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Extraction of Distinctive Antigens from Neoplastic Tissue

Abstract. Antigens have been obtained from HeLa and J111 cells by treatment with fluorocarbon. Cross absorption studies revealed that the antigen from HeLa cells had no serologic relationship to normal human uterus. Antibody for HeLa extract coated red blood cells was found in 25 percent of the sera from patients with malignant diseases.

Taylor *et al.* (*1*) and DeCarvalho (*2*) reported that antigens were obtained from both fresh tumor tissues and tumor cell lines of human and chicken origin by treatment of extracts with fluorocarbon. These antigens were specific for the tumor of origin when assayed by complement-fixation and gel-diffusion against immune rabbit sera. Neither chick muscle, primary monkey kidney cells, nor primary human amnion cells contained similar antigenic material.

This report describes the finding of antibody to HeLa cell extracts treated with Genetron, in the sera of human beings with malignant metastatic disease.

HeLa cells and J111 cells grown in medium 199 supplemented with 10 to 15 percent normal calf serum were used as the source of antigenic material. Uterine tissue was obtained from a normal human uterus freshly removed. These tissues were homogenized at 4°C for 5 minutes at high speed in a Vir-Tis 45 homogenizer in distilled water to yield 20-percent suspensions. The aqueous extracts were centrifuged at 3000*g* at 4°C for 10 minutes and the pellets were discarded. These extracts were designated as crude antigens (C). Rabbits were immunized with a total of 6 ml given in six intravenous injections.

One-half volume of Genetron 113 was added to each of the crude extracts and mixed for 1 minute at 4°C at high speed in a Vir-Tis 45 homogenizer. Each Genetron-treated extract was centrifuged at 3000*g* at 4°C for 10 minutes and the supernatant was saved. This process was repeated with the supernatant fluids for a total of five extractions. This procedure apparently removes nonspecific protein, leaving a specific antigen in the aqueous phase. Only the HeLa and J111 extracts contained material which was precipitable with trichloroacetic acid. Rabbits were given a series of six intravenous injections of the extracts, equal to a total of 250 μg of protein nitrogen. Other rabbits were injected with a like volume of the final extract of normal uterus even though no protein was present. These extracts were designated as Genetron antigens (G) (*3*).

The diluent for the complement-fixation test was 0.85 percent saline. The hemolytic system was composed of 0.3 ml of complement containing two full units and 0.6 ml of 1 percent optimally sensitized sheep red cells. Optimal dilutions of antigens were determined by box titrations, and sera were titrated with these optimal dilutions. Titers are expressed as the reciprocal of the highest dilution of serum showing 100-percent fixation.

Table 1 shows the magnitude of cross reactivity among the crude antigens (C) and their antisera (C) as determined by complement-fixation. The cross reactions were virtually eliminated by extraction with Genetron. It is noteworthy that all antigenicity was lost from uterus (G antigens and antisera). These data are in agreement with the findings of Coriell *et al.* that common antigens are present in long-term tissue-culture cell lines (*4*). The presence of common antigens was also observed in the crude uterus extract. While the results given in Table 1 are confined to data obtained by using a normal human uterus, similar results were obtained with normal human skin, muscle, and liver.

Table 1. Specificity of cell extracts obtained by Genetron treatment. Symbols: C, crude antigens and their antisera; G, Genetron antigens and their antisera.

Antigen	Complement-fixation titers with antisera					
	HeLa(C)	J111(C)	Uterus(C)	HeLa(G)	J111(G)	Uterus(G)
HeLa(C)	320	80	80	640	<5	<5
J111(C)	40	80	40	<5	80	<5
Uterus(C)	40	80	320	<5	<5	<5
HeLa(G)	320	10	<5	1280	10	<5
J111(G)	<5	320	<5	<5	640	<5
Uterus(G)	<5	<5	<5	<5	<5	<5

Table 2. Specificity of antisera as determined by absorption.

Antigen used for immunization	Absorption of antiserum	Antiserum titer*	
		Uterus (C)	HeLa (G)
Uterus(C)	None	320	<5
Uterus(C)	Uterus(C)	<5	<5
Uterus(C)	HeLa(C)	10	<5
Uterus(C)	HeLa(G)	320	<5
HeLa(G)	None	<5	640
HeLa(G)	HeLa(G)	<5	10
HeLa(G)	HeLa(C)	<5	40
HeLa(G)	Uterus(C)	<5	640

*Determined by complement-fixation.

Antisera to the crude uterus extract and to the Genetron HeLa extract were absorbed as indicated in Table 2. Since the Genetron-treated HeLa extract was not particulate, it was added to each antiserum in equal volumes, incubated at 4°C overnight, and centrifuged at 30,000*g* for 30 minutes. Each absorbed serum was titered against an optimal dilution of each antigen used for immunization. It is evident that an antigen is present in HeLa cells which is serologically distinct from antigens in normal uterus.

Using the tanned red cell hemagglutination technique as modified by McKenna (*5*), we sensitized red cells with the product obtained by Genetron treatment of HeLa cells, or with normal human uterus treated in the same way. A total of 366 human sera have been assayed by this technique (*6*). Of the 193 sera from patients with various malignancies, 49 (25 percent) showed evidence of antibody capable of reacting with the HeLa extract, and nine (5 percent) other sera reacted with the J111 extract. None of the sera from patients with malignancy reacted with the uterine material and none of the remaining 173 sera from patients without malignancy reacted with extracts of HeLa, J111, or uterus. Of 49 patients

whose sera showed evidence of antibody to HeLa extract, 75 percent were from patients with known metastatic disease. The titers observed (1:10 to 1:80) were of the same order as those found by Aizawa and Southam (*7*) following implantation of viable cell lines derived from tumor tissue.

