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PREFACE

An alteration of membrane components is one of the obvious events following malignant transformation of a cell. An expression of new antigens on the cell surface which can be detected by antibodies or phytohemagglutinins, a deletion of components which existed in the normal state, or a quantitative rearrangement of cell surface composition are only a few of the possible results of transformation. Aside from the morphological and genetic changes which the cell undergoes, alteration of the surface is also accompanied by biological abnormalities, such as loss of contact inhibition, which can be monitored. Thus a science has been founded which deals with the nature of these changes and how they reflect the differentiation state of a cell whether normal or abnormal.

Many of the approaches undertaken in the past few years have provided a conceptual means of assessing the state of a cell. For example, it is now possible to determine whether a cell has become infected with an oncogenic virus by the expression of new membrane antigens, even though the cell does not manifest other malignant properties. It is the hope of medical research that these advances will aid in the early detection and eradication of cancer. Aside from this knowledge it has also become apparent that there is a unique architecture to the cell surface membrane during various stages of differentiation. By relating these changes, scientists envision a solution which will allow one to prevent or rescue a cell from the pathologic deviation of cancer as well as aid in diagnosis.

The series of articles contained within these volumes reflects this widespread interest and activity. A major goal in the selection of the subject matter was to provide readings which deal with normal cell surfaces as well as those aberrant components which arise following transformation. Although there are sections on viral antigens, as well as those induced by carcinogens, the reader should be aware that this classification is only tentative and the phenomena of antigenic expression may be the same. Some of the work on the chemical nature of membrane components has been included and represents only a prelude to the new information which is coming out of laboratories throughout the world. From these readings one should emerge with an increased awareness of the advances being made to explain membrane phenomena of normal and abnormal cells.

Ronald T. Acton, Ph.D.
October, 1972

Viral Specific Surface Alterations

Cross-reacting Tumor-specific Transplantation Antigens in Tumors Induced by Adenoviruses 3, 14, and 12[1]

Jaro Ankerst and Hans O. Sjögren

INTRODUCTION

Viral neoplasms usually possess TSTA[2] which are common for all neoplasms induced by the same virus but different for tumors induced by different viruses (13, 15, 18). There are 2 known exceptions to this rule, the cross-reacting TSTA of Friend, Moloney, and Rauscher leukemias and murine sarcoma virus, induced sarcomas (2, 3) and the cross-reaction between the TSTA of tumors induced by the different human adenovirus types 7, 12, and 18 (8, 21). The present study was performed in order to investigate further the cross-reactions between the TSTA of tumors induced by virus types belonging to Group A and B adenoviruses.

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[1] This work was supported by grants from the Swedish Cancer Society, A. Jönsson's Foundation, the Swedish Society for Medical Research, and the Medical Faculty, University of Lund, Lund, Sweden.

[2] The abbreviations used are: TSTA, tumor-specific transplantation antigen; E10F, Eagle's medium containing 10% fetal bovine serum; TPD_{50}, cell dose causing tumor growth in 50% of the recipient group; CI, colony inhibition; LNC, lymph node cell.

MATERIALS AND METHODS

Mice. Mice of strains A/Sn and CBA and (A/Sn × CBA)F_1 and (A/Sn × C57BL)F_1 hybrids were used. All mouse strains were maintained by continuous, single-line, brother-to-sister mating. All mice were kept on a standard pellet diet and water *ad libitum*.

Tumors. The various tumors used in the present investigation are listed in Table 1. All the mouse tumors have previously been demonstrated to possess the TSTA specific for the virus type in question (1, 11, 19, 21). The YAA-CI and YAA-CI-RC2 are clones of a cell line carrying the Moloney and the polyoma-specific TSTA as well (19). The BHK-C13 cells (clone 13 of the BHK-21 cell line) were obtained from Dr. I. MacPherson. They were grown in E10F. The BHK-Rous cells derived from BHK-C13 cells that were transformed by RSV-SR *in vitro* (20). They were grown in E10F. The A3Hall, A7Hall, and A14Hall were obtained from Flow Laboratories, Inc., Rockville, Md., as established *in vitro* cell lines of 3 hamster tumors induced by adenovirus types 3, 7, and 14, respectively. They were carried in E10F. TAD III (14) and A12H (R. M. McAllister, unpublished data), adeno 12 hamster tumor cells were obtained from Dr. L. Berman and Dr. MacAllister, respectively. They were carried in E10F.

