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Increase of normal myeloblast viability and multiplication without blocking differentiation by type C RNA virus from myeloid leukemic cells

(bone marrow/macrophage- and granulocyte-inducing protein/leukemic cell competence for differentiation/virus from mutant cells)

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ABSTRACT Clones of mouse myeloid leukemic cells that differ in their competence to be induced for normal cell differentiation by the protein inducer MGI produce type C virus. These viruses have been studied for their effect on the viability, multiplication, and differentiation of normal bone marrow cells either with or without the addition of MGI. Virus from leukemic clones that can differentiate normally to mature macrophages and granulocytes (MGI^+D^+ clones) induced some multiplication of myeloblasts in the bone marrow, but the cells did not differentiate without adding MGI. In the presence of MGI, this virus then induced an increased number of colonies whose cells differentiated to mature macrophages or granulocytes as in colonies of uninfected cells. Virus infection also resulted in a decrease in the amount of MGI and fetal calf serum that was required for colony formation. Virus from MGI^+D^+ clones, in the presence of MGI, was 500-fold more effective in increasing colony formation than virus from the differentiation-defective MGI^-D^- clones, although both types of virus replicated with equal efficiency in the normal bone marrow cells. No such increase was obtained after infection with the Friend leukemic virus complex or the Moloney murine leukemia virus. Infection with virus from a MGI^+D^+ clone that was differentiated by MGI mainly to macrophages induced a higher percentage of macrophage colonies than virus from MGI^+D^+ clones that were differentiated by MGI to granulocytes and macrophages.

Studies with isolated myeloblast colony-forming cells from the bone marrow have indicated that these are the target cells for the virus. Infection of these isolated myeloblasts with virus from MGI^+D^+ clones induced some multiplication without differentiation in the absence of MGI, and increased the viability and multiplication of the myeloblasts without inhibiting their ability to differentiate in the presence of MGI. The results, therefore, indicate that virus from MGI^+D^+ cells can increase the viability and multiplication of normal myeloblasts in the bone marrow without blocking the ability of these cells to be induced to differentiate by MGI, and that this effect was directly related to the competence of the leukemic host cells to be induced for normal differentiation. It is suggested that the difference between the effect of virus from MGI^+D^+ and MGI^-D^- cells may be due to a difference in their integration sites in relation to the genes that control cell viability, multiplication, and differentiation.

Normal myeloblasts require the macrophage- and granulocyte-inducing protein that we now call MGI (1, 2) for cell viability, cell multiplication, and cell differentiation to mature macrophages and granulocytes (2, 3). The study of myeloid leukemic cell clones has shown that myeloid leukemia can originate by a genetic loss of the requirement for MGI for cell viability and multiplication without a loss of the ability to be induced by MGI to differentiate normally to mature cells (1–4). This type of leukemic cell we call MGI^+D^+ (D^+ for differentiation to mature cells) (1, 2). Results obtained with different myeloid leukemic clones have indicated that the change that allows the leukemic cells to be viable and multiply in the absence of MGI can then be followed by other genetic changes that produce a partial (MGI^+D^-) or complete (MGI^-D^-) block in the competence of the leukemic cells to be induced to differentiate by MGI (2, 4, 5). Cell competence to be induced for normal differentiation by MGI (6) and the induction and termination of the differentiation process (7) are associated in mouse myeloid leukemic cells with differences in the production of type C RNA virus.

Cell behavior can be changed in normal mouse erythroid cells by *in vitro* infection with Friend virus (8), in mouse B lymphocytes by *in vitro* infection with Abelson virus (9–11), and in human B lymphocytes by infection with Epstein–Barr virus (12, 13). In view of the relationship between virus production and myeloid leukemic cell differentiation, which suggests a role of the virus in the process of cell differentiation (7), the present studies were undertaken to determine (*i*) whether infection of normal myeloblasts in the bone marrow with virus from myeloid leukemic cells can change their normal MGI requirement for cell viability, cell multiplication, and differentiation, and (*ii*) whether viruses from myeloid leukemic cells that differ in their competence to be induced to differentiate by MGI have different abilities to change these properties of normal cells. The mouse strains used in these studies, SJL/J and SL, have an $Fv\text{-}1^{nn}$ genotype, and virus produced by all the leukemic clones studied has been identified as N-tropic (7).

