Successful Replication of Parvovirus B19 in the Human Megakaryocytic Leukemia Cell Line MB-02

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The pathogenic human parvovirus B19 has been shown to undergo productive replication in the erythroid lineage in primary normal human hematopoietic progenitor cells. However, none of the established erythroleukemia cell lines has allowed B19 virus replication in vitro. The remarkable erythroid tissue tropism of B19 virus was evaluated with a human megakaryocytic leukemia cell line, MB-02, which is dependent on the growth factor granulocyte-macrophage colony-stimulating factor but can be induced to undergo erythroid differentiation following treatment with erythropoietin (Epo). Whereas these cells did not support B19 virus DNA replication in the presence of granulocyte-macrophage colony-stimulating factor alone, active viral DNA replication was observed if the cells were exposed to Epo for 5 to 10 days prior to B19 virus infection, as detected by the presence of the characteristic B19 virus DNA replicative intermediates on Southern blots. No replication occurred if the cells were treated with Epo for 3 days or less. In addition, complete expression of the B19 virus genome also occurred in Epo-treated MB-02 cells, as detected by Northern blot analysis. B19 progeny virions were released into culture supernatants that were biologically active in secondary infection of normal human bone marrow cells. The availability of the only homogeneous permanent cell line in which induction of erythroid differentiation leads to a permissive state for B19 virus replication in vitro promises to yield new and useful information on the molecular basis of the erythroid tissue tropism as well as parvovirus B19-induced pathogenesis.

Parvovirus B19, discovered serendipitously in sera of asymptomatic blood donors (6), is now known to be the etiologic agent of a variety of clinical disorders in humans that include transient aplastic crises associated with various hemolytic anemias (17, 20, 22), a common childhood exanthem—erythema infectiosum—also called fifth disease (3), postinfection polyarthralgia (18, 30), some cases of thrombocytopenia (2, 25), chronic bone marrow failure (9, 11), and hydrops fetalis (1, 4). The virus has a remarkable tropism for the erythroid lineage in human hematopoietic progenitor cells, and only primary cells from human bone marrow (14-16, 25, 26), fetal liver (28), peripheral blood (21), and umbilical cord blood (28) have thus far been shown to support B19 virus replication in vitro. However, the heterogeneous nature of hematopoietic cells as well as the low-frequency occurrence of the erythroid progenitor cells has prompted the search for a permanent cell line that is permissive for B19 virus replication in vitro. Interestingly, despite the erythroid tropism of B19 virus, none of the human erythroleukemia cell lines tested thus far has allowed B19 virus replication. We examined a cell line, MB-02, from a patient with megakaryocytic leukemia, which can be induced to undergo erythroid differentiation in vitro (13), and hypothesized that the putative erythroid differentiated state of these cells might render them permissive for B19 virus replication. In this report, we document that MB-02 cells,

only when induced to undergo active erythropoiesis, do indeed support active and complete B19 viral replication.

(A preliminary report of this work has been presented previously [13a].)

Erythroid differentiation of MB-02 cells. A detailed account of this cell line has recently been published (13). Briefly, these cells are dependent on the growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF) but can be induced to undergo erythroid differentiation by treatment with erythropoietin (Epo). For example, over 90% of these cells can be committed to terminal erythroid maturation leading to progression from primitive erythroblasts to production of late erythroblasts and normoblasts after a 10-day treatment with Epo (13). We carried out a similar study in which MB-02 cells were cultured in RPMI medium supplemented with 10% human serum and 200 U of GM-CSF (a generous gift from Immunex Corp., Seattle, Wash.) per ml and were either untreated or deprived of GM-CSF and treated with 4 U of Epo (Toyobo, New York, N.Y.) per ml at 37°C for up to 10 days. Cells were collected on glass slides by cytocentrifugation and stained with Wright-Giemsa stain as previously described (13). A composite morphology of these cells is shown in Fig. 1.

