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Spontaneous Transformation of Human Brain Cells Grown in vitro and Description of Associated Virus Particles

Abstract. *A human brain cell culture grown in vitro has spontaneously transformed, as determined by morphology, growth characteristics, and karyotype analysis. Virus particles morphologically akin to oncogenic RNA viruses are present in the transformed cells, which are now in subculture 60.*

In our studies of the subacute spongiform viral encephalopathies, numerous human and primate brain tissues have been grown in vitro (*1, 2*). In this report we present data on the spontaneous transformation of one of the human brain cultures and a description of the morphological characteristics of the virus particles observed in this cell culture.

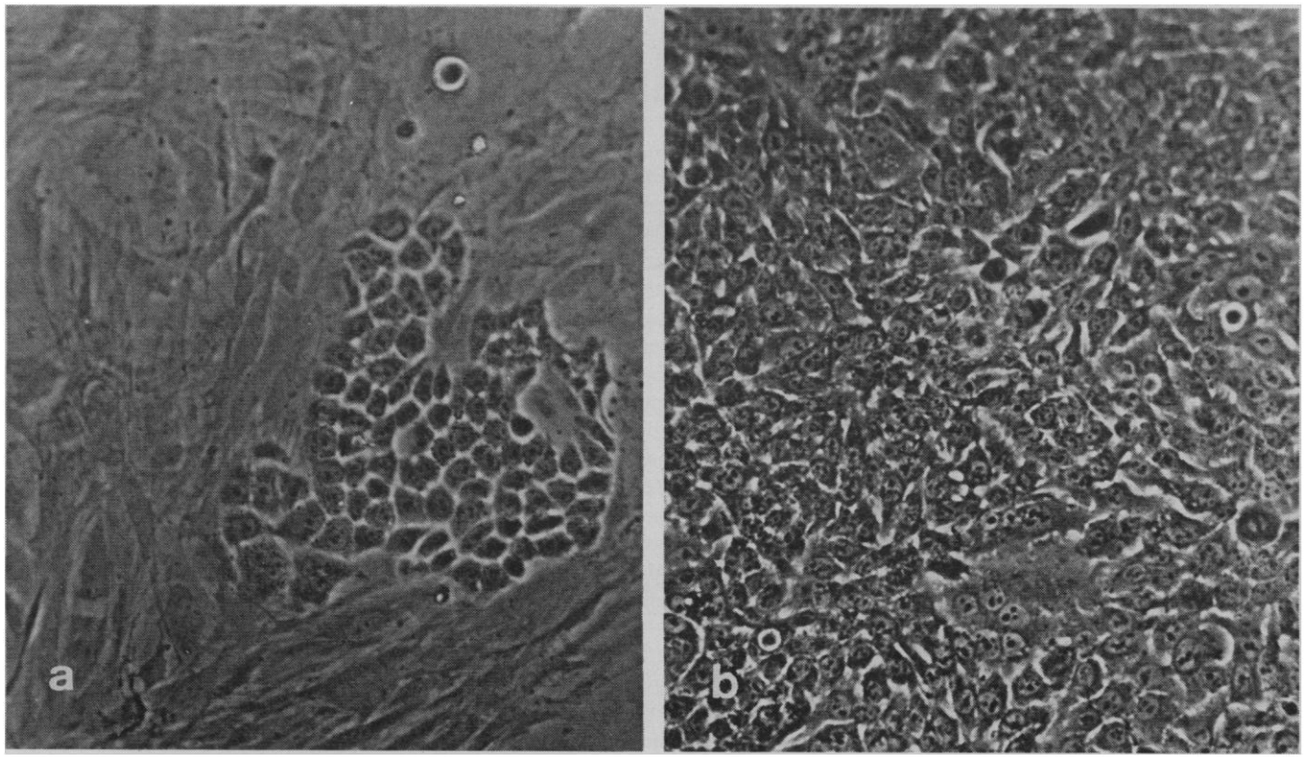
A specimen of human brain was obtained at biopsy from a patient (S.G.) with Creutzfeldt-Jakob disease; the cell line that was derived from this tissue is termed SG. The tissue was placed in Eagle's minimal essential medium supplemented with penicillin, 100 unit/ml; streptomycin, 100 μg/ml; and 20 percent fetal bovine serum inactivated at 56°C for 60 minutes. The tissue was cut into small pieces and divided into two samples. The first sample was explanted into Falcon flasks according to the technique described by Rogers *et al.* (*2*). The second sample was washed with medium not containing bovine serum, treated with 0.025 percent trypsin, and seeded into Falcon flasks. Both kinds of cultures were incubated at 35°C in atmosphere containing 5 percent CO_2 and observed and fed at approximately 4-day intervals. When the cell sheets were about three-fourths confluent, the cells were treated with a mixture of 0.05 percent trypsin and 0.02 percent ethylenediaminetetraacetic acid and subcultured into new flasks.