MATERIALS AND METHODS

Source of Cells and MGI. The three MGI^+D^+ clones (nos. 9, 11, and 12) and two MGI^+D^- clones (nos. 5 and 13) of myeloid leukemic cells were derived (4) from a spontaneous myeloid leukemia in a SL mouse, and the two MGI^-D^- clones (nos. 1 and 6) were derived from two different x-irradiation-induced myeloid leukemias in SJL/J mice (4). Before induction, all the clones grew in suspension as myeloblasts to promyelocytes, and cells of all clones produced myeloid leukemia after inoculation into isologous adult mice. Normal bone marrow cells were taken from the femurs of 2 to 3-month-old SL or SJL/J mice. Normal myeloblasts from the bone marrow of mice injected with sodium caseinate were isolated by isolating the nonrosette-forming cells after two cycles of C3 rosette formation and Ficoll-Hypaque density centrifugation as described (14). Unless otherwise stated, Krebs ascites cell conditioned medium was used as a source of MGI (4).

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Abbreviations: MGI, protein inducer of differentiation in normal and leukemic myeloid cells; MGI^+ cells, cells inducible by MGI for some differentiation-associated properties; D^+ cells, cells that can be induced to differentiate by MGI to mature macrophages or granulocytes; MuLV, murine leukemia virus.

Cell Cultures and Colony Formation in Agar. The myeloid leukemic and normal bone marrow cells were cultured in modified Eagle's medium and 10% heat-inactivated fetal calf serum (4). The normal cells were seeded in mass culture at 10^5 cells per ml and for colony formation in agar (15) at 10^5 cells per 50-mm petri dish in medium with 20% fetal calf serum and 0.2 mM dithiothreitol (16), unless otherwise stated. Myeloblast clusters, containing 8–16 cells, were counted 3 days after seeding. Colonies of >50 cells, which contained mature cells, were counted at 7 days after seeding. Cell morphology was determined after staining with May–Grunwald–Giemsa. The reproducibility of the number of colonies, myeloblast clusters, and morphological cell types varied up to ±15%, and the results represent the means of at least three independent experiments.

Viruses, Infection of Bone Marrow Cells, and Virus Infectivity Assay. Partially purified virus obtained from culture fluid as described (6) was generally used for infection of bone marrow cells. Virus was further purified when needed by banding in a 15–45% sucrose equilibrium gradient. The NB-tropic strain of the Friend leukemia virus complex and the NB-tropic Moloney murine leukemia virus (MuLV) grown in NIH/3T3 cells were compared with the N-tropic virus from the myeloid leukemic cells. Bone marrow cells were infected at a final concentration of 10^7 nucleated cells per ml, in the presence of 16 μg of polybrene per ml, by incubation at 37°C for 1 hr with shaking every 15 min. Polybrene by itself had no detectable effect on the ability of the bone marrow cells to form myeloblast clusters or colonies, or on differentiation by MGI. Uninfected cells were treated with the infection procedure without adding virus. Virus infectivity assays were carried out by an infectious center assay using fluorescent staining with anti MuLV-group specific antigen antiserum (17) as described (18). The virus titer is expressed in immunofluorescence units at 4 days after infection. Infectious titers of the virus used to infect bone marrow cells were determined by infecting SC-1 mouse fibroblasts that are sensitive to both N- and B-tropic viruses (19). Production of infectious virus by the bone marrow cells was determined by using filtered medium, 5 days after infection, to infect SIM and SIM.R mouse fibroblasts that are sensitive to N-tropic and B-tropic viruses, respectively (20).

