nal, but why the orthogonal edges are offset by transform faults. With one additional assumption, namely, that the transform faults have essentially zero strength to sliding motion, this also can be understood. On the basis of this assumption, the orthogonal boundary may be offset in many places and still preserve the symmetry of the system. Thus the cooling effects which tend to straighten curved sections, the unidirectional symmetry of the tensile stresses, and the lack of shear strength of the transform faults are sufficient conditions to explain why the observed pattern would be stable and in fact would evolve from an originally irregular configuration.

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8. The discovery of the ridge transform fault phenomenon in wax was stimulated by a seminar given by Dr. G. Thompson and by the fact that the family of one of us (J.N.B.) was making candles at home at the same time. Our initial attempts at pulling the freezing layer of candle wax apart resulted in the formation of a ridge transform fault system. Numerous other persons have contributed valuable suggestions both to the design of the final experiment and to the report, among them: T. Atwater, H. W. Menard, P. Molnar, H. Bradner, and B. Parker. We thank L. Ford and W. Walston for advice in photographic techniques; B. Winsett for creating the hand drawings; and R. L. Parker, H. W. Menard, and P. Molnar for critically reading this manuscript. We also thank Drs. A. Lachenbruch and G. Thompson for a preprint of their paper.

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Cytotoxic Antibody in Normal Human Serums Reactive with Tumor Cells from Acute Lymphocytic Leukemia

Abstract. *Serums showing complement-dependent cytotoxic reactions to acute lymphocytic leukemia cells were detected in three normal unimmunized subjects. These serums were reactive with tumor cells from 514 (514 tested) acute lymphocytic leukemia patients, and three (12 tested) patients with acute myelocytic leukemia; they did not react with tumor cells from patients with acute monocytic leukemia (two tested), with chronic lymphocytic leukemia (two tested) or with leukolymphosarcoma (two tested); nor did they react with normal lymphocytes from 52 different donors. These reactive serums appear to recognize antigens primarily associated with acute lymphocytic leukemia.*

Herberman and Fahey reported finding cytotoxic human antibodies reactive to cultured lymphoid cells, but their studies indicate that the antigen being detected on the lymphoid cell lines is not tumor specific (*1*). However, these antigens may be virus induced inasmuch as Diehl *et al.* (*2*) were unable to initiate cultures of lymphoid cells unless Epstein-Barr virus was present. Mann *et al.* (*3*) produced rabbit antiserum to a purified cell membrane component from a tissue culture cell line of Burkitt's lymphoma, which was cytotoxic to tumor cells derived from patients with both acute lymphocytic and acute myelocytic leukemia. The antiserum was not cytotoxic to peripheral cells of normal individuals but was cytotoxic to peripheral cells of 5 of 41 relatives of leukemia patients. This serum was thought to be detecting antigen or antigens associated with acute leukemia. We have now found in three normal unimmunized individuals complement-dependent cytotoxic serums that appear to be relatively specific for acute lymphoblastic leukemia cells.

In our laboratory, two-way lymphocytotoxicity cross matches are routinely performed between leukemia patients and their parents and sibs; that is, the lymphocytes of each person are cross matched for cytotoxicity with the serum of each of the other persons. The negative serum control (N.N.) was occasionally reactive against leukemic cells. The serum of the father (C.B.) of one of our patients was cytotoxic to the leukemia cells of his son (C.B., Jr.) who had typical acute lymphoblastic leukemia with a peripheral leukocyte count of 80,000 mm^3 of which 90 to 95 percent were blast cells. Before any other therapy was applied this patient demonstrated a precipitous decrease in leukocyte count to 5000 mm^3 after receiving a blood transfusion from an unrelated male donor (M.W.). The individuals C.B., M.W., and N.N. gave no history of prior transfusions, and N.N., a 23-year-old unmarried female, had never been pregnant.

Cytotoxicity was measured by incubating 2 μl of each undiluted serum sample with 1 μl of cell suspension (10^6 cell/ml) for 30 minutes at room temperature in the Falcon microtest tray. Excess antibody was removed by washing (*4*). The cells were then incubated for 1 hour at room temperature with 4 μl of rabbit complement. Trypan blue was added, and reactivity was determined by dye exclusion. A reaction was considered positive if a minimum of 20 percent of the cells were killed. These serums were toxic to 20 to 50 percent of the cells.

