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Detection of Cancer by Culturing Cancerous Tissue *In Plastico*

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In a previous report, it was felt that the rat tumor cell line, T-333, was a mixture of heterogeneous cells with different characteristics with respect to karyotype, tumorigenicity, and response to Rous Sarcoma virus (RSV) infection. These characteristics of heterogeneous cell subpopulations could be selected by use of different culture substrates.

In this experiment, diversity of the cells in response to complement mediated cytolysis employing syngeneic rat anti-sera was studied. More than 50% of the glass grown cells were lysed while only 19% of the plastic grown cells were lysed by the specific immune sera of syngeneic rats. This finding suggests that growth in plastic culture wares selects cells with resistance to complement mediated cytolysis.

It seems likely that the previously reported enhanced tumorigenicity of plastic grown T-333 cells is due to clonal selection of cell subpopulations which can better tolerate at least one arm of the *in vivo* immune surveillance system.

Key Words: *in vitro, in plastico,* ploidy, microcytotoxicity, heterogeneity, tumorigenicity, immunogenicity.

The cellular heterogeneity of tumors has been known since the last century when histologic studies first identified cytologic differences among cells within the same tumor. Since then, the use of various methods to study neoplastic cells *in vivo* and *in vitro* has revealed significant heterogeneity in the phenotypic characteristics of tumors from the same host. This includes differences in metastatic behavior (Fidler, 1977, Fidler, 1978, Poste, and Fidler 1980, Lotan. and Nicolson 1979, Tsuruo. *et al.*, 1981), karyotype (Ohno 1971, Mitelman 1972, Becker, 1973, Nowell 1976, Lee 1977, Dexter, *et al.*, 1978, Wai-Kwan *et al.*, 1982), susceptibility to anticancer therapy (Baranco. *et al.*, 1972, 1973, Hakanssen *et al.*, 1974 Heppner, *et al.*, 1978, Homo 1980, Suruo *et al.*, 1981, Wai-Kwan, 1982), antigenicity, immunogenicity, growth behavior *in vitro* (for general overview, Ponten, 1973), suceptibility to viral infection (Lee, 1977), and biochemical properties. More recently, studies done by Lee (1976) revealed that different subpopulations of T-333 cells could be selected by use of glass and plastic substrates. T-333, a spontaneously trans-

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formed rat cell line which has both hypo- and hyperploid cells, grew well on plastic but lost hypoploid cells and gained in tumorigenicity. Analysis of the chromosomes of T-333 cell lines grown on plastic, glass and immediately after culture of tumors produced by cell inoculation showed that one of the marker chromosomes was present only in the hyperploid cells which were the major population in plastic ware. This chromosome was paired in glass cultures, but was single in the highly tumorigenic plastic grown cells and *in vivo* tumor. Thus it appeared that the enhanced tumorigenicity of the plastic grown cells was due to selection of a cell subpopulation identifiable by its chromosome constitution.

In the present study, responsiveness to complement mediated cytoxicity of the cells selected by the above methods was studied.

MATERIALS AND METHODS

1. Animals and cell line

Philadelphia albino rats (PAG rats Gey strain) which were extensively inbred and syngeneic to the selected cells were used in this study. The cell line was T-333, a cell line established in 1938 by explanting a bit of normal subcutaneous areolar tissue from PAG rats. The cells have been cultured in glass tubes for routine passage.

