BIOCHIMICA et BIOPHYSICA ACTA(CR)

REVIEWS ON CANCER

Vol. CR2 · Nos. 1-4 March 1975

BIOCHIMICA et BIOPHYSICA ACTA

REVIEWS ON CANCER

INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND BIOPHYSICS
REVUE INTERNATIONALE DE BIOCHIMIE ET BIOPHYSIQUE
INTERNATIONALE ZEITSCHRIFT FÜR BIOCHEMIE UND BIOPHYSIK

VOL. 417 (1975)

ELSEVIER SCIENTIFIC PUBLISHING COMPANY
AMSTERDAM

Vol. 417 No. 1 BIOCHIMICA ET BIOPHYSICA ACTA March 28, 1975

REVIEWS ON CANCER

Vol. CR 2 No. 1

CONTENTS

Forthcoming articles

Temperature sensitive mutants of avian sarcoma viruses
by J. A. Wyke (London, U.K.)

Carcinoembryonic antigen (CEA). Molecular biology and clinical significance
by A. Fuks, C. Banjo, J. Shuster, S. O. Freedman, P. Gold (Montreal, Canada)

BIOCHIMICA ET BIOPHYSICA ACTA
International Journal of Biochemistry and Biophysics

REVIEWS ON CANCER

Vol. CR 2 No. 2

CONTENTS

BIOCHIMICA ET BIOPHYSICA ACTA
International Journal of Biochemistry and Biophysics

Vol. 417 No. 3/4 BIOCHIMICA ET BIOPHYSICA ACTA December 31, 1975

REVIEWS ON CANCER

Vol. CR 2 No. 3/4

CONTENTS

Forthcoming articles

Chromatin structure and function in proliferating cells
by R. Baserga and C. Nicolini (Philadelphia, Pa., U.S.A.)
Cell surface proteins and malignant transformation
by R. O. Hynes (Cambridge, Mass., U.S.A.)

BIOCHIMICA ET BIOPHYSICA ACTA
International Journal of Biochemistry and Biophysics
Founded in 1947 by H. G. K. Westenbrink, Managing Editor, 1947-1964

Biochimica et Biophysica Acta, 417 (1975) 1–23
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

BBA 87012

AVIAN RNA TUMOR VIRUSES

A MODEL FOR STUDYING TUMOR ASSOCIATED CELL SURFACE ALTERATIONS

REINHARD KURTH[a] and HEINZ BAUER[b]

[a]Imperial Cancer Research Fund Laboratories, London (U.K.) and [b]Institut für Virologie, Fachbereich Humanmedizin, Justus Liebig-Universität Gießen (G.F.R.)

(Received September 23rd, 1974)

CONTENTS

Abbreviations: TSSA, tumor-specific cell surface antigen; TSTA, tumor-specific transplantation antigen; CEA, carcino-embryonic antigen; EA, embryonic antigen; CEC, chick embryo cell; ATV, avian tumor virus; ASV, avian sarcoma virus; ALV, avian leukosis virus; WGA, wheat germ agglutinin; Ve, virus envelope antigen.

I. INTRODUCTION

A property characteristic of a cancer cell is its relative release from homeostatic growth control mechanisms. This becomes manifest in vivo by invasive growth and the capacity to metastasize, and in vitro a correlation may be seen in the relative release from contact inhibition. If one accepts the assumption that tumor-specific cell surface changes are at least in part responsible for the altered social behaviour of cancer cells, in that the responsible surface receptors no longer recognize or properly transduce the signals mediating cell-to-cell recognition, it becomes obvious that the investigation of the cancer cell surface is of utmost importance for the understanding of the process of malignant transformation and, in particular, for the evaluation of the growth pattern of cancer cells.

Of the several possible approaches to detection and assay of tumor cell surface changes, immunological methods seem to be particularly useful. On the assumption that most cell surface constituents are immunogenic, animals can be immunized with tumor cells, preferably syngeneic, and the immunological response of the host can be investigated in vivo. In addition, the humoral and cellular immune reactions against newly appearing tumor specific antigens can be used to study in vitro specificity, structure, biosynthesis and function of tumor-specific cell surface antigens (TSSA).

Since the growth capacities of tumors in vivo seem to be decisively influenced by the host immune response against the tumor cells, the study of TSSA is certainly also of practical value for considerations of tumor immune prophylaxis and therapy.