The cells of all members of this family were not killed (negative) with the serums of C.B., N.N., and M.W., except for the leukemic cells of C.B., Jr., which were killed at a rate of 50, 50, and 30 percent, respectively. These negative reactions included two HL-A identical sibs. Peripheral lymphocytes obtained from C.B., Jr., while he was in clinical remission after chemotherapy were also negative in the cytotoxicity test, as were peripheral lymphocytes of a normal control transformed by phytohemagglutinin in culture. The cytotoxic reactions were all complement dependent. Normal human serums as well as rabbit serums were effective complement sources. The titers of the cytotoxic serums, expressed as the reciprocal of the last dilution resulting in a positive reaction, were 32 for N.N. and 16 for both C.B. and M.W. All three serums showed slightly increased reactivity when diluted one-eighth. Whether this is a prozone or anticomplement activity is unclear.

Absorption with the previously frozen leukemic cells of C.B., Jr., removed the cytotoxic activity of all three serums. The serums were absorbed by mixing equal volumes (approximately 0.1 ml) of packed cells and undiluted serum, incubating for 30 minutes at room temperature, and centrifuging at 4°C. The undiluted absorbed serums were then tested against the leukemic lymphocytes of C.B., Jr., which had been frozen in liquid nitrogen. The negative control was serum from an unimmunized AB (blood type) donor. The positive controls were the unabsorbed test serums.

A quantity of morphologically normal cells from C.B., Jr., sufficient to absorb the serum of the father, C.B., was obtained very early in remission. These cells greatly attenuated the cytotoxic activity of the serum, but gave a

negative reaction in the cytotoxic test with all three serums. The serums of N.N. and M.W. were absorbed 3 months later with this patient's remission lymphocytes but, contrary to the results obtained with the father's serum, the cytotoxic activity was not removed by absorption. It was not possible to absorb the father's serum on the same date because the quantity of cells obtained was insufficient.

All three serums were absorbed with lymphocytes from the mother and one of the HL-A identical sibs of the patient. None of these removed the cytotoxic activity. The patient's leukemic cells were tested with the serums after having been incubated with his own serum for 30 minutes. This absorption did not alter the activity of the serums on the cells, suggesting little or no blocking factor in the patient's own serum.

The serum of C.B. was fractionated on a Sephadex G-150 column (2.5 cm by 90 cm) in a buffer consisting of 0.05*M* tris, 0.002*M* EDTA, and 0.1*M* NaCl buffer at *p*H 8.0. The course of fractionation was followed by measuring the optical density at 280 nm, and the peak eluting at a volume corresponding to the IgM immunoglobulins (approximately 900,000 molecular weight) was pooled separately from the peak corresponding to the IgG and IgA immunoglobulins (about 150,000 molecular weight). The immunoglobulin fractions, the albumin fraction, and a nonfractionated sample were dialyzed against deionized water and were concentrated by lyophilization. They were reconstituted in barbital buffer to half the original volume. The 7*S* (IgG and IgA) fraction of the serum of C.B. had no cytotoxic activity, but the 19*S* (IgM) fraction and the dialyzed, lyophilized whole serum control gave the same reactivity as the starting material. The other two serums have not been fractionated.

Serum N.N. was tested with cells of six acute lymphocytic leukemia patients and C.B. and M.W. serums were tested with five. All gave positive cytotoxicity reactions. All three serums were positive with the white cells of one patient with acute myelocytic leukemia; equivocal (+/−) results were obtained with two others. They were all negative with cells from nine other patients with acute myelocytic leukemia. They were also negative with tumor cells from two patients with acute monocytic leukemia, from two patients with leukolymphosarcoma, and from two patients with chronic lymphocytic leukemia. Peripheral lymphocytes from one patient with Hodgkin's disease, one with aplastic anemia, one with lung carcinoma, and one with ovarian carcinoma were negative. No reactivity was noted when the serums were tested against peripheral lymphocytes from four patients with acute lymphocytic leukemia patients in remission, in addition to C.B., Jr. Peripheral tumor cells from one of these patients while in relapse were killed by the three serums. Our standard testing procedure described above was used for all tests.