As can be seen, untreated or Epo-treated MB-02 cells on day 0 (Fig. 1a and d) showed the characteristic features of undifferentiated megakaryocytic leukemia cells, whereas by day 5 of Epo treatment (Fig. 1b and e), primitive erythroblasts were readily visible, and by day 10 of Epo treatment (Fig. 1c and f), a majority of these cells showed mature erythroid cell differentiation. Replicate cultures were used in all subsequent B19 virus infection studies.

Parvovirus B19 DNA replication in MB-02 cells. MB-02

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FIG. 1. Morphology of MB-02 cells cultured in the presence of Epo. Cells were incubated in the presence (4 U/ml) of Epo for 0 (a and d), 5 (b and e), and 10 (c and f) days, and cytocentrifuged preparations were stained with Wright-Giemsa stain. Original magnifications, $\times 160$ (a to c) and $\times 400$ (d to f).

cells, with and without prior treatment with Epo, were infected with a B19 virus-positive serum (B19-SH) obtained from a patient with sickle cell anemia experiencing aplastic crisis at the Indiana University Hospitals, Indianapolis, Ind. (25), as previously described (26–28). After infection, cells were washed, and low- M_r DNA was isolated at various times postinfection (p.i.) by the method described by Hirt (10). Equivalent amounts were digested to completion with *Bam*HI restriction endonuclease (Boehringer Mannheim Biochemicals, Indianapolis, Ind.); electrophoresed on 1% agarose slab gels; transferred to Southern blots (24); probed with a B19 virus DNA-specific probe, pYT103 (7), radioactively labeled with [32 P]dCTP by the random hexanucleotide primer labeling method of Feinberg and Vogelstein (8); and analyzed essentially as previously described (25–28). The results are depicted in Fig. 2.

In Fig. 2, lane 1, low- M_r DNA isolated from B19 virus-infected normal human bone marrow (NBM) cells, which have previously been shown to be permissive for B19 virus replication as determined by the presence of the characteristic *Bam*HI doublets (3.9/3.8 kb and 1.5/1.4 kb) representing the viral DNA replicative intermediates (15, 16, 25, 27–29), was electrophoresed to serve as a positive control. It is interesting to note that MB-02 cells that were maintained only in GM-CSF did not support B19 virus DNA replication 48 h p.i. (Fig. 2, lane 2). Low- M_r DNA isolated from either untreated (not shown) or Epo-treated MB-02 cells 0 h p.i. showed a strong hybridization signal from the input virus (Fig. 2, lane 3), indicating that these cells allowed entry of infecting B19 virions. The unique 1.5- and 3.9-kb bands result from digestion with *Bam*HI restriction endonuclease at a single site in the B19 virus genome after reannealing of the two viral DNA strands (15, 16). More interestingly, however, is that when Epo-treated cells were incubated for 48 h p.i., the characteristic *Bam*HI doublets representing the

viral DNA replicative intermediates were clearly detectable, which is similar to what is observed for primary human hematopoietic cells (15, 16, 25, 27–29). These data suggest that MB-02 cells, when induced to undergo erythroid differentiation, become permissive for the viral DNA replication, although the efficiency of replication is less than 10% of that in NBM cells (data not shown).

Correlation of B19 virus DNA replication with erythroid differentiation. Since recent studies by Takahashi et al. (29) have suggested that susceptibility to B19 virus infection of the human primary erythroid progenitor cell increases with differentiation, it was of significant interest to next examine whether MB-02 cells also exhibited a similar correlation. MB-02 cells were grown and maintained in the presence of either GM-CSF or Epo for up to 7 days, infected with B19 virus, and analyzed on Southern blots for the viral DNA replication exactly as described above. These data are shown in Fig. 3.

