Response of Bone Marrow to MC29 Avian Leukosis Virus in Vitro


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SUMMARY

Infection of avian bone marrow with strain MC29 leukosis virus in vitro resulted in infection, elaboration of virus, and growth of characteristic cells. Newly liberated virus with the infectious and neutralization properties of strain MC29 agent and growth of the cells were demonstrable 48 hr and 5-6 days respectively after infection. Studies by light and electron microscopy revealed similarities between the bone marrow cells and the myeloid elements (myelocytes) of myelocytomatosis notable in the relatively low nucleus/cytoplasm ratio; very large nucleolus; and ribosome-rich cytoplasm with a dense gray (protein) matrix and a grainy or “ground glass” appearance. Such cells differed markedly in these morphologic aspects from the myeloid cells (myeloblasts) of myeloblastic leukemia induced by BAI strain A. They also differed in their short-lived growth of 3—4 weeks when compared with myeloblast proliferation indefinitely in culture. The cells were similar to chick embryo cells morphologically altered by strain MC29 because of the characteristically large nucleolus, the high concentration of cytoplasmic ribosomes, and the slight rough endoplasmic reticulum. A notable difference was the high content of diffuse chromatin in the chick embryo cells and the reverse proportion of condensed chromatin in the myelocytes. The various aspects of strain MC29 specificity of influence on cell response are discussed.

INTRODUCTION

Infection of the chicken with strain MC29 avian leukosis virus (21, 28) results in the induction of a broad spectrum of myeloid hematopoietic tissue neoplasms varying from tumors of granulated myelocytes to growths of nongranulated myeloid cell derivatives of indeterminate differentiation. In this influence on myeloid cells, the action of strain MC29 is analogous to that of BAI strain A, but, in contrast, the BAI strain A disease consists invariably of simple, though often profound, myeloblastic leukemia (9, 13, 14). Further evidence of strain MC29 specificity with respect to the effects of avian leukosis agents is the rapid infection and massive, characteristic morphologic alteration (11) of CEC* in vitro (19, 26). In addition, the CEC response, under suitable conditions, is marked by occurrence of foci (24) of circumscribed cell growth numerically proportional to strain MC29 virus concentration. Although other avian leukosis strains induce CEC infection, morphologic alteration is slow and uncertain, and other strains do not induce CEC foci (10, 19, 26, 33).

Singular aspects of strain MC29 influence on host-cell response were again evident in recent studies with avian bone marrow treated with the agent in vitro. Exposure of cultures to the virus resulted in elaboration of the agent and cell growth as seen with other leukosis virus strains (10, 22, 23, 33). Cell morphology and behavior of the cultures, similar to that of the myeloblastic BAI strain A disease, were clearly different from the attributes of bone marrow cultures (1) infected with the BAI strain A agent responsible for myeloblastic leukemia. This report describes the characteristics of the strain MC29 virus-infected bone marrow cultures with respect to cell growth, liberation of virus into the culture fluid, and cell morphology as seen in light and electron micrographs. The results are compared with relevant findings with chickens diseased with MC29 and BAI A strains and with bone marrow cultures infected with BAI strain A.

MATERIALS AND METHODS

Cultures

Bone marrow was from White Leghorn chickens of Line 15 (36) hatched from eggs from the laboratory laying flock. Femoral and tibial bones from exsanguinated 90- to 345-day-old chickens were crushed, pooled, and shaken vigorously in growth medium. The supernatant was decanted, spun at 150 X g for 10 min, and the sedimented cells were pooled and centrifuged once more at 75 X g for 5 min and then the speed was increased to 150 X g for another 5 min. The upper third

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4Abbreviations used are: CEC, chick embryo cells; PBS, phosphate-buffered saline; VP, virus particles; FFU, focus-forming units; PDT, population doubling time.
of the packed cell layer was resuspended in appropriate concentration in growth medium, and 5-ml volumes containing 1 X 10^7 to 2 X 10^7 non-red blood cells were introduced into 60-mm Petri dishes (Falcon Plastics, Los Angeles, Calif.).

Ten to thirty cultures prepared for each trial were incubated at 38.5°C in an approximately 5% CO₂ humidified atmosphere. Four to five days later, the medium was decanted, and the nonconfluent cell layers were washed with PBS, pH 7.4 (12), and inoculated (10, 25) with 0.1 ml of undiluted or 10-fold diluted stock virus. After incubating the cultures for 30 min, 5 ml of growth medium were added to each. Half of each group of the cultures were kept for controls. Cultures containing coverslips were terminated at daily intervals. The cells washed with PBS and fixed in absolute methanol were stained with May-Grünewald-Giemsa.