For investigations of TSSA, virus-induced tumors seem to be more advantageous than chemically induced tumours because all tumors of a given virus etiology contain common TSSA [1], thus facilitating reproducible induction of given TSSA in different cells and allowing their characterization under various experimental conditions.

Of the various tumor viruses, the avian RNA tumor virus system offers specific advantages that make it a unique model for immunological investigations of tumor cell surface alterations. These advantages include: (1) the existence of defined tumor virus strains exerting different biological effects on a given host cell, i.e. leading to cell transformation and/or virus replication, (2) the ability of most avian sarcoma virus strains to replicate in the absence of any helper virus, enabling cell transformation by monoclonal virus strains, (3) the occurrence of different responses after infection by a given virus strain in different host target cells, (4) the availability of virus mutants defective in functions needed either for cell transformation, virus replication or for both, (5) the availability of assays for expression of endogenous virus functions and (6) the possibility of manipulating selectively the humoral and cellular immune responses in the natural host, e.g. through thymectomy or bursectomy.

II. DESCRIPTION OF THE AVIAN TUMOR VIRUS SYSTEM

Avian RNA-containing tumor viruses (ATV) are C-type viruses according to

Bernhard's (1960) morphological criteria. They are assembled during a budding process at the cell surface and the various virus strains are morphologically indistinguishable. Their structural constitution is essentially identical, the only significant difference being in the size of the RNA genome [3,4]. The virus strains, on the other hand, differ in the immunological properties of the virus envelope (Ve) antigens [5,6]. The latter are glycoproteins in nature [5,6] and define the host range, cross neutralization and interference patterns of these viruses [7]. On this basis, ATV were divided originally into two [8,9], and now comprise at least 7 subgroups, A through G.

The biological properties of individual ATV strains are distinct with respect to the histological type of target cell that can be transformed by a given virus. Therefore, ATV can be classified into avian sarcoma (ASV) and avian leukosis viruses (ALV). This distinction serves purely operational purposes, because both ASV and ALV are highly oncogenic in the natural, genetically susceptible host, the chicken, but under tissue culture conditions only ASV transform chicken embryo fibroblasts (CEF), ALV is replicating in these cells.

Because of these properties and also taking advantage of the fact that ATV can transform mammalian cells without virus replication, one could hope to be able to distinguish between cell surface alterations that are specific for virus replication and those intrinsic to transformed cells.

Table I summarizes the ASV and ALV-strains of different subgroups, which have been studied in vitro with regard to their induction of cell surface changes.

TABLE I

ASV AND ALV STRAINS OF DIFFERENT SUBGROUPS AS USED IN STUDIES ON VIRUS-INDUCED CELL SURFACE ANTIGENS

Subgroup	ASV	ALV
A	Schmidt-Ruppin strain 1 (SRV-1)	Non-converting (NC) SRV-1[a]
	Prague strain A (Prague-A)	Rous-associated virus-1 (RAV-1)
B	Prague strain B (Prague-B)	Rous-associated virus-2 (RAV-2)
		Avian myeloblastosis virus-B (AMV-B)
C	Bratislava-77 strain (B-77)	Rous-associated virus-49 (RAV-49)
	Prague strain C (Prague C)	
D	Schmidt-Ruppin strain-H (SRV-H)	Rous-associated virus-50 (RAV-50)
		NC-SRV-H[a]

[a] Non-converting mutants were obtained after hydroxylamine-treatment of SRV-1 or SRV-H, respectively [10].

III. BIOCHEMICAL DETECTION OF MEMBRANE CHANGES IN ATV-TRANSFORMED CELLS

Intensive efforts have been made to establish consistent differences in the biochemical composition of malignant and the corresponding normal cells. One of the main difficulties in these studies has been the lack of a truly corresponding normal cell. For instance, after viral transformation of a permanent cell line, it is virtually impossible to define what should be its normal counterpart: the uninfected permanent cell line, which often is oncogenic itself, or a newly started embryonic cell culture comprising a mixed cell population and often possessing a different chromosomal karyotype. Prolonged cell passage will also alter the biochemical composition of the cell membrane [11]. Also important, only in recent years has it become clear that the cell cycle plays a decisive role in the composition of the cell surface, and only the more recent studies take the cell cycle dependent alterations into full account. The above mentioned problems can be overcome in the ATV system, because sarcoma viruses are able to transform 80–90% of CEC within two days after infection [12], minimizing an influence of prolonged tissue culture on the plasma membrane. Furthermore, transformation-defective mutants have been isolated which are temperature sensitive in their oncogenic function [13]. This enables the comparison of freshly infected cells from the same chicken embryo growing either at permissive or non-permissive temperature.