Again, B19 virus-infected NBM DNA was included as a positive control (Fig. 3, lane 1); it is noteworthy that no B19 virus DNA replicative intermediates were detected in cells not treated with Epo (lanes 2, 4, 6, and 8) or in cells that were treated with Epo for less than 3 days or less (lanes 3 and 5), although Epo was included during B19 virus infection. The characteristic *Bam*HI doublets were clearly detected in cells treated with Epo for 5 and 7 days (Fig. 3, lanes 7 and 9). Interestingly, the early primitive erythroblasts are detected in these cultures after 5 days of Epo treatment (13) (Fig. 1). We conclude from these studies that exposure of MB-02 cells to Epo for at least 5 days is required to achieve a permissive state. These data, therefore, suggest that the MB-02 cell culture faithfully mimics the primary human erythroid progenitor cell in its susceptibility to B19 virus permissivity (29).

B19 virus genome expression in MB-02 cells. We next

FIG. 2. Southern blot analysis of parvovirus B19 DNA replication in NBM and MB-02 cells. Untreated NBM and MB-02 cells and MB-02 cells treated with Epo were infected with B19 virus, and low- M_r DNA samples cleaved with *Bam*HI restriction endonuclease were analyzed as described in the text. Lane 1, B19 virus-infected NBM cell DNA; lane 2, untreated, B19 virus-infected MB-02 cell DNA (48 h p.i.); lane 3, Epo-treated, B19 virus-infected MB-02 cell DNA (0 h p.i.); lane 4, Epo-treated, B19 virus-infected MB-02 cell DNA (48 h p.i.). *Hind*III-cleaved PM2 DNA fragments were coelectrophoresed to serve as size markers. ss, single strands of B19 virus DNA.

wished to examine whether Epo-treated MB-02 cells also allowed complete transcription of the B19 virus genome. Equivalent amounts of total RNA samples isolated by the method described by Chomczynski and Sacchi (5) from B19 virus-infected MB-02 cells pretreated with either GM-CSF or Epo were analyzed on Northern blots as previously described (14, 25, 28). These results are shown in Fig. 4.

Mock-infected and B19 virus-infected NBM cells were included to serve as appropriate controls. As is evident, no B19 virus RNA transcripts were detected in mock-infected NBM cells (Fig. 4, lane 1), whereas abundant B19 virus genome expression was seen in infected NBM cells (Fig. 4, lane 2). Interestingly, the three B19 virus major RNA transcripts of 3.1, 2.3, and 0.8 kb were also detectable in B19 virus-infected MB-02 cells pretreated with Epo (Fig. 4, lane 3), but not in untreated cells (Fig. 4, lane 4). These data indicate that complete transcription of the B19 virus genome also occurs in MB-02 cells, although the level of expression is less than 10% of that observed in B19 virus-infected NBM cells.

Biological activity of the B19 progeny virions. In preliminary experiments, we also documented by DNA dot blot analysis (25, 28) complete assembly and release of the B19

FIG. 3. Southern blot analysis of the kinetics of B19 virus DNA replication in Epo-treated MB-02 cells. Cells were treated with either GM-CSF or Epo for various indicated times, infected with B19 virus, and incubated in the presence of Epo for 48 h. The rest of the steps were the same as described in the legend to Fig. 2. Lane 1, B19 virus-infected NBM cell DNA; lanes 2, 4, 6, and 8, DNA from B19 virus-infected MB-02 cells cultured in the presence of GM-CSF alone; lanes 3, 5, 7, and 9, DNA from B19 virus-infected MB-02 cells cultured in the presence of Epo alone. ss, single strands of B19 virus DNA.

virus progeny virions into culture supernatants of MB-02 cells only when these cultures were pretreated with Epo (data not shown). It was of interest to next examine whether the progeny virions produced in MB-02 cells were infectious for NBM cells. This was carried out by a two-cycle infection assay essentially as previously described (25, 28). Equivalent amounts of low- M_r DNA isolated from mock-infected NBM cells or NBM cells infected with culture supernatants from MB-02 cells, with or without pretreatment with Epo, at various times p.i., were analyzed on Southern blots as described above. These data are depicted in Fig. 5.

