tute of Aeronautics and Astronautics Conference on Space Manufacturing Facilities (American Institute of Aeronautics and Astronautics, New York, in press); H. Kolm, *ibid.*; K. Fine, *ibid.*; F. Chilton, *ibid.*
24. B. O'Leary, in preparation; abstract, American Astronomical Society Division of Planetary Science meeting, Honolulu, January 1977; paper presented at the Lunar Science Conference, Houston, March 1977; in *Proceedings of the Third Princeton–American Institute of Aeronautics and Astronautics Conference on Space Manufacturing Facilities* (American Institute of Aeronautics and Astronautics, New York, in press).
25. G. W. Driggers and J. Newman, in *The 1976 NASA Ames/OAST* (Office of Aeronautics and Space Technology) *Summer Study on Space Manufacturing of Nonterrestrial Materials*, to be published as part of the series *Progress in Aeronautics and Astronautics* (American Institute of Aeronautics and Astronautics, New York, in press).
26. G. W. Wetherill, *Annu. Rev. Earth Planet. Sci.* 2, 203 (1974); J. G. Williams and G. W. Wetherill, *Astron. J.* 78, 510 (1973).
27. This work has been supported by NASA grant NSG-2062. I thank G. K. O'Neill, E. M. Shoemaker, E. F. Helin, J. Niehoff, and G. Veeder for providing calculations and other data prior to publication; I also thank the reviewers for their constructive criticisms.

12 July 1976; revised 14 March 1977

Antibody-Induced Antigen Redistribution and Shedding from Human Breast Cancer Cells

Abstract. *Cell surface antigens of human breast cancer cells undergo a rapid redistribution when bound by antibodies from cancer patients. The subsequent shedding of these antigen-antibody complexes and free antigen may be instrumental in tumor survival.*

Tumor-specific cell surface antigens are potent sources of tumor protection from host defense mechanisms and may be intimately involved in the metastatic process. Surface antigens that are shed by tumor cells may compete with the tumor for the effector processes of the immune system, thereby allowing tumor survival (*1*). In the present study we show that antibodies from breast cancer patients induce the redistribution and subsequent shedding of cell surface antigens from cultured BOT-2 human breast cancer cells (*2*). To our knowledge, this is the first demonstration of this phenomenon in epithelial tumors in humans, although similar shedding of membrane antigens from human melanoma cells (*3*) and 7*S* IgM from Burkitt lymphoma cells has been reported (*4*).

Antibody-induced redistribution and shedding of mammary tumor cell surface antigens were studied by living cell membrane immunofluorescence. The patient serum used for demonstration of antigen redistribution and shedding was known to have complement-dependent cytotoxicity to BOT-2 human breast tumor cells and was representative of a bank of 10 serums with identical reactions. This serum contained immunoglobins of the G class that bound the surface of BOT-2 cells as identified by a positive reaction with IgG specific antiserum. The immunofluorescence technique was applied to the living cell membrane by incubating for 1 hour at 4°C 5×10^6 tumor cells in 3.0 ml of patient serum from which the complement had been removed by heat. The cells were then rinsed for 1 hour in three changes of phosphate-buffered saline (PBS) at 4°C. The specimen was then coupled with fluorescein-labeled goat antiserum to human IgG for 30 minutes at 4°C and again washed for 1 hour in three changes of PBS at 4°C. This stage was considered 0 time and a sample was taken for microscopic examination. Four similar samples were taken, and in each of them the PBS was replaced with Eagle's minimum essential medium containing 10 percent calf serum. These samples were then incubated in an atmosphere of 5 percent CO_2 and air at 37°C for intervals of 2, 4, 6, and 8 hours. In a second series of experiments, BOT-2 cells were treated with patient serum at 4°C as above, incubated at 37°C for 26 hours, then reincubated in the same patient serum to determine if antigens were replaced. At the end of each incubation period, the samples were extracted from the medium as pellets by centrifugation at 1000*g* for 10 minutes and mounted in buffered glycerin. Specimens were photographed on an Olympus FLM-UV microscope fitted with an FITC interference filter and a Y-52 barrier filter. Controls consisted of (i) samples from which patient serums had been omitted and (ii) samples to which serums from normal human volunteers were added.