The primary cultures prepared by both methods initially grew out as monolayers of fibroblast-like cells. After 60 days, foci of cells with altered morphology (Fig. 1a) were noted in subculture 3 of the cultures that had been treated originally with trypsin. During the next 30 days foci of similarly changed cells appeared in subcultures from all flasks of the culture treated originally with trypsin, and in subcultures from two of the three flasks of primary explants. By subculture 5 the transformed cells were the only cell types observed in all cultures (Fig. 1b). (Subcultures derived from the one untransformed flask of primary explant and from transformed cultures at various subculture levels had earlier been frozen and stored in liquid nitrogen.) The transformed cells appeared initially in small focal areas of altered morphology. These cells were larger than the original fibroblast-like cells and were epithelial in appearance; they rapidly overgrew the untransformed cell sheet. They displayed the characteristics of transformed cells; loss of contact inhibition was shown by continued growth of confluent cultures, which caused overlapping and piling. Subcultures derived from the lines prepared originally with or without trypsin were maintained as two cell lines until subculture 10, then the subcultures from both lines were pooled.

The transformed SG cell line has now been maintained for 14 months and is in subculture 60. The culture was diluted 1 : 3 for subcultures 1 to 5, 1 : 4 for subcultures 6 to 35, and 1 : 5 for subcultures 36 to 60. The cells now show a rapid rate of growth with enormously increased capacity to persist in serial subcultures; subculture 60 is a 10^{38} dilution of the primary cultures. The cells have a 60 percent plating efficiency, and as few as ten cells are needed to initiate cell growth on a surface area of 25 cm^2.

Chromosome studies on subcultures 47 and 54 (*3*) indicate that the cells are of human origin and contain a chromosome number ranging from 70 to 80. The majority of cells contain 75 chromosomes; one is a large accrocentric marker with a long arm longer than that in the No. 13 to 15 group, and one or two chromosomes are minutes. The incidence of chromosomal aberrations is less than 1 percent.

Virus particles morphologically resembling the known oncogenic RNA viruses were detected in all cultures examined since the transformation occurred. At various subculture levels, SG cells were removed from the flasks with rubber policemen and sedimented at 600*g* for 10 minutes. The cell pellets were fixed in 2 percent glutaraldehyde, post-fixed in Dalton's chrome osmium, dehydrated in alcohol, and embedded in Epon. The cells were stained with lead citrate and examined in a Siemens electron microscope. Virus particles were not observed when the brain culture prepared with trypsin was harvested after 40 days of incubation and before morphological changes had occurred. Virus particles were found in all of the brain cultures examined after morphological changes—that is, in subculture 4 of the explant culture, subcultures 4 and 6 of the culture prepared with trypsin, subcultures 14, 26, and 36 of the pooled culture. Three morphological forms of virus particles were observed in the transformed SG brain cultures (Fig. 2). Intracellular particles about 60 to 90 nm in diameter were observed only within the cytoplasm and contained an electron-transparent center, giving a doughnut-shaped appearance (Fig. 3a). Particles that were budding from the plasma membrane (Fig. 3b) were also seen; the particles appeared to acquire an outer envelope in this process. Finally, extracellular virus particles were observed; these were oval or spherical, about 100 to 120 nm in diameter (Fig. 3c). Most of the extracellular virus particles contained a partially dense nucleoid. The virus forms observed appear to represent one type of particle that shows dif-



