Isolation of a Non-focus-forming Agent from Strain MC29 Avian Leukosis Virus

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SUMMARY

Strain MC29 avian leukosis virus induces a broad spectrum of cancers principally of the myeloid hematopoietic system, but it also causes primary tumors of the kidney and liver and, not infrequently, erythroblastosis. An unusual feature for a leukosis virus is the capacity of the strain to produce marked and very rapid morphological alterations of chick embryo cells with formation of foci in the cell monolayers resembling those associated with Rous sarcoma virus infection. This report describes isolation from terminal dilution clones of a component of strain MC29 with properties distinctly different from those of the standard strain. This agent does not produce foci or cause morphological alteration of chick embryo cells typical of MC29 infection. Further, it induces erythroblastosis in the chicken to the exclusion of myeloid hematopoietic tissue response and does not cause cancers of other organs affected by MC29. In other properties, the agent behaves as a leukemia virus in the activation of Rous sarcoma virus non-producer cells, in the interference with Rous sarcoma virus infection of chicken embryo cells, and in ultrastructural and sedimentation properties and RNA component constitution.

INTRODUCTION

Strain MC29 avian leukemia virus (20, 28) causes a broad spectrum of cancers (22) of principally myeloid hematopoietic tissue origin: myelocytomatosis, a widespread dissemination of myelocytes in organs and tissues; and myelocyte tumor masses, myelocytomas (28). Less frequent cancers are primary renal (28) and liver (17) tumors and occasionally erythroblastosis (28). Under suitable conditions, the agent induces a high incidence of mesotheliomas (5) arising from peritoneal, epicardial, and pericardial epithelium. A further singular property of strain MC29 is infection of CEC2 with massive morphological conversion within 72 to 80 hr (3, 25) and formation of distinct foci (23) in CEC monolayers applicable to assay by FFU enumeration (23).

A variety of avian sarcoma and leukemia virus strains consist either of mixtures of etiologically different agents or of subgroups or subtypes [A, B, C, or D (7)] differing by immunological or other properties or both. RSV preparations contain a sarcoma-inducing component together with a number of RAV's (7, 15, 19, 31), some with the etiological activity of leukemia agents (31). Leukosis viruses [BAI A, RPL 12, and R strains (1, 2)] likewise exhibit properties of subgroups A or B or both (37). It has been suggested that the BAI strain A may be a mixture of different etiological agents (29, 34), and recent studies indicated heterogeneity of strain R (18).

As an essential aspect of characterization of strain MC29, studies were made of the “standard” strain as a possible mixture of different agents. Previous work showed that MC29 strain contained both subgroups A and B (10). Further investigations have now resulted in the isolation from strain MC29 of an agent lacking capacity to induce foci or to cause morphological alterations of CEC (16) like those produced by the standard preparations. The agent, designated MC29-AV, causes erythroblastosis without myeloid or other growths induced by the standard strain which it, nevertheless, resembles in other properties. Isolation and preliminary characterization of MC29-AV are described in this report.

MATERIALS AND METHODS

Viruses. The origin of strain MC29 has been described (28). The principal experiments here (Table 1) were done with virus in 1 pool of Virginia chicken blood plasma from birds with typical myelocytomatosis and with 3 separate specimens of tissue culture virus. Blood drawn from 10 birds by heart puncture (9) was freed of cells by centrifugation, and the pooled plasmas were passed through a Selas filter of 0.2 porosity (Selas Corporation of America, Philadelphia, Pa.). Derivatives of this virus passed continually in CEC cultures (24) provided the culture virus pools, TC 11567 I and TC 11567 II (Table 1).

Virus designated CL-8 was obtained in a separate end point dilution experiment yielding a single CEC focus under an agar overlay containing homologous immune serum from the chicken. Agar above the clone was removed and broken up in phosphate-buffered saline solution (8), and the suspension containing virus without cells was inoculated onto fresh CEC. Although virus was liberated into the culture, morphological alteration was delayed for 7 days. Subsequent repeated passage yielded CL-8 agent of behavior similar to that of strain MC29.
Cultures. Eggs yielding embryos were from a flock of resistance-inducing factor-free Leghorn chickens. Preparation and maintenance of the CEC cultures in 15- x 60- or 20- x 100-mm dishes (Falcon Plastics, Los Angeles, Calif.) were the same as the corresponding conditions applied earlier (24).