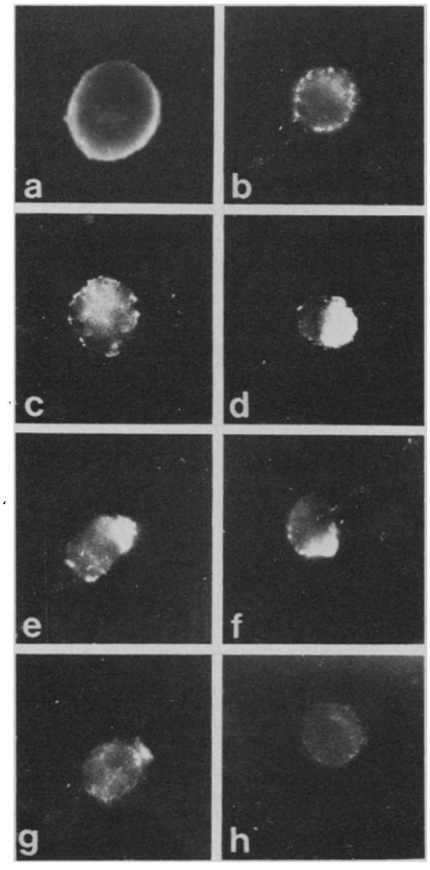

Fig. 1. Immunofluorescence of cell surface antigens. (a) Zero time. (b) After 2 hours. (c) After 4 hours. (d–f) After 6 hours. (g, h) After 8 hours. (Original magnification, ×400)

At 0 time, immunofluorescence of cell surface antigens bound by patient serum IgG appeared as a single bright halo on the tumor cell membrane (Fig. 1a). This type of localization remained unchanged up to 8 hours when the cells were maintained at 4°C. However, when the temperature was raised to 37°C a redistribution of antigens rapidly occurred. After 2 hours of incubation at 37°C the membrane antigens were redistributed into small aggregates that formed a uniform speckled pattern over the whole cell surface (Fig. 1b). Four hours of incubation at 37°C allowed further aggregation into large clumps irregularly distributed on the cell surface (Fig. 1c). After 6 hours of incubation, most cells had single fluorescent clumps with considerable fluorescent intensity (Fig. 1, d to f). After 8 hours of incubation at 37°C most cells had little or no fluorescence; however, between cells there was considerable highly fluorescent debris floating free (Fig. 1, g and h). In the second experimental series, where antibody-labeled cells were incubated an additional 26 hours, and then reexposed to the same patient serum, no binding occurred. At the end of this incubation period, almost 100 percent of the cells remained viable and could be plated for continued growth. Omission of breast cancer serum or the substitution of normal serum abolished all fluorescence.

The redistribution and shedding response of cell surface antigens to anti-

bodies has been shown to be effective in the blockage of cytotoxicity. This blockage apparently occurs during the last step of the immunological response as a result of the fixation of cytotoxic antibodies and paralysis of primed effector cells. Currie and Basham (*5*) demonstrated this blocking phenomenon in vivo by showing an increase in cell mediated cytotoxicity after tumor resection, and by washing lymphocytes in vitro to remove the attached tumor antigen or antigen-antibody complexes, thus restoring their cytotoxicity. Gentile and Flickinger (*6*) have demonstrated the presence of circulating breast tumor antigens and antibody complexes of this type in the serums of breast cancer patients. Calafat *et al.* (*7*) have shown that the redistribution and shedding of tumor antigens can be induced by the fixation of antibodies, and that this phenomenon results in an antigen-denuded cell which is not recognized by a second wave of effector agents.

Our studies confirm the antibody-induced redistribution and shedding of cell surface antigens, and show that this mechanism is active in our breast tumor system. The finding that after complete shedding the antigen was not replaced within 26 hours could indicate a basic method of tumor survival, since the antigen-denuded cell was viable and able to replicate but was not recognized by subsequent effector agents. Preliminary studies in our laboratory indicate that the speed and efficiency of antibody-induced antigen shedding may be related to immunoglobulin type. It appears that antibodies of the type that do not bind complement are redistributed at a different rate than complement-binding antibodies and are more slowly shed.

These findings suggest that tumor success or failure in the host is a complex and dynamic series of events, and that attention must be directed to tumor cell defense mechanisms in concert with the study of host defense mechanisms.

