Isolation of Revertants from a Factor-Independent Myeloid Cell Line

M. Kawai[1], T. Mori[1], C. Stocking[2], and K. Notake[1]

Mouse myeloid stem cell line D35 was obtained from a primary cell culture of C3H bone marrow cells. This cell grows completely dependent on the multi-lineage colony-stimulating factor (multi-CSF; IL-3) or the granulocyte macrophage colony-stimulating factor (GM-CSF).

By serial dilution of the medium every 3 days, autonomously growing mutants were obtained spontaneously at the rate of 10^{-8}–10^{-7}. Among 18 mutants, 11 produce GM-CSF by themselves, three produce multi-CSF, and two do not secrete any growth factors that stimulate the parental cell line. All of those mutants that were checked were tumorigenic in nude mice. Four of the GM-CSF-producing mutants turned out to be the result of the insertion of an intracisternal A particle (Dind 1), Rauscher murine leukemia virus (Dind 4), or Friend spleen focus-forming virus (F-SFFV; Dind 5, Dind 9) at either 3′ or 5′ of GM-CSF locus.

To clarify whether aberrant expression of a CSF gene is sufficient for autonomous growth and tumorigenicity, and to investigate possible mechanisms of gene activation, as well as insertion and excision of these retroviruses, we have attempted to obtain revertants that again have a growth dependency on factor. After cultivation of these autonomous mutants with multi-CSF, 10^6 cells were washed and incubated for 24 h without any growth factor. A final concentration of 10^{-5} *M* BrdU was then added to the culture. After another 21 h, Hoechst dye 33258 (final 1 μg/ml) was added for 3 h to enhance the killing by light. All dishes were exposed to fluorescent light at a distance of 10 cm for 30 min. Cells were

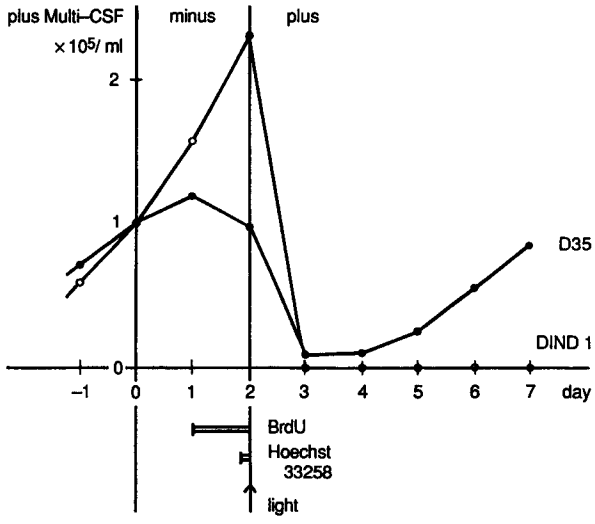

Fig. 1. Counter selection of Revertants from Dind 1 cells by BrdU-H33258-light killing

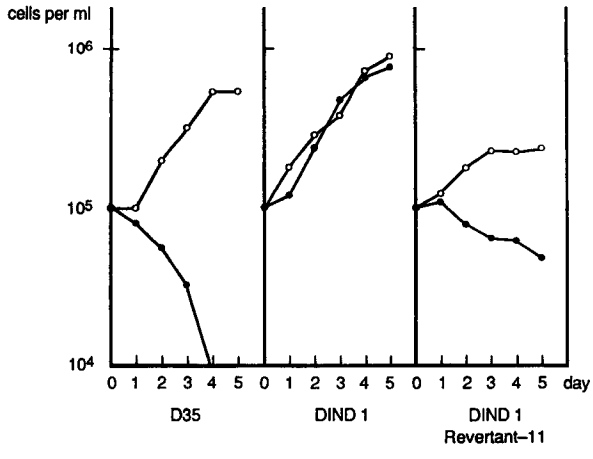

Fig. 2. Cell growth curve with and without multi-CSF. Open circles show the growth with multi-CSF (5% v/v of WEHI-3 cell conditioned medium), filled circles show the growth curve without multi-CSF

[1] Aichi Medical University, Aichi, Japan
[2] Heinrich-Pette-Institut, Hamburg, Federal Republic of Germany

subsequently washed three times with fresh media. The next day, as shown in Fig. 1, almost all the Dind 1 cells had been killed, but the control D35 cells had survived. After 7 days we saw 20–100 clonal clumps of the surviving Dind 1 cells in each dish. Clumps were directly transferred with Eppendorfer pipettes to the 96-well plates and cultivated with multi-CSF. After reaching confluency, each of three aliquots were shifted to 24-well plates to check their dependence of multi-CSF or GM-CSF. Among the 44 clumps, two were clearly dependent again on either factor. The other 42 were similar to normal or slow-growing Dind 1 cells. Figure 2 shows the growth curve of recloned revertant II in the presence or absence of multi-CSF, compared with D35 and Dind 1. As revertant II is shown to be a true revertant of Dind 1, it would be of interest to determine whether the IAP is still in the GM-CSF locus or not, and whether this revertant has lost the tumorigenicity in nude mice.

We have also shown here a more effective method for cell selection using a combination of H33258 with BrdU and light. This method decreases the cell survival rate to 10^{-5} from $\sim 10^{-2}$ obtained with standard systems using BrdU and light alone.