Inoculation of Chickens. Host response to MC29-AV was studied by i.v. and i.p. inoculation into 1- or 3-day-old line 15 White Leghorn chicks (38). Blood smears were made on each chicken at daily or appropriate intervals. Sections of tissue from birds dying of disease or killed after a suitable period of observation were fixed in Zenker's fluid and stained with hematoxylin and eosin.

Resistance-inducing Activity. By usual procedures (30), primary cultures in 20- x 100-mm dishes were incubated for 24 hr and inoculated with serial dilutions of MC29-AV. Cultures from each dilution were treated with trypsin, replated, and challenged after 3 days with 100 to 200 MC29 or RSV FFU. A corresponding series of cultures were challenged at 6 days. The cells were stained for focus count 7 days after inoculation of the respective cultures.

Cell and VP Counts. Infectivity of MC29 virus and RSV were measured by focus formation (23), and MC29 and MC29-AV VP were counted by electron microscopy (24, 32). CEC fixed with 2% glutaraldehyde were counted in a hemocytometer.

Staining with Fluorescein-conjugated Antibody. The materials and procedures were the same as those used in earlier work (11) with cells infected with strain MC29 in Sykes-Moore culture chambers (35) carried out during the course of the present experiments.

Activation of RSV NP Cells by MC29-AV. MC29-AV was derived from the 10^-7.3, 10^-7.3, and 10^-7 dilution series, respectively, of Experiments 19568, 5969, and 9169 (Table 1) taken at 18, 55, and 54 days of culture. RAV-1 was obtained from Dr. R. Ishizaki. Tests were made by usual procedures (15) with RSV NP cells kindly provided by Dr. H. Hanafusa. Samples of medium taken daily from 5 to 9 days after addition of virus were centrifuged, filtered through 0.45-μ Millipore filters (Millipore Corp., Bedford, Mass.), and stored at —78° for later VP count and RSV infectivity estimate.

Radioisotope-labeled Virus. Cultures of CEC infected with MC29-AV were treated with 10 mCi/ml of uridine-5-3H (26 mCi/μmole, New England Nuclear Corporation, Boston, Mass.). Culture fluid containing 8.2 X 10^8 VP/ml was harvested 24 hr later, filtered through a 0.45-μ Millipore filter, and stored at —78°.

Virus equilibrium sedimentation studies with the labeled MC29-AV and the isolation and characterization of MC29-AV RNA were effected by procedures used in analogous investigations of BA! strain A virus (36).

RESULTS

Demonstration of MC29-AV. Occurrence of an agent associated with, but behaving unlike, strain MC29 virus has been observed in a variety of experiments during the past 2 years. The major attribute of MC29-AV initially recognized was the infection of CEC with consequent virus synthesis but without focus production or changes in cell morphology or growth rates produced by strain MC29. These characteristics are illustrated by the findings (Table 1) in experiments with successive dilutions of 2 tissue culture virus pools (11567 I and 11567 II) and of Virginia chicken blood plasma virus from chickens diseased with strain MC29. Other details of Experiment 19568 are given in Table 2.

In Experiment 19568, described in part in Tables 1 and 2, cultures treated with decreasing doses of MC29 virus from 8.3...
Response by morphological alteration of CEC to strain MC29 maintained 62 days in culture after inoculation with virus (TC 115671, Experiment 19568, Table 1)

<table>
<thead>
<tr>
<th>Culture series</th>
<th>Virus dose/culture (particles)</th>
<th>FFU</th>
<th>Morphological alteration (day)</th>
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<tr>
<td>1</td>
<td>$10^{-2}$</td>
<td>$8.3 \times 10^6$</td>
<td>3,400</td>
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<tr>
<td>7</td>
<td>$10^{-1.3}$</td>
<td>42</td>
<td>0.017</td>
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<tr>
<td>8</td>
<td>$10^{-1.6}$</td>
<td>21</td>
<td>0.008</td>
</tr>
<tr>
<td>9</td>
<td>$10^{-1.9}$</td>
<td>10</td>
<td>0.004</td>
</tr>
<tr>
<td>10</td>
<td>$10^{-2.2}$</td>
<td>5</td>
<td>0.002</td>
</tr>
<tr>
<td>13</td>
<td>$10^{-1}$</td>
<td>$8.3 \times 10^7$</td>
<td>34,000</td>
</tr>
<tr>
<td>14</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Table 2

a Tissue culture fluid contained $8.3 \times 10^8$ particles corresponding to $3.4 \times 10^6$ FFU/ml. Inoculums were 0.1 ml of respective dilutions per dish.

b AT, altered atypically; NA, not altered.

