Reports

Common Antigens in Tissue Culture Cell Lines

Gey (*1*) has pointed out that pure lines of cancer cells and of normal cells from the same species can be grown in test tubes in identical media free from tissue juices and multiple cell types present in fresh tissue. In such a controlled environment, one would expect the peculiarities of the cancer cell to become readily apparent. While many differences have been described, critical analysis shows that none are unique to the cancer cell (*2*). This is hard to explain since cancer cells can cause death when they are injected into a suitably prepared animal, while fresh normal cells so injected promptly disappear (*3*). Apparently the animal can readily distinguish between normal and cancer cells.

We wish to report the results of antigenic analysis of various tissue-culture cell lines by means of the complement-fixation test. In these studies, experimental animals were injected with pure cultures of normal or malignant cells grown in tissue culture, and differences in the immunologic response were sought. The results of these tests indicated extensive sharing of common antigens by long-term tissue-culture cell lines even between cells from different species, and this is the subject of this report (*4*).

Cell lines were grown in synthetic medium 199 (*5*) to which 10 or 20 percent horse serum was added and were injected into 4- to 6-lb white New Zealand rabbits. Immune sera from these rabbits were reacted with cell antigens grown in medium 199 containing serum from a different species. A modified Kolmer technique was used with overnight fixation, two exact units of complement titrated in the presence of antigen, and two optimal units of antigen as determined by block titration with homologous antiserum.

As is shown in Table 1, similar antigens were found in many long-term tissue-culture cells. Antisera prepared against HeLa (human epidermoid carcinoma of the cervix), human embryo intestine (derived from normal embryo tissue), and cynomolgus monkey heart (derived from normal adult monkey heart) gave almost identical complement-fixation titers with all three antigens.

These same sera either did not react or gave titers eightfold less than against the homologous antigen when they were tested with antigens prepared from first-generation monkey kidney tissue culture or fresh monkey heart as shown in Table 1. Control antigens showed that the cross reactions were not due to traces of foreign serum or to the tissue-culture medium. No agglutinins for sheep red blood cells were found in the antisera. Furthermore, Forssmann antigen was ruled out as the common antigen by adsorption of the immune sera with sheep red blood cells. Adsorption of sera with group A, Rh-positive human cells reduced the titer but did not remove the common complement-fixing antibodies.

Additional complement-fixation studies indicate that other long-term tissue-culture cell lines share common antigens with the three mentioned above. These include Hep-2, a human epithelial carcinoma of the larynx; C-3, a cell line derived by Salk from a benign fibromyoma of man: and ERK, embryo rabbit kidney established in tissue culture by Westwood. Two additional long-term tissue-culture lines, human kidney (Chang) and human conjunctiva (Chang), are less closely related to this group of long-term tissue-culture cells. On the other hand, additional fresh primary tissue-culture cells reacted poorly with antisera against the long-term tissue-culture cells. These include tissue-culture cells from rabbit kidney and human heart, and rabbit kidney suspension. In this group also fall fresh human sympathicoblastoma suspension and the long-term tissue-culture mouse L cell (Earle).

The significance of these observations is given some support by the fact that three separate investigators have observed similar antigenic crossings between HeLa cells and one or more long-term tissue-culture lines from other species (*6*). Interpretation of our findings must be guarded because the antigenic crossings observed were with sera of homologous high titer against a long-term cell line, while antisera against fresh tissue-culture cells were of homologous lower titer. Repeated injection of fresh primary tissue-culture cells into several rabbits has not produced a single high-titer antiserum, in contrast to the ease with which high-titer antisera were produced by injection of long-term cell lines.

Tissues taken directly from the animal, or first-generation tissue-culture cells, do not contain in high concentration the common antigens found in long-term tissue-culture cells. This suggests that the common antigens have been acquired, accentuated, or changed in avidity during long-term cultivation in tissue culture. The fact that one fresh human cancer suspension (sympathicoblastoma) does not contain the common antigen suggests that it is not associated with malignancy per se, and the fact that Earle's L cell does not contain it suggests that it is possible to maintain long-term tissue cultures without their acquiring common antigenic components.

Several possible theories can be suggested to explain how long-term tissue cultures might acquire common antigens: (i) contamination of all antigenically related cell lines by a common tissue culture cell, for example, HeLa cells; (ii) loss of species specificity by the cell or increase of a component essential for the free living state upon prolonged multiplication in vitro; (iii) contamination with a common virus or bacterium; or (iv) possibly, errors in the test, or in its interpretation.

1) Contamination of all the antigenically related tissue cultures with a common tissue-culture cell is a possible explanation. Past experience in bacteriology and virology has demonstrated many instances in which accidental contamination of laboratory stocks has occurred, and this can obviously occur with tissue cultures. However, there is evidence that this is not the case since the antigenically related cell lines differ from each other in chromosome number and pattern and in tumor formation in treated rats (*3*). It is also possible that the cell lines are all contaminated with a common cell from the serum used in the culture medium. This too is unlikely for the reasons listed. It could be eliminated as a possible source of error in future studies by heat inactivation of all sera used in the feeding of tissue cultures.