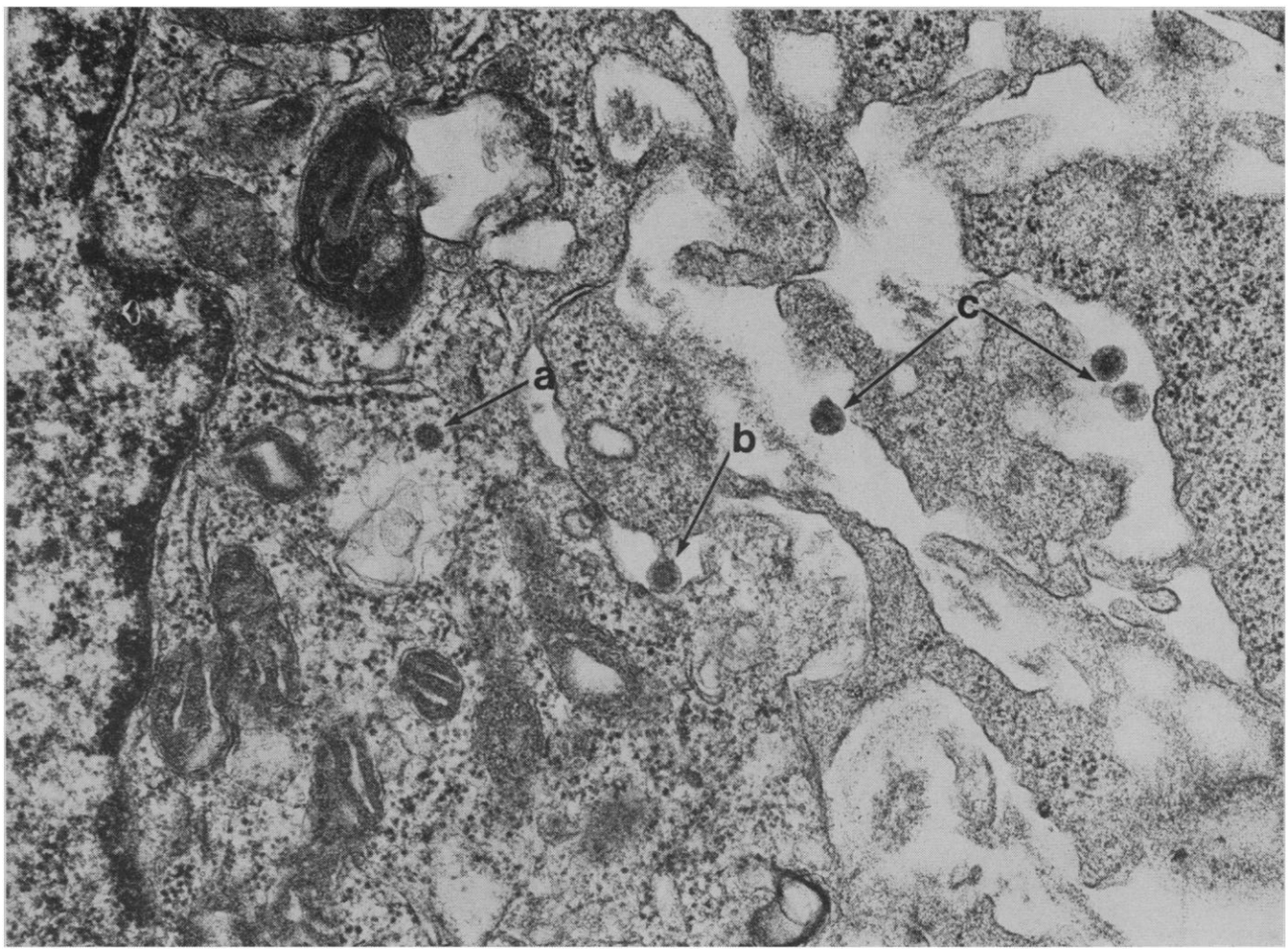


Fig. 1 (top). SG brain cell cultures, showing (a) foci of cells with altered morphology and (b) transformed brain cells at subculture 10 (×130). Fig. 2 (bottom). Electron micrograph of the SG transformed cells with three forms of virus particles: (*a*) intracytoplasmic, (*b*) budding, and (*c*) extracellular (× 42,000).

ferent stages of development in the cytoplasm, in the budding process, and in extracellular spaces. Although the virus particles morphologically resemble the oncogenic RNA viruses, they do not appear to fulfill the criteria for classification as true B or C type viruses but more closely resemble the Mason-Pfizer monkey virus and visna and foamy viruses (*4*).

Attempts were made to pass this virus by inoculation of live transformed SG cells and cell-free fluids into the following cell lines: human embryonic kidney (HEK), Vero, African green monkey kidney, BHK-21, WI-38, normal squirrel monkey brain, normal chimpanzee brain, human foreskin (MA-184), human skin and muscle (MA-337), and human embryonic brain cells (MA-332). In no instance did we observe a cytopathic effect or detect virus particles by electron microscopic examination of these inoculated cell lines. Further, SG transformed cells failed to induce a cytopathic effect in WI-38 or primary African green monkey kidney cells after cocultivation or fusion with inactivated Sendai virus. Supernatant fluids from cell cultures do not hemagglutinate red blood cells from guinea pig or chicken, nor has hemadsorption been observed with these erythrocytes. Because the detected virus particles morphologically resemble the oncogenic RNA viruses, cell suspensions were screened for gS 3 interspecies determinants (present in the cat, mouse, rat, hamster, gibbon ape, and woolly monkey) by a radioimmune precipitation assay, and were found to be negative (*5*). The cells also do not fluoresce with antiserums prepared against SV40 and polyoma T antigens.