Sera. Sera for the cytotoxic tests were obtained from mice. The immunization procedure has been described (1).

Control Sera. C1, C2, C3, C4, C5, and C6 are different pools of serum obtained from untreated CBA mice.

Serum pool MC1 derived from mice immunized with 8 doses of syngeneic M1S57 tumor cells.

Serum pool HC1 was obtained from (A/Sn × CBA)F_1 hybrid mice after 11 doses of BHK-C13 hamster cells.

Antiadeno Tumor Sera. Serum pools M12-1, M12-2, M12-3, M12-4, and M12-5 were obtained from mice immunized with 5 to 13 doses of syngeneic adeno 12 tumor cells. Pool H14-1 derived from (A/Sn × CBA)F_1 hybrid mice treated with 13 doses of A14Hall hamster tumor cells.

All sera were heated to 56° for 30 min, sterilized by filtration, and stored at −20° until use.

Cytotoxic tests by the ^{51}Cr release technique were performed as described previously (1). The target cells to be tested were incubated with sodium chromate (obtained from the Radiochemical Centre, Amersham, England) for 12 to 24 hr at 37°. All tests were performed in duplicate tubes and the significance of differences in release

Table 1

Tumors used in the present investigation

Designation of tumor	Inducing agent	Animal strain	Serial passage
A12B2	Adenovirus type 12	CBA mouse	*in vivo*
A12B3	Adenovirus type 12	CBA mouse	*in vivo*
A12B4	Adenovirus type 12	CBA mouse	*in vivo*
A12B5	Adenovirus type 12	CBA mouse	*in vivo*
A12B6	Adenovirus type 12	CBA mouse	*in vivo*
A12B8	Adenovirus type 12	CBA mouse	*in vivo*
A12B10	Adenovirus type 12	CBA mouse	*in vivo*
A12B22	Adenovirus type 12	CBA mouse	*in vivo*
A12SBA	Adenovirus type 12	(A/Sn × CBA)F_1 hybrid mouse	*in vivo*
SEBD	Polyoma virus	CBA mouse	*in vivo*
YAA-CI	Moloney virus/polyoma virus	A/Sn mouse	*in vivo*
YAA-CI-RC2	Moloney virus/polyoma virus	A/Sn mouse	*in vivo*
RSC	RSV-SR virus	A/Sn mouse	*in vivo*
RBD	RSV-SR virus	CBA mouse	*in vivo*
MIS57A	Methylcholanthrene	(A/Sn × C57B1)F_1 hybrid mouse	*in vivo*
BHK-CI3		Syrian hamster	*in vitro*
BHK-Rous	RSV-SR virus	Syrian hamster	*in vitro*
A3Hall	Adenovirus type 3	Syrian hamster	*in vitro*
A7Hall	Adenovirus type 7	Syrian hamster	*in vitro*
A14Hall	Adenovirus type 14	Syrian hamster	*in vitro*
TAD III	Adenovirus type 12	Syrian hamster	*in vitro*
A12H	Adenovirus type 12	Syrian hamster	*in vitro*

was calculated according to Student's *t* test. The radioactivity was measured in a scintillation counter (Selektronik, Horsholm, Denmark), and the percentage of isotope release was calculated as described previously (1).

Transplantation Immunity Tests. The previously described procedure (1) was used. Groups of CBA mice were treated with hamster tumor cells as indicated in Table 3. The mice were whole-body-X-irradiated with 350 R 7 to 10 days after the last immunizing dose, and 24 hr later they were challenged with known numbers of viable (as judged by the trypan blue exclusion test) cells of a syngeneic adeno 12 tumor. Each cell dose was inoculated into 5 to 10 animals. The growth of the inoculated tumor cells was recorded for 60 days and the TPD/50 was calculated by the method of Kärber (12).

CI Tests. The CI technique was used to demonstrate cellular immunity *in vitro* following procedures previously described (6–8).

Trypsinized suspensions of target tumor cells were added to 60-mm Falcon plastic Petri dishes, and the cells were allowed to attach to the bottom. After 12 to 24 hr, the culture medium (Eagle's medium containing 30% fetal bovine serum) was discarded, and 0.5 ml suspended LNC (containing 2×10^7 cells/ml) was added. After 1 hr, 4 ml Eagle's medium with 15% fetal bovine serum were added to each Petri dish. The dishes were incubated for 3 to 5 days at 37° in an atmosphere containing 5% CO_2. They were stained with crystal violet and the number of colonies per dish was counted. Three to 5 dishes were used for each type of LNC.