\(\times 10^7\) to \(8.3 \times 10^2\) VP (\(3.4 \times 10^6\) to 0.34 FFU)/culture changed morphologically within 3 to 23 days. Cultures treated with 83 to 21 VP or 0.03 to 0.008 FFU/culture were not altered in the same manner during the 62-day period. In Experiment 5969, 830 VP/culture caused definite changes, whereas lesser doses did not induce typical alteration. MC29 virus from the chicken (Experiment 9169, Table 1) induced a similar response but, on the basis of particle number, was less effective than tissue culture virus, and as many as 1320 particles/culture did not alter the cells. CL-8 agent, about 47 days after inoculation, the VP counts were 1.1 X 10^8, 6.4 X 10^8, and 3.6 X 10^8, respectively.

Uninfected cells (Fig. 1) vary morphologically from long spindle shapes to large elements of irregular contours with many pseudopodia and filapodia (16, 25) and oval and lenticulate nuclei with distinctly staining chromatin and 1 or more small nucleoli. Pronounced changes in MC29-infected cells result (Fig. 3) in angulated forms (16, 25) with deeply stained cytoplasm and spherical or spheroidal nuclei with relatively homogeneous, faintly stained nucleoplasm containing 1 or more very large dense nucleoli. Although morphological response to MC29-AV is much less than that to MC29, CEC infected with MC29-AV exhibit (Fig. 2) definite differences from the normal structure. The cells (Fig. 2) seem larger and more angular and exhibit fewer spindle shapes than normal. The nuclei appear somewhat more rounded and relatively more homogeneous and less dense with perhaps some increase in the size of the nucleoli. It is notable that the cells of Fig. 2 were from the same source and in culture for the same period as those of Fig. 1.

Despite the small morphological changes with the lower virus dose, virus synthesis was demonstrable by direct particle count and other criteria, and concentrations of agent were essentially the same as with cells typically altered by MC29. Virus elaboration evident within 3 days (Chart 1) in Culture 13 exposed to the large dose of MC29 virus increased rapidly to about 10 days and continued at a variable but high output through 30 days. In contrast, virus increase in Culture 7 (MC29-AV) with the lower initial MC29 dose was measurable only at 7 days, but, within 8 to 9 days more, it closely approximated that of Culture 13. Since later findings showed that the virus in Culture 7 was MC29-AV, it was thus evident that the rate of liberation of this agent alone was essentially the same as that of the standard MC29 containing also MC29-AV in Culture 13. No particles unequivocally identifiable as virus were observed in Culture 14 not exposed to virus.

Response to fluorescein-labeled Antibodies. Fluorescein-labeled antibody studies yielded results paralleling the VP counts. Uninfected CEC from Culture 14 did not stain (Fig. 6a), but Culture 13 CEC (Fig. 6b) inoculated with a high dose of MC29 virus fluoresced strongly. In Cultures 7 and 8, most of the slightly changed CEC were markedly stained (Fig. 6, c and d) in granular cytoplasmic masses and at the cell periphery. CEC from Cultures 9 and 10 (Fig. 6, e and f) inoculated with the smallest virus doses showed no morphological alteration and were not stained. No stained nuclei were seen.

Response of CEC to Passage of MC29-AV. In an experiment on further passage of MC29-AV yielding typical results, fluid from a preparation derived from Culture 7 (Table 2) containing 1.2 X 10^9 VP/ml 53 days after initial infection was inoculated on CEC in 1.0-, 0.5-, 0.2-, and 0.1-ml volumes in 20- x 100-mm dishes (5 X 10^6 cells/dish). Morphological alteration was like that illustrated in Fig. 2, and electron microscopy demonstrated virus synthesis in all cultures. At 8 days after inoculation, the VP counts were 1.1 X 10^6, 6.4 X 10^5, 3.5 X 10^6, and 3.6 X 10^6, respectively.