Cultures were also prepared from the primitive myeloid cells occurring occasionally in the circulating blood of chickens diseased with strain MC29. Buffy-coat cells from blood were suspended in culture medium. Volumes of 5 ml containing 2 X 10^7 to 5 X 10^7 cells were plated as was the bone marrow material, and, in addition, cultures were prepared and incubated under the conditions employed for growing the myeloblasts of BAI strain A-induced leukemia (3, 4). For comparative studies, cultures were prepared in the usual way (3, 4) with primitive cells from birds with myeloblastic leukemia and incubated at 37°C in shaking-suspension cultures.

Medium for the Petri dish bone marrow cultures was that already described (10, 25). The same medium was also used for tests of growth of the primitive blood cells from birds with strain MC29 myelocytomatosis in shaking-suspension cultures. Myeloblasts were cultured as usual (3, 4) in fluid of equal parts of M199 and chicken serum with added glucose and folic acid. Tests were also made with this medium in shaking-suspension cultures of the blood cells of myelocytomatosis.

Results were first noticed 5 days after exposure of the culture to virus. On the 9th, 10th, and 11th days respectively 2.8 X 10^5, 8.3 X 10^5, and 24.8 X 10^5 cells per retained culture were discarded. In a typical experiment (Chart 1), the characteristic cells were first noticed 5 days after exposure of the culture to virus. On the 9th, 10th, and 11th days respectively 2.8 X 10^5, 8.3 X 10^5, and 24.8 X 10^5 cells per retained culture were discarded. The PDT in this period of 9-12 days—calculated from the curve constructed on points representing the number of cells which, theoretically, would have accumulated had the cultures

**RESULTS**

**Occurrence and Growth of Specific Cells**

Experiments were made with 7 preparations of bone marrow cells (Table 1). Ten to thirty cultures were used in each trial, half of which were treated with from 1.7 X 10^4 to 3.5 X 10^4 FFU, 4.1 X 10^7 to 8.3 X 10^7 particles, of virus per culture. Despite the differences in donor ages, the cultures were closely similar in all of the studies. At the beginning, almost all of the cells adhering to cover glasses resembled plasmacytes (Fig. 1) of normal adult avian bone marrow [Figs. 391-397 of Ref. 27]. Other distinctive elements were few, large or giant osteoclasts containing varying numbers of small, oval nuclei [Figs. 398, 399 of Ref. 27]. Entirely different cells with a morphology resembling that of myelocytes occurring in myelocytomatosis (Fig. 2) were notable only in virus-infected cultures within 5-6 days after exposure to the MC29 agent. Increase in these cells, which were adherent to the culture dish or free in the medium, was rapid for a few days but declined quickly thereafter. Efforts to pass the culture by transfer of cells to new dishes in fresh medium were unsuccessful. Growth was prolonged, however, by returning half of the number of sedimented cells in fresh medium to the original dish in which many of the elements were attached to the culture dish—and by discarding the others used for cell count.

**Cell Growth**

When the free-floating cells numbered about 5 X 10^4 to 7 X 10^4, the supernatant fluids were drawn off and centrifuged. The cells were resuspended in growth medium as before, and half of the number was returned in fresh medium to the original culture vessel. The remaining cells were counted and discarded.

**Morphology**

Blood smears and other cell preparations were stained with May-Grünewald-Giemsa. Cells from cultures and blood were also examined by phase contrast microscopy. Tissues were fixed in Zener's solution, and sections were stained with hematoxylin and eosin.

For electron microscopy, cells from bone marrow cultures were fixed either in pellets with 3% glutaraldehyde, or in suspension by mixing with equal volumes of 5% glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.4 (30, 32). After 2 or 6 hr at 2°C, suspended cells were sedimented and these, as well as specimens fixed as pellets, were kept overnight at 2°C in 0.1 M cacodylate buffer, pH 7.4, containing 0.3 M sucrose (32). All specimens were postfixed in buffered 1% osmic acid, pH 7.4 (29) and embedded in Maraglas (15). For samples from the chicken, blood drawn from the heart in heparin was spun at 150 X g for 10 min, and the buffy coat was resuspended in plasma and treated like the specimens from the cultures. Ultrathin sections were cut with glass knives on a Porter-Blum microtome, transferred to copper grids, stained with uranyl acetate (37) for 1 hr and lead citrate (31) for 15 min, and examined in a Siemens Elmiskop I with an objective aperture of 50 μ at 60 kv.
Table 1

