Differential Induction of Tumour Antigens by Transformation-defective Virus Mutants

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SUMMARY

Normal rat kidney cells infected by a variety of transformation-defective temperature-sensitive avian leukosis sarcoma virus mutants were tested for the expression of transformation characteristics at permissive and restrictive temperature. Morphology, growth behaviour and agglutinability by concanavalin A corresponded fully to the phenotype of the infected cells: at permissive temperature the cells resembled wild type virus transformed cells, whereas when grown under restrictive conditions they became virtually indistinguishable from normal cells.

The quantitative expression of allo- or xenogeneic cell surface antigens was not significantly affected by the phenotype of the cells. Two out of the five tested mutants induced tumour antigens in the expected temperature-dependent manner, whereas the other three mutants were able to induce tumour-specific cell surface antigens even in the revertant cells cultured at the restrictive temperature.

These findings extend previous results about tumour antigen induction in mutant-infected cells of the natural host, the chicken embryo fibroblasts. The value of transformation-defective tumour antigen-positive mutants for vaccination purposes will be discussed.

INTRODUCTION

In animals, oncogenic viruses are known to induce tumour-specific cell surface antigens (TSSA; Klein, 1966; Beveridge, 1973; Bauer, 1974), which may elicit a cytotoxic immune reactivity in immunized hosts and in certain situations enables the host to reject the tumour. Cell transformation by all tested avian leukosis sarcoma virus (ALSV) strains results in the expression of a common TSSA (reviewed in Bauer, 1974; Kurth & Bauer, 1975). This holds true not only for transformed cells in the natural host, i.e. chicken embryo fibroblasts or myeloblasts, but also for avian sarcoma virus (ASV)-transformed mammalian cells (Kurth & Bauer, 1972a; Gelderblom & Bauer, 1973). TSSA is a 100 000 mol. wt. glycoprotein (Bauer et al. 1975). It is not known whether these molecules are virus- or cell-coded, nor is anything known about their function. TSSA is inserted in the plasma membrane but is distinct from virus envelope components also present at this location (Gelderblom, Bauer & Graf, 1972; Kurth & Bauer, 1972b). As in several other experimental tumour virus systems (Alexander, 1972), the retrogenetic expression of tumour associated embryonic antigens has also been demonstrated in ASV-transformed cells, but these antigens are distinct from TSSA (Kurth & Bauer, 1973a).

Immunization against cancer, already a reality in two animal neoplastic diseases (Beveridge, 1973) may also be possible for some forms of human malignancy if viruses inducing
TSSA are eventually implicated in their causation. The history of vaccine development has shown that the most effective and reliable vaccination procedures involve the use of attenuated, but living and replicating bacteria or viruses. The recent isolation of temperature-sensitive mutants defective in their transforming function(s) (Toyoshima & Vogt, 1969; Martin, 1970; Kawai & Hanafusa, 1971; Wyke, 1973; Wyke & Linial, 1973) but not in their ability to replicate, provided an animal model to investigate the temperature-sensitivity of TSSA-induction and of other transformation markers in mutant-infected cells.

In a recent report we described TSSA-expression in chicken fibroblasts by some, but not all tested mutants under conditions non-permissive for the expression of other parameters of transformation (Kurth et al. 1975). This result prompted the present investigation of the phenotype of mutant infected mammalian cells. For these cells, the transforming potential of the mutants at the permissive temperature and the temperature-sensitivity of the induced transformation-characteristics had to be demonstrated first, before the effect on antigen, and in particular tumour antigen expression could be tested.

The studies may elucidate host control mechanisms of the expression of the transforming virus gene(s). Furthermore the transformation-defective but tumour-antigen positive mutants may be useful for vaccination procedures against tumour antigens.