Chart 1. VP liberation by CEC infected and morphologically altered by MC29 virus (Culture 13), by cells infected with MC29-AV (Culture 7), and by cells not exposed to virus (Culture 14) in the experiment of Table 2. Number of particles in the uninfected culture was at the limit of the counting method and represents particles in the general size range of avian tumor viruses.
Cell Growth. CEC infected and morphologically altered by MC29 usually exhibit a PDT approximately one-half that of uninfected cells (3, 24), but CEC infected with MC29-AV in Experiment 19568 (Chart 2) proliferated exponentially at essentially the same rate, 2.4 days PDT, as uninfected cells in the 30-day period. Growth of MC29-infected cells was not notably more rapid than that of the other culture series until about 10 days after infection. Thereafter, however, the PDT of cells morphologically completely altered at 3 days decreased to about 1.6 days.

MC29-AV Interference with RSV and Standard MC29 Virus. Tests of the sensitivity of CEC exposed to MC29-AV to infection with RSV and MC29 (Table 3) demonstrated complete interference with focus formation by both RSV and MC29 virus. Electron microscopy showed growth of MC29-AV at 3 days in the cultures of Experiment 1, Table 3, initially receiving $1.9 \times 10^7$ and $1.9 \times 10^6$ MC29-AV VP derived from pool TC 11567 but not in those exposed to $1.9 \times 10^5$ particles. Release of the agent in 3 days was likewise small in cultures (Experiment 2, Table 3) derived initially with virus from blood plasma (Experiment 9169, Table 1). Nevertheless, interference at 3 days, although small in the dishes treated with the smallest doses of MC29-AV, was evident in all inoculated cultures. At 6 days, however, the MC29-AV particle count was about $10^5$ in all cultures, and interference was complete in all.

Activation of RSV NP Cells. Table 4 illustrates activation of RSV NP cells resulting in the liberation of infectious agent inducing typical RSV foci in CEC monolayers. The concentration of FFU in MC29-AV-treated cultures was of the same order, $10^5$/ml, as that observed with RAV-1. The control culture not treated with either MC29-AV or RAV likewise contained typical VP, $8.8 \times 10^7$/ml, but contrary to the findings with the activated cultures, these particles, like those observed with other NP cells (6), did not induce foci.

Buoyant Density of MC29-AV in Sucrose Gradient. The equilibrium sedimentation peak of uridine$^3$H-labeled MC29-AV (Chart 3A) indicated a buoyant density of 1.16 which was in good agreement with the density reported for other avian tumor viruses measured under like conditions (1). This value, however, is in no way a reflection of the probable density of the fully hydrated agent in a normal environment (33).

Sedimentation Profile of MC29-AV RNA. BAl strain A virus RNA consists of at least 4 components of different molecular sizes (4). Comparison with the sedimentation profile (Chart 3B) of BAl strain A virus RNA indicated the same components in MC29-AV RNA: the main peak of 60 S material; 2 small peaks of 28 S and 18 S, presumably rRNA's; and the peak of 4 S components. Thus, by these criteria, MC29-AV RNA was indistinguishable from BAl strain A leukosis virus RNA.

Response of Chickens to MC29-AV. A study of 155 chicks gave results (Table 5) similar to those observed in other less extensive experiments. Cancers induced in DG 1 by MC29

### Table 3

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Infecting dose of MC29-AV (particles/culture)</th>
<th>VP/ml</th>
<th>Challenge after infection with MC29-AV</th>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 Days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RSV</td>
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<tr>
<td>1</td>
<td>None$^b$</td>
<td>0</td>
<td>1.00$^e$</td>
</tr>
<tr>
<td></td>
<td>$1.9 \times 10^7$</td>
<td>$2.0 \times 10^9$</td>
<td>0.02</td>
</tr>
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<td>$1.9 \times 10^6$</td>
<td>$5.4 \times 10^7$</td>
<td>0.00</td>
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<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>None$^b$</td>
<td>$0.5 \times 10^7$</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>$1.9 \times 10^7$</td>
<td>$62.0 \times 10^7$</td>
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</tr>
<tr>
<td></td>
<td>$1.9 \times 10^6$</td>
<td>$7.6 \times 10^7$</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>$1.9 \times 10^5$</td>
<td>$2.3 \times 10^7$</td>
<td>0.36</td>
</tr>
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</table>

$^a$ Concentration of MC29-AV particles (VP) in the cultures at time of challenge.

$^b$ Cells in control cultures were not infected with MC29-AV. The number of "particles" in these cultures represents images in the general size range of virus (see Chart 1).