The SG brain cells grown in vitro have altered cell morphology, chromosome abnormalities, loss of contact inhibition, and a high plating efficiency, and show no diminution of growth by subculture 60. Associated with these growth changes is the presence of virus particles morphologically resembling the oncogenic RNA viruses. A number of facts suggest that the spontaneous transformation and associated virus particles are not due to an outside source of contamination. The transformed cultures are of human origin, as determined by chromosome analysis and complement fixation reactions of human brain antigens with hyperimmune rabbit antiserum prepared against the transformed SG cell line. When the transformation occurred, our laboratory had no cell lines that could have been sources of contamination, such as other transformed cell lines or cell lines that contained similar virus particles. Six other brain cultures from patients with Creutzfeldt-Jakob disease were grown in vitro at the same time as the SG brain cultures. These six cell lines remain morphologically unaltered, and similar virus particles have not been observed in the two lines examined by electron microscopy.

We demonstrated earlier that the infectious agent that elicits Creutzfeldt-Jakob disease in primates is able to persist for long periods of time in cell cultures derived from the brains of patients with this disease; these infectious cell cultures are not transformed (*1*). Until the effects of inoculating subhuman primates with frozen SG brain biopsy suspensions, SG brain cells grown in vitro, and purified virus are known, no conclusions can be drawn about the etiological relation between Creutzfeldt-Jakob disease and the virus observed in SG transformed cells.

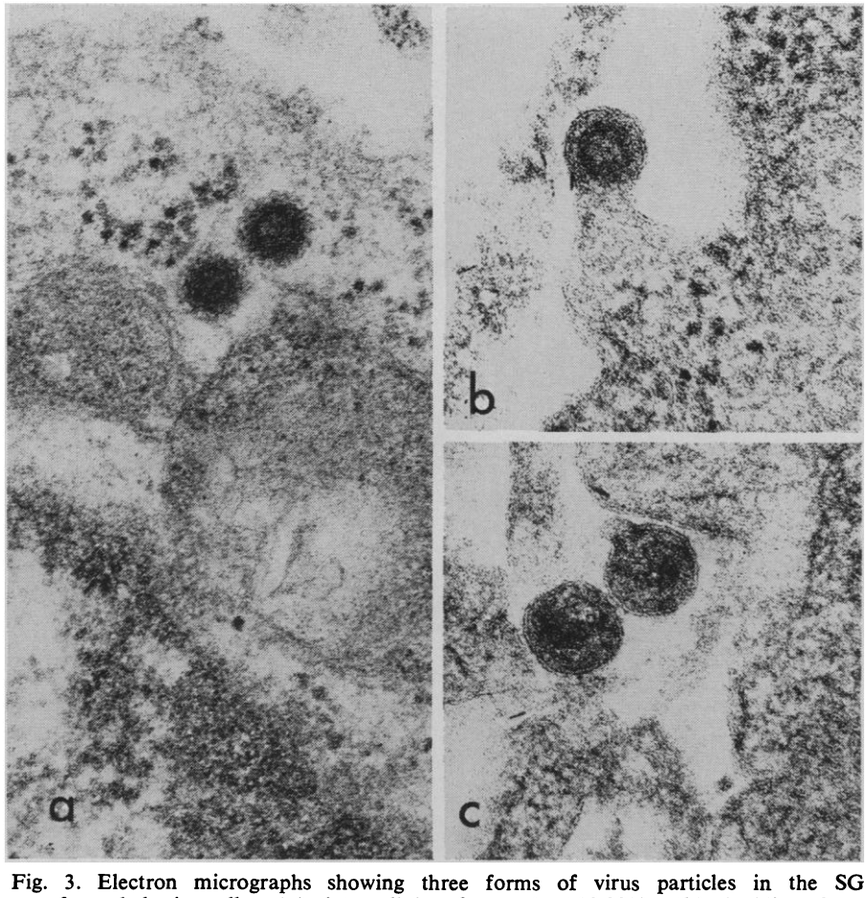


Fig. 3. Electron micrographs showing three forms of virus particles in the SG transformed brain cells: (a) intracellular forms (× 118,000), (b) budding form (×115,000), and (c) extracellular form (×115,000).

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5. The assay for gS 3 antigens was done by W. Parks of the National Cancer Institute.
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