LBL-11868 c.2
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CHANGES IN THE ELECTRICAL SURFACE CHARGE AND TRANSPLANTATION PROPERTIES OF TA3 ASCITES TUMOR CELLS DURING SHORT-TERM MAINTENANCE IN AN ISOTONIC SALT SOLUTION

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December 1980

Prepared for the U.S. Department of Energy under Contract W-7405-ENG-48

DISCLAIMER

LBL-11868

CHANGES IN THE ELECTRICAL SURFACE CHARGE AND TRANSPLANTATION PROPERTIES OF TA3 ASCITES TUMOR CELLS DURING SHORT-TERM MAINTENANCE IN AN ISOTONIC SALT SOLUTION*

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December, 1980

* Research support was received from United States Department of Energy Contract No. W-7405-ENG-48 with the Lawrence Berkeley Laboratory.

SUMMARY

TA3 ascites tumor cells maintained *in vitro* as a dilute suspension in 0.9% NaCl solution (physiological saline) were found to undergo time-dependent degenerative processes leading to alterations in both membrane characteristics and tumor transplantation properties. A 30% decrease in the negative cellular surface charge density occurred within 2 hr. when TA3 cells were incubated in a 0.9% NaCl solution at 23 °C. A similar reduction in negative surface charge density occurred within 0.5 hr. when the medium was maintained at 37 °C. This time-dependent reduction in surface charge was prevented when cellular metabolism was blocked either by maintaining the medium at 4 °C, or by adding 1 mM cyanide ion to a 23 °C medium.

TA3 cells incubated as a dilute suspension in 0.9% NaCl solution at 23 °C also exhibited a large, time-dependent reduction in proliferative capacity in isogeneic LAF_1/J hosts, as indicated by an increase in the tumor dose for 50% mortality (TD50). Lowering the temperature of the medium to 4 °C was observed to slow the onset of the degenerative processes that lead to a decreased transplantability of TA3 cells. The modification in growth properties of TA3 cells maintained *in vitro* was found to be attributable in part to an alteration in tumor histocompatibility. This effect was demonstrated by comparing the tumor growth kinetics and TD50 values in normal hosts versus hosts that had been immunosuppressed by whole-body irradiation.

Following the *in vitro* maintenance of TA3 cells, nigrosin dye exclusion tests were performed as a means of assessing cell viability. Evidence obtained in this series of experiments indicated that vital staining is an inadequate criterion for judging either the extent of cell membrane damage or the loss of cellular proliferative capacity.

INTRODUCTION

Several experimental procedures with tumors require *in vitro* maintenance of the cells under conditions that represent only an approximation to the physiological milieu. Examples of such procedures include the preparation of inocula for tumor transplantation and immunological studies on tumors in a cell culture system. Another example is the *in vitro* - *in vivo* cytotoxicity test in which the proliferative capacity of tumor cells is studied *in vivo* following incubation with a chemotherapeutic agent *in vitro*. As a means of characterizing several of the degenerative processes that can occur when tumor cells are maintained *in vitro* under relatively nonphysiological conditions, we have examined the influence of a 0.9% NaCl suspending medium on the surface charge and transplantation properties of TA3 ascites adenocarcinoma cells.

MATERIALS AND METHODS

Suppliers. Female adult (12-to 16-week-old) LAF_1/J mice from The Jackson Laboratories (Bar Harbor, Maine) were used in all experiments. Sterile saline (0.9% NaCl solution) was obtained from Cutter Laboratories (Berkeley, California), Medium 199 from Microbiological Associates (Bethesda, Maryland), phosphate-buffered saline from Gibco (Grand Island, New York), ^{131}I-labeled human serum albumin from Mallinkrodt (St. Louis, Missouri), nigrosin dye from Metheson, Coleman and Bell (Norwood, Ohio), V. cholerae neuraminidase (500 units/ml, where 1 unit of activity is defined as the amount of enzyme that releases 1 μg of N-acetylneuraminic acid from human α_1- glycoprotein in 15 min. at 37 °C) from Calbiochem-Behring Corporation (LaJolla, California), and crystalline N-acetylneuraminic acid from Sigma (St. Louis, Missouri).

Tumor Lines and Transplantation Procedures. Two TA3 ascites tumor sublines were propagated in isogeneic LAF_1/J mice by the weekly i.p. injection of 10^5 cells in a 0.1 ml volume of 0.9% NaCl solution at 4 °C: (1) the near-diploid TA3-Ha subline, which originated in the laboratory of Dr. T. Hauschka (1), was supplied by Dr. B. Sanford (Department of Pathology, Harvard Medical School); (2) the hypotetraploid TA3-L subline, which arose during serial transplantation of TA3-Ha tumors, was provided by Dr. G. LaPage (Cancer Research Unit, University of Alberta). Both TA3-Ha and TA3-L cells had 12 hr. doubling times during exponential growth in LAF_1/J mice.

TD50 Measurements. Tumor doses for a TD50 determination were prepared by serial twofold dilutions starting from a stock cell suspension with a concentration equal to the highest dose level. Sterile saline (0.9% NaCl solution) was used as the diluent. In all cases, the highest dose level was chosen to produce 100% mortality, and a total of 6 to 8 graded dose levels were used for a TD50 determination. Each tumor dose in a 0.1 ml volume was injected i.p. into 10 recipient mice. The TD50 value was calculated by probit analysis from the percent mortality observed at the various dose levels over an 8-week period following tumor inoculation. As a test of reproducibility, TD50 determinations were performed twice.

Tumor Growth Kinetics. The kinetics of ascites tumor growth were measured by determining the total number of TA3 cells in the peritoneal cavity as a function of time after tumor cell inoculation. The total population of TA3 cells was determined from the product of peritoneal volume and tumor cell concentration. Volume was determined by the isotope dilution technique using ^{131}I-labeled human serum albumin dissolved in 0.9% NaCl solution. Cell concentrations were measured with an electronic counter (Counter Electronics, Hialeah, Florida). Each point of a tumor cell growth curve was determined from the average population of TA3 cells in the peritoneal cavities of 10 mice.

Immunosuppression of Tumor Hosts. LAF_1/J mice were immunosuppressed by administering a 500 rad whole-body dose of ^{60}Co radiation two days prior to inoculation with ascites tumor cells. Irradiation was performed with a 1400 Ci ^{60}Co source at an exposure dose rate of 20 R/min. A factor of 0.96 was used to convert the exposure dose rate in R/min to the absorbed dose rate in rad/min.

Vital Staining. The membrane integrity of TA3 cells was assessed by their ability to exclude nigrosin dye. Cells were examined in a hemocytometer immediately following the addition of 0.5% (wt/vol) nigrosin to the suspending medium. The percentage of cells excluding nigrosin was based on an observation of the number of stained cells in a field containing 200 to 300 total cells. The superior staining characteristics of this vital dye with ascites tumor cells have been discussed by Kaltenbach et al. (2).