2. Antisera preparation

The cells grown in glass ware (*in vitro*) were used as the source of antigen. The cells harvested from the glass culture flasks were washed three times with primary growth media by centrifugation at 150g for 30 minutes at room temperature. The number of cells were adjusted to be 10^7 cells per 0.5 ml of phosphate buffered saline (PBS). The cells were then mixed with an

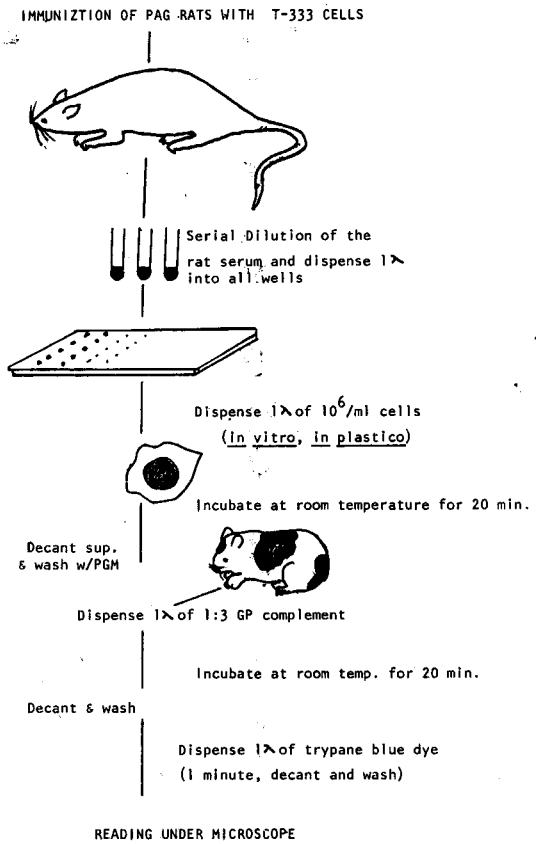

Fig. 1. Summarized Protocol of Microcytotoxicity Test.

equal volume of Complete Freund Adjuvant (CFA). This mixture was injected into two sites of the animals, i.e., 0.1ml into the foot pads and 0.2ml intramusculary in hind legs. The booster immunizations were done one month after the primary immunization by injecting 2.85×10^7 cells/0.5ml of PBS and CFA. The animals received a second booster injection of 5.84×10^6 cells by the same method one month following the first booster injection.

The immunized animals were bled by cardiac puncture one month after the last booster injection. The sera was absorbed into guinea pig liver in refrigerator overnight.

Guinea pig sera were freshly prepared and absorbed into normal PAG rat liver in the same way as above to provide complement.

3. Target cell preparation

T-333 cells were selectively prepared by culture in glass culture flasks (*in vitro*) or in plastic flasks (*in plastico*) according to the methods of previous studies (Lee, 1976).

The culture wares were Corning glass and Falcon plastics supplied by Corning and Microbiological Associates in U.S.A., respecitvely. These cells were gently trypsinized (0.05%, GIBCO, U.S.A.) to harvest and adjust the number of cells.

Cell preparations with more than 95% of viable cells were used as the source of target cells for the tests. The cells were maintained in medium McCoy'5A (Microbiological Associates, U.S.A.) containing 20% Fetal Bovine Serum (FBS, M.A., U.S.A.).

4. Microcytoxicity assay

For these assays, microcytotoxicity plastes for HLA studies (Linbro, Flow, Lab., U.S.A) were used. One lambda of diluted antisera was dispended into each well of the test plastes. One lambda of the target cells (10^6 cell/ml) was added to the wells, followed by incubation at room temperature for 15 munutes. Following this incubation, the fluids were decanted by flicking and the wells were washed with fresh medium. One lambda of GP sera (1:3 solution) was then added and incubated at 37'C in a humidified 5% CO_2 incubator for 20 minutes. Finally, following decanting the fluid in the wells, one lambda of trypan blue dye solution was dispensed for one minute at room temperature. The cytotoxicity was scored by examining the plates under 100x light microscope.

