Differential Expression of Relevant Rous Sarcoma-associated Antigens in Cultured Cells

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SUMMARY

These studies compared the abilities of each of normal chicken embryo fibroblast (CEF) cells, Rous sarcoma virus-transformed CEF cells, and Rous sarcoma (RS) tumor cells derived from the wings of chickens with growing neoplasms, as well as extracts and supernatant fluids of each of these cell types, to interact with the lymphocytes of chickens bearing tumors induced by Rous sarcoma virus. Cultured RS cells were more susceptible in Cr-release cytotoxicity assays to killing by the splenic lymphocytes of RS-bearing chickens than were Rous sarcoma virus-transformed CEF cells which, in turn, were more susceptible than normal CEF cells. In contrast, extracts (3 M KCI derived) of both RS cells and transformed CEF cells were equally capable of eliciting blastogenic responsiveness of circulating lymphocytes obtained from birds with tumors, while, in the case of supernatant fluids, significant reactivity was detected only when material derived from cultures of transformed CEF cells was used. Similar levels of responsiveness against such culture supernatant fluids were observed when circulating lymphocytes of chickens infected with avian leukosis viruses were studied. Lymphocytes of normal chickens served as control. These results point to a different expression of relevant detectable tumor-associated antigens in these various cell types. Scanning electron microscopy revealed few morphological differences between RS cells and transformed CEF cells, although both displayed considerably greater numbers of surface ruffles and microvilli than did normal CEF cells.

INTRODUCTION

In recent years, a number of laboratories have carried out extensive immunological studies on the avian RS system as a means of understanding antigenic changes that accompany virus-induced oncogenesis. Most of this work has been carried out with cultures of normal CEF cells and with CEF cells infected and/or transformed by various members of the ALSV group. On the basis of such studies, it has been shown that at least 2 distinct cell surface antigens are associated with CEF cells of virus-transformed as opposed to normal phenotype: an ALSV subgroup-specific envelope (Ve) antigen and a group-specific TSSA (6, 13, 14). More recent experimentation has revealed that these antigens represent the major viral glycoprotein of M.W. 85,000 and a nonviral cell surface glycoprotein of M.W. 100,000, respectively (4, 22).

In contrast, most of the studies from our laboratory have been performed with cultured Rous tumor cells derived from the wings of birds with growing neoplasms rather than in vitro-transformed CEF cells as targets. While not providing additional information on the respective roles of Ve and TSSA antigens in the antitumor immune response, we have shown that the antitumor cellular and humoral immune response is more effective as measured in vitro, when assayed against autochthonous as opposed to allogeneic tumor cells (29, 30). These observations suggested the existence of unique, individual-specific antigenic configurations on the surfaces of these neoplasms, in addition to the previously described antigens which reflect the activities or presence of the oncogenic agent.

In this communication, we present evidence that the effectiveness of the cellular in vitro response may vary considerably with the type of target tissue used. Specifically, we have shown that Rous tumor cells, grown in vitro, are more susceptible to killing in cell-mediated cytotoxicity tests than are RSV-transformed CEF cells. In contrast, supernatant fluids but not 3 M KCI extracts of RSV-transformed CEF cells were far more stimulatory in lymphocyte blastogenesis assays than were similar materials obtained from cultures of tumor cells. These findings provide evidence for a differential expression of relevant RS-associated antigens in these various cell types.