Microelectrophoresis. Microelectrophoretic measurements were made with a Zeiss Cytopherometer (Carl Zeiss, New York) equipped with a $Zn/ZnSO_4$ electrode assembly (3). The rectangular microelectrophoresis chamber (700 μm thick) was immersed in a circulating water bath maintained at 4.0 ± 0.1 °C, 23 ± 0.1 °C or 37.0 ± 0.1 °C. The cellular electrophoretic mobility was determined from a series of velocity measurements on individual cells at each of the two stationary levels within the microelectrophoresis chamber. All measurements were made with the polarity of the applied field in alternate directions, thereby cancelling out any effect of mechanical fluid drift on the net electrophoretic velocity. Mobility measurements were made on TA3 cells suspended in a 0.9% NaCl solution at a temperature identical to that of the in vitro holding medium (4, 23 or 37 °C). The pH of the cell suspension was adjusted to 7.0 ± 0.1 by the dropwise addition of an isotonic (0.15 N) aqueous solution of NaOH or HCl.

The surface charge density (σ) of TA3 cells was calculated from the measured electrophoretic mobility (μ) using an approximation of the Gouy-Chapman equation (4): $\sigma = \mu \cdot \eta \cdot \kappa$. In this equation, η is the solution viscosity and κ is the Debye-Hückel constant. The reciprocal of κ is defined as

the Debye length, and is a measure of the thickness of the ionic double layer associated with fixed charges at the membrane surface (5). In the present experiments, the ionic strength of the electrophoresis solution was 0.15 and the associated Debye length was approximately 8 Å. The above equation relating σ and μ provides an accurate estimate of the surface charge density for intact cells suspended in a univalent salt solution at physiological ionic strength (4).

Neuraminidase Treatment and Sialic Acid Measurements. Removal of sialic acid residues was carried out by incubating TA3 ascites cells in phosphate-buffered saline (pH 7) containing 100 units/ml of V. cholerae neuraminidase. Prior to incubation with the enzyme, the cells were centrifugally washed four times (300 x g, 5 min.) in 15 volumes of 0.9% NaCl solution. Erythrocytes were removed by aspiration from the top of the cell pellet after each wash. The washed TA3 cells were incubated at a concentration of 2×10^7 cells/ml in the 100 units/ml neuraminidase solution for 30 min. at 37 °C, after which the cells were pelleted by centrifugation and the supernatent solution was carefully removed and stored at 4 °C for a later assay of sialic acid content. The enzyme-treated cells were washed once and their electrophoretic mobility was then measured by procedures described above. Control cells were treated with neuraminidase that had been inactivated by heating at 100 °C for 10 min. (6).

The amount of sialic acid removed from TA3 cells by neuraminidase treatment was assayed by the thiobarbituric acid method of Warren (7). The supernatent solution from neuraminidase-treated cells was heated to 70-80 °C for 10 min. to flocculate the enzyme and any residual cells.

The solution was then clarified by two serial centrifugations at 1100 x g for 5 min., following which the colorimetric assay for sialic acid was carried out. Appropriate controls and blanks were processed in an identical manner. Crystalline N-acetylneuraminic acid dissolved in distilled water was used to construct a standard curve for the determination of sialic acid concentrations.

RESULTS

Cellular Surface Charge Density. A time-dependent decrease in the negative surface charge density of TA3 cells occurred during maintenance *in vitro* at 23 °C or 37 °C as a dilute 1% (vol/vol) suspension in 0.9% NaCl solution. As shown for hypotetraploid TA3-L cells in Fig. 1 and for near-diploid TA3-Ha cells in Fig. 2, the initial rate of decrease in the surface charge density was both rapid and temperature-dependent. Within 2 hr. at 23 °C and 0.5 hr. at 37 °C, the cellular surface charge densities of both TA3 sublines achieved a stable level that was approximately 30% lower than the initial value. Longer periods of incubation at either temperature did not lead to a further reduction in surface charge density. The initial rate of reduction in surface charge density was observed to be greater for TA3-Ha cells than for TA3-L cells. This difference in rates was quantitated by fitting the initial slope of the surface charge density *vs.* time curve to a single exponential. At 23 °C, the initial slope was characterized by a $t_{\frac{1}{2}}$ of 2.7 hr. for TA3-L cells and 1.6 hr. for TA3-Ha cells. At 37 °C, the $t_{\frac{1}{2}}$ values were 1.2 and 0.6 hr. for TA3-L and TA3-Ha cells, respectively.

Maintaining the temperature of the TA3 cell suspension at 4 °C was found to prevent the time-dependent decrease in surface charge density that occurred at the higher incubation temperatures. In addition, a gradual increase in charge density of approximately 15% for TA3-L cells and 10% for TA3-Ha cells occurred during a 4 to 5 hr. incubation at 4 °C. The increase in the density of charge was not attributable to a shrinkage of the cell surface area during a prolonged incubation at 4 °C, since microscopic measurements demonstrated that the mean diameter of TA3-L

cells did not change from its initial value of 14 μm. The possibility was also considered that the time-dependent increase in charge density at 4 °C may result from a gradual release of ascites fluid proteins that were absorbed onto the cell membrane surface *in vivo*. An attempt was therefore made to accelerate the desorption of surface-bound materials by subjecting TA3-L cells to four centrifugal washes (300 x g, 5 min.) in 15 volumes of 0.9% NaCl solution at 4 °C. The cells were then resuspended (1% vol/vol) in 0.9% NaCl solution at 4 °C for measurements of the surface charge density as a function of time. As shown in Fig. 3, the washed cells exhibited a constant, i.e. time-independent, surface charge density that was initially 15% greater than that of the unwashed cells. As also shown in Fig. 3, TA3-L cells that were subjected to an identical washing procedure at 23 °C did not exhibit a significant difference in their surface charge density *vs.* time characteristics relative to unwashed cells. These findings suggest that a slow desorption of ascites fluid proteins from the surface of TA3-L cells occurs during incubation in 0.9% NaCl solution at 4 °C, and that the rate of this desorptive process is significantly greater at an incubation temperature of 23 °C.

The similarity of the surface charge density *vs.* time profiles for washed and unwashed TA3-L cells at 23 °C also suggests that the time-dependent decrease in charge density observed at this temperature is not attributable to the gradual desorption of acidic sialomucoid materials from the cell surface. However, the possibility was also considered that, following the desorption of surface-bound ascites fluid proteins, a loss of sialoglycoproteins may occur from the outer surface of the native cell membrane.