**METHODS**

*Cells and viruses.* Normal rat kidney cells from an outbred Osborn-Mendel rat (Duc-Nguyen, Rosenblum & Zeigel, 1966) infected by the transformation-defective, temperature-sensitive avian oncornavirus mutants *ts LA* 23 Prague (PR); *ts LA* 24 PR; *ts LA* 25 PR and *ts LA*29 PR (abbreviated *ts* 23, *ts* 24, *ts* 25, and *ts* 29 respectively) were obtained from Dr J. A. Wyke, Imperial Cancer Research Fund Laboratories, London. The biological and transforming properties of these transformation-defective (T-class) mutants have been described in detail (Wyke & Linial, 1973). Each of the above mutants belongs to a different cooperative-transformation group (Wyke, Bell & Beamand, 1975). Mutant-infected cells are designated NRK-23, NRK-24, NRK-25 and NRK-29, respectively. The Prague strain of ASV represents the mutants’ wild-type parental virus strain, the respective transformed NRK cells being designated NRK-PR. The expression of the transformed phenotype of mutant-infected cells is temperature dependent. They display the characteristics of transformed cells at 37 °C, and revert to a normal phenotype at 40 °C. It is crucial to maintain exactly the non-permissive temperature of 40 °C ± 0.5 °C, because higher temperatures are not tolerated by rat cells and lead to facilitated agglutination by concanavalin (con) A and an increased non-specific absorption of immunoglobulins, especially the iodinated preparations. The latter observation is in agreement with the general finding that iodinated proteins tend to stick non-specifically to other proteins, in particular cell debris (Nossal *et al.* 1972).

Cloned NRK cells infected by the transformation- and replication-defective (coordinately defective: C-class) ALSV-mutant *ts LA* 339 B77 were generously provided by Dr T. Graf, Max Planck-Institut für Virusforschung, Tübingen. Established cell lines from the two subclones NT3 (NRK-339-3) and NT4 (NRK-339-4) are temperature-sensitive in their expression of transformation functions (Graf & Friis, 1973) and were included in the studies on tumour antigen expression at restrictive (37 °C) and permissive (33 °C) temperature. NRK-339-r3/2 is a revertant of the NRK-339-3 line which had reverted to a temperature independent expression of the transformed phenotype.

Mutant- or wild-type-infected NRK cells do not synthesize infectious avian oncornavirus particles (Graf & Friis, 1973; J. Wyke, personal communication).


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_Sera._ Three types of antisera were prepared, namely against xenogeneic, allogeneic, and tumour-specific cell surface antigens.

For the recognition of xenogeneic antigens, adult male New Zealand white rabbits were immunized with $10^7$ NRK cells given intravenously three times a week for 2 weeks. The rabbits were bled from the ear 1 week later. Pre-immunization sera provided the controls.

For immunization against alloantigenic determinants, cloned uninfected NRK cells derived from an Osborn-Mendel rat were maintained overnight in serum-free medium, harvested by EDTA-treatment and injected into inbred Wistar rats. The rats received weekly intraperitoneal injections of $10^8$ cells each for 4 weeks. After a further 6 days the rats were bled out. Normal Wistar rat serum from the same inbred stock served as a negative control.

The chicken serum (no. 64) obtained after immunization with a sub-tumourigenic dose of the Schmidt–Ruppin strain H of ASV (subgroup D) was described previously (Kurth & Bauer, 1972a, 1973a). This serum possesses a high titre of anti-TSSA antibodies and also recognizes tumour-associated retrogenetically expressed embryonic antigens (Kurth & Bauer, 1973a). It furthermore contains antibodies against the subgroup-and group-specific virus envelope glycoproteins (L. Rohrschneider, personal communication). An absorption of this antiserum to the infected NRK cells via virus envelope components is, however, unlikely because these cells are firstly non-producers and are secondly infected by virus strains derived from the subgroups A or C. Furthermore, rabbit antisera prepared against isolated ALSV-group and subgroup-specific envelope antigens had no cytotoxic effect on mutant-infected NRK cells. Normal and anti-TSSA chicken sera were absorbed on monolayers of glutaraldehyde-fixed normal NRK cells and cleared by low (300g/45 min) and high (200 000g/45 min) speed centrifuging.

The indirect antiglobulin-technique. For the indirect antiglobulin-technique, rabbits were immunized with 1.5 mg purified chicken or rat immunoglobin emulsified in FCA and injected subcutaneously at different sites. Anti-rabbit IgG was produced in sheep by injecting 25 mg rabbit IgG emulsified in FCA subcutaneously, followed 4 weeks later by an intravenous booster with 15 mg. The IgG fractions were isolated as described before (Kurth & Bauer, 1973a).

Interest was focused on the quantitative expression of different types of antigens in the plasma membrane of _ts_ mutant infected cells grown under permissive or restrictive conditions. The indirect antiglobulin-technique (sandwich-test) was preferred to the cytotoxic assays used previously, because in cytotoxic tests only transplantation type antigens will be recognized, and because the antiglobulin-technique is more sensitive than cytotoxic tests based on $^{51}$Cr release (Kurth & Bauer, 1973a).