MATERIALS AND METHODS

Virus. The following strains of avian tumor viruses were used in these experiments: Schmidt-Ruppin, subgroup A (SR-A), kindly provided by Dr. G. S. Martin, Imperial Cancer Institute, London, England; PrB, subgroup B, kindly provided by Dr. D. Blair, University of Western Ontario, London, Ontario, Canada; and B77, subgroup C, RAV-1, subgroup A, RAV-49, subgroup C, all kindly supplied by Dr. E. R. Phillips, Lady Davis Institute for Medical Research, Montreal, Quebec, Canada. All viruses were propagated in cultures of CEF according to a previously described procedure (27). In the case of the PrB virus, polybrene (2 μg/ml) was used. In recent years, a number of laboratories have carried out extensive immunological studies on the avian RS system as a means of understanding antigenic changes that accompany virus-induced oncogenesis. Most of this work has been carried out with cultures of normal CEF cells and with CEF cells infected and/or transformed by various members of the ALSV group. On the basis of such studies, it has been shown that at least 2 distinct cell surface antigens are associated with CEF cells of virus-transformed as opposed to normal phenotype: a ALSV subgroup-specific envelope (Ve) antigen and a group-specific TSSA (6, 13, 14). More recent experimentation has revealed that these antigens represent the major viral glycoprotein of M.W. 85,000 and a nonviral cell surface glycoprotein of M.W. 100,000, respectively (4, 22).

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added to the medium during infection. Supernatant fluids containing infectious virus were collected from cultures of almost completely transformed cells, clarified by low-speed centrifugation and frozen at -70° until use.

**Eggs, Chickens, and Tumor Induction.** COFAL-negative (24), pathogen-free C/O eggs and chickens were purchased from the breeding colonies of either SPAFAS, Inc., Norwich, Conn., or the Institut Armand Frappier, Laval, Quebec, Canada. Both male and female animals, 8 to 12 weeks of age, received injections in the right wing webs of transformed culture supernatant fluid containing approximately 10^3 to 10^4 focus-forming units of virus. After 3 or 4 weeks, by which time sizeable neoplasms (approximately 2 cm in diameter) had developed, in most instances, pieces of tumor tissue were removed surgically from some of the animals. Three days after surgery, some of these birds were killed, and their spleens were harvested. In some experiments, chickens infected with avian leukemia viruses (a dosage lethal to 100% of the chickens) were used as lymphocyte donors between 2 and 3 weeks after viral inoculation. Age-matched normal chickens that received injections of supernatant fluid of normal CEF cells served as controls.

**Cell Cultivation.** The Rous tumors were excised aseptically from ether-anesthetized birds which were then maintained in individual cages. The tumor tissue was cut up, subjected to trypsinization, and plated for growth in tissue culture in MEM (supplemented with 4% fetal calf serum; penicillin, 100 units/ml; and streptomycin, 100 units/ml) as previously described (30). Normal CEF cells were secondary cultures (2 to 3 days old) derived from 11-day-old embryos and grown in MEM. RSV-transformed CEF were secondary cultures (2 to 3 days old) derived from 11-day-old embryos and grown in MEM. RSV-transformed CEF were secondary cultures derived from the same embryos and were maintained in the same way.

**Microcytotoxicity Assay.** These tests were performed by incubating 91^Cr-labeled target cells with suspensions of splenic lymphocytes at 37° and measuring the amounts of label released. Splenic lymphocytes were prepared by teasing the spleens of normal and tumor-bearing chickens in BSS. After large fragments and adherent cells had settled out, the remaining cells were washed 3 times by low-speed centrifugation. Cell counting in a hemocytometer revealed over 95% of the cells thus obtained to have the appearance of lymphocytes. For the purpose of determining cytotoxicity, 6 x 10^4 target cells in 0.2 ml culture medium were seeded into each microwell of a tissue culture microtest plate (Microtest II; Falcon Plastics, Oxnard, Calif.), and incubated at 37° as previously described (30). The cells of each well were labeled with 4 to 5 μCi of 91^Cr in 20 μl of BSS at 37° for 1 hr. Following extensive washing of the wells with BSS, lymphocytes were added in 0.2 ml of BSS at the splenocyte:target cell ratio of 100:1. After various times of incubation at 37°, culture fluids from each well were transferred to test tubes and counted for released radioactivity. All tests were carried out with 4 replicate samples. Total incorporated radioactivity was determined by counting the cells removed by trypsinization from each of 4 control wells.