2) Reversion of cell lines to a common primitive cell which has lost part of its species specificity through long

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Table 1. Complement-fixation titer of rabbit antisera with various tissue-culture cell antigens. TC, tissue culture.

Antigen* and dilution	TC passage	Antiserum†				
		HeLa	Human embryo intestine	Cynomolgus monkey heart	Rhesus monkey	
					Kidney	Heart
		200	37	63	1	0
HeLa (1:8)	6	128‡	512	1024	0	4
Human embryo intestine (1:4)	36	64	512	1024	16	32
Cynomolgus monkey heart (1:8)	73	128	512	1024	0	8
Rhesus monkey kidney (Und.)	1	16	8	32	32	4
Rhesus monkey heart (1:16)	0	4	0	8	0	32
		Controls				
Tissue culture medium and antibiotics		0	0	0	0	0
Horse serum (1:64)		0	0	0	0	0
Calf serum (1:4 to 1:1024)		0	0	0	0	0
Human serum (1:1024)		4	0	4	0	0
Monkey serum (1:1024)		0	0	0	4	4

* All cells for CF antigens were grown in calf serum and medium 199 and were standardized to contain 2.5×10^6 washed cells per milliliter and diluted as indicated to obtain two units of antigen in the dilution showing greatest complement fixation with its homologous antiserum as shown by a block titration.
† All reactions were carried out in the presence of two exact units of complement titered in the presence of antigen. Cells for preparation of antisera were grown in horse serum.
‡ Endpoints read from highest dilution tubes showing 3 + or 4 + fixation.

growth in tissue culture is an intriguing concept which would readily explain the observed partial or complete antigenic cross reactions as well as the morphologic similarities of long-term tissue-culture cells and possibly their relationship to malignant cells. That is, if long-term tissue-culture cells from various species gradually acquire a preponderance of a common antigen or antigens as they adapt to independent growth in vitro as well as the property of unrestrained growth in vivo when transplanted back to cortisone- and x-ray-treated rats (*7*), then a new laboratory experimental approach to study of malignancy may be at hand.

This last hypothesis can be approached in two ways: (i) by ruling out all other causes of cross antigenicity and (ii) by starting new cell lines from normal tissue under rigidly controlled conditions to prevent contamination and observing whether these new lines also revert to the common antigenic cell. Such studies are now under way.

3) The third and most likely explanation for common antigenicity of long-term tissue-culture lines derived originally from different animal species is contamination with a common virus or bacterium. To escape detection, such a contaminant would have to be resistant to the antibiotics in the culture medium and should not cause obvious turbidity of the medium, should fail to grow in routine bacteriological culture media, and should cause no extensive cytopathogenic changes in the tissue-culture cells. We have searched for bacterial and viral contaminants by the usual methods, but it is not possible to rule out the presence of an unknown noncytopathogenic virus or bacterium by means of negative tests. A slow-growing diphtheroid-like organism in one of our tissue-culture lines did not share common antigens with the long-term cell lines. Pleuropneumonia-like organisms (PPLO) have been reported in tissue-culture cell lines by Robinson, Wichelhousen, and Raizman (*8*) and are present in our HeLa cell lines. Extensive search by Morton and Rothblat (*9*) has revealed PPLO in several but not all long-term cell lines maintained in this laboratory and in cell lines from other laboratories. CF tests with the PPLO antigen, T5, isolated from our HeLa culture and antisera against the long-term tissue-culture cells were all positive. Similar tests with a PPLO antigen prepared from a laboratory strain of human genital origin, O7, were all negative. These tests suggested that the contaminating PPLO may be one common antigen shared by some long-term cell lines. However, adsorption of the long-term tissue culture antisera with PPLO-T5 did not remove the common antibody for long-term tissue-culture antigens. This, combined with the fact that some of the cell lines did not contain PPLO, suggests that these contaminating organisms (PPLO) are not the explanation of the common antigens in long-term cell lines. However, cell lines may contain different antigenic types of PPLO, and this has not been investigated.

These studies raise more questions than they answer, but two points seem to be fairly well established: (i) The acquisition of common antigens by long-term tissue-culture lines occurs frequently and crosses species lines. The explanation for this is not clear although several possibilities are offered. (ii) Modern tissue-culture media plus antibiotics provide an ideal medium for PPLO, viruses, and some fastidious bacteria and their presence may not be revealed by routine bacteriological sterility tests (*10*).

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References and Notes

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Phosphorescence in Liquid Scintillation Counting of Proteins

Alcoholic solutions of the quaternary base Hyamine have been reported by Vaughan *et al.* (*1*) as being suitable solvents for amino acids and proteins in liquid scintillation counting systems. While investigating this Hyamine system for the counting of proteins and tissues, we encountered, when working with larger weights of such substances, a phosphorescence phenomenon, which, if it is not eliminated, can cause considerable trouble in the counting of samples of this magnitude. Fortunately methods are available whereby such interference can be eliminated or circumvented.

The substances used were dried rat bile, rat liver, and horse serum; whole rat muscle; egg albumin, gelatin, Bactopeptone, and trypsin. As much as 100 mg, and in some cases more, of these substances can be dissolved in 3.0 ml of