As demonstrated by the data presented in Table 1, the neuraminidase-sensitive sialic acid residues at the surfaces of washed TA3-Ha and TA3-L cells account for approximately 50% of their surface charge densities measured at 4 °C. A direct chemical test was therefore made to quantitate any change in the total amount of neuraminidase-sensitive sialic acid present at the surface of TA3-L cells during the course of a 2 hr. incubation at either 4 °C or 23 °C. The cells were centrifugally washed immediately prior to the release of sialic acid by neuraminidase in order to remove any adsorbed ascites fluid sialomucin materials. The results of these assays are presented in Table 2, and clearly demonstate that no decrease in the amount of neuraminidase-sensitive sialic acid occurred during the course of a 2 hr. incubation of TA3-L cells at either 4 °C or 23 °C. Furthermore, the amount of enzymatically released sialic acid was identical at the two incubation temperatures. This finding is in distinct contrast to the observed difference in the surface charge densities of centrifugally washed TA3 cells incubated at 4 °C and 23 °C, and suggests that the time-dependent decrease in charge density at 23 °C is not attributable to a major loss of acidic sialoglycoprotein materials from the outer membrane surface.

The stable surface charge characteristics of TA3 cells suspended at 4 °C relative to 23 °C or 37 °C suggests a possible link between cellular metabolism and the time-dependent changes in surface charge density that occur at the higher temperatures. Further support for a role of metabolism was obtained in the experiments shown in Fig. 4, in which TA3-L cells were incubated as a dilute suspension at 23 °C in a 0.9% NaCl solution containing

1 mM cyanide ion. The presence of this metabolic blocking agent served to inhibit the time-dependent decrease in cellular surface charge density. In addition, a large decrease in the surface charge density occurred immediately following the removal of cyanide ion from the 23 °C incubation medium. These results strongly suggest that the molecular structure of the cellular membrane surface is altered in a time-dependent manner under conditions where the cells are actively metabolizing.

Cellular Proliferative Capacity and Vital Staining. Using TD50 measurements as an end point, an assessment was made of the proliferative capacity of TA3-L cells incubated from 0 to 2 hr. in a 0.9% NaCl solution at either 4 °C or 23 °C. A comparison was also made of the TD50 values for these cells in normal and immunosuppressed hosts. In addition, a parallel set of measurements was carried out to assess the ability of TA3-L cells to exclude the vital dye nigrosin following exposure to the various incubation conditions. The results of these TD50 and dye exclusion studies are summarized in Table 3.

A TD50 value of 18 cells was obtained for TA3-L cells that were maintained *in vitro* as a concentrated suspension in ascites fluid at 4 °C prior to transplantation. When the cells were incubated as a dilute suspension (1% vol/vol) in 0.9% NaCl solution for 2 hr. at 4 °C, the TD50 increased to 1200 cells in normal hosts, and to 310 cells in hosts that were immunosuppressed by pre-irradiation. These TD50 values demonstrate that a substantial decrease in the proliferative capacity of TA3-L cells occurs during incubation at 4 °C. It is interesting to note, however, that no indication of lethal cellular damage was obtained on the basis of dye exclusion tests with nigrosin. The significantly lower TD50 value obtained with immunosuppressed relative to normal hosts suggests that an alteration occurs in the histo-

compatibility properties of TA3-L cells maintained in a dilute suspension at 4 °C, and that this factor plays a role in the decreased transplantability observed following incubation of the tumor cells in vitro.

TA3-L cells that were incubated as a 1% (vol/vol) suspension in 0.9% NaCl solution at 23 °C exhibited a time-dependent increase in the TD50 value. Following a 2 hr. incubation at this temperature, the TD50 was 3750 cells, which is three times larger than the TD50 obtained for TA3-L cells maintained for 2 hr. at 4 °C. The nigrosin dye exclusion test indicated that only a 9% decrease occurred in the number of viable cells at 23 °C relative to 4 °C. This observation provides further evidence that vital staining is a poor indicator for the loss of cellular proliferative capacity.

The TD50 value for cells maintained at 23 °C was found to be nearly identical in normal and immunosuppressed hosts, thereby indicating that the decreased transplantability of these cells results primarily from a reduced cellular proliferative capacity rather than from an active host immune response against sublethally damaged cells. An alteration in tumor histocompatibility properties occurs when TA3-L cells are maintained at 23 °C (as described below), but this effect appears not to be detectable by a TD50 measurement.

The introduction of a metabolic block through the addition of 1 mM cyanide ion to a 23 °C suspending medium served only to increase the TD50 value in both normal and immunosuppressed hosts. This observation indicates that the metabolic blocking agent had an irreversible effect on the proliferative capacity of TA3 cells.

Growth Kinetics. In vivo cell growth kinetics were measured in order to obtain a sensitive indication of changes in the histocompatibility properties

of TA3-L cells during maintenance in a 0.9% NaCl solution at 23 °C. As shown in Fig. 5, tumor cells incubated as a 1% (vol/vol) suspension at 23 °C for 0.5 hr. prior to transplantation immediately entered exponential growth only in immunosuppressed hosts. An immunologic response of normal LAF_1/J mice against the tumor cells is evident from the fact that a lag period of several days preceded the initiation of exponential growth. Inocula of less than 10^3 cells were found to be rejected in both normal and immunosuppressed hosts. These results clearly demonstrate that TA3-L cells maintained at 23 °C in a 0.9% NaCl solution undergo an alteration in histocompatibility properties. This fact was not evident from TD50 measurements alone, since a nearly identical cell inoculum was required for successful tumor growth in normal and immunosuppressed hosts.

Growth kinetics were also measured for TA3-L cells maintained at 23 °C in Medium 199, a balanced salt solution containing glucose, amino acids and other nutrients. The growth properties were found to be identical to those observed for cells maintained at 23 °C in a 0.9% NaCl solution (8), thus indicating that the markedly decreased transplantability of these cells is not attributable to a lack of metabolites in the medium.

DISCUSSION

The glycoprotein coat of the TA3 adenocarcinoma tumor has been extensively studied in an effort to relate the biochemistry of cell surface macromolecules to the antigen expression and transplantation properties of this ascites tumor. During the past decade, it has been demonstrated that a major cell surface glycoprotein termed "epiglycanin" masks the $H\text{-}2^a$ histocompatibility antigens of TA3-Ha tumor cells and thereby permits their transplantation into a wide variety of allogeneic mouse hosts, as well as into several species of rats (9, 10). Combined biochemical and electron microscopic studies have shown epiglycanin to be a high-molecular-weight (5×10^5 daltons), filamentous glycoprotein rich in terminal sialic acid residues (11 - 14). The removal of sialic acid from the TA3-Ha cell surface by treatment with neuraminidase serves to unmask the expression of histocompatibility antigens and thereby decreases the transplantability of these cells into allogeneic hosts (15 - 17).