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Donor birds No.</th>
<th>Age (days)</th>
<th>Cultures</th>
<th>Virus particles added per culture ($\times 10^7$)</th>
<th>Focus forming units added per culture ($\times 10^4$)</th>
<th>Altered cells first noted (days after infection)</th>
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<tbody>
<tr>
<td>128</td>
<td>2</td>
<td>120</td>
<td>Control</td>
<td>5</td>
<td>3.4</td>
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<td>135</td>
<td>Infected</td>
<td>5</td>
<td>3.4</td>
<td>6</td>
</tr>
<tr>
<td>208</td>
<td>3</td>
<td>90</td>
<td>Control</td>
<td>5</td>
<td>3.4</td>
<td>5</td>
</tr>
<tr>
<td>227</td>
<td>3</td>
<td>165</td>
<td>Infected</td>
<td>10</td>
<td>1.7</td>
<td>5</td>
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<tr>
<td>232</td>
<td>3</td>
<td>171</td>
<td>Control</td>
<td>10</td>
<td>1.7</td>
<td>5</td>
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<td>1368</td>
<td>3</td>
<td>250</td>
<td>Infected</td>
<td>10</td>
<td>3.5</td>
<td>6</td>
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<tr>
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<td>2</td>
<td>345</td>
<td>Control</td>
<td>10</td>
<td>3.5</td>
<td>5</td>
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</table>

Data from 7 experimental infections of bone marrow cultures with strain MC29 virus.

Chart 1. Growth of myelocytes (Figs. 2, 3) appearing in bone marrow cultures on the 5th day (arrow) after infection with strain MC29 virus. Ten cultures, each plated with $2 \times 10^7$ cells/60-mm dish, were treated with $1.7 \times 10^6$ focus-forming units of virus/dish. Culture fluids were changed on the 7th, 9th–22nd, and 24th days. Cells in the supernatant fluid were sedimented and counted on the days indicated by the vertical bars. Half of the cells were returned to the original dish in fresh culture fluid, and the other half were discarded. Each bar is the average from the 10 cultures and represents twice the number of cells counted. The curve of cell accumulation was calculated on the basis of hypothetical cell numbers that might have been attained per initial culture had no cells been discarded.

been divided without discarding cells—was 0.3 days. The PDT in the interval of 12 to 20 days, calculated in the same way, was 2.3 days. The total number of cells potentially derived per initial culture was about $3 \times 10^8$. The highest cell concentration (11th day) was about $5 \times 10^5$/ml of culture medium. Growth ceased after 20 days, although medium was changed on the 22nd and 24th days.

These growth attributes differed greatly from those of myeloblasts produced in a like manner by BAI strain A virus (1) which grow indefinitely in suspension in shaking cultures. In contrast, strain MC29 virus-infected cells failed to grow on transfer to shaking flasks. In an experiment to directly compare the responses to the respective agents, bone marrow cultures were prepared in the usual medium in Petri dishes as described and, likewise, with aliquots of the same cell suspension under the conditions employed for shaking cultures in M199 chicken serum mixture (3, 4). The cultures were then inoculated in parallel with strain MC29 or BAI strain A. Growth occurred as usual in the Petri dish cultures inoculated with strain MC29; in the shaking cultures, characteristic cells appeared in 5 days, but significant growth was not evident. In contrast, myeloblasts in the BAI strain A-treated cultures in shaking flasks grew as observed previously (1) but failed to proliferate in the static Petri dish preparations as was also found earlier (2).

The differences in the growth potentials of the 2 cell types were further emphasized by the failure to establish in vitro the primitive myeloid cells from the blood of chickens diseased with strain MC29. Such cells did not survive for more than a few days in either Petri dish or shaking cultures. Primitive cells of BAI strain A myeloblastic leukemia, however, proliferate readily and for indefinite periods under these conditions (3, 4).

Morphology of the Specific Cells and of Related Elements

As mentioned, plasmacytes were numerous and persisted without evident morphologic change in both control and virus-inoculated cultures. Mitotic figures were rare. Special studies were not done to differentiate mononuclear osteoclasts from plasmacytes.

Cells characteristic of cultures infected with strain MC29, similar to those previously described (35), were difficult to study by usual procedures of light microscopy. Those adherent to the cover glass (Fig. 2) remained essentially spherical without flattening, a characteristic, likewise, of myeloblasts. The deeply stained cells showed a vacuolated cytoplasm with a relatively small, eccentrically placed nucleus and a very large nucleolus. Mitoses were frequent (Fig. 2). Essentially the same features were displayed by cells sedimented from culture fluid, resuspended in chicken serum, and smeared as for a blood film. The differences in the relative sizes of the cytoplasm and...
nuclei appeared greater (Fig. 3) than those that were apparent in Fig. 2.