A total of 13 freshly excised human tumors of various origin and six adult normal human tissues including one each of liver, spleen, kidney, brain, skin, and muscle have been treated with Genetron as described. Protein material remained in all of the extracts from tumor but not in those from normal tissue. These proteins were serologically distinct from normal tissue, but were serologically interrelated.

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Possible Transpacific Contact on the Coast of Ecuador

Abstract. The earliest pottery-producing culture on the coast of Ecuador, the Valdivia culture, shows many striking similarities in decoration and vessel shape to pottery of eastern Asia. In Japan, resemblances are closest to the Middle Jomon period. Both early Valdivia and Middle Jomon are dated between 2000 and 3000 B.C. A transpacific contact from Asia to Ecuador during this time is postulated.

The earliest phase of the Valdivia culture, discovered on the coast of Ecuador in 1956, is dated by carbon-14 at 4450 ± 200 years ago (U.S. Geological Survey sample No. W-631), making it one of the earliest dated occurrences of pottery in the New World (*1*). The technical and artistic level of Valdivia pottery is too high, however, for it to represent a local invention of pottery making. An explanation for this anachronism has been suggested by new material recently recovered from large-scale excavations in the deepest portion of the Valdivia site.

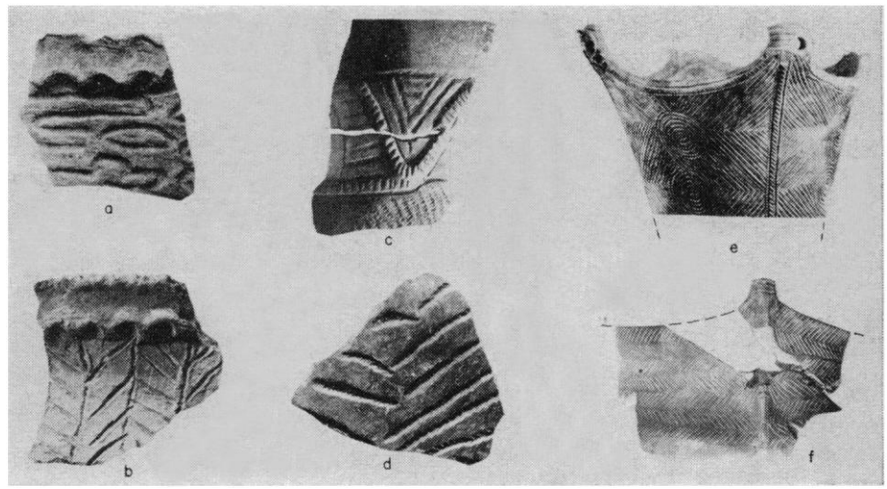

Fig. 1. Examples of resemblances between Jomon (*a, c, e*) and early Valdivia (*b, d, f*) pottery in form and decoration. *a–b*, Folded-over and finger-pressed rim; *c–d*, "braid" impression; *e–f*, castellations on rim and zig-zag arrangement of incised lines. Provenience: *a*, Iwasake type, Kyushu, Middle Jomon (*2*); *c*, Katuzaka type, Honshu, Middle Jomon (*3*); *e*, Horinouchi shell-mound, Chiba, Late Jomon (*4*); *b, d, f*, Valdivia site, Guayas Province, Ecuador.

The basic Valdivia cultural complex is part of an early shellfish-gathering subsistence pattern represented by sites along the Pacific coast of the New World from California to Chile. Shell fishhooks and crudely chipped stone tools are characteristic artifacts. Pottery making, and perhaps other cultural traits, was introduced into this horizon on the coast of Ecuador, and characteristics of vessel shape and decoration suggest that the introduction came from Asia across the Pacific Ocean.

Preliminary comparative analysis shows the following features shared by early Valdivia pottery and that of the Middle to Late Jomon period of Japan, also dated between about 3000 and 2000 B.C.: folded-over rims with finger-pressed edge (Fig. 1, *a–b*); "braid" impression (Fig. 1, *c–d*); castellated rims (Fig. 1, *e–f*); zoned punctation; incised lines embellished with nicks; shell stamping in rows; small rectanguloid areas with a central punctate; crude anthropomorphic faces on rim exterior of open bowls; finger-made grooves; incisions in zig-zag, crosshatch, and zoned parallel line patterns; undulating rims bordered by an incised line on the exterior; alternating incised lines and rows of punctates; small trianguloid excised areas incorporated into incised designs; ornamental unsmoothed coils; three parallel incised lines partly obliterated by later surface smoothing along the rim interior; red slipped surfaces; and small tetrapod supports.

Nonceramic traits found in early Valdivia culture include stone mortars, shell bracelets, and small stone figurines. Except for the figurines, these also occur in Middle Jomon, but it cannot yet be determined whether such similarities are traceable to the generalized ancestral shellfish-gathering complex from which both Valdivia and Jomon are derived, or to direct contact.

A transpacific introduction rather than a land route is postulated on several grounds: (i) the absence of any similar pottery complex on the Pacific coast of Central and North America, the expected route of a migrant people living on shellfish; (ii) the closeness of the similarities, which imply a direct and firsthand contact; and (iii) the location of Ecuador with respect to two major ocean currents. One is the Equatorial Counter Current, flowing from the Caroline Islands eastward just north of the equator; the other is the Japan or Black Current flowing from Japan toward the British Columbia coast, where it divides into the Alaska and California currents.

The latter current flows southward along the coast of Mexico and Cen-