RESULTS

Formation of Myeloblast Clusters without MGI and Increase of MGI-Induced Colony Formation with Myeloid Differentiation by Infection of Bone Marrow Cells with Virus from MGI$^+$D$^+$ Cells. In the absence of MGI, uninfected normal bone marrow cells from SL mice did not form any colonies (>50 cells) or smaller groups of cells after seeding in agar. However, bone marrow cells infected with virus from MGI$^+$D$^+$ cells formed small (8–16 cell) clusters of myeloblasts. These were most evident 3 days after seeding in agar (Fig. 1). At later times these clusters degenerated without differentiation. Because these clusters consisted only of myeloblasts, they will be referred to as myeloblast clusters. The highest number of these clusters, 150 per 10^5 cells seeded, was obtained when the bone marrow cells were seeded in agar 1 day after infection. In the presence of 10% MGI in the agar, there was a higher number of macrophage and granulocyte colonies in the infected than in the uninfected cells, and the highest number of colonies was also obtained when the bone marrow cells were seeded in agar 1 day after infection. With 10% MGI, virus infection gave about a 4-fold increase in number of colonies (Fig. 2A). The colonies formed after virus infection were also larger (Fig. 1) and survived longer than those formed by the uninfected cells. In the virus infected as in the uninfected colonies, there was

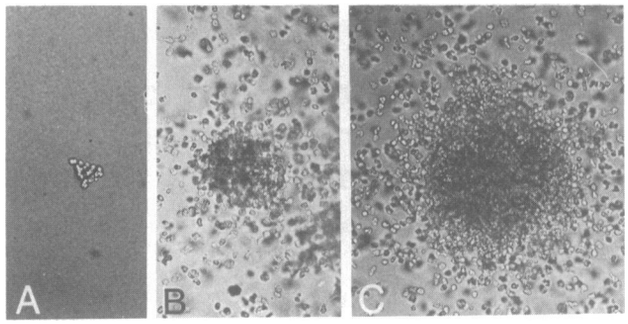

FIG. 1. Photographs of virus-infected myeloblast cluster and of uninfected and virus-infected colonies with differentiation from bone marrow cells. (*A*) Cluster of infected myeloblasts 3 days after seeding in agar without MGI. (*B*) Colony of uninfected bone marrow cells. (*C*) Colony of infected bone marrow cells. *B* and *C*, 7 days after seeding in agar with 10% MGI. Cells were infected with MGI$^+$D$^+$ virus. (×80.)

differentiation to mature macrophages and granulocytes. Similar results were obtained when the virus was further purified by sucrose equilibrium density centrifugation, and the colony enhancing activity of the virus was completely destroyed by incubation at 56°C for 30 min. This temperature treatment also destroyed more than 95% of the reverse transcriptase activity, whereas the MGI activity was not affected. The myeloblast cluster-inducing and colony-enhancing activity of partially purified virus pellets was also abolished by incubation for 1 hr at 37°C with anti-Rauscher MuLV antiserum produced against highly purified Rauscher MuLV (kindly supplied by S. A. Aaronson, National Cancer Institute, Bethesda, MD), but this incubation did not inactivate MGI. There was no measurable effect after infection of BALB/C bone marrow cells with the N-tropic virus from MGI$^+$D$^+$ leukemic cells.

Decrease in Requirement for MGI and Serum for the Formation of Colonies with Myeloid Differentiation after Infection with Virus from MGI$^+$D$^+$ Cells. Experiments with different concentrations of MGI have shown that the virus-infected SL bone marrow cells formed colonies at an MGI concentration (0.5%) that did not give colony formation by