Although cells from the blood of chickens with myelocyto-
matosis did not proliferate actively in culture, they did persist for a time. Fig. 4 shows a smear of such cells, prepared in the same way as that of Fig. 3, held in culture for 10 days. There was a definite resemblance to the bone marrow derivatives (Fig. 3), but the large nucleoli were indistinct though clearly evident.

Some features of the cells were more clearly demonstrable by phase-contrast micrography (Figs. 5, 7). The relatively small, eccentrically located nuclei and large nucleoli were especially prominent in flattened cells (Figs. 5, 6). The Golgi areas in both figures were extensive and showed vacuoles, and many mitochondria were adjacent to them. Rather large protrusions of the cell membrane were common in the preparation of Fig. 5. In Fig. 7, the myelocytes showed similarities to those of Figs. 5 and 6 immediately after removal from the blood, but they could not be flattened further without rupture of the nuclei and disruption of cell structure.

Myeloblasts from a bird with leukemia induced by BAI strain A (Fig. 8) showed marked differences from the strain MC29 virus-derived cells. The nuclei were relatively large, and the nucleoli were not visible in this preparation.

Figs. 9—12 showed further comparisons of strain MC29 cells and BAI strain A-induced myeloblasts. The blood smear from a bird with myelocytomatosis (Fig. 9) again showed indistinct, large nucleoli and illustrated frequently observed mitoses. The myeloblast nuclei (Fig. 10) were very large and granular, and nucleoli were not demonstrable. Many cells were broken, leaving the nuclei free.

Other differences between the myelocytes and myeloblasts induced by strains MC29 and BAI A respectively were emphasized by characteristics of the cells in diseased tissue. Typical myelocytomatosis of the liver (Fig. 11) showed crowding of the sinuses, invasion of acinar cords, and destruction and replacement of hepatocytes by closely packed myelocytes. In contrast, in BAI strain A leukemia, myeloblasts (Fig. 12) filled the sinuses as individual cells and caused damage to hepatocytes by interference with blood supply without active invasion of the cords. A principal attribute of the neoplastic myelocytes is formation of cohesive, organized, and invasive growths, whereas myeloblasts form only loose cell masses, either in the vascular system or in the tissues by extravasation.

Electron Microscopy

Figs. 13—16 demonstrate the ultrastructural aspects of myelocytes from bone marrow cultures treated with strain MC29, and Figs. 17—19 show the findings in parallel studies on primitive cells from the blood of birds with myelocytomatosis induced by the agent. The structural features of these cells, derived by strain MC29 virus infection, are compared with those of myeloblasts from bone marrow cultures (Figs. 20, 21) and the chicken (Figs. 22, 23) infected with the BAI strain A agent.

The electron micrographs substantiate and greatly extend the findings by light microscopy. Clearly illustrated are the relatively small nuclei of the bone marrow cells (Figs. 13—15) which are of irregular contour and indented or lobulated. Small amounts of condensed chromatin are deposited at the nuclear membrane or in small masses in the nucleoplasm. The high content of diffuse chromatin characteristic of CEC morphologically altered by strain MC29 (19) was not evident as such. The particularly prominent nucleoli (Figs. 13, 15, 16) exhibit no unusual structural characteristics. Such large nucleoli were also a feature of strain MC29 altered CEC (19).

A peculiar aspect of structure was the grainy or "ground glass" appearance of the cytoplasm which was common to several forms of neoplastic cells in myelocytomatosis as suggested in the light micrograph of Fig. 11 and clearly evident in the electron micrograph of Fig. 15. This might be attributed, in part, to the high concentration of ribosomes and to the amorphous gray matrix, both unevenly distributed throughout the cytoplasm (Figs. 15, 16). Rough endoplasmic reticulum was sparse and was represented chiefly by short, narrow channels. The mitochondria were small, and the Golgi apparatus was well developed. The plasma membrane showed numerous filiform processes (Figs. 13—15), and budding virus particles were frequently evident. Filamentous membrane processes like those in Fig. 15 were very numerous and prominent in some solid tumors in the liver which are to be described in another report.3

Myelocytes from the blood of chickens with myelocytomatosis (Figs. 17—19) exhibited the principal features characterizing the cells from bone marrow infected with MC29 virus. Notable differences evident in Figs. 17—19, compared with Figs. 15 and 16, were the distinctly larger quantities of condensed chromatin (Fig. 19), smaller numbers of mitochondria, much less rough endoplasmic reticulum, and less plasma-membrane activity as judged by the fewer filamentous processes. Some cells contained a few granules. These cells also showed the typical high concentration of ribosomes and gray matrix responsible for the characteristic appearance of the cytoplasm. A well-developed Golgi is illustrated in Fig. 19.