**Lymphocyte Stimulation.** These tests were performed by a modification of the procedure of Lopez et al. (15). Approximately 2 to 2.5 weeks after viral inoculation, circulating lymphocytes were obtained by drawing blood from both virus-injected and normal chickens into heparin by cardiac puncture. (In the case of sarcoma virus-injected animals, neoplasms had grown to about 2 cm diameter by that time.) The mononuclear fraction, consisting almost entirely of lymphocytes, was purified by Ficol-Isoopaque gradient centrifugation (5), collected by aspiration, and washed twice by centrifugation for 15 min at 500 x g in BSS. The cells were then resuspended in bicarbonate-buffered Roswell Park Memorial Institute medium (supplemented with 2% fetal calf serum; penicillin, 100 units/ml; and streptomycin, 100 units/ml) to a final concentration of 10^5/ml. Cultures containing 1 ml of this suspension in 17- x 100-mm tubes were incubated in the presence or absence of various test antigens for 72 hr at 37°. Each test was carried out with at least 3 replicate samples. Tritiated thymidine (1 μCi/tube; New England Nuclear, Boston, Mass.) was added to the culture tubes for the final 16 hr of incubation, following which the samples were processed by trichloroacetic acid precipitation onto filter pads, and the amount of incorporated radioactivity was determined. Lymphocyte stimulation indices were calculated as the ratio between amounts of radioactivity incorporated in the presence and absence of antigenic stimulus.

**Soluble Tissue Extracts.** Soluble tissue components were prepared by 3 M KCl extraction from each of normal CEF cells, RSV-transformed CEF cells, and Rous tumor cells by a modification of the procedure of Melzer et al. (17) as previously described (18), and stored at -20° until they were used. Protein concentrations were determined by the method of Lowry et al. (16).

**RESULTS**

**Cytotoxicity Studies.** The Schmidt-Ruppin strain of RSV, subgroup A, was used in all of the experiments discussed in this section. Splenocytes from both normal and tumor-bearing chickens were assayed for cytotoxic activity against monolayer cultures of each of 91^Cr-labeled RS tumor cells, RSV-transformed CEF cells, and normal CEF cells. After varying times at 37°, the radioactivity of the supernatant fluids was determined. Under the conditions of these experiments, normal spleen cells did not effect a release of 91^Cr above spontaneous release levels. The specific cytotoxic response obtained with splenocytes from tumor-bearing chickens is shown in Chart 1. The results of this experiment also confirm 2 previous findings from our laboratories, namely, that splenic lymphocytes of tumor-bearing chickens are more reactive against neoplastic target cells in autochthonous than in allogeneic in vitro combinations, and that splenocytes from animals with large necrotic tumors are ineffective in this sort of cytotoxicity test (30).

Subsequent studies were carried out to compare the ability of each of RS cells and normal and RSV-transformed CEF cells to serve as target tissues in this system. The results of a representative experiment are shown in Chart 2. Splenic lymphocytes from each of 2 normal and 2 tumor-bearing chickens were incubated for 8 hr with 91^Cr-labeled monolayer cultures of these various target types, and levels of 91^Cr release were calculated as described in "Materials and Methods." The most effective cytotoxic immune response was observed in those instances in which cultured tumor cells were used as targets. Again, preferential au-
tochthonous recognition was observed. RSV-transformed CEF cells appeared to be less susceptible to killing than were Rous tumor cells and somewhat more susceptible than normal CEF. Differences between levels of susceptibility of normal and transformed CEF, however, were not always significant.

The results of 4 other similar experiments in which effector cells from normal and tumor-bearing chickens were exposed to these various target types are summarized in Table 1. Including the results of Chart 1, interactions between the splenocytes of tumor-bearing chickens and normal CEF cells caused significant target damage in 0 of 9 cases, whereas such an effect was seen in 4 of 9 cases when RSV-transformed CEF cells were used. In contrast, specific killing of RS tumor cells was recorded at significant levels in 8 of 9 instances studied.