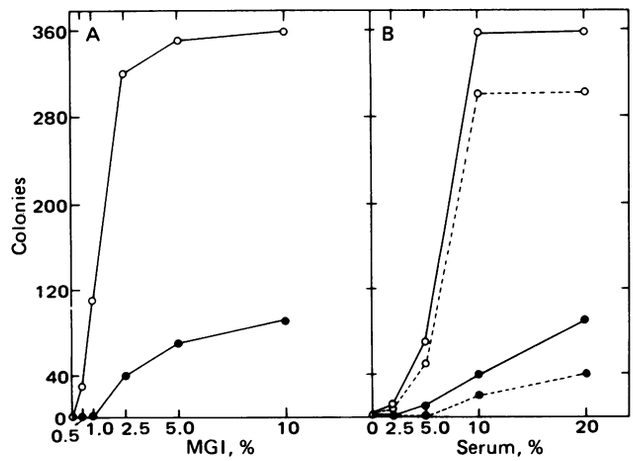

FIG. 2. MGI and serum requirement for the formation of colonies with differentiation. SL bone marrow cells were infected with 5×10^3 immunofluorescence units of MGI$^+$D$^+$ virus (clone 9) per 10^6 cells, washed, cultured for 1 day with 10% MGI in liquid medium, washed, and seeded in agar at 10^5 cells per petri dish. (*A*) Virus infected (○) or uninfected (●) cells were seeded in agar with different concentrations of MGI. (*B*) Virus infected (○) or uninfected (●) cells were seeded in agar with 10% MGI and different concentrations of fetal calf serum without (- - - -) and with (——) 0.2 mM dithiothreitol.

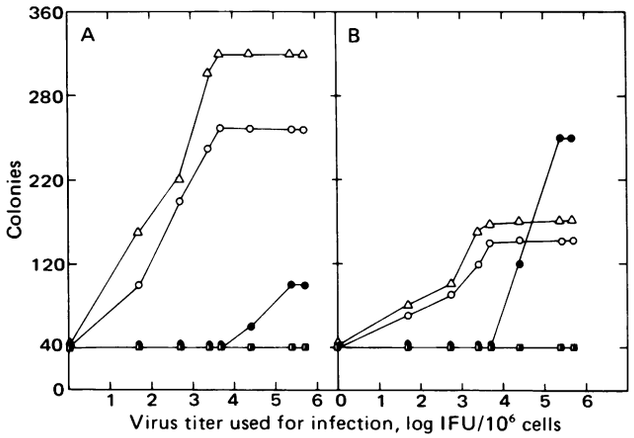

FIG. 3. Number of colonies with differentiation after infection of SL and SJL/J bone marrow cells with different titers of virus from MGI$^+$D$^+$, MGI$^+$D$^-$, and MGI$^-$D$^-$ cells, the Friend virus complex, or Moloney MuLV. Bone marrow cells were infected with different virus titers as in the legend to Fig. 2 and seeded in agar at 10^5 cells per petri dish with 2.5% MGI. (*A*) SL bone marrow cells. (*B*) SJL/J bone marrow cells. △, Virus from MGI$^+$D$^+$ clone 11; ○, virus from MGI$^+$D$^-$ clone 5; ●, virus from MGI$^-$D$^-$ clone 1; □, Friend virus complex; ■, Moloney MuLV. MGI$^+$D$^-$ clone 13 gave similar results to MGI$^+$D$^-$ clone 5, and MGI$^-$D$^-$ clone 6 gave similar results to MGI$^-$D$^-$ clone 1. IFU, immunofluorescence units.

uninfected bone marrow cells and that, after virus infection, a lower concentration of MGI was needed for optimal colony formation (Fig. 2*A*). The number of colonies formed by virus-infected cells with 2.5% MGI was 8-fold higher than the number formed by the uninfected cells with 10% MGI (Fig. 2*A*). There was also a lower requirement for fetal calf serum for colony formation after virus infection and in the absence of dithiothreitol the virus-infected cells formed colonies with 2.5% serum whereas 10% serum was required for the uninfected cells to form colonies (Fig. 2*B*). The addition of dithiothreitol produced a 2-fold increase in the number of colonies formed by the uninfected bone marrow cells with 20% serum and had almost no effect on the virus-infected cells (Fig. 2*B*).

Direct Relationship between the Ability of Myeloid Virus to Induce Macrophage and Granulocyte Colonies and Competence of the Host Leukemic Cells to Undergo Differentiation. Virus from the partially differentiable MGI$^+$D$^-$ clones showed almost the same enhancing effect on the formation of macrophage and granulocyte colonies as virus from the completely differentiable MGI$^+$D$^+$ clones. However, virus from both the MGI$^+$D$^+$ and MGI$^+$D$^-$ clones was 500-fold more effective than virus from the differentiation-defective MGI$^-$D$^-$ clones (Fig. 3). A similar difference was found in the ability of these viruses to induce myeloblast clusters in the absence of MGI. The N-tropic virus from MGI$^+$D$^+$ and MGI$^-$D$^-$ cells (7) replicated with an equal efficiency in the bone marrow cells. MGI$^+$D$^+$ clone 11 is differentiated by MGI primarily to macrophages whereas MGI$^+$D$^+$ clones 9 and 12 are induced by MGI to both granulocytes and macrophages. Results on the percentage of macrophage and granulocyte colonies after infection of bone marrow cells have indicated that virus from clone 11 gave 90% macrophage colonies, whereas virus from clones 9 and 12 gave 40% and 50% macrophage colonies, respectively.

