastoma	262	179	961	2.2
G.C.	III	Astrocytoma	196	59	1647	6.5
Z.W.	III	Astrocytoma	264	566	1535	1.8
J.J.	III	Astrocytoma	404	215	2698	4.4

Cells cultured in 20% fetal calf serum.

* cpm/6 $\times$ 10^5 unstimulated lymphocytes.

‡ cpm/4 $\times$ 10^5 tumor cells.

§ $\dfrac{\text{MLTC}}{\text{Unstimulated lymphocytes} + \text{tumor cells}}$

cific sensitization of autologous lymphocytes to tumor-associated antigens on the surface of tumor cells.

Table IV indicates the results of studies in which human serum was substituted for fetal calf serum. Autologous serum consistently inhibited lymphocyte activation by autochthonous tumor cells in comparison with simultaneous cultures with normal human serum. Inhibitory activity was greater in the serum of group I (anergic) patients than in group III patients, although these differences were not statistically significant.

Lymphocyte Tdr-^{3}H Incorporation in Response to Allogeneic Mononuclear Leukocytes.—Patient lymphocytes cultured in autologous plasma incorporated significantly less Tdr-^{3}H than control lymphocytes cultured in normal plasma in the one-way MLC ($P < 0.01$) (Fig. 1). However, reactivity of patient lymphocyte increased to normal values when cultured in normal plasma. Similarly, suppression of lymphocytes from healthy controls was consistently observed in cultures containing patient plasma (Fig. 1). Suppressor activity was not affected by heating plasma at 56° C for 30 min or by storage at −20° C for 2 months.

The suppression was proportional to the concentration of patient plasma

TABLE IV

The Effect of Autologous Serum on Cell-Mediated Reactivity to Tumor-Specific Antigens in Mixed Lymphocyte-Tumor Culture

Skin test		Autologous*	Normal*	Inhibition‡
Patient	Group			
			cpm	%
M.S.	I	715	1371	48
W.H.	I	22	909	97
N.T.	I	66	193	65
R.C.	I	991	1809	44
G.M.	I	518	6040	89
M.G.	I	24	156	85
A.D.	I	97	0	0
T.D.	II	0	180	100
J.H.	II	2625	4919	45
J.K.	III	110	344	67
G.C.	III	89	170	48
Z.W.	III	1122	1878	40
J.J.	III	722	1966	63

* Each value indicates net cpm as determined by subtracting cpm of unstimulated leukocytes in autologous or normal serum from cpm in culture containing the same serum. Tdr-^{3}H uptake by nonstimulated lymphocytes was the same in cultures with autologous serum (mean 260 cpm, range 112–378 cpm) and homologous serum (mean 295 cpm, range 124–415 cpm).

‡ $\%\ \text{inhibition} = \dfrac{\text{cpm in normal serum} - \text{cpm in autologous serum}}{\text{cpm in normal serum}}$.

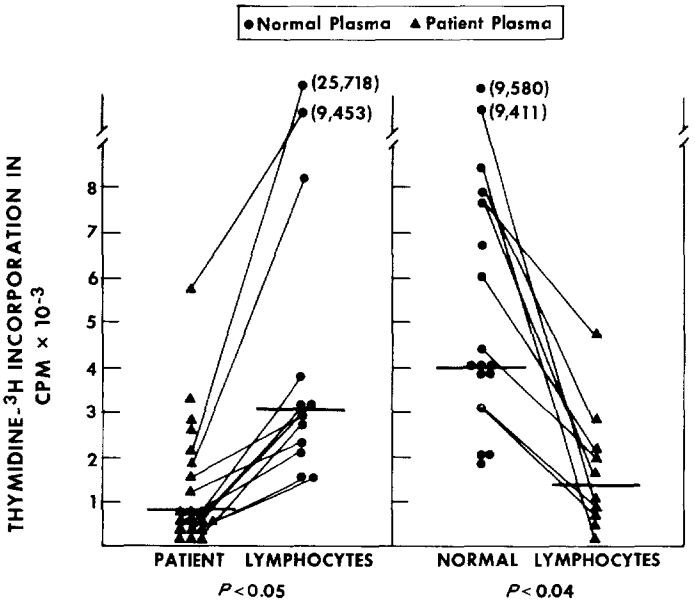

FIG. 1. The effect of plasma on cellular reactivity in the one-way mixed lymphocyte culture. W. H. B. "stimulator" cells were used in all studies. Solid lines show simultaneous cultures containing either patient or normal plasma. *P* values were determined by analysis of variance.