RESULTS AND DISCUSSION

1. Antigenic property of T-333 cells cultured *in vitro*.

Antigen has the ability to elicit host immune responses, which including both the humoral and cellular specific immune reactions. It has been reported that tumor cells are mixtures of heterogenous cell subpopulations, some of which can elicit strong immunologic responses while others cannot (Prehn 1970, Pimm, *et al.*, 1977). This variability to elicit an *in vivo* immune response could be determined by the genetic variability of the cells or by epigenetic factors involved the *in vivo* immunologic interaction (Hsu et al., 1958, Chow *et al.*, 1980). T-333 cells have been reported to by heterogeneous with respect to karyotype, a clear evidence for genotypic variability. Therefore, it might be supposed that part of the cell population is not antigenic or at best has weak antigenicity. It has also been reported that only the hyperploid cells are tumorigenic and that their growth is selectively enhanced by plastic culture wares. Thus, it might be assumed that the subpopulation of the cells are not equally responsible for immunogenicity.

The cells belong to hypoploid subpopulation might have genetically determined increased antigenicity and susceptibility to lysis. However, this remains to be studied.

2. Response of T-333 cells *in vitro* and *in plastic* to immune cytolysis.

It was cleraly demonstrated that the cells grown *in vitro* were lysed more extensively than

Table 1. Susceptibility of target cells to complement mediated cytotoxicity

Target \ Dilution	0	−1	−2	−3	−4
G cell	56	61	45	50	39
P cell	16	13	20	18	17

G cell : *cells grown in vitro*
P cell : *cells grown in plastico*

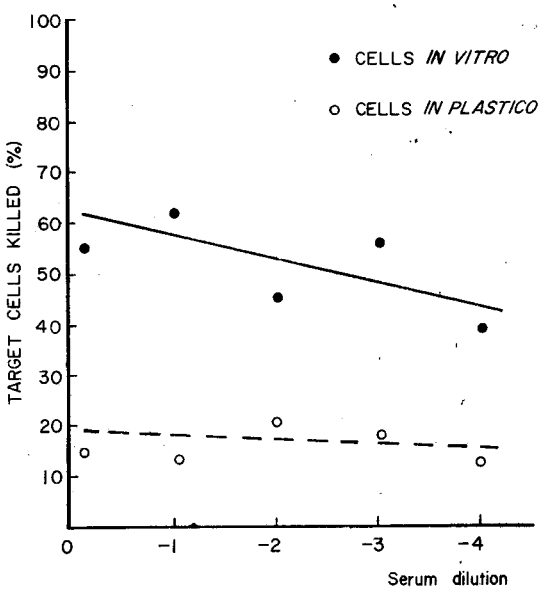

Fig. 2. Susceptibility of the Target Cells to Complement Mediated Cytotoxicity.

the cells grown *in plastico* by specific antisera. More than 50% of the *in vitro* adapted cells were destroyed by complement mediated cytotoxicity while only 18% of the *in plastico* adapted cells were lysed. Furthermore, the results revealed that T-333 cells are heterogeneous in response to complement mediated cytotoxicity. This diversity could be sharply segregated by culturing those cells in different culture wares; glass and plastic.

Cul.ivation of T-333 cells *in plastico* could select a subpopublation with resistance to lysis. *In plastico* adapted cells have been reported to be highly tumorigenic and to have a hypoploid chromosome constitution. These facts taken together support the possibility of *in vivo* immunologic "sneaking through" or a "dilution escape" mechanism (Mengerson *et al.*, 1975) of the hyperploid cells, the presence of which was demonstrated by karyologic analysis of tumor cells (Lee, 1977). The maximum cytolysis of *in vitro* cells was 50%. It might be supposed that this phenomena is due to lysis of the hypoploid cell subpopulation which constitutes about 50% of the total cell population *in vitro*.

The lysis of *in plastico* cells was not only low but also demonstrated a minimal result in the dose response relationship.

The direct relationship between the karyologic characteristics of the cells and their response to immune cytolysis remains for further analysis.

CONCLUSION

The heterogeneity of T-333 cells with respect to karyotype, tumorigenicity, and response to Rous Sarcoma virus was further analysed to determine their heterogeneity in response to complenent mediated cytotoxicity *in vitro*.

The cells grown *in plastico* were more resistant to immunologic lysis. This phenomenon is in agreement with the previously reported fact that plastic grown cells have increased tumorigenicity and hyperploidy.

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