IIIA. Lipids

Careful recent studies in the ATV-system allow some generalizations to be drawn about the effect of transformation on cell surface chemistry. Similar to the events following SV40 and polyoma virus transformation, ASV transformed CEC contain about 25% less sialic acid than uninfected CEC [14,15]. Avian leukosis virus infected, but untransformed CEC show the normal amount of sialic acid. Indirect evidence suggests that mainly the changes in glycolipids are responsible for the reduced sialic acid content. While infection of CEC with ALV causes only insignificant changes in the cellular glycolipid architecture, a decrease in some sialoglycolipids that parallels the rapid development of ASV-transformation in tissue culture has been described [16]. At the same time, the corresponding precursors, ceramide and lactosylceramide, accumulate, indicating a reduced activity of the specific sialyl transferases which normally would complete sialosyl-glycolipid synthesis. Studies with DNA tumor viruses showed that the enzyme sialyl transferase, which transfers sialic acid to lactosylceramide in the last step of hematoside synthesis, is greatly reduced to 15–30% of the normal value in the transformed cell membrane [17,18].

Hakomori et al. [16] furthermore observed that those glycolipids which are drastically reduced after transformation are the same that are highly increased in normal cells reaching confluency. These results could not, however, be confirmed by others [19], who found only a minor, if any, reduction in the profile of hematoside and higher gangliosides after ASV transformation. The cause of this contradiction is still unresolved.

A density dependent glycosyl extension response was also described for other normal cell lines, most detailed for normal hamster fibroblasts [11,20–24]. Longer neutral glycolipids are increasingly prominent as cultures reach confluency, paralleled by an increase in activity of the corresponding enzymes responsible for glycosyl chain elongation [22,23]. As mentioned above, transformed cells have a generally reduced amount of longer glycolipids and show no density-dependent glycosyl extension response [25,16].

As far as the phospholipids are concerned, Quigley et al. [26,27] found no significant differences in their content between normal and ASV-transformed CEC. The same authors stated furthermore that the phospholipid profile of the ASV-envelope qualitatively resembles that of the plasma membrane from which it is budding, showing only quantitative differences in the amount of individual phospholipid species in the viral and cellular membranes.

In summary, the glycolipid changes in transformed cells seem to reflect more the increased growth rate of the tumor cells than to represent a parameter specific for transformation.

IIIB. Glycoproteins

It has often been speculated that the relative presence or absence of glycoproteins might distinguish the cancer cell from its normal counterpart. The general approach used to characterize the possible differences was the isolation and purification of structural membrane components by treatment of either whole cells or cell membranes with mild detergents or with proteolytic enzymes. Less commonly, hypo- or hypertonic treatment of intact cells was also employed to dissociate external surface structures from the rest of the cell. The eluates were then usually submitted to chromatographic procedures or polyacrylamide gel electrophoresis for further analysis. It is very difficult to evaluate what degenerative changes occur to the membrane molecules during the various isolation procedures, especially those involving the actions of proteolytic enzymes. The comparison of putative differences in the glycoprotein profiles from normal and transformed cells should therefore always be related to the experimental techniques used [28].

The most consistent difference found in several tumor virus systems so far is the presence of larger fucose-containing glycopeptides in transformed cell membranes, which are, e.g., obtained after controlled trypsin and pronase digestion followed by Sephadex chromatography [29]. The association of these changes with the transformed phenotype of cells was clearly shown with the temperature-sensitive T5 ASV-mutant isolated by Martin [30]. T5 transforms and replicates in CEC at 35 °C, whereas at the non-permissive temperature of 41 °C it only replicates without inducing detectable changes in the normal phenotype of the cells. The larger fucose-containing glycopeptides can only be isolated from transformed, i.e. rounded and refractile CEC when grown at 35 °C, whereas at 41 °C the phenotypically normal CEC contain only minor quantities of these components, the elution profile being indistinguishable from that of uninfected CEC [31]. The transformation-associated glycopeptides seem to

contain also more sialic acid residues than their normal counterparts, as shown by their more extensive digestion by neuraminidase [31].