$^c$ The degree of interference in the infected cultures was calculated as the number of foci which appeared following challenge with RSV or MC29 divided by the respective number of foci on uninfected control CEC cultures. CEC cultures which showed a sensitivity of 0.1 or less were considered resistant.
Table 4
Activation of RSV NP cells by MC29-AV virus derived in Experiments 19568, 5969, and 9169 (Table 1)

<table>
<thead>
<tr>
<th>Activator</th>
<th>Activator dose (particles/plate)</th>
<th>Particles (x 10^9)</th>
<th>FFU (x 10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC29-AV 11567 I</td>
<td>1.8 x 10^8</td>
<td>9.4</td>
<td>2.2</td>
</tr>
<tr>
<td>MC29-AV 11567 II</td>
<td>1.8 x 10^8</td>
<td>21</td>
<td>1.8</td>
</tr>
<tr>
<td>MC-29-AV Virginia chicken plasma</td>
<td>1.0 x 10^7</td>
<td>5.5</td>
<td>3.0</td>
</tr>
<tr>
<td>RAV,</td>
<td>1.4 x 10^6</td>
<td>3.0</td>
<td>4.1</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>0.88^b</td>
<td>0</td>
</tr>
</tbody>
</table>

^a Supernatant fluid from 24-hr culture period 9 days after addition of activator virus.

^b Particles with a morphology typical of avian tumor viruses.

DISCUSSION

Evidence that strain MC29 avian tumor virus isolated initially from diseased chickens (1) contains more than a single distinct etiological entity has been demonstrated by a variety...
A Non-focus-forming Agent from MC29 Leukosis Virus

Table 5

Chicken response to MC29-AV within 102 days compared with that of MC29 in the same period

<table>
<thead>
<tr>
<th>Virus</th>
<th>DG</th>
<th>Volume (ml)</th>
<th>Route</th>
<th>Chicks inoculated</th>
<th>Correc ted no.</th>
<th>Blood</th>
<th>Liver</th>
<th>Mesotheli um</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC29</td>
<td>1</td>
<td>8.0</td>
<td>i.v.</td>
<td>V05–24</td>
<td>17</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.0</td>
<td>i.p.</td>
<td>V25–43</td>
<td>16</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>MC29-AV</td>
<td>3</td>
<td>17.8</td>
<td>i.v.</td>
<td>V44–61</td>
<td>16</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>35.6</td>
<td>i.p.</td>
<td>V62–81</td>
<td>16</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td></td>
<td>5</td>
<td>8.0</td>
<td>i.v.</td>
<td>V82–100</td>
<td>16</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>16.0</td>
<td>i.p.</td>
<td>V101–120</td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>4</td>
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<tr>
<td></td>
<td>7</td>
<td>4</td>
<td>i.v.</td>
<td>V121–139</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td></td>
<td>8</td>
<td>8</td>
<td>i.p.</td>
<td>V140–159</td>
<td>13</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* MC29 virus was from a pool of tissue culture fluid containing $4 \times 10^8$ particles/ml.

* MC29-AV derived through 3 passages of virus from Experiment 19568 (Tables 1 and 2) contained $8.9 \times 10^8$ particles/ml. DG 3 and DG 4 received undiluted fluid. For DG 5 and DG 6, the fluid was diluted to the same particle concentration as that of the MC29 preparation for Groups 1 and 2. The MC29-AV for Groups 7 and 8 was a further 2-fold dilution of the preparation of DG 5 and DG 6.

of criteria. Passage of cloned virus obtained by terminal dilution of MC29 yielded an agent distinguished from crude strain MC29 by: (a) lack of focus induction in CEC monolayers; (b) atypical morphological alteration of CEC in *vitro*; (c) induction of erythroblastic leukemia to the exclusion of characteristic myeloid tissue response (myelocytomatosis or myelocytosis); and (d) absence of neoplasms [mesothelioma (5), renal tumors, and others] associated in high incidence with MC29 infection (28). In other respects, MC29-AV exhibited properties common to other members of the leukemia family group. The agent liberated in CEC cultures exhibited ultrastructural, sedimentation, and RNA constitutional aspects indistinguishable from those of the RNA avian tumor viruses; reacted with leukemia virus (BAI strain A) fluorescein-conjugated antibody; interfered with CEC sensitivity to RSV infectivity; and activated RSV NP cells to the production of active virus.