The MGI$^+$D$^+$ and MGI$^+$D$^-$ clones originate from a leukemia in a SL mouse and the MGI$^-$D$^-$ clones, from leukemias in SJL/J mice. Both strains of mice have an *Fv-1^{nn}* genotype (7). However, infection with virus from MGI$^+$D$^+$ and MGI$^+$D$^-$ clones in the presence of MGI gave about a 2-fold higher number of colonies with SL than with SJL/J bone marrow, whereas virus from MGI$^-$D$^-$ clones, at a 500-fold higher titer, was more effective in increasing colony formation with SJL/J than with SL bone marrow (Fig. 3). This difference between the strains of mice was also observed for the induction of myeloblast clusters in the absence of MGI. Infection with the Friend leukemia virus complex or Moloney MuLV did not show any of the effects found with the MGI$^+$D$^+$ myeloid leukemia virus on either SL or SJL/J bone marrow cells (Fig. 3), although both these virus types replicated in the bone marrow cells.

Evidence That the Myeloblast Colony-Forming Cells in the Bone Marrow Are the Target Cells for the Virus from MGI$^+$D$^+$ Cells. Normal myeloblasts were isolated from SL bone marrow, and enrichment for these myeloblasts enriches for myeloblast colony forming cells (14). After infection with virus from MGI$^+$D$^+$ cells at various stages of enrichment, the number of myeloblast clusters in the absence of MGI and the increase in the number of macrophage and granulocyte colonies in the presence of MGI was related to the number of myeloid colony-forming cells (Table 1). At the final enrichment stage about 99% of the cells were myeloid, of which 93% were myeloblasts. About 4% of these cells formed colonies in the presence of MGI, and this colony formation was increased to 15% after virus infection (Table 1).

Effect of MGI$^+$D$^+$ Virus on Viability, Multiplication, and Differentiation of the Myeloblast Colony-Forming Cells. Experiments with isolated myeloblasts from normal SL bone marrow in liquid medium have indicated that, in the absence

Table 1. MGI$^+$D$^+$ virus induced formation of myeloblast clusters and colonies with differentiation at different stages of enrichment for bone marrow myeloblasts

Enrichment stage	Cell type, % of total					No. of myeloblast clusters per 10^4 cells seeded without MGI, + or − virus		No. of macrophage or granulocyte colonies per 10^4 cells seeded with MGI, + or − virus	
	Erythroid	Small lymphocytes	Mature granulocytes	Intermediate stages	Myeloblasts	+	−	+	−
Normal bone marrow	10 ± 3	13 ± 4	11 ± 3	48 ± 6	18 ± 3	10 ± 2	0	45 ± 6	15 ± 3
After sodium caseinate	2 ± 1	3 ± 1	2 ± 1	60 ± 6	33 ± 3	20 ± 3	0	220 ± 18	50 ± 6
After one cycle of C3 rosetting	1 ± 0.2	3 ± 1	0	20 ± 3	76 ± 6	100 ± 8	0	1050 ± 52	300 ± 30
After two cycles of C3 rosetting	0	1 ± 0.2	0	6 ± 2	93 ± 6	150 ± 16	0	1500 ± 60	420 ± 35

Bone marrow from SL mice was enriched for myeloblasts as described in *Materials and Methods* and ref. 14. The cells were infected with virus from MGI$^+$D$^+$ clone 9 and seeded in agar with or without 10% MGI as in the legend to Fig. 2. ± indicates SD.