ROBERT E. NORDQUIST
Cancer Research Program, Oklahoma Medical Research Foundation, Oklahoma City 73104, and
Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City 73190
J. HILL ANGLIN
Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center
MICHAEL P. LERNER
Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center

22 JULY 1977

References and Notes

1. G. C. Davey, G. A. Currie, P. Alexander, *Br. J. Cancer* **33**, 9 (1976); E. J. Leonard *et al.*, *Immunological Aspects of Neoplasia* (Williams & Wilkins, Baltimore, 1975).
2. R. E. Nordquist, D. R. Ishmael, C. A. Lovig, D. M. Hyder, A. F. Hoge, *Cancer Res.* **35**, 3100 (1975).
3. S. P. L. Leong, C. M. Sutherland, E. T. Krementz, *ibid.* **37**, 293 (1977).
4. E. Yefenof, I. P. Witz, E. Klein, *Int. J. Cancer* **17**, 633 (1976).
5. G. A. Currie and C. Basham, *Br. J. Cancer* **26**, 427 (1972).
6. J. M. Gentile and J. T. Flickinger, *Surg. Gynecol. Obstet.* **135**, 69 (1972).
7. J. Calafat, J. Hilgers, W. J. Van Bitterswijk, M. Verbeet, P. C. Hagman, *J. Natl. Cancer Inst.* **56**, 1019 (1976).
8. We thank H. K. Geis, P. L. Munson, and P. J. Riggs for their excellent assistance. Supported by American Cancer Society grant BC-230 and the Maizie Wilkinson fund.

11 January 1977

Impaired Regulation of Alveolar Ventilation and the Sudden Infant Death Syndrome

Abstract. *Infants who subsequently died of sudden infant death syndrome manifest alveolar hypoventilation during quiet sleep and abnormality of ventilatory response to carbon dioxide breathing in comparison to normal infants.*

Pathological evidence in infants dying of sudden infant death syndrome (SIDS) suggests that chronic hypoxia had been experienced (*1*). Physiological data have indicated prolonged sleep apnea (PSA) (*2*). Aaron *et al.* have suggested that the underlying mechanism might be abnormal cardiac conduction—prolongation of the repolarizing phase of cardiac electrical activity (QT interval) (*3*). In the course of conducting studies on infants who experienced aborted SIDS, we have obtained data on three who subsequently died of SIDS. We now compare the results of studies of these infants to those of normal controls. These studies were approved by the Human Studies Committee of the Massachusetts General Hospital. Written informed consent was obtained from parents.

The patients were three infants (two males) who were first seen at ages 7, 8, and 36 weeks after having experienced two, one, and multiple resuscitations, respectively. Chest radiographs, electrocardiograms, electroencephalograms, serum electrolytes, sugar, calcium, phosphorus, magnesium, and amino acids were normal.

The corrected QT interval (QT_c) was determined from the average of six randomly selected electrical complexes in standard lead II of electrocardiograms of awake infants and compared to 46 normal control infants whose ages spanned those of the study infants. The interpreter was unaware of the origin of each recording.

Ventilation at rest during quiet sleep and the ventilatory response to breathing 5 percent CO_2 in air were measured in each infant by the nasal pneumotachograph technique (*4*) at least 1 week after resuscitation. The results are compared with those for 12 normal infants of similar age also studied during quiet sleep.

Death occurred at 12, 36, and 39 weeks, respectively, when resuscitative efforts with bag and mask, initiated by the parents at home in response to an alarm from an electronic monitor, failed to restore vital functions. Patient 1 had experienced five and patients 2 and 3 had each undergone more than ten resuscitations prior to the final unsuccessful efforts. Autopsies of two failed to reveal a cause of death; an autopsy was not permitted on the third.

The QT_c interval was 0.39 seconds in

Table 1. The corrected QT interval in 3 SIDS infants compared with that in 46 normal infants. Mean ± standard deviation is presented for the normal infants.

Subjects	QT_c
Patient 1	0.38
Patient 2	0.40
Patient 3	0.40
Normal infants	0.39 ± .02

Table 2. Ventilation ($\dot{V}_E$) and the response to CO_2 breathing in 3 SIDS infants and 12 normal infants.

Subjects	$\dot{V}_E$ (ml min^{-1} kg^{-1}) (BTPS)	End tidal P_{CO_2} (mm-Hg)	$\Delta\dot{V}_E/\Delta P_{CO_2}$ (ml min^{-1} kg^{-1} mm-Hg^{-1})
Patient 1	215	40	20
Patient 2	177	37	20
Patient 3	112	44	10
Controls	206.4 ± 68.8	35.1 ± 1.9	63.1 ± 19.1