in the culture (Fig. 2). In the instance illustrated, plasma J.J. suppressed the activation of patient T.B.'s lymphocytes in MLC.

MLC suppressor activity in patient plasma disappeared shortly after the removal of the tumor. Fig. 3 indicates that the plasma of patient R. C. completely inhibited his lymphocyte reactivity to allogeneic cells before craniotomy. One month after surgery, suppressor activity was no longer present. In this study the reactivity of washed lymphocytes after surgery was almost twofold greater than before the tumor was removed.

Lymphocyte Suppressor Activity In Vitro and Delayed Hypersensitivity In Vivo.—Leukocytes from five patients were stimulated with PHA in cultures containing either autologous or normal serum. In all instances autologous serum inhibited Tdr-^{3}H incorporation (mean inhibition 44%, range 23–66%).

Plasma from five group I (anergic) patients strongly suppressed MLC reactivity of autologous lymphocytes (mean inhibition 92%). Significantly less suppressor activity was observed when mononuclear leukocytes of group III patients were cultured in autologous plasma (mean 63%) ($P < 0.05$) (Table V).

Immunochemical characterization of a Suppressor of Lymphocyte Activation.—The effect of patient and normal IgG fractions on the reactivity of normal

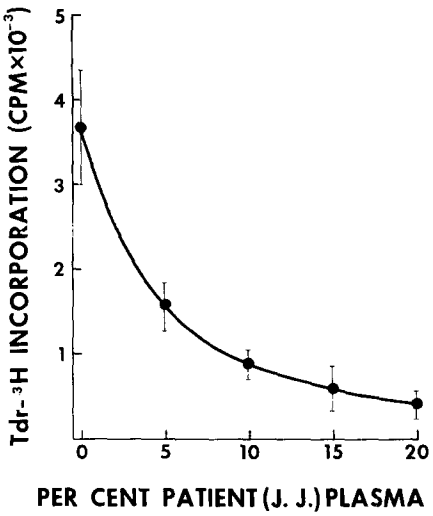

FIG. 2. The effect of increasing concentrations of patient plasma on the reactivity of homologous mononuclear cells in the one-way mixed lymphocyte culture.

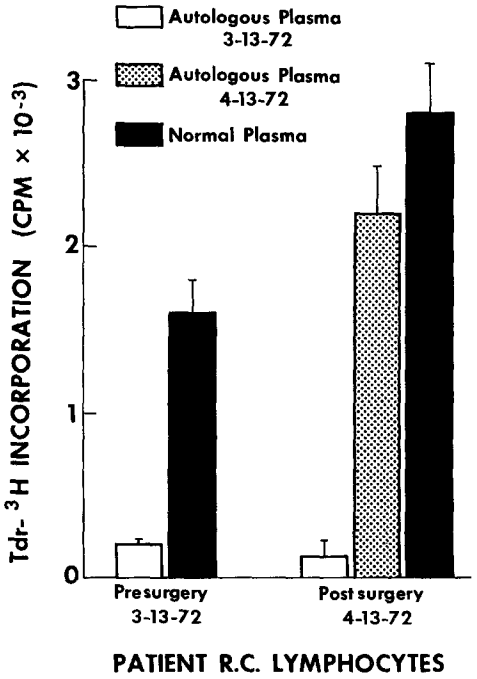

FIG. 3. Reactivity of patient (R. C.) lymphocytes in one-way mixed lymphocyte culture before and after removal of his ependymoma. Bars indicate mean and one standard deviation of triplicate cultures.