**Lymphocyte Stimulation Studies.** These studies were performed by measuring incorporation of [3H]thymidine by peripheral lymphocytes of normal and tumor-bearing chickens, following exposure in vitro to either 3 M KCl extracts or supernatant fluids of cultured RS tumor cells, normal CEF cells, and RSV-transformed CEF cells. All experiments were carried out between 3 and 5 weeks following inoculation of chickens with RSV. Chart 3 depicts the results of typical experiments in which the B77 strain (subgroup C) of RSV was used both for tumor induction in vivo as well as cellular transformation in vitro. In each of 3 separate experiments, chickens bearing tumors induced by RSV were capable of mounting a specific antitumor immune response as measured by this technique. Degree of responsiveness, however, varied between experiments, and different antigenic extracts and/or culture supernatants appeared to be more stimulatory to the circulating lymphocytes of some tumor-bearing birds than others. For example, the infectious supernatant of B77 virus-transformed CEF cells was considerably more reactive than extracts of the same cells or of tumor cells in Experiment 1 of Chart 3, less reactive in Experiment 2, and reactive at intermediate levels in Experiment 3. Similarly, 3 M KCl extracts of each of B77-transformed CEF and B77 tumor cells were capable of provoking a significant blastogenesis response in Experiments 2 and 3, but not in Experiment 1. No important differences were detected between the capacities of extracts of either of these 2 cell types to effect lymphocyte stimulation.

These results on the lymphocyte stimulatory capacity of cell extracts were confirmed in 6 other experiments using chickens inoculated with any of the SR-A, B77, or PrB (subgroup B) strains of RSV. In each experiment, circulating lymphocytes of such animals were exposed to extracts of normal CEF as well as transformed and tumor cells induced by viruses of both homologous and heterologous subgroups. Few differences, if any, were detected between the capacities of homologous and heterologous extracts to elicit a significant blastogenesis response (unpublished observations). The results obtained with homologous extracts are summarized in Table 2.

In all, including the results of Chart 3 and Table 2, significant levels of stimulation were obtained with 18 of 20 chickens in each of 9 experiments. In certain cases, the extracts prepared from transformed CEF cells were more reactive than were extracts of tumor cells, while in other experiments the reverse was true. However, no clear-cut picture of the relative stimulatory capacities of extracts of either of these 2 cell types emerged. Under similar circumstances, extracts of normal CEF were occasionally stimulatory to the circulating lymphocytes of RS-bearing animals, but to a lesser degree than extracts of either RS cells or transformed CEF. In contrast, such normal CEF extracts were never found to be significantly stimulatory to the lymphocytes of normal chickens.

Frequently, the stimulation indices obtained in these experiments were less than 1, indicating that less isotope had been incorporated in such cultures than in the case of normal medium-incubated controls. This suggested that the cell extracts used may have been somewhat toxic for chicken lymphocytes, thus resulting in a decreased cell population under conditions of non- or minimal stimulation. A more consistent picture emerged when the supernatant
Immunity against Rous Sarcomas

Table 1

Cytotoxic activity of splenocytes from normal and tumor-bearing chickens

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Target cells</th>
<th>% 51Cr release when effector cells were</th>
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<td>1</td>
<td>Normal CEF</td>
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<td>Transformed CEF</td>
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<td>23.0 Normal 30.6 Sensitized</td>
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<td>Normal CEF</td>
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<td>Transformed CEF</td>
<td>22.7 Normal 23.5 Sensitized</td>
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<tr>
<td></td>
<td>RS</td>
<td>13.5 Normal 28.4 Sensitized</td>
</tr>
<tr>
<td>3</td>
<td>Normal CEF</td>
<td>23.9 Normal 21.7 Sensitized</td>
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<td>Transformed CEF A</td>
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<td>Normal CEF B*</td>
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<td>Transformed CEF B</td>
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<tr>
<td></td>
<td>RS</td>
<td>19.9 Normal 29.2 Sensitized</td>
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</table>

* Probability of significant difference between normal and sensitized splenocytes; Student's t test.