The series of studies reported here demonstrate the extremely labile character of the TA3 ascites tumor cell surface. Incubation of the cells as a dilute suspension in 0.9% NaCl solution at either 23 °C or 37 °C was found to produce a time-dependent decrease in the negative surface charge density that could be inhibited by lowering the solution temperature to 4 °C. A similar behavior was demonstrated for both the near-diploid TA3-Ha subline (modal chromosome number = 41) and the hypotetraploid TA3-L subline (modal chromosome number = 77). Although the negative surface charge density of the TA3-L subline is twice that of the TA3-Ha subline (Table 1 and Figs. 1 and 2), both the kinetics and the extent of the temperature-dependent

reduction in charge density were nearly indentical for the two tumor sublines. Additional studies with the TA3-L subline demonstrated the following features of this phenomenon: (1) the decrease in charge density is not attributable to a desorption of acidic mucoproteins that are present in the ascites fluid during tumor growth *in vivo*; (2) the membrane content of sialic acid residues, which account for approximately 50% of the surface charge density, does not decrease in parallel with the charge density at an incubation temperature of 23 °C; (3) the time-dependent decrease in surface charge density can be inhibited by blocking cellular metabolism, either by lowering the solution temperature to 4 °C or by introducing cyanide ion into the medium at 23 °C .

The histocompatibility properties of TA3 cells were also observed to change during maintenance of the cells as a dilute suspension in 0.9% NaCl solution. The alteration in tumor histocompatibility reflects a change in the expression of transplantation antigens at the cellular surface, and thus provides further evidence for the occurrence of alterations in membrane integrity. It is interesting to note that nigrosin dye exclusion tests, which indicate gross alterations in membrane structure and permeability, failed to reflect significant membrane damage in TA3 cells maintained *in vitro*.

The mechanisms underlying the observed changes in membrane properties of TA3 cells incubated in a 0.9% NaCl solution are not clear. The results of the present series of experiments suggest that TA3 cells undergo a rearrangement of membrane surface structures that occurs rapidly when the cells are metabolizing. As a consequence, the cellular electric charge

density and the expression of surface antigens are irreversibly altered. The possibility that these alterations result from a loss of sialoglycoproteins from the TA3 cell membrane during *in vitro* maintenance appears unlikely based on the observation that the cellular content of neuraminidase-susceptible sialic acid residues did not undergo either time-dependent or temperature-dependent changes.

In parallel with changes in their surface properties, TA3 cells maintained in a 0.9% NaCl solution exhibited a significantly reduced tumorigenicity in isogeneic LAF_1/J hosts. Although the antigenicity of TA3 cells was found to increase during maintenance *in vitro*, evidence was also obtained that this phenomenon alone cannot account for the observed loss of tumorigenic potential *in vivo*. It is conceivable that a decreased proliferative capacity of TA3 cells incubated in a 0.9% NaCl solution may result from the leakage of electrolytes or other cytoplasmic components that are essential for cell division. The release and/or activation of degradative enzymes that lead to cellular autolysis could also severely restrict the proliferative capacity of TA3 cells.

In conclusion, the maintenance of TA3 ascites cells in a nonphysiological medium composed of an isotonic salt solution promotes the rapid development of alterations in cellular membrane structure, histocompatibility, and proliferative capacity. Although the mechanisms underlying these changes have not been fully elucidated, it is evident from the results presented here that both the rate of onset and the extent of these adverse cellular effects can be minimized by the use of a low temperature during short-term tumor cell maintenance *in vitro*.

REFERENCES

1. Hauschka, T. S. Cell population studies on mouse ascites tumors. Trans. N.Y. Acad. Sci. 16: 64-73 (1953).

2. Kaltenbach, J. P., Kaltenbach, M. H. and Lyons, W. B. Nigrosin as a dye for differentiating live and dead ascites cells. Exp. Cell Res. 15: 112-117 (1958).

3. Tenforde, T., Clark, E. J., MacGregor Jr., R. D., Glaeser, R. M., Streibel, M. J. and Todd, P. W. A convenient microelectrophoresis assembly. Med. Biol. Engin. 11: 236-238 (1973).

4. Tenforde, T. Microelectrophoretic studies on the surface chemistry of erythrocytes. Adv. Biol. Med. Phys. 13: 43-105 (1970).

5. Haydon, D. A. The electrical double layer and electrokinetic phenomena. Recent Prog. Surface Sci. 1: 94-158 (1964).

6. Landaw, S. A., Tenforde, T. and Schooley, J. C. Decreased surface charge and accelerated senescence of red blood cells following neuraminidase treatment. J. Lab. Clin. Med. 89: 581-591 (1977).

7. Warren, L. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234: 1971-1975 (1959).

8. Richards, W. R. and Kelly, L. S. *In vivo* studies of radiation potentiation by iodoacetamide and observations on tumor transplantation immunity. Lawrence Radiat. Lab. Rep. No. UCRL-20110, Univ. Calif., Berkeley, California (1970).

9. Hauschka, T. S., Holdridge, B. A. and Planinsek, J. A. Loss of species specificity in mouse carcinoma TA3, lethal for adult rats. Proc. Amer.

Assoc. Cancer Res. 9: 29 (1968).

10. Hauschka, T. S., Weiss, L., Holdridge, B. A., Cudney, T. L., Zumpft, M. and Planinsek, J. A. Karyotypic and surface features of murine TA3 carcinoma cells during immunoselection in mice and rats. J. Natl. Cancer Inst. 47: 343-359 (1971).

11. Codington, J. F., Sanford, B. H. and Jeanloz, R. W. Cell-surface glycoproteins of two sublines of the TA3 tumor. J. Natl. Cancer Inst. 51: 585-591 (1973).

12. Slayter, H. S. and Codington, J. F. Size and configuration of glycoprotein fragments cleaved from tumor cells by proteolysis. J. Biol. Chem. 248: 3405-3410 (1973).

13. Codington, J. F., Linsley, K. B., Jeanloz, R. W., Irimura, T. and Osaura, T. Immunochemical and chemical investigations of the structure of glycoprotein fragments obtained from epiglycanin, a glycoprotein at the surface of the TA3-Ha cancer cell. Carbohydrate Res. 40: 171-182 (1975).

14. Codington, J. F., Klein, G., Cooper, A. G., Lee, N., Brown, M. C. and Jeanloz, R. W. Further studies on the relationship between large glycoprotein molecules and allotransplantability in the TA3 tumor of the mouse: studies on segregating TA3-Ha hybrids. J. Natl. Cancer Inst. 60: 811-818 (1978).

15. Sanford, B. H. An alteration in tumor histocompatibility induced by neuraminidase. Transplantation 5: 1273-1279 (1967).