TABLE V

Plasma Suppression of Cellular Reactivity In Vitro Compared with Cutaneous Reactivity to DNCB and Common Antigens

Patient	One-way mixed lymphocyte culture		Inhibition‡	
	Autologous*	Normal*		
	cpm		%	
Group I skin reactions (nonresponders)				
W.H.	593	2937	80	
A.D.	23	2674	99	
R.C.	72	1409	95	
G.M.	188	2539	93	
M.G.	176	1886	91	
Mean	210	2289	92	$P < 0.05$
	$P < 0.01$			
Group III skin reactions				
J.K.	407	1328	67	
J.J.	3279	8069	59	
H.S.	5732	8982	36	
R.C.	1266	2805	55	
W.D.	232	3585	98	
Mean	2183	4954	63	
	$P > 0.05$			

Groups defined in Table I.

P values determined using Wilcoxon Rank Sum Test (41).

* Each value indicates net cpm as determined by subtracting cpm of unstimulated leukocytes in autologous or homologous plasma from cpm in stimulated culture containing the same plasma.

‡ $\%$ inhibition $= \dfrac{\text{cpm in normal plasma} - \text{cpm in autologous plasma}}{\text{cpm in normal plasma}} \times 100.$

responder cells in MLC is shown in Fig. 4. The blastogenic response of W.J. cells was completely suppressed by 100 μg/ml of IgG from patient J.H. Other normal cells (E.P.) were equally suppressed by this concentration of this IgG. Similar results were obtained with the IgG fractions of three other patients when compared with equivalent amounts of normal IgG isolated at the same time.

Patient IgG also modified lymphocyte stimulation by PHA. 1 mg/ml of IgG from J.H. significantly reduced the response of normal (W.J. and W.H.B.) lymphocytes to PHA. Lymphocyte activation by PHA was totally suppressed by increasing the level of patient IgG to 2.5 mg/ml, whereas this concentration of normal IgG resulted in only 30% suppression (Fig. 5).

To test for possible cytotoxic effects of the fractions, we added suppressive concentrations of patient IgG to normal lymphocytes and rabbit complement, using a standard macromethod (18). Trypan blue viability studies, after incubation at 37°C for 1 hr, indicated less than 5% stained cells.

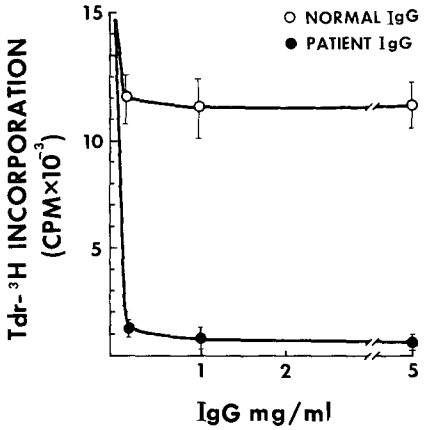

FIG. 4. The effect of patient (J. H.) IgG on the reactivity of normal mononuclear leukocytes in the one-way mixed lymphocyte culture. The concentration of IgG per culture is illustrated.