As already demonstrated by light microscopy, the myeloblasts of myeloblastic leukemia induced by BAI strain A differed substantially, but by no means profoundly, from the strain MC29 virus-derived cells (Figs. 8, 10, 12). This was made particularly evident, as shown in Fig. 20, by the relatively small nucleolus and the character of the cytoplasm (Figs. 20, 21) of myeloblasts from bone marrow in culture with BAI strain A agent. The nuclei in the thin section of Fig. 20 appear to be much smaller than suggested by light microscopy (Figs. 8, 10). As in the cultures infected with strain MC29 virus, the nuclei were irregular in shape; some were indented others lobulated. As evident in Fig. 20 and more so in Fig. 21, the cytoplasm of the myeloblasts did not show the unusual appearance evident in the strain MC29 cells; ribosomes were fewer, and the grayish ground substance was much less dense.

Myeloblasts from the leukemic chicken (8, 18), however, showed (Figs. 22, 23) the large nuclei seen by light microscopy and, in addition, variation in contour and lobulation of the organelle. In these blood cells, there was much condensed chromatin, a more plentiful and highly developed rough endoplasmic reticulum, and fewer apparent ribosomes.
Virus Elaboration

Virus output in the bone marrow cultures was followed both by counts of physical particles and by infectivity titration (FFU estimate). Chart 2 shows the characteristics of liberation of physical virus particles by the cultures in the experiment of Chart 1. Virus particle production was slightly more than $10^8$/ml for the 7th to 10th days but increased thereafter to about $10^9$ particles/ml. The average total potential virus output, calculated with consideration of discarded cells, was about $1.6 \times 10^{12}$ particles/ml of the initial cultures in the 24-day period.

In this same experiment, counts were made on the cells discarded and on virus particles in the corresponding culture medium. Calculations based only on the number of free-floating cells, i.e., not counting those remaining attached to the dish, revealed the rates of particle liberation/cell/hr given in Table 2. These rates, representing only broad approximations, varied from the extremes of 17 to 184 with an average of 82 particles/cell/hr, a value not greatly different from the numbers found in most of the estimates.

Virus output in terms of FFU/ml of culture fluid from another experiment is shown in Chart 3. The newly formed infectious agent was detectable at 48 hr, and the successive increases in FFU output/ml in the respective intervals followed a sigmoid curve to a level of about $10^5$ FFU on the 10th day.

Infectivity of virus from bone marrow cultures was very low relative to that from CEC cultures (25). This attribute was not studied in detail, but in estimates on culture fluid at the 12-, 16-, and 20-day intervals, in the experiment of Chart 2, virus particle concentrations were $1.18 \times 10^9$, $0.58 \times 10^9$, and $2.07 \times 10^9$/ml in comparison with respective FFU values of $8.6 \times 10^4$, $6.4 \times 10^4$, and $5.4 \times 10^4$. The corresponding ratios of virus particles/FFU were $1.4 \times 10^4$, $0.9 \times 10^4$, and $3.8 \times 10^4$ compared with approximately $10^3$ particles/FFU of virus taken at 6-hr intervals from CEC cultures (25). Whether this was due to differences in culture conditions or to real properties of the agent from the 2 sources was not determined.

Neutralization of Virus from Bone Marrow Cultures

Similarity between strain MC29 virus from bone marrow cultures and the agent elaborated by CEC was tested further by comparative neutralization studies (16). Infectivity of the preparation of bone marrow virus employed was $1.4 \times 10^4$

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**Table 2**

<table>
<thead>
<tr>
<th>Total time of infection (days)</th>
<th>Virus particles/cell/hour</th>
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<tr>
<td>9</td>
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<tr>
<td>10</td>
<td>17</td>
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<tr>
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<tr>
<td>18</td>
<td>77</td>
</tr>
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<td>20</td>
<td>184</td>
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</table>

Virus particles released in strain MC29-infected bone marrow cultures.
virus particles/FFU and that of the CEC agent was $1.28 \times 10^3$ virus particles/FFU. In the experiment summarized in Chart 4, the concentration of bone marrow virus was $1.5 \times 10^6$ virus particles/inoculum (1.12 $\times 10^2$ FFU/inoculum) and that of CEC-derived agent was $1.4 \times 10^6$ virus particles/inoculum (1.1 $\times 10^3$ FFU/inoculum) in the respective serum virus mixtures. Chart 4 reveals no significant differences between the 2 groups of data obtained with the same quantities of immune serum in mixtures with essentially identical numbers of particles of the respective agents.