16. Simmons, R. L. and Rios, A. Immunotherapy of Cancer: Immunospecific rejection of tumors in recipients of neuraminidase-treated tumor cells

plus BCG. Science 174: 591-593 (1971).

17. Simmons, R. L., Rios, A., Ray, P. K. and Lundgren, G. Effect of neuraminidase on the growth of a 3-methylcholanthrene-induced fibrosarcoma in normal and immunosuppressed syngeneic mice. J. Natl. Cancer Inst. 47: 1087-1094 (1971).

TABLE 1

Surface charge characteristics of TA3-Ha and TA3-L cells

Subline	Treatment	4 °C electrophoretic mobility ± 1 S.D.[a] ($\mu m \cdot sec^{-1}/V \cdot cm^{-1}$)	Negative surface charge density[b] (Coulomb·m^{-2})
TA3-L	None	-1.056 ± 0.072	2.089×10^{-2}
	Neuraminidase[c]	-0.537 ± 0.114	1.062×10^{-2}
TA3-Ha	None	-0.499 ± 0.109	9.87×10^{-3}
	Neuraminidase	-0.255 ± 0.068	5.04×10^{-3}

(a) Electrophoretic mobilities are the average values from measurements on 100 individual cells at 4.0 ± 0.1 °C. The cells were centrifugally washed prior to the mobility measurements, as described in Materials and Methods.

(b) If the surface charge is treated as being uniformly distributed, then the center-to-center spacing between charges is 25 Å and 17 Å on TA3-Ha and TA3-L cells, respectively. After neuraminidase treatment to remove sialic acid residues, the average center-to-center spacing between the remaining charges on TA3-Ha and TA3-L cells is, respectively, 35 Å and 24 Å.

(c) Neuraminidase incubation conditions are described in the text. Incubation of control TA3-Ha and TA3-L cells with heat-inactivated neuraminidase had no significant effect on the electrophoretic mobility of either cell line.

TABLE 2

Sialic acid released by neuraminidase from TA3-L cells following in vitro maintenance at 4 °C and 23 °C

Temperature	Incubation Time[(a)]	No. Measurements	Sialic acid released (μmole/10^9 cells ± 1 S.D.)
4 °C	0 hr.	2	0.60 ± 0.04
	2 hr.	4	0.62 ± 0.05
23 °C	0 hr.	2	0.56 ± 0.03
	2 hr.	4	0.63 ± 0.04

(a) Following the incubation of TA3-L cells as a 1% (vol/vol) suspension in 0.9% NaCl solution at pH 7, the cells were centrifugally washed four times and treated with neuraminidase by methods described in the text.

TABLE 3

Growth properties and vital staining of TA3-L cells maintained in vitro

Incubation conditions[a]	Dilution factor[b]	Incubation time (hr.)	Percent cells excluding nigrosin	Host irradiation[c] (rad)	TD50
4 °C	1	0	97	0	18
4 °C	100	2	97	0	1200
4 °C	100	2	97	500	310
23 °C	100	0.5	91	0	2400
23 °C	100	0.5	91	500	2310
23 °C	100	2	88	0	3750
23 °C	100	2	88	500	3760
23 °C + 1mM KCN	100	2	94	0	7000
23 °C + 1mM KCN	100	2	94	500	5630

(a) The cells were suspended in 0.9% NaCl solution adjusted to pH 7.0 ± 0.1.

(b) The dilution factor is the volume of the suspension relative to the cell volume in undiluted ascites fluid.

(c) The 500 rad whole-body dose of ^{60}Co radiation was administered two days prior to the injection of TA3-L cells.

FIGURE LEGENDS

Fig. 1 The measured electrophoretic mobility (panel A) and the calculated negative surface charge density (panel B) are shown as a function of time for a 1% (vol/vol) suspension of hypotetraploid TA3-L cells in 0.9% NaCl solution at 4, 23 and 37 °C. The solution pH was maintained at 7.0 ± 0.1 throughout the course of the incubation. Each point represents the average value of mobility measurements on 10 individual cells carried out within a time interval of approximately 5 min.

Fig. 2 The mobility and surface charge density are shown as a function of time for a 1% (vol/vol) suspension of near-diploid TA3-Ha cells in 0.9% NaCl solution at 4, 23 and 37 °C.

Fig. 3 The mobility and surface charge density are plotted as a function of incubation time at 4 °C and 23 °C for TA3-L cells subjected to four centrifugal washes (300 x g, 5 min.) prior to suspension in a 0.9% NaCl solution. For comparison, the mobility and surface charge density curves obtained for unwashed TA3-L cells are shown as dashed lines.

Fig. 4 The mobility and surface charge density are plotted as a function of incubation time for TA3-L cells suspended in a 0.9% NaCl solution containing 1 mM KCN. A time-dependent reduction in mobility is also shown for TA3-L cells that were suspended at 23 °C in the presence of KCN for 2 hr., following which the cells were centrifuged and resuspended in 0.9% NaCl solution without added KCN.

Fig. 5 The growth kinetics of TA3-L cells are shown for i.p. inocula of 6×10^3 and 6×10^4 cells in normal LAF_1/J hosts, and in hosts that were immunosuppressed by administering a 500 rad whole-body dose of ^{60}Co radiation prior to tumor transplantation. The TA3-L cells were incubated as a 1% (vol/vol) suspension in 0.9% NaCl solution at 23 ^{o}C for 0.5 hr. prior to injection. Each point on the growth curves represents the mean value $\pm$ 1 S.E. of the number of TA3-L ascites cells determined from measurements on 10 tumor-bearing mice.