Isolation and iodination of immunoglobulins was described earlier (Kurth & Bauer 1973a; Kurth _et al._ 1975). The anti-antibody immunoglobulin preparations of rabbit or sheep origins were iodinated with $^{131}$I by the chloramine T method and absorbed with fixed normal and wild-type Prague-ASV-infected NRK cells before use.

Normal and infected NRK target cells were seeded into the 96 wells (16 mm diam.; 2 cm² culture area) of Linbro 96 CV-TC plates and maintained for usually three days at the desired test temperature. Care was taken that all target cell types had reached a density of about $1 \times 10^6$ cells/cm² on the day of test, i.e. were still dividing. The use of Linbro 96 CV-TC plates offers the advantage that all cell types to be tested can be grown on one or two plates under very similar culture conditions, with the possibility of using parallel cultures for serum titrations and for cell counts.

Prior to incubation with antisera, cultures were washed once by immersing the entire plate in PBS and rocking it gently for 5 min on a platform before pouring off the PBS. Cell
density for each cell type was then determined by trypsinization of cells from two parallel cultures. The counts usually differed by less than 10%.

 Cultures to be incubated with antibodies were fixed with 0.125% glutaraldehyde (5 min) and again washed twice in PBS. These cells received dilutions of unlabelled normal serum or antisera and were incubated at room temperature for 60 min on a rocking platform. After three washes, the [\(^{125}\)I]-labelled second IgG preparation recognizing those antibodies of the first serum which had bound to the cells, was added in 0.1 ml volumes. This second incubation was terminated after another 30 min by aspirating the supernatant fluid and four washes in PBS, each for 5 min as described above. The fifth and final wash consisted of 0.4 ml KCl/HCl buffer (pH 1.0; 5 min) to break up antigen-antibody interactions (Anderson & Dresser, 1970; Sjögren et al. 1971). 0.3 ml samples of this buffer were transferred to scintillation vials containing 5 ml Aquasol (NEN, Boston, U.S.A.) and their radioactivity was assessed. The number of antibody molecules bound per cell was calculated according to the average cell number per culture, knowing the specific radioactivity of the second immunoglobulin preparation and assuming a mol. wt. of 150,000 for the IgG. The difference of binding of specific IgG minus binding of normal IgG was defined as specific adsorption.

Agglutination by concanavalin A. Concanavalin A (con A) was obtained from Sigma Chemicals Co. (St Louis, Mo., U.S.A.). We followed the experimental procedure described previously (Kurth & Bauer, 1973 b), i.e. harvesting subconfluent, growing cells with 0.02% EDTA, and, after appropriate washings, adding 2 × 10⁴ cells in 5±l volumes to 5 ±l con A-dilutions pre-dispersed into the 20 ±l wells of Falcon microplates I (Falcon Plastics, Los Angeles, U.S.A.). The plates were inverted and hanging drops were inspected microscopically after 15 min incubation at room temperature. Agglutination was scored as described in Table I.

RESULTS

The growth behaviour of ts-mutant infected NRK cells

At non-permissive temperature, the ts-mutants used in this study are known to be defective in a transformation function in chicken embryo fibroblasts (Toyoshima & Vogt, 1969; Wyke, 1973; Wyke & Linial, 1973). Similarly, the most striking characteristic with mutant-infected NRK cells is the disappearance of the transformed phenotype under restrictive conditions. Phenotypically reverted NRK-cells are generally less refractile, more elongated and exhibit a more evident cytoplasm than their transformed counterparts. The morphological changes of NRK cell clones infected by the C-class mutant ts 339 have been described by Graf & Friis (1973). Additional studies with these cells have demonstrated that other parameters characteristic for the transformed state of a cell, e.g. sugar uptake, agglutinability by con A, and growth in agar suspension are likewise temperature-dependent (Graf & Friis, 1973). The phenotypes of normal and wild-type-infected NRK cells are not affected by such temperature shifts.

The growth behaviour of infected NRK cell lines is illustrated in Fig. 1. At the permissive temperature (Fig. 1 a), all mutant-infected cells show exponential growth after an initial lag phase of one day. It became obvious, however, that some transformed cells grow considerably more slowly than others, e.g. comparing NRK-339 with NRK-23. In contrast to all transformed cells, uninfected NRK cells stop dividing after reaching a saturation density of about 8 × 10⁶ cells/cm².