Chart 4. Comparative neutralizing activities (measured on CEC monolayers) on strain MC29 virus from chick embryo cell cultures and that from bone marrow cultures of antiserum from chickens immunized with virus from CEC cultures. The amounts of virus/0.1 ml inoculum in the virus-serum mixtures were 1.5 $\times 10^6$ particles (1.12 $\times 10^2$ FFU/inoculum) and 1.4 $\times 10^6$ particles (1.1 $\times 10^3$ FFU/inoculum) of bone marrow and CEC culture agents respectively. BM virus, bone marrow virus; CEC virus, chick embryo cell virus; FFU, focus-forming units.

**DISCUSSION**

As already noted, the most outstanding aspects of the findings with strain MC29 virus have been the differences between host responses to this agent and those responses to other leukemia viruses. This was evident, first, in the broad spectrum of hematopoietic tissue neoplasms, the renal growths, and hepatocyte tumors (20, 28) which were so different from myeloid neoplasia (13) in the form of myeloblastic leukemia caused by the BAI strain A. A second difference, and this from all other leukemia strains, was the rapid infection and uniform morphologic alteration (7, 19, 26) of chick embryo cells with formation of foci permitting rapid and accurate assay (24) of the agent *in vitro*. Studies with bone marrow in culture have revealed strain MC29 effects similar, in principle, to those of other leukemia viruses in the induction of infection, initiation of cell growth, and elaboration of the etiologic virus. On the other hand, however, comparison of the individual features of these responses with those to BAI strain A (1) has demonstrated strain MC29 specificity of influence *in vitro* paralleling that observed *in vivo*.

Cells proliferating in strain MC29 bone marrow cultures were morphologically essentially indistinguishable from myelocytes in the blood and tissues in myelocytomatosis. Features exceptional in comparison with BAI strain A myeloblasts produced either in the bird or bone marrow cultures were very large nucleoli, ribosome-rich cytoplasm, and an unusually dense gray matrix with the staining reaction of protein imparting a characteristic grainy or "ground glass" appearance to the cytoplasm. Other marked differences were the transient growth of myelocytes compared with enduring proliferation of myeloblasts in culture (1, 3, 4) and the behavior of the 2 cell types in the respective diseases of myelocytomatosis and myeloblastosis. Rough endoplasmic reticulum was poorly developed in the myelocytes.

The differences between the cells were the more remarkable in view of the closely related, if not identical, precursor myeloid elements. Brief ultrastructural study (28) indicated direct one-step differentiation of myeloblasts from the most primitive intersinusoidal bone marrow elements, with the morphologic aspects of cells designated as hemocytoblasts (5, 6). A different bone marrow precursor of the myelocytes of myelocytomatosis was not discernible. In the BAI strain A disease, neoplastic myeloblasts, morphologically indistinguishable from normal myeloblasts (8, 18), crowded the marrow, but, in myelocytomatosis, myeloblasts were scarce among the predominant myelocytes with most showing granules. There was no indication that myeloblasts were a transitional stage, certainly not as neoplastic elements, in the formation of myelocytes but, instead, myelocytes seemed to differentiate directly from the same primitive precursor elements as the myeloblasts. In all studies of myelocytomatosis, the myelocyte, with or without granules, was the cell with the neoplastic properties of proliferation and tumor formation. This was contrary to the earlier view (17) that the cells in the circulating blood in myelocytomatosis were of lymphoid character. Cells of the morphologic and growth characteristics of myeloblasts have not been observed in strain MC29 myelocytomatosis.

It is interesting to note that CEC morphologically altered by strain MC29 exhibited characteristics (19) in common with the myelocytes of both cultures and diseased birds. The similarities were marked by very large nucleoli, a high concentration of cytoplasmic ribosomes, and poorly differentiated rough endoplasmic reticulum. Particularly striking were the nucleoli which are a special attribute common to almost all cells of foci permitting rapid and accurate assay (24) of the agent *in vitro*. Studies with bone marrow in culture have revealed strain MC29 effects...
the most rapid proliferation either in tissue culture or in the respective diseases.