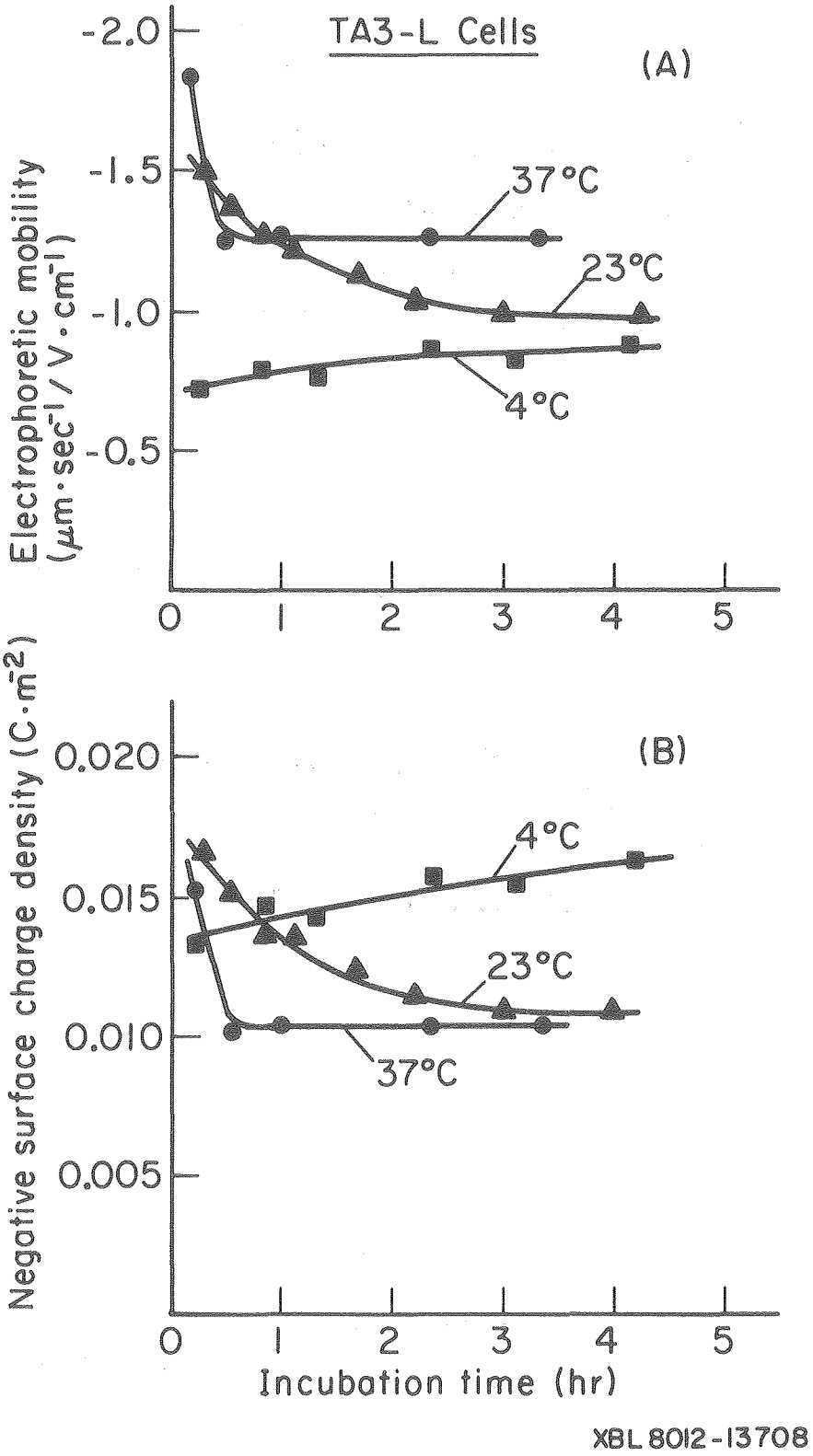
TA3-L Cells
(A)
-2.0
-1.5
-1.0
-0.5
Electrophoretic mobility
(μm·sec⁻¹/V·cm⁻¹)
37°C
23°C
4°C
0
1
2
3
4
5
(B)
0.020
0.015
0.010
0.005
Negative surface charge density (C·m⁻²)
4°C
23°C
37°C
Incubation time (hr)
XBL 8012-13708

FIGURE 1

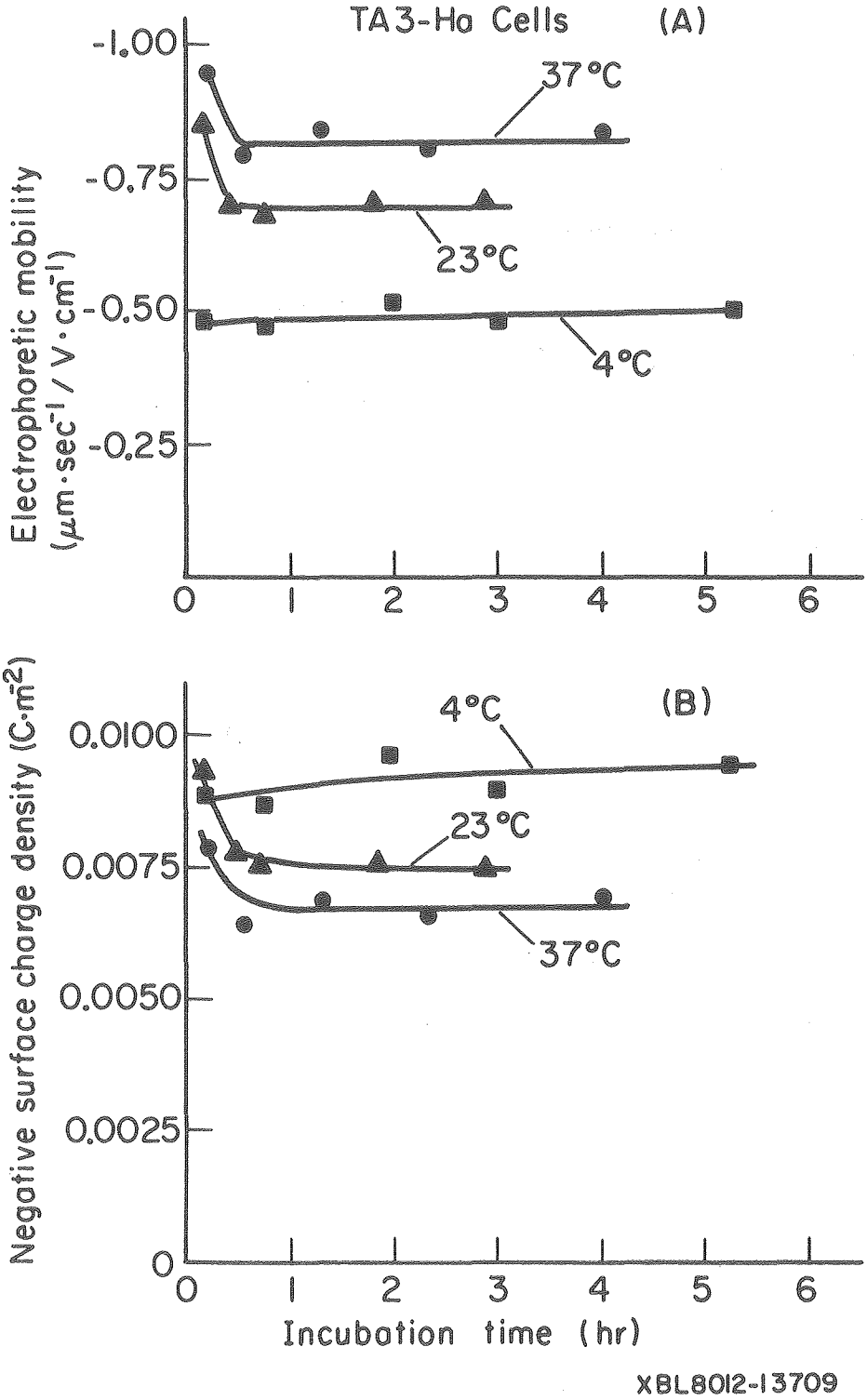
TA3-Ha Cells
(A)
37°C
23°C
4°C
Electrophoretic mobility
(μm·sec⁻¹ / V·cm⁻¹)
-1.00
-0.75
-0.50
-0.25
0
1
2
3
4
5
6
(B)
4°C
23°C
37°C
Negative surface charge density (C·m⁻²)
0.0100
0.0075
0.0050
0.0025
0
Incubation time (hr)
XBL8012-13709