of MGI, the number of uninfected cells decreased rapidly due to cell death. In contrast, the virus infected cells showed an initial increase in the number of cells, followed by a much slower decrease in cell number (Fig. 4A). Neither the uninfected nor infected cells showed differentiation in the absence of MGI as judged by cell morphology, induction of Fc rosettes, C3 rosettes (4), or lysozyme (21). In the presence of MGI, uninfected cells showed an initial decrease in cell number during the first day in culture, whereas in the virus-infected cells there was an immediate increase in cell number. After 7 days in culture, there was about a 2-fold higher number of cells in the infected cultures (Fig. 4A). Both the infected and uninfected cultures showed differentiation to mature cells in the presence of MGI (Fig. 4B). After 7 days, there was a 10-fold higher number of myeloblasts and about a 30% higher number of mature cells in the infected cultures. At this time, the infected cells gave 180 macrophage and granulocyte colonies per 10^5 cells seeded, compared to 12 colonies with the uninfected cells.

DISCUSSION

The present results have shown that infection of normal bone marrow myeloblasts with type C virus from MGI^+D^+ myeloid leukemic cells resulted in increased myeloblast viability and multiplication, but not differentiation, in the absence of MGI. Upon the addition of MGI, which is required for viability, multiplication, and differentiation of uninfected myeloblasts (2, 3), the virus caused an increase in viability and multiplication without blocking the capacity to differentiate, which then led to an increase in the number of macrophage and granulocyte colonies in agar. These results indicate that MGI^+D^+ virus infection can either induce some production of MGI or can to some extent substitute for the effect of MGI on the viability and multiplication of normal myeloblasts. This was also substantiated by the results showing that colony formation after virus infection required a lower concentration of MGI than colony formation by uninfected myeloblasts. Assuming that virus infection can to some extent substitute for MGI, a further stage

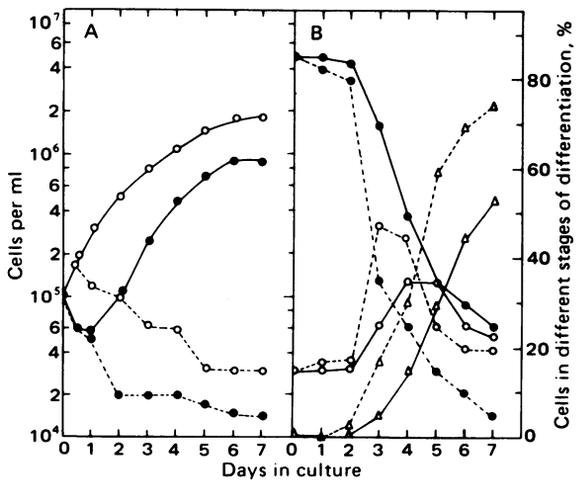

FIG. 4. Effect of MGI^+D^+ virus on the viability, multiplication, and differentiation of isolated myeloblasts cultured in liquid medium. Isolated myeloblasts from SL bone marrow were infected as in the legend to Fig. 2 and seeded at 10^5 cells per ml with or without 2.5% MGI from endotoxin serum (14). (A) Total number of cells. Virus-infected (○) or uninfected (●) cells were cultured without (- - - -) or with (——) MGI. (B) Percentage of cells on different stages of differentiation in cultures with MGI. ●, Myeloblasts; ○, intermediate stages; △, mature cells; ——, virus-infected cells; - - - -, uninfected cells.

of this substitution can then occur in myeloid leukemia, in which the malignant cells seem to have become completely independent for MGI for viability and continued cell multiplication (2, 3).

This conclusion regarding the possible substitution of the effect of a normal regulator protein required for cell viability and proliferation by virus infection may also apply to the results obtained with other viruses that effect hematopoietic cells. Normal T lymphocytes require for cell viability and multiplication the regulatory protein TCI (T cell colony inducer) (22) and normal B lymphocytes presumably require another protein, BCI (23). The formation of cell lines after infection of mouse B lymphocytes with Abelson virus (9–11) and of human B lymphocytes with Epstein-Barr virus (12, 13) may thus be due to the substitution of the normal B lymphocyte requirement by these viruses. The results showing that normal mouse erythroid cells infected with the Friend spleen focus-forming virus can form erythroid colonies without erythropoietin (8) indicate that such a substitution can also occur in erythroid cells. But, in contrast to the results obtained with the normal myeloid cells, in which after infection with virus from MGI^+D^+ cells the myeloblasts still required MGI for differentiation, the Friend virus-infected erythroblasts also differentiated without erythropoietin (8). Both the infected erythroblasts (8) and the infected myeloblasts showed a lower than normal requirement for fetal calf serum for colony formation.