Chart 3. Lymphocyte stimulation by 3 mM KCl extracts and supernatant fluids of normal and RSV (B7)-transformed CEF cells and RS (B7) tumor cells. Significance of comparisons with unstimulated control cultures (Student's t test): Experiment 1, supernatant fluid of B7-transformed cells with sensitized lymphocytes, p < 0.01; all others not significant; Experiment 2, extracts of each of B7-transformed CEF cells and B7 tumor cells with sensitized lymphocytes, p < 0.05; all others, not significant; Experiment 3, supernatant fluid of B7-transformed cells with sensitized lymphocytes, p < 0.05, p < 0.01; extract of B7-transformed cells with sensitized lymphocytes, p < 0.05, p < 0.01; extract of B7 tumor cells with sensitized lymphocytes, p < 0.05, p < 0.01; all others not significant.

The results of Table 3 indicate specific reactivity against culture fluids of transformed CEF to have occurred with each of 5 animals tested, while stimulation by fluids of cultured RS cells or normal CEF was observed with 1 of 5 and 0 of 5 tumor-bearing chickens, respectively. This observation, when considered in concert with recent findings from our laboratory (31) that cultured RS cells produce relatively little transforming virus in comparison with transformed CEF, suggests that a major portion of this lymphocyte stimulation response may be directed against virion components.

In order to assess this possibility, we investigated the capacity of circulating lymphocytes from each of avian sarcoma virus- and avian leukosis virus-injected animals to react against supernatant fluids of cultures of CEF cells infected and/or transformed by various viral agents. The results (Table 4) indicated generally similar levels of reactivity against both leukosis and sarcoma virus-containing supernatant fluids on the part of lymphocytes derived from the various virus-inoculated animals tested. This was true regardless of whether a sarcoma or leukosis virus had been used for inoculation. In contrast, far lower levels of responsiveness against such supernatant fluids were obtained with lymphocytes from each of 4 normal controls. Since infection of CEF cells with leukosis viruses does not cause morphological transformation or apparently the expression of nonvirion tumor-specific surface antigens (8, 13, 14), these data are consistent with the hypothesis that the lymphocyte stimulation test measures reactivity directed largely against viral components in this system. No evidence of viral subgroup specificity was found in these experiments.

The above results indicating a differential expression of relevant tumor-associated antigens in RSV-transformed CEF cells and tumor cells suggested the possibility that these 2 cell types might differ from one another in cell...
surface characteristics. This subject was investigated by examining the surface morphology of each of normal and transformed CEF cells and RS tumor cells by scanning electron microscopy.

The results showed that the fusiform normal CEF cells have relatively smooth surfaces and a paucity of microvilli. In contrast, both transformed CEF cells and RS cells appeared to be rounded and contained many more surface

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**Table 2**

<table>
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<tr>
<th>Experiment</th>
<th>Chicken</th>
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<th>Tr-CEF&lt;sup&gt;4&lt;/sup&gt;</th>
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* Chickens were given injections of supernatant fluids from cultures of either normal or virus-transformed CEF cells.
* Incorporated in absence of stimulant.
* N-CEF, normal CEF cells.
* Probability of significant difference from non-stimulated cultures; Student's t test.
* Tr-CEF, RSV-transformed CEF cells.
* RS tumor cells.
* NS, not significant.

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**Table 3**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Chicken</th>
<th>cpm</th>
<th>N-CEF</th>
<th>p&lt;sup&gt;4&lt;/sup&gt;</th>
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<th>p</th>
<th>RS&lt;sup&gt;4&lt;/sup&gt;</th>
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* Chickens were given injections of supernatant fluids from cultures of either normal or virus-transformed CEF cells.
* Incorporated in absence of stimulant.
* N-CEF, normal CEF cells.
* Probability of significant difference from nonstimulated cultures; Student's t test.
* Tr-CEF, RSV-transformed CEF cells.
* RS tumor cells.
* NS, not significant.
**Table 4**

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<th>Experiment</th>
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<th>RAV-1-in-fected CEF</th>
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* Chickens were given injections of supernatant fluids from cultures of either normal or virus-inoculated CEF cells.