Virus elaborated by the myelocytes in strain MC29 bone marrow cultures was morphologically like that of other RNA avian tumor agents. Behavior of the virus was also the same as that of agent from CEC cultures with respect to influence on CEC monolayers and neutralization reaction. An explanation of the low infectivity relative to physical particle number, in contrast to that of agent from CEC cultures, was not apparent, although, obviously, virus synthesized by myelocytes may differ basically in activity from that derived from CEC. Difficulties in enumeration of total cells in the cultures precluded accurate estimates of virus output in relation to cell number. Approximations, however, indicated that the rates of particle liberation were similar to those for myeloblasts (3, 4), but they were much less than the rate for CEC (7, 25).

Specificity of virus influence, evident by the greatly different responses of the same cells to the BAI A and MC29 virus strains, has been stressed. It should be emphasized, however, responses of the same cells to the BAI A and MC29 virus particle liberation were similar to those for myeloblasts (3,4), although the myeloid cells of BAI A disease do not differentiate beyond the myeloblast stage, those in myelocytomatosis occur in a broad spectrum of differentiation stage from cells closely resembling granulocytes through cells with no granules to the extreme of elements with no resemblance to myelocytes or other identifiable myeloid derivatives.3

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Avian Leukosis Virus and Bone Marrow


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Fig. 1. Plasmacytes from bone marrow cell suspension adherent to the cover glass in culture 1 day after plating. X 750.

Fig. 2. Myelocytes characteristic of bone marrow preparations appearing 5–6 days after treatment with strain MC29 virus. Ten days after virus infection, the densely stained cells (adherent to 2 sites on the same cover glass) were essentially spherical, contained relatively small nuclei (N) and large, prominent nucleoli (NU), and showed frequent mitotic figures (MT) (compare with Fig. 5). X 750.

Fig. 3. Smear of cells sedimented from supernatant fluid of bone marrow culture 14 days after infection with strain MC29 virus. Large nucleoli (NU) were less prominent under these conditions of staining than those of the corresponding adherent cells of Fig. 2 (compare with Figs. 2, 5). X 750.

Fig. 4. Smear of cells from peripheral blood of strain MC29-diseased bird (Z 891, see Fig. 6) kept in tissue culture for 10 days. Only indefinite outlines suggested large nucleoli (NU) seen in phase-contrast pictures of cells from the same cultures shown in Fig. 6. X 750.

Fig. 5. Phase-contrast micrograph of cells from supernatant fluid of bone marrow cultures 8 days after infection with strain MC29 virus (see Figs. 2, 3). The vacuolated cytoplasm was voluminous relative to the nucleus, contained occasional fat droplets (L), and often showed large wavy membranes (ME) on one side. Nucleoli (NU) were very large and prominent in the eccentrically situated nuclei (N). X 1500.

Fig. 6 Phase-contrast micrograph of cells kept in culture for 10 days after recovery from the buffy coat of blood obtained from Bird Z 891 102 days after inoculation with strain MC29 virus. The greatly flattened cells (from the same culture as those of Fig. 4) were similar to the cells from cultures of strain MC29-infected bone marrow (Fig. 5). The nuclear membranes of some cells (arrow) were ruptured by pressure under the cover glass. X 1500.

Fig. 7. Phase-contrast micrograph of buffy-coat cells from blood of Bird Z 974 24 days after infection with strain MC29 virus. The moderately flattened cells showed 1 or 2 prominent nucleoli (NU). X 1500.

Fig. 8. Phase-contrast micrograph of myeloblasts from buffy coat of Bird O 323 with myeloblastic leukemia 17 days after inoculation with BAI strain A virus. The cytoplasm was small relative to the size of the nucleus (N), and nucleoli were not evident. X 1500.

Fig. 9. Blood smear from strain MC29-diseased Bird Z 974 made at the same time that the specimen was taken for the phase-contrast micrograph of Fig. 7. Nucleoli (NU) were evident but not distinct. X 750.

Fig. 10. Blood smear from BAI strain A-diseased Bird O 323 with myeloblastosis 16 days after inoculation with the agent. The granular nuclei were large, enclosed in scant cytoplasm, and showed no nucleoli. Numerous nuclei were free. Mitotic figures were numerous. X 750.

Fig. 11. Distribution of nongranulated myelocytes (MYC) in liver of Bird Y 109 30 days after inoculation with strain MC29 virus. The primitive cells with typical large nucleoli filled the sinuses and actively invaded the parenchymal cell cords (P), destroying and replacing the hepatocytes. X 280.