The experiments with virus from mutant clones of myeloid leukemic cells that differ in their competence to be induced to differentiate by MGI have shown that similar titers of virus from MGI^+D^+ and MGI^+D^- cells were needed to obtain an optimum increase in colony formation in the presence of MGI, where virus from both these cell types was 500-fold more effective than virus from the differentiation-defective MGI^-D^- clones. This suggests that the virus from MGI^-D^- cells contains a very small fraction of MGI^+D^+-like virus. The viruses used in the present studies were derived from leukemic cells that originated in either SL or SJL/J mice. Virus from the SL cells gave a higher increase of myeloblast multiplication and colony formation on SL myeloblasts than on SJL/J myeloblasts and vice versa. The SL and SJL/J mice differ in their H-2 antigens (unpublished results). There may thus be some H-2 restriction for the effect of these myeloid viruses.

Infection with the Friend virus complex or the Moloney MuLV did not in the present experiments increase colony formation in the presence of MGI. It still remains to be determined, whether this was because these viruses were not derived from myeloid cells. A study of much longer-term cultures of bone marrow cells has reported that, after infection with the Friend virus complex, a longer survival of myeloid colony-forming cells was detected several months after initiation of the cultures. However, it is not yet known whether this late survival represents a direct effect of Friend virus infection on the myeloblasts or an effect of the virus on the feeder cells that are present in these long term cultures (24). It also remains to be determined whether infection with MGI^+D^+ virus can produce continuously growing lines of myeloid cells and whether, as in the case of Abelson virus (11, 25), this requires certain helper viruses.

The isolation of myeloblasts from normal bone marrow has indicated that myeloblast colony-forming cells that can be induced to differentiate by MGI to macrophages and granulocytes are the target cells for the virus from MGI^+D^+ cells. Infection with virus from a MGI^+D^+ clone that was differentiated by MGI mainly to macrophages induced a higher percentage of macrophage colonies than virus from MGI^+D^+ clones that were differentiated by MGI to granulocytes and to macrophages. This indicates that, depending on the myeloid differ-

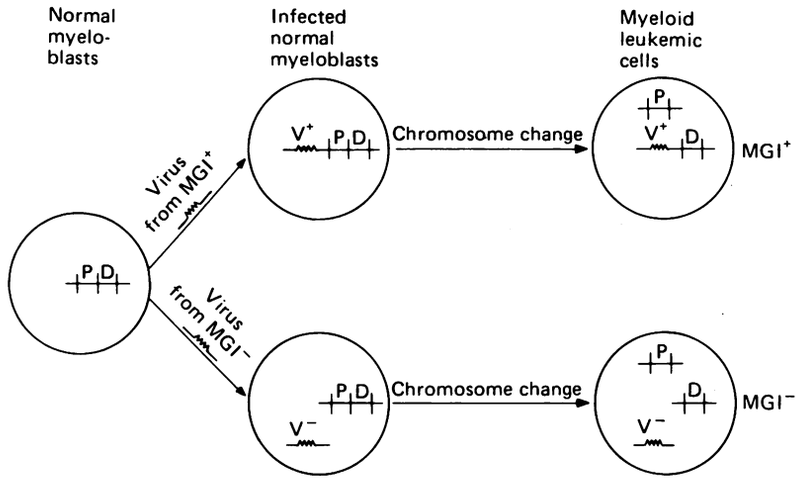

FIG. 5. Model that assumes different integration sites for the virus from MGI^+D^+ cells (V^+) and MGI^-D^- cells (V^-) in relation to the genes for cell viability and proliferation (P) and for cell differentiation (D).

entiation pattern of the virus host cells, these myeloid viruses may differentially effect the pattern of cell differentiation.