**DISCUSSION**

These studies compared the abilities of each of normal CEF cells, RSV-transformed CEF cells, and RS tumor cells, as well as extracts and supernatant fluids of each of these cell types, to interact with the lymphocytes of chickens bearing tumors induced by RSV. Normal chickens served as controls. Most of the tumor-bearing animals that we examined were able to mount a specific cell-mediated antitumor response as measured by the techniques of target cell cytoxicity and peripheral lymphocyte stimulation. In this regard, these studies confirm and extend earlier observations obtained by Lopez et al. (15) who demonstrated, by lymphocyte stimulation technique, the existence of cell-mediated immunity to avian oncornaviruses in chickens infected with any of several Rous-associated viruses. In addition, evidence for a preferential autologous recognition by the immune system for RS target cells was demonstrated, thus confirming earlier findings obtained with this tumor model (29, 30).

The data presented here also indicate that the ability of lymphoid cells from tumor bearers to interact with either intact target cells, target cell extracts, or target cell supernatant fluids is highly dependent on the source of the antigen and the technique used in each instance. This points to the complexity of the circumstances in which studies on antitumor immunity with this model are carried out, and may suggest a possible explanation for some of the conflicting interpretations of experimental results obtained by various laboratories in the past. We have demonstrated that RS tumor cells are far more susceptible to the cytotoxic activity of sensitized chicken lymphoid cells than are CEF cells transformed by RSV of the same subgroup (identical virus preparation) as that used for tumor induction. In contrast, the supernatant fluids of RSV-transformed CEF cells were consistently more effective at stimulating the peripheral lymphocytes of birds with tumors than were similar fluids derived from either normal CEF cells or cultured tumor cells (derived from growing neoplasms approximately 3.5 weeks following inoculation with RSV). Moreover, we were not able to detect differences between levels of responsiveness against supernatant fluids of each of avian sarcoma virus-transformed CEF cells or leukemia virus-infected CEF cells on the part of lymphocytes from birds given injections of either type of viral agent. Since infection with leukemia viruses does not apparently lead to the expression of nonviral tumor-associated antigens (8, 13, 14), these data are consistent with the hypothesis that the lymphocyte blastogenesis procedure primarily measures viral antigens in...
this system. Alternatively, reactivity may be directed against cell surface antigens that are associated with either ALSV infection or transformation, or both. In any case, the relevant antigens appear to be group specific, as we have been unable to distinguish among various viral subgroups. Lopez et al. have reported similar findings in their lymphocyte stimulation studies. This does not imply that other immunological assay methods (e.g., colony inhibition testing, immunoprecipitation) need have similar or identical targets. Our efforts to resolve further the issue of whether viral or nonviral antigens are primarily responsible for the development of antitumor immunity in this system proved frustrating. Work with gradient-purified or pelleted-resus- pended virus in lymphocyte blastogenesis experiments yielded positive data in some instances but not in others (unpublished observations). These studies are being con- tinued. When 3 M KCl extracts of the various cell types described were used as stimuli, no significant differences were observed between the abilities of such preparations from either RSV-transformed CEF or RS tumor cells to effect lymphocyte responsiveness, although both were more efficient at this than extracts of normal CEF cells. These results suggest a differential expression of relevant RS-associated antigens in these various cell types and indicate that levels of observed antitumor immunity may vary considerably, even when single animals are examined, accor- ding to the source of antigen used.