FIGURE 2

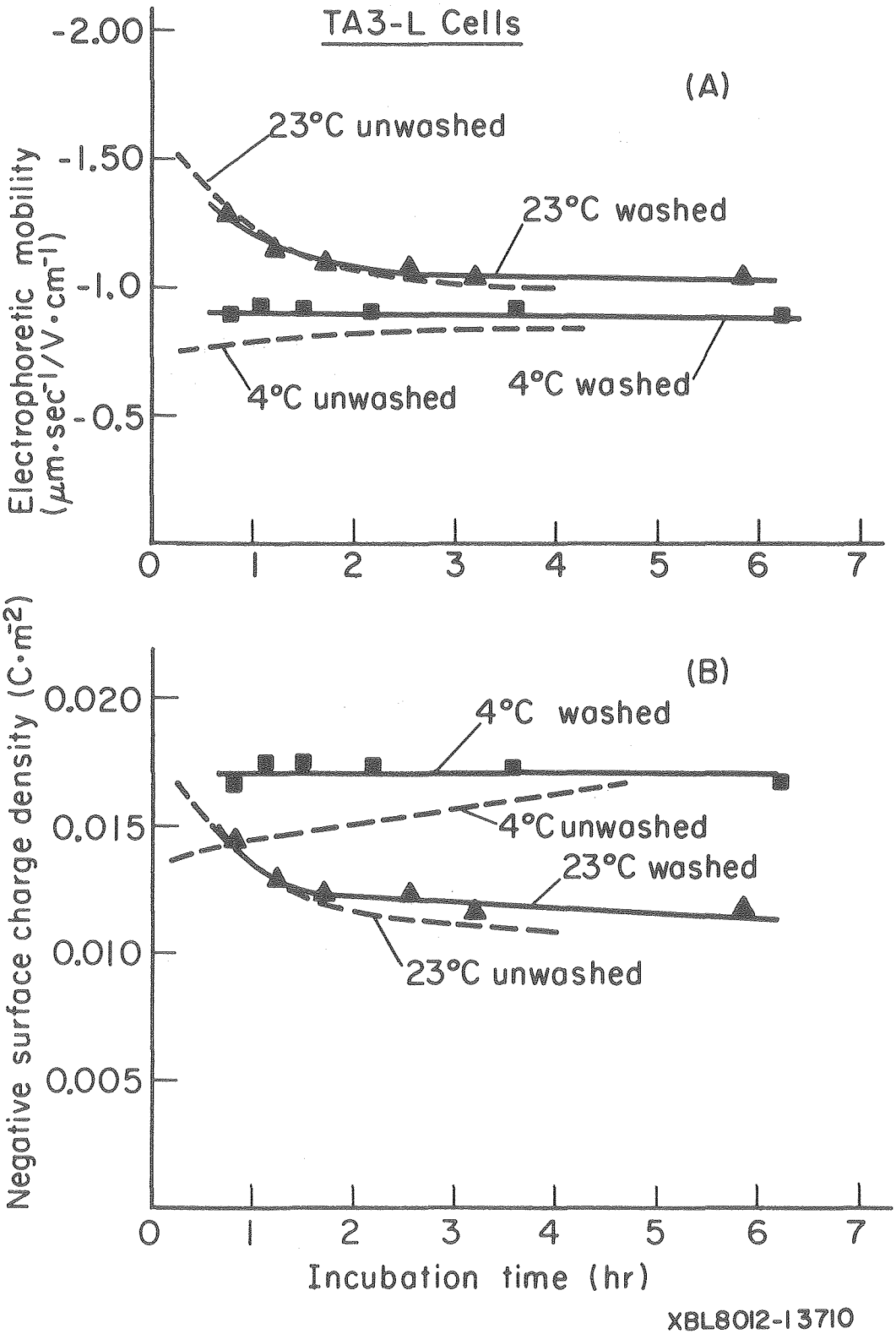
TA3-L Cells
(A)
23°C unwashed
23°C washed
4°C unwashed
4°C washed
Electrophoretic mobility (μm·sec⁻¹/V·cm⁻¹)
-2.00
-1.50
-1.0
-0.5
0
1
2
3
4
5
6
7
(B)
4°C washed
4°C unwashed
23°C washed
23°C unwashed
Negative surface charge density (C·m⁻²)
0.020
0.015
0.010
0.005
Incubation time (hr)
XBL8012-13710