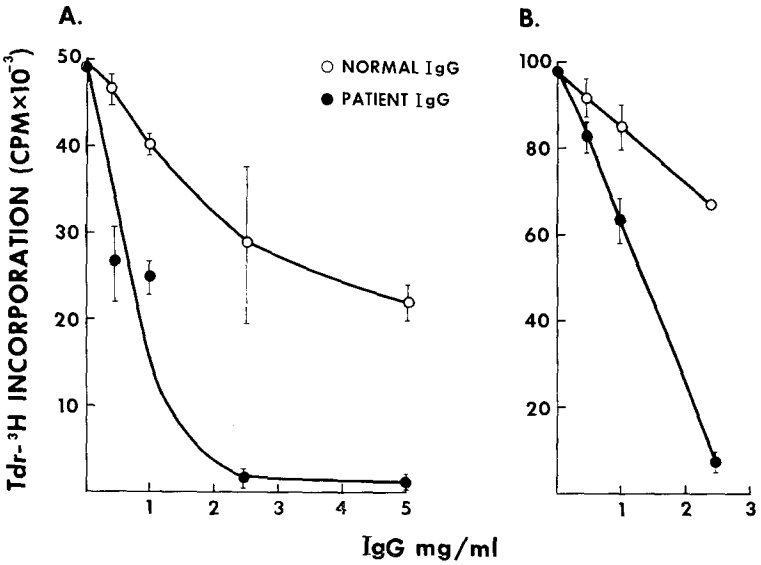

FIG. 5. The effect of IgG on the proliferative response of normal lymphocytes to phytohemagglutinin. (*A*) Increasing concentrations of patient J.H. IgG and normal IgG were added to cultures containing W.J. cells. (*B*) Similar studies with W.H.B. mononuclear cells.

DISCUSSION

The most significant finding to emerge from the present experiments is that the expression from cell-mediate dimmunity may be broadly modified by IgG fractions of serum from patients with primary intracranial tumors. Initially we found impaired delayed hypersensitivity in patients with benign or malignant

tumors. Experiments were than designed to test cellular reactivity and humoral modifying factors separately.

In the first series of experiments, cellular reactivity to a variety of stimulants was tested in cultures containing a single source of normal human serum. Lymphocyte transformation to soluble antigens was decreased and this finding correlated with cutaneous anergy. Leukocytes from one-third of patients who could not be sensitized to DNCB (groups I and II) responded poorly to PHA, but all patients in group III responded normally to this mitogen. In contrast to the findings in studies with soluble antigens, the mixed lymphocyte cultures were normal when patients' mononuclear cells reacted to membrane antigens on allogeneic cells.

Secondly, we demonstrated sensitization of patients' lymphocytes to tumor-associated antigens in the mixed lymphocyte-tumor cell reaction. These findings are in agreement with those of Levy and coworkers, who used another method (19). In addition, the present studies indicated that lymphocytes from group I patients responded to tumor cells as well as did those from group III patients. Although the expression of delayed hypersensitivity was impaired, there was no intrinsic defect in the patients' lymphocytes, at least in ability to recognize and respond to membrane antigens.

The next series of experiments indicated that patients' sera consistently blocked cell-mediated tumor immunity. This specific blocking or "enhancing" activity occurs in animal and human neoplasms, and has been recently reviewed by the Hellströms (20). Of greater interest, we found that the IgG fraction of patients' sera suppressed the blastogenic response of both patients' and normal lymphocytes to PHA and to histocompatiblity antigens on allogeneic mononuclear cells. Further, quantitatively more MLC suppressor activity was found in anergic patients than in others with milder degrees of cutaneous hyporeactivity. These studies suggest that enhancing sera, in addition to specifically blocking tumor immunity, may broadly and nonspecifically suppress mononuclear cellular reactivity. A sufficient serum concentration of suppressor factor may thus eventually impair host immunocompetence.

The cell type inhibited by the humoral agent is not known. Macrophages are required for the activation of lymphocytes in vitro (21). Human blood contains macrophages (monocytes) and other macrophage precursors as well as lymphocytes (22). Humoral factors interfering with the function of either lymphocytes or macrophages may suppress the MLC. The possibility of dysfunction of macrophages is not excluded by these studies. A recent report, however, indicates that macrophages obtained from anergic persons permit purified lymphocytes from healthy persons to function normally (23). It is thus likely that the inhibitor in patients' sera directly suppresses lymphocyte activation.

Serum inhibitors of lymphocyte activation in cancer patients have been

described previously (7, 24, 25), but these have not been characterized. Alpha globulin has been found to inhibit lymphocyte activation (26). In one study, serum alpha globulin concentration correlated with depressed PHA reactivity in patients with cancer of the colon (27), but this finding was not confirmed (28). In our studies to date, we have found significant suppressor activity only in the first peak of DEAE cellulose chromatography where IgG was the only protein detectable.