LNC suspensions were prepared by pressing lymph nodes (axillary, cervical, inguinal, and mesenteric) through a 60 mesh stainless steel screen into Eagle's medium containing 2% bovine serum. The lymph nodes were harvested 6 to 12 days after the last immunizing cell dose. The suspended cells were washed with the same medium and used in CI tests.

RESULTS

Cytotoxic Activity of Mouse Antiadeno 12 Tumor Sera against Hamster Adeno 14 and 3 Tumor Cells. Four serum pools of mice hyperimmunized against 3 different syngeneic adeno 12 tumors were tested against A14Hall hamster adeno 14 tumor cells by the ^{51}Cr release technique previously used successfully for demon-

stration of TSTA in adeno 7 tumor cells (1). Serum M12-4 was tested against A14Hall and A7Hall hamster tumor cells and BHK-C13 hamster cells and against A12B22 syngeneic mouse adeno 12 tumor cells (Table 2). It had a strong cytotoxic effect against both of the hamster adeno tumors and was active also against the syngeneic adeno 12 tumor cells, but it was completely inactive against the BHK-C13 control cells. The hyperimmune sera directed against 2 other syngeneic mouse adeno 12 tumors were also shown to have a cytotoxic effect against A14Hall tumor cells (Table 2).

The cytotoxic activity of mouse serum M12-4 tested against A14Hall target cells was demonstrable up to a dilution of approximately 1:700 (Chart 1). The titration was performed twice with almost identical results. The found titer is remarkably high and is higher than cytotoxic titers previously found by various techniques against the TSTA(s) of other adeno tumors or other viral neoplasms.

Antiserum of (A × CBA)F_1 hybrids immunized against A14Hall tumor cells was tested against semisyngeneic A12B10 adeno 12 tumor cells in parallel with a similar serum against BHK-C13 cells (Table 2). The anti-A14Hall serum was clearly cytotoxic, while anti-BHK-C13 serum had no effect.

Two different mouse antisera against syngeneic adeno 12 tumor cells were tested against A3Hall target tumor cells. Both sera were cytotoxic in dilutions of 1:12 (Table 2).

Induction of Isograft Immunity to Adeno 12 Tumors by A14Hall and A3Hall Hamster Tumor Cells. CBA mice pretreated with 10 doses of 10^5 A14Hall cells exhibited a clear cut immunity toward isografts of A12B3 adeno 12 tumor cells (Table 3). The level of immunity was about the same as that induced by adeno 12 hamster tumor cells. The A3Hall cells induced a weak transplantation resistance, as did A7Hall tumor cells. Animals similarly treated with BHK-C13 hamster cells were tested as controls. They showed no immunity to the adeno 12 tumor isografts.

Cross-Reactivity between Adeno 12 Mouse Tumor Cells and A3Hall and A14Hall Hamster Tumor Cells Demonstrated by Colony Inhibition Tests for Cell-mediated Immunity. LNC of CBA mice immunized with 4 different syngeneic adeno 12 tumors inhibited the colony formation of A14Hall (Table 4) and A3Hall (Table 5) tumor cells, while the LNC of mice similarly immunized against polyoma and Rous tumor cells had no effect.

Table 2

Cross-reactivity between various adeno 12 mouse tumors and hamster tumors induced by adenovirus types 3, 7, and 14 demonstrated by ^{51}Cr-cytotoxic tests