It is not quite clear to what extent an altered activity of sialyl transferases may be responsible for this change, particularly since seemingly conflicting results on its activity have been obtained, ranging from decreased [17,18,34], to unchanged [32] to increased [31,33] activity. The most likely explanation for these discrepancies is the possibility that the various laboratories have measured different kinds of the donor and acceptor specific sialyl transferases.

Very recently several reports have appeared correlating ASV-induced cell transformation with the disappearance or the significant diminution of cell membrane (glyco-) proteins [35–38]. It will be of great interest to characterize further the biochemistry of these molecules and, above all, to try to associate a function specific for normal cells with those cell surface structures.

IIIC. The differential interaction of cell surfaces with lectins

Lectins comprise a group of proteins which can be isolated from a variety of plant and animal tissues [39] and which share the property of causing cell agglutination. They are usually glycoproteins and have gained widespread attention since the discovery that some of them preferentially agglutinate transformed cells [40]. This effect, however, is strictly dose-dependent, and normal cells can also readily be agglutinated with higher doses of the corresponding lectin.

By using the two so far best characterized lectins, Concanavalin A isolated from Jack beans [41] and wheat germ agglutinin [42], transformed CEC could be agglutinated with low doses of Concanavalin A and wheat germ agglutinin [43–46]. The previous failure to see differential agglutination [47] could well have been due to the increased cellular mucopolysaccharide secretion paralleling transformation by some ASV-strains, since Burger and Martin [45] also found that pretreatment of the cells with hyaluronidase was necessary to detect the dose-dependent agglutinability. In their experiments, as well as in the study by Biquard and Vigier [44], temperature-sensitive virus mutants defective in their transforming ability were employed to demonstrate that increased agglutinability was strictly linked to the transformed phenotype of the cells.

It is not the aim of this review to discuss in detail the cause and implications of the facilitated agglutination of tumor cells by lectins, especially since several competent reviews have appeared very recently [48,49]. A few remarks, however, should be added here to outline the relevance of these investigations and to relate the surface alterations to the other changes described in this chapter.

Earlier assumptions that normal cells expose less agglutinin receptors on their cell surface turned out to be invalid when it was shown that normal cells bind approximately as much labeled Concanavalin A or wheat germ agglutinin as their transformed counterparts [50–54]. Topological studies by electron microscopy originally showed a different surface distribution of the bound lectins, which were evenly distributed on normal cells instead of the clusters observed on the surface of agglutinated tumor cells [51].

In more recent studies [54,55] target cell membranes were stained by labeled lectins immediately before fixation and examination by electron microscopy . A homogeneous surface distribution of the bound lectins was observed on both normal and tumor cell surfaces. This indicates that the patch-formation of cell-bound lectins is a secondary effect of the cell membrane, the mechanism of which is not yet clear.

In this context, it is important to note that mild protease treatment renders normal cells also agglutinable with low doses of lectins [129] and leads to a clustering of membrane-bound lectins [130]. The suggestion by Nicholson [130] that protease treatment increases the fluidity of the normal cell membrane has recently gained indirect experimental support by the demonstration of increased lateral diffusion of labeled Concanavalin A in the tumor cell membrane as compared to its mobility in the unmodified normal cell membrane [54,55].

It is of interest in this connection to remember that some new protease activities have been found to be associated exclusively with the tumor cell surface [33,56–59]. By autodigestion of cell surface glycopeptides these enzymes might lower the restraint on the lateral diffusion of agglutinin receptors, facilitating clustering and subsequently lectin agglutination.

Exceptions have been reported that broaden the concept of specificity of facilitated agglutination for transformed cells. Cells lytically infected with polyoma virus [60], SV40 virus [61], Adenovirus 12 [62], and Herpes simplex virus [63] all show increased lectin agglutinability within 18 h after infection. Likewise, the non-oncogenic Newcastle disease virus [63,64] and Vaccinia virus [65] render infected cells agglutinable a few hours after infection.