Isolation of the suppressor factor in the IgG fraction of patients' sera suggests that the active material is an antibody. These molecules may directly inhibit lymphocyte function. Antibodies directed against light chain determinants of immunoglobulin (29) and against HL-A antigens suppress the activation of responder lymphocytes in MLC. Poor correlation was noted between the HL-A specificity of antibodies and the HL-A antigens leukocyte membrane in cultures in which suppression was observed (30, 31). Inhibitory antibodies may be directed to sites other than HL-A, such as antigens controlled by a postulated MLC locus (32, 33) or to other receptors on the lymphocyte.

Brain cells and lymphocytes may share certain membrane determinants. It is known that in mice the theta antigen of thymus-derived lymphocytes is shared by brain cells (34). If tolerance to brain is broken, isoantibodies elicited may cross-react with receptors on lymphocytes. The brain has been considered to be an immunologically privileged site (35). The development of a tumor may allow immunologically competent cells to penetrate the blood-brain barrier and incite an immune response. Certain studies on the termination of tolerance suggest an alternative possibility (36). When brain cells acquire tumor antigens, tolerance is broken and antibody is synthesized against normal membrane determinants as well as tumor antigens. Some of the antibodies elicited may cross-react with determinants on the lymphocyte membrane. The appearance of suppressor isoantibody may reflect a protective compensatory response to antigens that elicit a population of lymphocytes sensitized to host determinants. This class of antibody, then, may serve as a regulator of cell-mediated immunity.

If we assume that an antibody is the humoral suppressor, several modes of action should be considered. Certain antibodies are lymphocytotoxic in the presence of complement. In our studies lymphocytes incubated with patient IgG fractions and rabbit complement were not killed. In addition, suppressor activity was observed even after heat-inactivation of complement. Cytotoxicity is therefore unlikely. The possibility that inhibitory serum contains sufficient antibodies of such diverse specificity as to mask receptor sites on all stimulants is also unlikely. Consistent with many recent observations on the mechanism of immunologic enhancement, it is most suitable to consider a direct effect on the lymphocyte membrane (31, 37–39). Cross-reacting antibody molecules may suppress lymphocyte activation either by altering the lymphocyte surface or by directly blocking a membrane receptor site.

The present studies, then suggest that impaired delayed hypersensitivity in patients with intracranial tumors follows the development of the neoplasm instead of preceding it. This suggestion is supported by the absence of an intrinsic cellular defect and the rapid disappearance of the humoral suppressor after removal of the tumor. Other workers have reported that tumor-specific blocking activity disappeared shortly after extirpation (40). This observation supports the close association between the enhancing and immunosuppressive activities in serum. If, in fact, IgG suppressor antibodies regulate the level of cell-mediated immunity, it is likely that similar immunosuppressive activity will be found in the sera of patients with other neoplastic or inflammatory diseases who have depressed cellular immunity.

SUMMARY

Tumor immunity in patients with primary intracranial tumors was assessed in relation to the general status of host immunocompetence. Lymphocyte sensitization to tumor-specific membrane antigens was demonstrated by the proliferative response of lymphocytes in the presence of autochthonous tumor cells. Paradoxically, one-half of the patients could not be sensitized to a primary antigen, dinitrochlorobenzene; existing delayed hypersensitivity was also depressed, as measured by skin tests and lymphocyte transformation in response to common antigens.

A heat-stable factor in patients' sera blocked cell-mediated tumor immunity. In addition, these "enhancing" sera consistently suppressed the blastogenic response of autologous and homologous lymphocytes to phytohemagglutinin and to membrane antigens on allogeneic cells in the one-way mixed lymphocyte culture. When patients' leukocytes were washed and autologous plasma replaced with normal plasma, reactivity in the mixed lymphocyte culture increased to normal values.

In vitro immunosuppressive activity in patients' plasma or sera correlated with depressed delayed hypersensitivity. After removal of the tumor, suppressor activity disappeared. IgG fractions of patient sera contained strong immunosuppressive activity. These data suggest that the suppressor factor may be an isoantibody elicited by the tumor that also binds to receptors on the lymphocyte membrane. In addition to specifically blocking cell-mediated tumor immunity, enhancing sera may broadly depress host immunocompetence.

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