The present data obtained with the myeloid virus from MGI^+D^+ and MGI^-D^- cells and previous results obtained with normal myeloblasts and myeloid leukemic cells (2) can be used to suggest the following model for the difference in the effect of these viruses. Normal myeloblasts require MGI (2, 3) for cell viability and proliferation and for differentiation. The genes that regulate proliferation and differentiation may thus be linked in normal cells (Fig. 5) so that in the presence of sufficient MGI the cells will proliferate and then differentiate. We suggest that the MGI^+D^+ virus, but not the MGI^-D^- virus, is integrated next to the proliferation site P (Fig. 5). This integration of MGI^+D^+ virus could then change the regulation of this site to give some viability and multiplication in the absence of MGI in the normal myeloblasts. Both the MGI^+D^+ and MGI^-D^- myeloid leukemic cells seem to be completely independent for MGI for viability and continued proliferation (2). Clones of both leukemic cell types show chromosome changes, and there are specific chromosome differences detectable by chromosome banding between MGI^+D^+ and MGI^-D^- cells (26). These chromosome changes could lead to a break of the postulated linkage between proliferation and differentiation (Fig. 5) so that both types of leukemic cells would then be independent of MGI for proliferation, but MGI^+D^+ cells could still respond to MGI for differentiation. The coregulation of virus production and differentiation in MGI^+D^+ cells (7) suggests that, in the myeloid leukemic cells, the virus may be integrated next to the differentiation regulating site in MGI^+D^+ but not in MGI^-D^- cells (Fig. 5). This may also explain the ability of the MGI^+D^+ but not of the MGI^+D^- cells to undergo differentiation in response to MGI. This model can be experimentally tested by studies on the integration sites of MGI^+D^+ and MGI^-D^- viruses.

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1. Sachs, L. (1974) *Harvey Lect.* **68,** 1–35.
2. Sachs, L. (1978) *Nature (London)* **274,** 535–538.
3. Fibach, E. & Sachs, L. (1976) *J. Cell. Physiol.* **89,** 259–266.
4. Lotem, J. & Sachs, L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3781–3785.
5. Hoffman-Liebermann, B. & Sachs, L. (1978) *Cell* **14,** 825–834.
6. Liebermann, D. & Sachs, L. (1977) *Nature (London)* **269,** 173–175.
7. Liebermann, D. & Sachs, L. (1978) *Cell* **15,** 823–835.
8. Clarke, B. J., Axelrad, A. A., Shreeve, M. M. & McLeod, D. L. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3556–3560.
9. Rosenberg, N., Baltimore, D. & Scher, C. D. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 1932–1936.
10. Rosenberg, N. & Baltimore, D. (1976) *J. Exp. Med.* **143,** 1453–1463.
11. Rosenberg, N. & Baltimore, D. (1978) *J. Exp. Med.* **147,** 1126–1141.
12. Henle, W., Diehl, V., Kohn, G., Zur Hausen, H. & Henle, G. (1967) *Science* **157,** 1064–1065.
13. Klein, G. (1975) *New Engl. J. Med.* **293,** 1353–1357.
14. Lotem, J. & Sachs, L. (1977) *J. Cell. Physiol.* **92,** 97–108.
15. Pluznik, D. & Sachs, L. (1965) *J. Cell. Comp. Physiol.* **66,** 319–324.
16. Toohey, J. I. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 73–77.
17. Hartley, J. W. & Rowe, W. P. (1976) *J. Virol.* **19,** 19–25.
18. Hilgers, J., Nowinski, R. C., Geering, C. & Hardy, W. (1972) *Cancer Res.* **32,** 98–106.
19. Hartley, J. W. & Rowe, W. P. (1975) *Virology* **65,** 128–134.
20. Ware, L. M. & Axelrad, A. A. (1972) *Virology* **50,** 339–348.
21. Krystosek, A. & Sachs, L. (1976) *Cell* **9,** 675–684.
22. Gerassi, E. & Sachs, L. (1978) *J. Immunol.* **121,** 2547–2553.
23. Douer, D. & Sachs, L. (1979) *J. Immunol,* in press.
24. Dexter, T. M., Scott, D. & Teich, N. M. (1977) *Cell* **12,** 355–364.
25. Scher, C. D. (1978) *J. Exp. Med.* **147,** 1044–1053.
26. Azumi, J. I. & Sachs, L. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 253–257.