By test at 2-fold increments, MC29-AV in mixture with MC29 was approximately 4- to 8-fold the concentration of MC29 *per se* as demonstrated by detection of virus liberation in culture by electron microscopy and conjugated antibody examination (Table 2 and Fig. 6). The results obtained by electron microscopy closely paralleled those found with labeled antibody. Rate of growth of MC29-AV-infected CEC was not increased above that of normal cells as by MC29 but was approximately the same as that of uninfected CEC under parallel conditions. In cultures, in well-established infection, the rate of MC29-AV particle liberation into the culture fluid was essentially equal to that of the sum of MC29 and MC29-AV in cultures initially inoculated with MC29 agent. Results of particle counts by electron microscopy gave definite evidence that the differences between the etiological activities of MC29 and MC29-AV in the chicken were not due to virus concentration or dose in the inoculums. This was attested by the data of Table 5 showing that $8 \times 10^7$ and $16 \times 10^7$ particles of MC29 given i.v. and i.p., respectively, gave the typical MC29 response, whereas inoculation of equivalent doses of MC29-AV given in parallel DG's (5 and 6) yielded the limited response characteristic of MC29-AV. Lymphomatosis response to either MC29 or MC29-AV was not regarded as significant, since the lymphoid tissue exhibits no evidence of specificity of reaction to any RNA avian tumor virus.

Complexity of strain MC29 is of special interest, since both chicken and CEC responses have appeared to be specific and uniform in numerous experiments in the past decade. More striking, however, has been the apparent etiological identity of strain MC29 with Furth's strain 2 (12, 13) isolated in New York in the early 1930's. Nevertheless, some aspects of behavior of MC29, as well as of Furth's strain 2, are contrary to those of other well-studied leukosis strains (1, 2). These differences in attributes are manifested particularly by the induction of foci in CEC culture (23) resembling those associated with sarcoma viruses and, equally unusual, the production of both myeloid and erythroid leukosis by the same inoculums. Such activity was observed also (14, 21) by the original BAI strain A from which a component affecting only myeloid tissue was separated by chance (1, 2), a component not observed to cause erythroblastosis in studies in many laboratories since 1950. Demonstrations of naturally occurring mixtures of genetically differing avian tumor viruses have been limited. Mixing of RSV and RAV may have occurred on frequent experimental passages of RSV (31). Apparently analogous to the complexity of MC29 and the original BAI strain A is the association of different entities in strain R (18) which, with respect to the hematopoietic system, is etiological only for erythroblastosis.

The significance or basis for inhomogeneity of at least some avian tumor virus strains is uncertain. The simplest explanation is fortuitous mixing or contamination of 1 strain by another. It seems strange, however, that strains such as MC29, Furth's strain 2, and the less well-studied CM II isolated (26) more recently in Germany should yield seemingly uniform responses. There is some possibility (for which there is no evidence) of genetic variation or mutation giving rise to different etiological entities within an initially homogeneous virus population. The problem of strain inhomogeneity as manifested by MC29 and R is distinct from that (29, 34) of potentially different agents responsible for multiplicity of host...
response to individual strains investigated in recent years, particularly with respect to BAI strain A.

REFERENCES

Figs. 1 to 3. Morphology of CEC in Experiment 19568, Tables 1 and 2. × 375.
Fig. 1. Uninfected cells (Series 14) in culture 34 days after infection.
Fig. 2. Cells (Series 7) showing slight morphological changes 34 days after infection with a low dose of MC29 (MC29-AV).
Fig. 3. Cells (Series 1) 13 days after infection with a large dose of MC29 virus, illustrating marked morphological alterations characteristic of MC29 influence.
Fig. 4. Myeloid cells with mitotic figure (M) in blood smear of V7, DG 1, 24 days postinoculation, with disease induced by i.v. inoculation of 8 × 10⁷ MC29 VP. × 750.
Fig. 5. Erythroblasts of most primitive morphology in circulating blood of V114, DG 6, 76 days postinoculation injected i.p. with 16 × 10⁷ MC29-AV particles. × 750.
Fig. 6. Responses to fluorescein-labeled antibody of cells of Experiment 19568, Table 2. a, cells not infected (Culture 14) are unstained; b, cells infected by standard MC29 virus (Culture 13) are morphologically altered and specifically stained; c and d, cells infected by low dose of MC29 (MC29-AV) (Cultures 7 and 8) producing virus but not characteristically altered are stained; e and f, cells neither altered nor producing virus (Cultures 9 and 10) are not stained. × 775.
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