It is evident that no B19 virus DNA replication could be seen either in mock-infected NBM cells (Fig. 5, lane 1) or in NBM cells that were infected with culture supernatants from B19 virus-infected MB-02 cells grown in GM-CSF alone at 48 h (lane 2) and 96 h (lane 3). However, culture supernatants from MB-02 cells pretreated with Epo followed by B19 virus infection 48 h (Fig. 5, lane 4) and 96 h (lane 5) p.i. were indeed infectious for NBM cells. In Fig. 5, lane 6, low- M_r DNA isolated from primary infection of Epo-treated MB-02 cells was electrophoresed to serve as a positive control. On the basis of these data, we conclude that MB-02 cells that are induced to undergo erythroid differentiation allow complete replication of human parvovirus B19 and produce progeny virions that are infectious.

One of the advantages of developing a culture system with an established cell line for the *in vitro* propagation of parvovirus B19 is its relative homogeneous nature compared with primary hematopoietic progenitor cells. The availability of such a system also obviates the need for bone marrow or blood donors as well as the need for fetal liver cultures which

FIG. 4. Northern (RNA) blot analysis of B19 virus genome expression in NBM and MB-02 cells. Equivalent amounts of total cellular RNA samples isolated from NBM and MB-02 cells 48 h p.i. were analyzed as described in the text. Lane 1, mock-infected NBM cell RNA; lane 2, B19 virus-infected NBM cell RNA; lane 3, Epo-treated, B19 virus-infected MB-02 cell RNA; lane 4, untreated, B19 virus-infected MB-02 cell RNA.

must take into account certain ethical considerations. Although the currently observed level of viral replication in MB-02 cells is significantly lower than that in NBM cells, the molecular basis of which remains unclear, it may be feasible to manipulate this system to make it more efficient (33). The relative homogeneous nature of this culture system may, in turn, also allow identification and isolation of the putative host cell factor(s) that may play a role in the remarkable tissue tropism of B19 virus for the erythroid progenitor cells. Furthermore, it has become increasingly clear that a persistent B19 virus infection may lead to pathogenesis of chronic bone marrow failure (9, 11) as well as polyarthralgia (32). The availability of this cell line should facilitate the molecular dissection of the underlying mechanism of viral persistence, although we were unable to document integration of the viral genome in MB-02 cells (data not shown).

Although it is interesting to note that B19 virus gene expression (14) is limited to human erythroid progenitor cells that permit active viral replication (15, 16, 26–29, 31), we have recently documented a low-level expression of the viral genes in the absence of viral DNA replication in primary human megakaryocytic lineage cells (25). It is perhaps not surprising, therefore, that a cell line from the megakaryocytic lineage that can also undergo erythroid differentiation is permissive for B19 virus replication in view of the fact that the differentiation pathways of the two lineages share certain common features (12, 19). In this context, it is also noteworthy that Shimomura et al. (23) have recently described continuous propagation of B19 virus in a human megakaryocytic leukemia cell line, UT-7/Epo, that has been adapted to grow in Epo. The culture system described here more closely resembles the natural target cell for B19 virus, since Epo treatment of MB-02 cells leads to terminal maturation

FIG. 5. Southern blot analysis of B19 virus DNA replication in secondary infections of NBM cells. NBM cells were either mock infected or exposed to the culture supernatants from B19 virus-infected MB-02 cells with and without treatment with Epo. The rest of the steps were the same as described in the legend to Fig. 2. Lane 1, DNA from mock-infected NBM cells; lanes 2 and 3, DNA from NBM cells exposed to the culture supernatants obtained from untreated, B19 virus-infected MB-02 cells at 48 and 96 h p.i., respectively; lanes 4 and 5, DNA from NBM cells exposed to the culture supernatants obtained from Epo-treated, B19 virus-infected MB-02 cells at 48 and 96 h p.i., respectively; lane 6, DNA from Epo-treated MB-02 cells in primary infection with B19 virus.

and, as a consequence, cessation of further cell division. It is not clear whether the isolation of a clonal population of UT-7/Epo cells adapted for growth in Epo leads to erythroid differentiation, because apparently these cells continue to proliferate with or without B19 virus infection. Whether subtle differences exist in the ways that B19 virus replication cycle is accomplished in these two culture systems remains to be investigated.

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