It has been proposed that lectin binding sites play a crucial role in cellular growth control. This hypothesis is indirectly supported by several lines of evidence. In their investigations of SV40-transformed 3T3 cell lines, Pollack and Burger [66] tested transformants and revertants for their saturation densities and agglutinability by wheat germ agglutinin and found both parameters to be inversely correlated. Similarly, Py-3T3 cells [67] or ASV-CEC [68,69] grown in the presence of phosphodiesterase inhibitors, assume normal morphology, exhibit increased growth control and become less agglutinable by lectins. Most support could be drawn from results reported by Burger and Noonan [70], who obtained evidence that addition of what was believed to be monovalent Concanavalin A to growing Py-3T3 cells restores the cells phenotypically to normal growth control. However, their reported procedures for splitting the normally tetrameric Concanavalin A molecules into monomeric subunits in fact led to a more drastic digestion [71], so that the Py-3T3 cells had in fact been treated with various-sized Concanavalin A fragments instead of with monovalent subunits. It would be desirable if other laboratories could substantiate this very important observation, making use of the same approach and also testing additional lectins and cell systems.

In summary, at the moment it seems still too early to draw any firm conclusions about the biological meaning of the tumor cell surface alterations that are detectable by the differential lectin agglutinability.

IV. IMMUNOLOGICALLY DETECTABLE CHANGES ON ATV-TRANSFORMED CELLS

IVA. In vivo studies of tumor antigens

Soon after the detection of tumor specific transplantation antigens (TSTA) in Polyoma virus-transformed cells [72,73], a similar cell surface antigen was also described for the ATV system. By using a variety of immunization procedures with tumor cells, transplantation immunity against challenge with viable syngeneic avian sarcoma virus (ASV)-transformed cells was obtained in mice [74–76]. The ASV specificity of the TSTA was proven by showing that the ASV tumors cross reacted immunologically with each other but not with tumors of chemical or different viral etiology [74,77,132]. Since ASV tumors in mammalian cells do not in general produce virus particles and since no virus-neutralizing antibodies could be detected, the antigens in question were not identical with virus envelope (Ve) antigens [77–79].

In vivo transplantation experiments also provided suggestive evidence that TSTA is group-specific for various ASV strains [80,131]. However, the three virus strains used in those early studies, namely SR-RSV, PR-RSV and BH-RSV, were not clone-purified and the possibility that a common virus was present in each stock was thus not excluded. Further support of the group-specificity of TSTA came from experiments in which mice could be immunized against challenge by syngeneic ASV tumor cells not only by transformed cells but also by avian myeloblastosis virus (AMV) injected soon after birth [79].

These results in mice were in contradiction to conclusions drawn from experiments in chicken which indicated that transplantation immunity was only induced by the Ve-antigen [81,82]. More recent experiments in chickens are, however, in full agreement with the data obtained in mice. Meyers et al. [83] were able to immunize chickens against challenge with ASV by injection with leukosis viruses of heterologous subgroup, i.e. possessing immunologically unrelated Ve antigen. Thus, ASV-transformed chicken cells, like mammalian ASV tumor cells, express ATV group-specific TSTA not identical with Ve-antigen.

Whether or not TSTA induced by ATV in different animal species were identical could not be clearly resolved by in vivo transplantation experiments. Cross reactions were observed between mouse and rat but not between mouse and hamster, rabbit or chicken ASV-tumors [77,84]. As outlined below, this question was clarified only after more controlled in vitro techniques became available.

IVB. In vitro studies of tumor antigens

1. Demonstration of two kinds of virus-directed cell surface antigens. After the in vivo transplantation experiments described above had demonstrated the existence of TSTA in ATV-transformed cells, in vitro experiments were started to investigate in greater detail the nature of the virus-induced cell surface changes. For purposes of this review, the term TSTA will be used for the tumor antigens detectable by in vivo experiments, whereas the cell surface alterations found by in vitro techniques will be designated tumor-specific surface antigens (TSSA). It can be assumed that

TABLE II

CYTOTOXIC EFFECT ON ATV-INFECTED CEC BY SPLEEN LYMPHOCYTES ISOLATED FROM ATV-IMMUNIZED CHICKENS

Refer to Table I for explanation of Abbreviations. ASV and ALV respectively indicate whether the virus strain does or does not transform CEC in vitro; the capital letters A through D indicate the subgroup of the respective virus strain.