All cells cultured at the restrictive temperature (Fig. 2 b) start growing only after a longer lag period and multiply generally at a slower rate. The uninfected as well as all mutant-infected NRK cells show a density-dependent growth behaviour, ceasing to divide at
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Fig. 1. Growth of NRK cells infected by transformation-defective ts mutants at permissive (a) and restrictive temperature (b). Cells were seeded at a density of $0.5 \times 10^6$ cells/cm$^2$ in 16 mm Linbro plates on day 0 and counted every 24 h thereafter. Counts represent the average of two parallel cultures: ×--x, NRK; ●—●, NRK-PR; □—□, NRK-23; ■—■, NRK-24; △—△, NRK-25; ▲—▲, NRK-29; ◆—◆, NRK-339-3; ○—○, NRK-339-4.

densities between 2 and $5 \times 10^5$ cells/cm$^2$, when cell layers have just reached confluency. This plateau in cell number is not due to cell loss from the substrate, since practically no cells can be detected in the supernatant medium. Under restrictive conditions, only the wild-type-infected NRK cells display an exponential growth capacity, suggesting an intrinsic density-independent growth stimulus. The cells resume growth within 48 h after downshift (not shown).

Agglutinability by concanavalin A

Increased agglutinability by plant lectins is another criterion of tumour cells (for a recent review see Burger, 1973). In the avian oncornavirus system, the temperature-sensitivity of facilitated agglutinability was previously demonstrated for chicken fibroblasts infected by the transformation-defective mutant T5 (Burger & Martin, 1972) and for NRK cell lines infected by ts 339 (Graf & Friis, 1973). In Table 1, these observations are extended to NRK cell lines infected by T-class mutants. Without exception, phenotype reversion to normal morphology at restrictive temperature is accompanied by a loss of preferential agglutinability by a given dose of con A.

The expression of cell surface antigens

The above observations indicate a correlation between the expression of phenomena characteristic of cell transformation and the function of the transforming gene product(s) of ts mutants. It remained to be seen whether the quantitative expression of different types of cell surface antigens, in particular of tumour-specific plasma membrane antigens was also affected by the phenotype of the cell.

Fig. 2a shows a typical titration curve obtained by incubating a constant number of
<table>
<thead>
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<th>Cell type</th>
<th>Temp. (°C)</th>
<th>Concentration of concanavalin A (μg x ml⁻¹)</th>
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<tr>
<td>NRK</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>NRK-PR</td>
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<td>0</td>
</tr>
<tr>
<td>NRK-29</td>
<td>37</td>
<td>0</td>
</tr>
</tbody>
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* The degree of agglutination is scored as follows: = 90 to 100 %, + = 70 to 90 %, ++ = 50 to 70 %, +++ = 30 to 50 %, ++++ = 0 to 30 %, single cells counted per incubation volume.

Fig. 2. Indirect antiglobulin-technique: detection of (a) xenogeneic or (b) allogeneic cell surface antigens. The same NRK cells infected by transformation-defective, temperature-sensitive ALSV mutants as listed in Fig. 1 were used as target cells. (a) Absorption of antibodies from normal or anti-NRK rabbit sera. (b) Absorption of antibodies from normal or anti-NRK Wistar rat sera. The serum quantities were all diluted to 200 μl using veronal buffer and added to the 16 mm parallel culture plates. Absorption was quantitative by staining the immunoglobulins of the first sera by [¹²⁵I]-labelled anti-immunoglobulin (20 μg [¹²⁵I]-IgG in 0.1 ml veronal buffer/16 mm plate). (a) Sheep anti-rabbit IgG immunoglobulin, (b) rabbit anti-rat IgG immunoglobulin. Specific absorption = binding of antibodies from antisera minus binding of antibodies from pre-immunization sera. The first standard deviation is given. ■■■, target cell grown at permissive temperature (37 °C); □□□□, target cells grown at restrictive temperature (40 °C).
Fig. 3. Indirect antiglobulin-technique: detection of tumour-specific cell surface antigens. Absorption of antibodies from normal and anti-TSSA chicken immunoglobulin preparations to parallel cultures of NRK cells infected with transformation-defective, temperature-sensitive avian oncornavirus mutants. The symbols for the cell types are the same as listed in Fig. 1. Target cells were either grown at permissive (a) or restrictive temperature (b). The immunoglobulin dilutions were all brought to a final volume of 100 μl using veronal buffer and added to the 16 mm culture plates. Absorption was measured by staining the chicken immunoglobulins with $^{125}$I-labelled rabbit anti-chicken IgG immunoglobulin (2 μg rabbit IgG in 0.1 ml veronal buffer/16 mm plate). Specific absorption = binding of antibodies from anti-TSSA serum minus binding of antibodies from normal chicken serum.

cells with increasing amounts of normal or anti-NRK rabbit sera until maximum absorption is reached. The rabbit antiserum recognizes the xenogeneic antigens expressed on the rat kidney cell surface, and no gross difference can be detected in the absorption pattern of cells infected with various virus mutants and grown either at permissive or restrictive temperature, with the possible exception that maximum absorption of antibodies at 37 °C is generally slightly higher than at 40 °C.