Experiment	Target tumor	Serum[a]		Complement-dependent ^{51}Cr release ± S.E. (%)[b]	Difference to control serum[c]
1	A14Hall	Control	C3	13.42 ± 0.64	
		Anti-A12B2	M12-4	47.78 ± 1.50	34.36[d]
	A7Hall		C3	4.20 ± 1.22	
			M12-4	25.22 ± 0.91	21.02[d]
	BHK-C13		C3	−0.68 ± 0.73	
			M12-4	1.10 ± 0.37	1.78 ($p > 0.05$)
2	A14Hall	Control	MCl	6.14 ± 0.41	
		Anti-A12B3	M12-2	15.16 ± 0.46	9.02[d]
		Control	C2	14.40 ± 1.24	
		Anti-A12SBA	M12-1	43.00 ± 1.16	28.60[d]
		Control	C3	2.42 ± 0.79	
		Anti-A12B2	M12-5	13.66 ± 0.60	11.24[d]
3	A3Hall	Control	C6	28.94 ± 1.31	
		Anti-A12B3	M12-3	38.50 ± 0.76	9.56[e]
		Control	C6	22.06 ± 0.95	
		Anti-A12B2	M12-5	29.10 ± 0.10	7.04[d]
4	A12B10	Control	C4	2.26 ± 0.22	
		Anti-A14Hall	H14-1	5.02 ± 0.13	2.76[e]
		Anti-BHK-C13	HC-1	1.60 ± 0.74	−0.66 ($p > 0.05$)
5	A12B22	Control	C5	6.30 ± 0.20	
		Anti-A12B2	M12-5	9.94 ± 0.14	3.64[d]
		Control	C3	32.62 ± 0.63	
		Anti-A12B2	M12-4	41.44 ± 0.71	8.82[d]

[a] Sera were used in final dilutions of 1:12 in Experiments 1, 2, and 3 and in dilutions of 1:6 in Experiments 4 and 5.

[b] Percentage of complement-dependent release means the difference in release of ^{51}Cr after incubation with active, as compared to inactivated, complement (heated for 30 min at 56°). Human complement was used in final dilution of 1:6 in Experiments 1, 2, 4, and 5 and in dilution of 1:12 in Experiment 3.

[c] The probability that difference is due to chance was calculated by Student's t test.

[d] $p < 0.01$.

[e] $p < 0.05$.

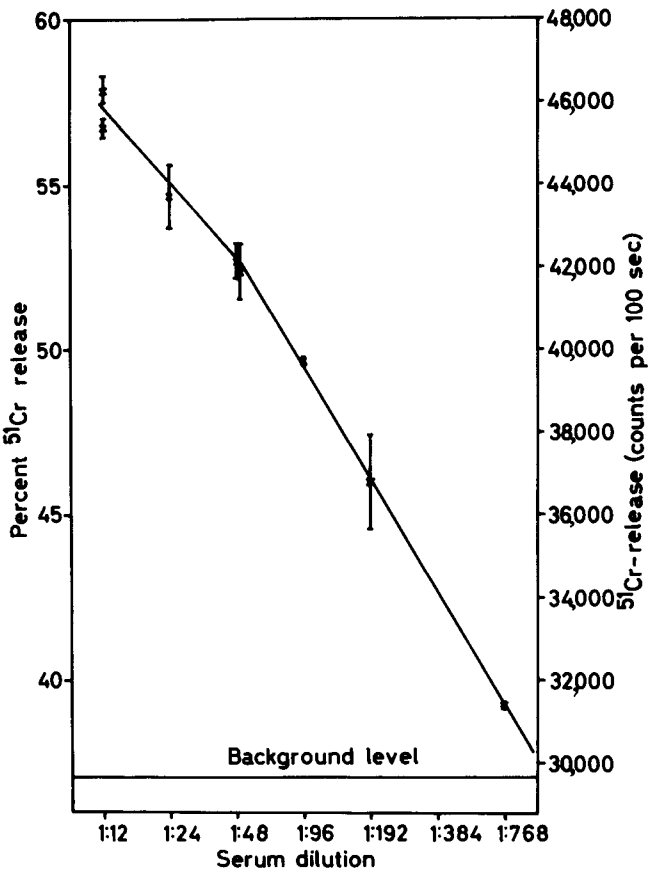

Chart 1. Titration by the ^{51}Cr release technique of the cytotoxic activity of a syngeneic adeno 12 tumor serum M12-4 tested against A14Hall hamster adeno 14 tumor cells. The same antiserum was tested in 2 different experiments and the variation in ^{51}Cr-release in duplicate tubes in the tests is indicated. Background level means release obtained by incubation of target cells in the presence of diluent and active complement in a final dilution of 1:6.

The inhibition was of approximately the same degree as that demonstrated against A12H adeno 12 hamster tumor cells tested in parallel with the A3Hall cells. The immune LNC had no effect against BHK-C13 hamster cells.

The LNC of CBA mice immunized against A3Hall,