Target CEC infected by virus strain	Lymphocyte effector cells isolated from chickens immunized with:				
	RAV-I (ALV-A)	NC-SRV-I (ALV-A)	SRV-I (ASV-A)	B-77 (ASV-C)	SRV-H (ASV-D)
uninfected	—[a]	—	—	—	—
RAV-1 (ALV-A)	+	+	+	—	—
NC-SRV-1 (ALV-A)	+	+	+	—	—
SRV-1 (ASV-A)	+++	+++	++++	+++	+++
MAV-B (ALV-B)	—	—	—	—	+
B-77 (ASV-C)	++	++	+++	++++	+++
NC-SRV-H (ALV-D)	—	—	—	—	+
SRV-H (ASV-D)	++	++	+++	+++	++++

[a]The score corresponds to a usual target cell killing rate of:

—: < 10%
+: 10–50%
++: 50–70%
+++: 70–100%
++++: 90–100%

100% survival = target cell number after incubation of uninfected CEC with immune chicken spleen cells.

TSTA are at least in part identical with TSSA, however, formal proof was lacking until explanted tumor cells were immediately characterized in vitro as will be outlined below.

The expression of ATV-induced TSSA on transformed CEC was first shown in vitro by the immunoferritin-technique [85] and by cellular microcytotoxicity tests [86]. The basic experimental approach with the two methods was similar. Chickens were appropriately immunized with ALV- or ASV-strains of different subgroups and their immunglobulins or their spleen or blood lymphocytes were isolated. The IgG fraction was used in an indirect immunoferritin-technique [87] to stain cultured CEC infected with different ALV- or ASV-strains (Fig. 1) [85]. Sensitized lymphocytes were likewise incubated with virus-infected CEC and their cytotoxic effect was measured [86]. Both studies independently reached similar conclusions, which for the cellular cytotoxic tests are summarized in Table II. The pattern of reaction can best be explained by postulating two sets of antigens which mediate the reactions: (a) the subgroup-specific viral envelope antigens, and (b) ATV group-specific TSSA expressed on all cells transformed by any of the tested ASV-strains.

(a) The Ve-antigens. The responsibility of Ve-antigens for some of the immunological reactions was directly seen with the electron microscope. In the same