The quantitative characterization of avian oncornavirus-induced TSSA turned out to be technically considerably more difficult than the measurement of xeno- or alloantigens. Using chicken immunoglobulins instead of whole sera in the first incubation in order to lower non-specific absorption, between 35 to $45 \times 10^3$ labelled antibodies bound maximally to each mutant transformed cell grown under permissive conditions (Fig. 3a and 4a). Uninfected NRK cells generally displayed a residual absorption of anti-tumour immunoglobulins despite pre-absorption of the immunoglobulin preparations as described above. The situation with mutant-infected NRK cells grown and tested under non-permissive conditions was considerably more complex (Fig. 3b and 4b). Under these circumstances TSSA expression in the cells was: (a) NRK-29 and NRK-339-3 cells adsorb only few, if any, anti-TSSA antibodies, that is absorption returns almost to the non-specific level shown by uninfected NRK cells; (b) NRK-25 and NRK-339-4 cells show a lowered but clearly positive absorption of anti-TSSA antibodies; (c) wild-type infected NRK-PR, the revertant NRK-339-r3/2 as well as NRK-23 and NRK-24 cells all exhibit a temperature-independent expression of TSSA.
DISCUSSION

The T-class mutants of ASV investigated here for their effect on cell surface antigen expression are phenotypically indistinguishable from the wild-type parental strains at 37 °C, whereas at 40 °C they do not transform NRK cells.

The transforming potential of all tested T-class mutants is demonstrable by comparing morphology (not shown here), growth pattern (Fig. 1) and agglutinability by Con A (Table 1) of infected NRK cells grown either under permissive or restrictive conditions. At the permissive temperature the cells display the transformed characteristics of wild-type infected NRK cells, whereas at the non-permissive temperature cells become more or less indistinguishable from uninfected NRK cells. The temperature-dependence of the cells' phenotype was furthermore underlined in temperature-shift experiments (not shown here).

The quantitative expression of xenogeneic (Fig. 2a) and allogeneic (Fig. 2b) antigens on exponentially growing, mutant-infected NRK cells does not seem to be grossly affected by the phenotype of the cells. The only generalization that may be made is the slightly lowered expression of both types of antigens on cells grown under restrictive conditions.

For the studies on tumour antigen expression, NRK cell lines infected by the C-class ts 339 mutant were included. At permissive temperature, all tested T- and C-mutants are able to induce approx. as many tumour-specific surface antigen determinants as the respective parental wild-type strains, leading to an absorption of 35 000 to 45 000 labelled antibodies per cell. This level of absorption is slightly higher than found for transformed CEF (Kurth et al. 1975). For technical reasons discussed elsewhere (Kurth et al. 1975), it probably represents a minimum estimate.

Similar to the results obtained with infected chicken embryo cells, absorption of anti-TSSA antibodies at restrictive temperature did not return to background values in all mutant-infected cells (Fig. 3b and 4b). Only NRK-29 and NRK-339-3 cells showed no clear
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TSSA expression under non-permissive conditions. In these cases, phenotypic normalization corresponded well with the relative absence of tumour antigen expression.

The NRK-25 and NRK-339-4 cell lines absorbed a lowered, but still clearly demonstrable amount of anti-TSSA antibodies. This finding is not altogether surprising since it has been shown before that ts 25 is a leaky mutant (Wyke & Linial, 1973) and that both CEF infected with ts 25 (Kurth et al. 1975) and NRK-339-4 cells (Graf & Friis, 1973) display some, but not all characteristics of transformed cells even at non-permissive temperature. Thus both NRK-25 and CEF-25 cells grown under restrictive conditions show a residual expression of tumour antigens as a marker for the residual function of the temperature-sensitive mutant gene product(s).