The foregoing observations suggest a tumor-specific antigenic determinant on the leukemic cell surface. This antigen is unrelated to HL-A inasmuch as the cells of sibs that were identical to the leukemic patient with respect to HL-A were unreactive in the cytotoxic test and were also incapable of removing the cytotoxic activity by absorption.

Table 1. "Extra" reactions with HL-A typing serums. Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia; AMMoL, acute myelomonocytic leukemia; ASCL, acute stem cell leukemia; APGL, acute progranulocytic leukemia.

Patient	Diagnosis	Antiserum	Specificity
C.B., Jr.	ALL	Suskie	HL-A2,12
		Tuckerman	HL-A2+
		Madison	HL-A13
		Shiley	HL-A1,W15
		JCa	Ao78
D.B.	ALL	Tuckerman	HL-A2+
B.D.	Aplastic	SA	HL-A5,W5
D.Co.	ASCL	BC	HL-A3,11
T.P.	APGL	JAC	Multi
		JOC	Multi
H.H.	AMMoL	Melnikoff	HL-A7,W22
		Cutten	HL-A7
G.I.	AML	D.W.	Multi
		Chayra	HL-A8
		SA	HL-A5,W5
R.A.	ALL	Chayra	HL-A8
		JAC	Multi
		BM	Multi
		Jones	HL-A9
D.Ca.	AML	DMI	HL-A9,W5
		DJ	HL-A1+
W.E.	AML	Te415	Multi
N.L.	Hodgkins	JAC	Multi
N.S.	Hodgkins	BC	HL-A3,11
		JAC	Multi
N.R.	AMMoL	JOC	Multi
		Storm	HL-A3+
A.W.	AML	Tuckerman	HL-A2+
		Cremen	Multi
		SA	HL-A5,W5
		SD	HL-A12+

Histocompatibility antigens other than HL-A are not responsible for the observations because the cells of the patient's mother are also cytotoxic negative and absorption negative with all three serums. Negative reactions obtained with normal lymphocytes transformed by phytohemagglutinin suggest that the response is not specific for blast cells.

The presence of antibodies to leukemia cells may not be a rare phenomenon. During our investigation of N.N., C.B., and M.W., we found four other serums which may react similarly. Three of these were from blood donors whose serums appeared responsible for a decrease in leukocyte count in other patients with acute lymphocytic leukemia. The fourth was from a fraternal twin of an acute lymphocytic leukemia patient whose serum was cytotoxic for his brother's tumor cells. These serums have not been tested as extensively as those of N.N., C.B., and M.W.; to date, they have all reacted to the cells of only one other leukemic patient (acute myelocytic), which were also reactive with N.N., C.B., and M.W. The second group of serums were negative with cells from one patient with di Guglielmo's disease, one with acute monocytic leukemia, two with myelocytic leukemia, one with myelomonocytic leukemia, and 22 normal subjects.

Another indication that the phenomenon might be common are observations of "extra" HL-A reactions of leukemic cells (*5*). In our study, 14 of 64 patients had "extra" reactions with typing serums not found in parents or other sibs (Table 1), which may be directed at tumor-specific antigens rather than against HL-A antigens known to be reactive with the "misfit" serums.

Absorption studies indicate that the cytotoxic activity can be removed by incubation with leukemic cells, but not with the remission leukocytes. The cytotoxicity of serum C.B. was attenuated by his son's cells early in remission. It is possible that the early remission cells retained a sufficient number of antibody binding sites to reduce the serum's reactivity but an insufficient number to be killed in the cytotoxic test. On the other hand, the later remission cells, which failed to remove cytotoxic activity from N.N. and M.W., may have been more "normal" with respect to antigenic determinants on their cell membranes.

Klein (*6*) reported in 1966 that cells transformed by oncogenic viruses de-

velop specific antigens at the cell surface and, further, that the antigens induced by a given virus in different hosts are similar or identical. Tompkins *et al.* (*7*) using serums from rabbits with regressed fibroma-induced tumors were able by immunofluorescence to detect a surface antigen on cells infected with fibroma virus. This antibody was specific for fibroma and unreactive with cells infected with other viruses. Their studies showed that the appearance of the antigen was dependent on synthesis of viral DNA. The nonreactivity of C.B., Jr.'s, remission cells in this study could be an indication that viral nucleic acid was not being synthesized.