FIGURE 3

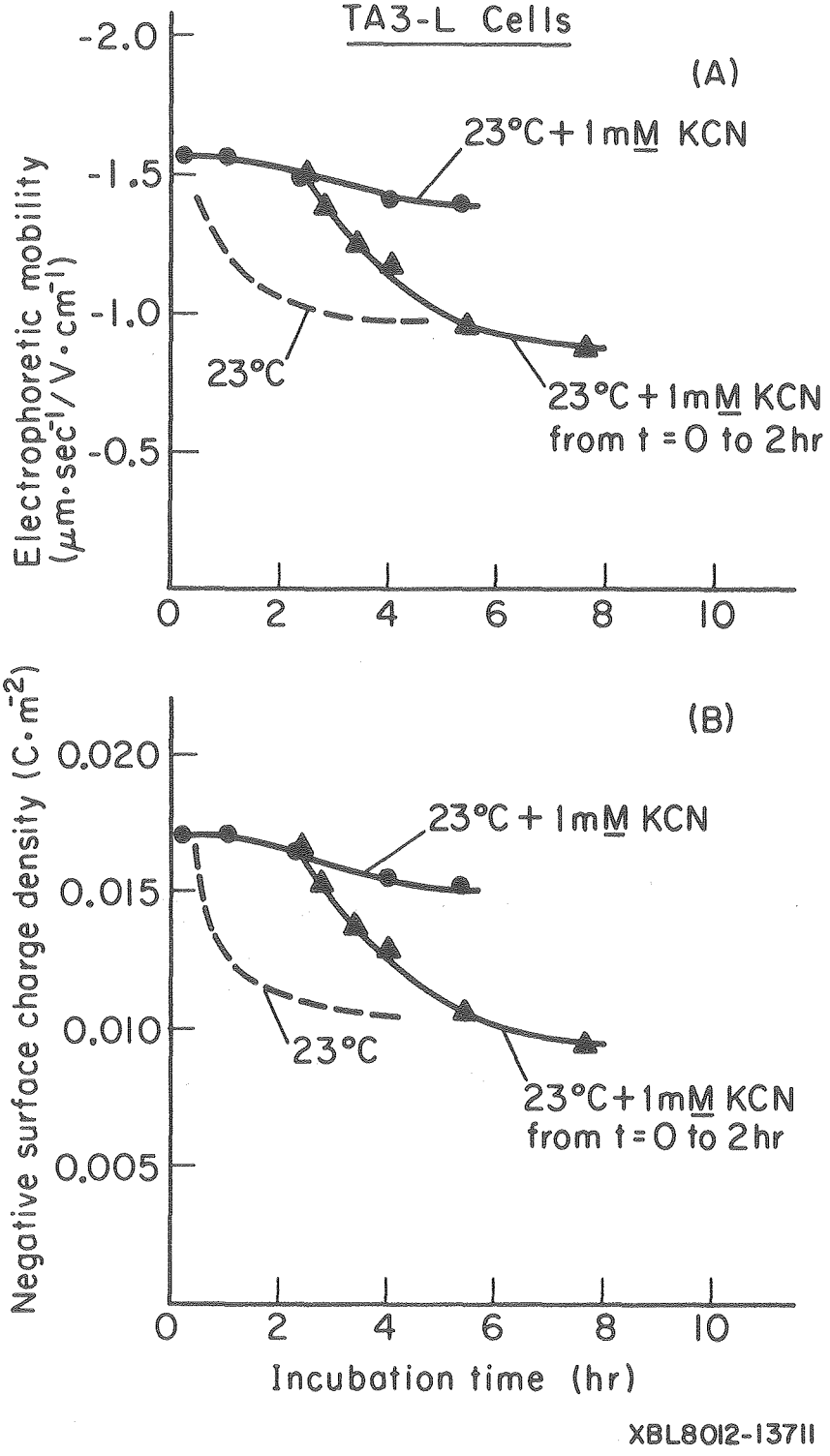
TA3-L Cells
(A)
23°C + 1 mM KCN
23°C
23°C + 1 mM KCN
from t = 0 to 2 hr
Electrophoretic mobility
(μm · sec⁻¹ / V · cm⁻¹)
-2.0
-1.5
-1.0
-0.5
0
2
4
6
8
10
(B)
23°C + 1 mM KCN
23°C
23°C + 1 mM KCN
from t = 0 to 2 hr
Negative surface charge density (C · m⁻²)
0.020
0.015
0.010
0.005
Incubation time (hr)
XBL8012-13711

FIGURE 4

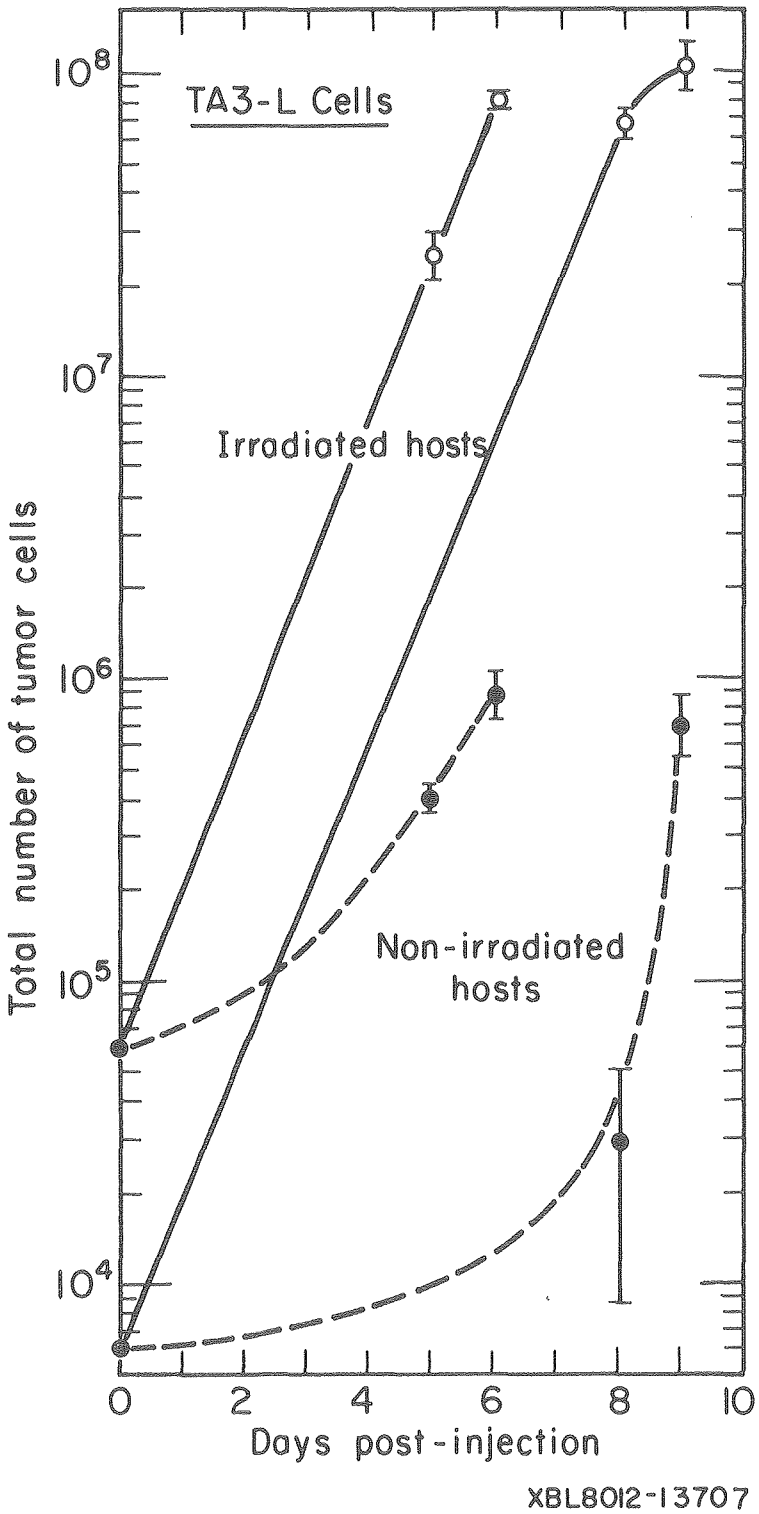
TA3-L Cells
Irradiated hosts
Non-irradiated hosts
Total number of tumor cells
10^8
10^7
10^6
10^5
10^4
0
2
4
6
8
10
Days post-injection
XBL8012-13707

FIGURE 5