NRK cells infected by the wild-type Prague strain virus, the revertant NRK-339-r3/2 cell line as well as the NRK lines infected by the two mutants ts 23 and ts 24 seem to express an unaltered level of TSSA at both permissive and restrictive temperature. Whereas in the case of the wild-type and revertant infected cell lines this result could have been anticipated, it was unexpected for the ts 23 and ts 24 infected NRK cells. These two mutants are also able to sustain TSSA-expression in infected CEF at non-permissive temperature, whereas 5 other T- or C-class mutants induce TSSA in the expected temperature-sensitive manner (Kurth et al. 1975). Thus, in the case of rat kidney and chicken embryo fibroblast cells, the origin of the host cell does not seem to influence or regulate differentially the expression of this temperature-sensitive transforming virus function(s). This is further evidence that TSSA expression is under control of a virus gene function and may be even a virus-coded product.

TSSA antigen isolated from transformed chicken fibroblasts, however, is a glycoprotein molecule, mol. wt. 100 000 (Bauer et al. 1975), which, if directly coded by the virus, would require a considerable portion of the virus genome (mol. wt. possibly as low as 3.4 x 10^6; Beemon, Duesberg & Vogt, 1974; Billeter, Parsons & Coffin, 1974). On the other hand, the virus may code only for a smaller part of the TSSA molecule or for a smaller protein which in turn induces the transformed cell to synthesize TSSA. The latter mechanism would have to be a very common one to allow the induction of cross-antigenic TSSA in such different species as chicken, mouse, rat, or hamster cells (Kurth & Bauer, 1972a; Gelderblom & Bauer, 1973). The number of mutants and of cell types investigated so far is, however, still too small to decide whether tumour antigen induction by wild-type viruses or by transformation-defective mutants can vary in different host cells. For practical reasons concerning anti-tumour immunization (see below), this question should be studied further by infecting histologically different cells of allo- or xenogeneic origin. The inverse correlation between ALSV-transformation and TSSA-expression is stringent, i.e. no transformed cells have yet been detected which do not express TSSA.

On the other hand, the demonstration of TSSA-induction in phenotypically normal cells shows that these antigens are not restricted to transformed cells. Two interpretations can be put forward to explain the discrepancy between phenotype and antigenicity. Ts 23 and ts 24 are late mutants which, in the natural host, are not replication-defective (Wyke & Linial, 1973). Even though infected NRK cells do not synthesize infectious oncornavirus particles, the virus mutants may well induce some, e.g. TSSA, but not all transforming proteins. However, in the NRK-23 and NRK-25 cells the presence of TSSA alone is obviously not sufficient for cell transformation, and the temperature-sensitive block may occur at a later stage in the transforming process.

An alternative explanation would be that the TSSA molecules themselves are the ts virus gene products. In that case the block in transformation would be caused by the temperature-
sensitivity of the configuration of the TSSA molecules themselves. Thus TSSA may still be immunologically recognizable, but functionally defective.

In animals, vaccination against tumours has become possible in at least two neoplastic diseases: Marek's disease of chickens, using live virus, and fibropapillomatosis of cattle, using killed virus (reviewed by Beveridge, 1973).

The isolation of tumour antigen-positive transformation-defective mutants may provide an approach for the development of vaccine virus strains against neoplasia. Temperature-sensitive mutants of oncogenic viruses, however, are obviously not suitable to be used for vaccination, because of the relatively high frequency of revertants and the possibility that endogenous virus functions may repair the mutant's defect by complementation or recombination.

There are two possibilities that might overcome these complications. First, as described in this report, transformation-defective mutants could be introduced into allo- or xenogeneic cells where they still induce immunologically cross-reactive tumour antigens. The use of histoincompatible TSSA-positive cells and of mutants instead of wild type virus may exclude the possibility of rescue of the transforming virus function(s) by endogenous viruses. Since histoincompatible cells would be eventually eliminated, they might be useful for vaccination in such a way as to avoid antigenic competition (Pross & Eidinger, 1974; Taussig, 1974) and immunological enhancement (Hellström & Hellström, 1974) and to produce protective immunity against tumours.

Alternatively, TSSA-positive transformation-defective deletion mutants may be useful if they are not repaired by endogenous virus functions. The two deletion mutants of ASV tested so far, originally selected for their inability to transform chicken fibroblasts in vitro (Graf et al. 1971), were, however, also unable to induce tumour antigens in fibroblasts (Gelderblom, Bauer & Graf, 1972; Kurth & Bauer, 1972b). Experiments have been started to select specifically for transformation-defective, but replicating deletion mutants that are still able to induce tumour-specific surface antigens in infected cells.

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REFERENCES

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