The hybridization experiments of Spiegelman *et al.* (*8*), Kufe *et al.* (*9*), and Hehlmann *et al.* (*10*) demonstrated sequence homologies between RNA's from various human tumors (including leukemia) and DNA synthesized from mouse RNA tumor viruses and reverse transcriptase. Their experiments showed that a particular tumor contains RNA homologous to the animal virus from the same type of tumor but not to other oncogenic RNA viruses.

A further suggestion of viral etiology is the report of Gallo *et al.* (*11*) of the presence of RNA-dependent DNA polymerase in the lymphoblasts of three patients with acute lymphocytic leukemia. This enzyme, analogous to that of RNA tumor viruses, was not found in the lymphocytes of 48 normal subjects.

Of interest also is the report of Failkow *et al.* (*12*) of the recurrence of leukemia in a female patient who had received a marrow graft from her brother. In this case, the leukemic cells possessed the male karyotype of the donor.

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Herpesvirus hominis: Isolation from Human Trigeminal Ganglion

Abstract. Herpesvirus hominis *was isolated from the trigeminal ganglion obtained at autopsy from 1 of 22 patients with no clinical evidence of active herpetic disease, and from one patient with malignant lymphoma who died with herpes zoster on the abdomen, pulmonary cytomegalic inclusion disease, and possible oral herpes simplex. Virus was isolated by cocultivation of explants of ganglion with monolayers of Vero green monkey kidney cells and required 3 weeks of culture before viral cytopathic effects were evident. These observations support the concept that latent infection of sensory ganglia may be the source of virus in recurrent herpetic disease in man.*

Recurrent infections with *Herpesvirus hominis* (herpes simplex virus, HSV) in man are thought by some investigators to result from virus released from latently infected sensory ganglia (*1*, *2*). Although inflammatory changes were described in the trigeminal ganglia in association with active facial herpetic disease (*3*, *4*), several attempts to isolate virus from the ganglia were unsuccessful (*4*, *5*), and virus particles were not identified in ganglia by electron microscopy (*2*). However, Stevens and his associates provided evidence for latency in sensory ganglia. They showed that HSV can induce latent infection in spinal ganglia of mice (*6*) and in the trigeminal ganglia of rabbits (*7*). Although virus could not be recovered from samples of mouse or rabbit ganglia ground in a homogenizer, it could be isolated from supernatant fluids of organ cultures of the ganglia. Virus could also be isolated by cocultivating explants of mouse ganglia with monolayers of rabbit kidney cells (*6*). In order to determine whether HSV can produce a similar latent infection in man, we attempted to isolate virus from the trigeminal ganglia obtained at autopsy of 23 humans. We placed explants of the ganglia directly on monolayers of cells that are susceptible to lytic infection by HSV and observed these cultures for cytopathic effects. To determine whether viruses might be latent in the human choroid plexus, we obtained samples of choroid plexus and

Table 1. Viral isolation studies from human trigeminal ganglia. Positive isolates were obtained after 3 weeks in culture with Vero cell monolayers.

Patient			Diagnosis	Virus isolation	Serum antibody to HSV
No.	Age (years)	Sex			
1	72	M	Cirrhosis, nutritional	−	
2	70	F	Multiple sclerosis	−	
3	54	M	Histiocytic medullary reticulosis	−	
4	65	M	Trauma	−	
5	43	F	Atrial septal defect	−	
6	17	M	Primary pulmonary hypertension	−	
7	18	F	Acute myelocytic leukemia	−	
8	24	M	Acute myelocytic leukemia	−	
9	29	M	Chronic myelocytic leukemia	−	
10	45	M	Astrocytoma	−	
11	51	F	Adenocarcinoma, breast	−	
12	47	F	Acute myelocytic leukemia	−	+
13	57	M	Saphenous vein bypass	−	
14	60	F	Rheumatic heart disease	−	+
15	72	M	Brain tumor	−	
16	59	F	Ovarian carcinoma	−	+
17	56	M	Cirrhosis, nutritional	+	
18	16	M	Ependymoblastoma	−	
19	11	M	Cystic fibrosis	−	−
20	27	M	Hodgkin's disease	−	+
21	23	F	Malignant lymphoma, histiocytic	−	
22	70	F	Parkinson's disease	−	
23	54	F	Malignant lymphoma, unclassified	+	