The existence of a cell-mediated antitumor response in RS-bearing chickens was first demonstrated by Sjögren and Jonsson (25), who showed by a colony inhibition test that thymic lymphocytes from tumor-bearing animals specifically inhibited the growth of target cells prepared from a transplantable murine Rous tumor. This confirmed the presence of Rous-associated antigens in tumors resulting from the inoculation of either RSV or RSV-transformed CEF cells into species of other than avian origin (12). The murine target cells used in this instance did not synthesize de novo RSV, leading to the conclusion that a nonvirus antigen played an important role in the development of immunity against avian sarcomas. Subsequently, it was shown by Gelderblom et al. (8) and Kurth and Bauer (13, 14) that at least 2 distinct tumor-associated antigens are present on the surface of RSV-transformed CEF cells: a subgroup-specific viral envelope antigen(s) found on the surface of cells actively engaged in the synthesis of progeny virus particles, and a virus group-specific tumor-associated antigen occurring on all cells transformed by members of the avian leukemia-sarcoma group. The latter antigen, termed TSSA, has since been shown on the basis of immunoprecipitation experiments to be a glycoprotein with an apparent molecular weight of 100,000 (4, 22). It now appears, how- ever, that the major viral glycoprotein gp 85 contains group- specific as well as subgroup-specific determinants (21). Thus, it would appear possible that the group-specific activity of TSSA may be due to that entity being either a glyco- protein product or precursor of viral glycoprotein, a possi- bility supported by the inability of Phillips and Perdue (19, 20) to demonstrate antigens of nonviral specificity in this system. The ability of certain types of nonvirus-producing RS cells (e.g., murine) to serve as effective targets in this system could then be explained if it were found that such cells are able to express the group-specific determinants of viral glycoproteins at their surface in the absence of virus production. Further study is necessary to clarify this point, which is especially relevant to our finding that cultured RS tumor cells serve as more susceptible targets in lymphocyte cytotoxicity assays than do RSV-transformed CEF cells. This result could perhaps have been predicted, since cul- tured RS tumor cells, which might be expected to be more representative of in vivo tumor growth than RSV-trans- formed CEF, could likewise be expected to express the more relevant antigenic configuration at their surface of the 2 cell types. Rubin (23) has documented that RSV-trans- formed cells are far more efficient producers of de novo virus than are RS tumor cells, a finding consistent with recent observations in our laboratory (31). These data could therefore be interpreted as supportive of the importance of nonviral antigens in this system. However, Dougherty et al. (7) have shown the presence of avian leukemia viral antigens and particles in chicken liver and pancreas tissues devoid of detectable infectious virus. Thus, the failure of cells to produce virus does not necessarily indicate an absence of viral antigen expression. In contrast, the fact that the culture fluids of RSV-transformed CEF cells are far more stimu- latory to the lymphocytes of animals with tumors than are culture fluids of RS cells may be the result of the presence of large quantities of complete virus particles in the former cell type only.

Our efforts at detecting morphological differences be- tween transformed CEF and RS cells by scanning electron microscopy revealed similarities, instead. We found that both of these typically rounded cell types contained numer- ous ruffles and microvilli at their surfaces, in contrast to the smooth fusiform shape of normal CEF cells. In this regard, our micrographs yielded information similar to that of other investigators who compared normal and transformed CEF cells only (1, 9, 26).

Last, our in vitro stimulation studies revealed that the circulating lymphocytes of normal chickens were occasion- ally responsive to both extracts and supernatant fluids of RSV-transformed CEF cells and that extracts of normal CEF cells were sometimes stimulatory to the lymphocytes of birds with tumors. These findings may be related to the fact that recent investigations have indicated that some of the antigens located on the surface of RSV-transformed CEF cells resemble normal cellular antigens presumably coded by an endogenous virus genome. Specifically, Hanafusa et al. (10) showed that many normal CEF cells could absorb antiviral neutralizing antibodies. Furthermore, antibodies against Rous-associated virus, when complexed to ferritin, were able to tag the noninfected CEF surface, as demon- strated by electron microscopy. More recently, Chen et al. (6) demonstrated the presence of the 2 internal ALSV, pro- teins p 15 and p 19 in normal CEF cells. Vaheri and Roos- lahti (28) have shown by radioimmunoassay that viral group- specific antigenic components can be detected in normal chicken cells and tissues. The presence of such antigens may, in the case of at least some animals, be responsible for the development of a state of natural antitumor immunity. This, in turn, could lead to a
secondary immune response and occasional tumor regression following inoculation of the oncogenic agent (11). Significantly, Andrewes (2) and Bang and Haley (3) have shown the presence of virus-neutralizing antibodies in the sera of young normal chickens. Our data suggest the existence of such natural immunity at a cellular level as well.

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REFERENCES

Differential Expression of Relevant Rous Sarcoma-associated Antigens in Cultured Cells


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