Fig. 12. Distribution of myeloblasts (MYB) in the liver of Bird A 427 with myeloblastic leukemia 19 days after inoculation with BAI strain A virus. Primitive cells (myeloblasts) crowded the sinuses but did not actively destroy hepatocytes or invade the parenchymal (P) cell cords. X 280.
Figs. 13–15. Cells from fluids from the same bone marrow cultures 10 (Fig. 13) and 9 (Figs. 14, 15) days after exposure to strain MC29 virus. Predominant micromorphologic features were: Nuclei (N) with highly irregular contours due to invaginations of the nuclear membranes. Some of these appeared to be lobulated (Fig. 13). Condensed chromatin (CH) was sparse and situated mostly at the nuclear membrane or less so in small masses throughout the nucleoplasm. Large nucleoli (Fig. 16) with normal component distribution (NU). An abundant cytoplasm with a large number of irregularly distributed free ribosomes embedded in a dense matrix stained characteristically like protein. An unusual feature of the cytoplasm was a granary or "ground glass" appearance due to the mixture of ribosomes in the background matrix. The rough endoplasmic reticulum (RER) was poorly developed and showed only very narrow channels, but the Golgi (G) complex was well developed (Figs. 5, 6). Many long filiform processes extended from the plasma membrane. Fig. 13, X 11,600; Fig. 14, X 10,500; Fig. 15, X 17,500.

Fig. 16. High magnification of the large nucleolus and a portion of the nucleus of the immature cell shown in the inset. Except for size, the nucleolar structure was not unusual but was essentially identical with these organelles in chick embryo cells (19) morphologically altered by strain MC29. Condensed chromatin was scattered in small masses in the nucleoplasm or at the nuclear membrane. Diffuse chromatin as seen in altered chick embryo cells (19) was not a prominent component of the nucleoplasm. A portion of cytoplasm (CY) with a mitochondrion (MI) protrudes into the nucleus. X 55,000; Inset X 7,700.

Figs. 17, 18. Electron micrograph surveys of myelocytes from the blood of a strain MC29 virus-diseased Bird U 775 30 days after inoculation with the agent. The nuclei were small in relation to the surrounding cytoplasm, and contained much dense chromatin mostly attached to the nuclear membrane. The RER was extremely scarce and mitochondria were few, but the Golgi area was well developed. Numerous free ribosomes were distributed evenly throughout the cytoplasm. Some granules (GR) characteristic of myelocytes were adjacent to the Golgi zone. Fig. 17, X 5,900; Fig. 18, X 11,000.

Fig. 19. Higher magnification of cell from the same preparation as that of Figs. 17 and 18 illustrates condensed chromatin (CH) and lack of demonstrable diffuse chromatin, numerous ribosomes, dense, homogeneous cytoplasm matrix, several granules (GR), and well-developed Golgi zone with centriole (G). Rough endoplasmic reticulum (RER) is very sparse. X 56,000.

Fig. 20. Survey of myeloblasts in bone marrow cultures infected with BAI strain A virus. Characteristics (Fig. 21) differing notably from those of strain MC29 myeloid cells from bone marrow were less homogeneity of the cytoplasm, small, dense nucleoli, and meager rough endoplasmic reticulum. Several granules (GR) were in the cytoplasm. The irregular, indented, or lobulated nuclei were placed to one side of the cell opposite the prominent Golgi complex and associated mitochondria. X 7,800.

Fig. 21. Myeloblast from bone marrow treated in culture with BAI strain A virus, in comparison with elements from analogous strain MC29 cultures (Figs. 13–15), shows relatively few ribosomes embedded in a matrix of low density. The nucleolus was comparatively large in this cell. X 45,000.

Figs. 22, 23. Myeloblasts of blood from bird with leukemia induced by BAI strain A virus. The survey picture (Fig. 22) shows relatively large, irregular, indented, or lobulated nuclei with small nucleoli (NU), characteristic of the myeloblasts, from the chicken or held in tissue culture. The mitochondria (MI) and ribosome contents of the cytoplasm are not unusual, and the cells lack the dense, gray background common to strain MC29-derived myeloid cells. The rough endoplasmic reticulum (RER) is relatively well developed. The chromatin (CH) appears to be definitely less condensed than in the cells of the blood from strain MC29-diseased birds (Fig. 18). Fig. 22, X 6,600; Fig. 23, X 23,200.
Response of Bone Marrow to MC29 Avian Leukosis Virus in Vitro


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