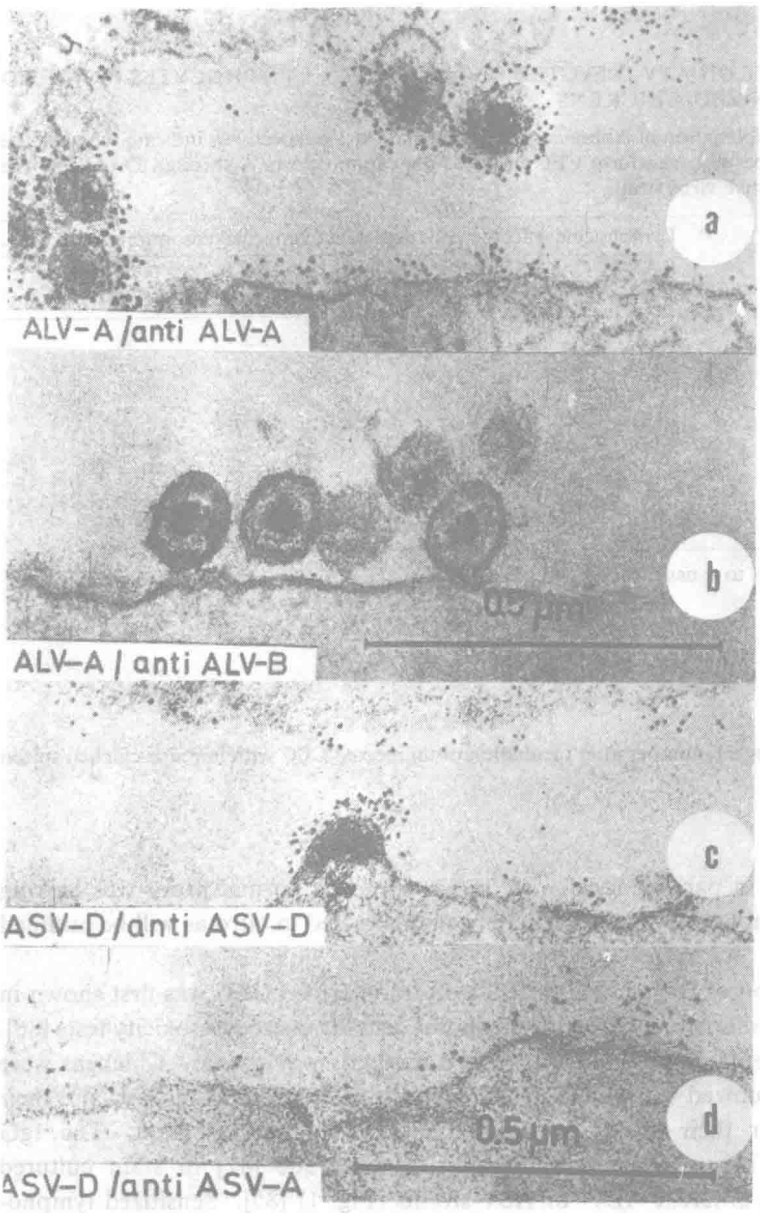

Fig. 1. Immuno-ferritin staining [85] of chicken fibroblasts either infected by ALV or transformed by ASV. (a) Detection of Ve antigen by homologous ALV antiserum; (b) negative control for (a); (c) detection of Ve antigens as well as TSSA by homologous ASV antiserum; (d) detection of TSSA by ASV antiserum of heterologous subgroup specificity.

picture both budding viral particles and Ve-antigen located at budding-free cell surface sites of ALV-infected cells were stained by antisera prepared against viruses of the homologous (Fig. 1a) but not of heterologous subgroup (Fig. 1b) [85]. Likewise,

the cytotoxic effect of immune lymphocytes when tested on ALV-infected cells followed the subgroup pattern (Table II).

(b) The TSSA-system. ASV antisera or immune lymphocytes from immunized chickens both showed the same subgroup specific reaction when tested in vitro against ALV-infected chicken cells. However, they reacted group-specifically when tested against ASV transformed cells (Fig. 1d) [85,86]. This was taken as proof for the existence of group-specific TSSA present on transformed but absent from productively infected untransformed cells.

There was, however, one discrepancy in the results obtained by the two methods. IgG from ALV-immunized chickens did not stain the surface of target cells transformed with ASV of other subgroups. This means that the IgG recognized only the Ve-antigens of the subgroup of the ALV used for immunization. In contrast, ALV-sensitized lymphocytes exerted a destructive effect on all transformed target cells irrespective of the subgroup of the in vitro transforming virus strain (Table II). Two reasons may account for this extended specificity.

First, TSSA are cell membrane antigens which might induce a predominantly cellular immune response. Second the lymphocytes were taken from animals injected usually three times with ASV over a period of three months, whereas the IgG in the other study came from animals bled four weeks after a single virus infection. It must be kept in mind that ALV [88] are oncogenic in vivo after a long incubation period of up to one year. The detectability of anti-tumor immunity in the leukosis virus-infected host months before the clinical manifestation of the tumor underlines the value of immunological techniques in the diagnosis of small, progressively growing tumors.

The cytotoxic effect via the Ve-antigen alone, e.g. by Rous-associated virus-1 sensitized spleen lymphocytes on CEC infected by non-converting mutants of Schmidt-Ruppin strain 1 obtained after hydroxylamine-treatment, as shown in Table II, is weak compared to the massive cell destruction seen whenever the target cells are transformed. This is the more surprising since the number of Ve-antigenic sites expressed on the fibroblastic cell membrane is much higher than the number of TSSA sites (unpublished observations). The less pronounced cytotoxic effect observed when only Ve-antigens are involved can in part be explained by the fact that free virus particles synthesized by the target cells in culture absorb to the sensitized lymphocytes during the incubation period of the cytotoxic assay. The weak cross-antigenicity of viral envelope antigens of subgroups B and D, first seen in neutralization experiments [90,91], is also demonstrable by the moderate killing effect of Schmidt-Ruppin strain H (subgroup D) sensitized lymphocytes on MAV-B infected target cells.

2. Common tumor antigens on ATV-transformed cells of different species. Evidence for a cross-antigenicity of TSTA induced by the same virus in cells of different species was first obtained for the DNA-containing tumor viruses, SV40 [92] and Polyoma [93]. Evidence accumulated since then by several laboratories can be interpreted to show that papovaviruses induce virus type-specific as well as virus group-specific tumor transplantation and/or surface antigens. The relationship between the