Friend Erythroleukaemia Cell Mutants Defective in Viral Gene Expression

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SUMMARY

Cellular mutants defective in the expression of viral polypeptides were isolated from the Friend erythroleukaemia cell line 745a by immunoselection with anti-ecotropic murine leukaemia virus serum in the presence of complement. The mode of appearance of the antiserum-resistant cells showed an interesting pattern which is discussed in the text. One of the mutants obtained contained no detectable gp70 or p15(E) while retaining gPr90 env; defects appeared to reside in the processing of the gPr90 env to gp70 and p15(E). In another type of mutant, gPr90 env was not detected. All these mutants retained spleen focus-forming virus (SFFV)-specific gp55 and gag precursor Pr68gag. The mutants superinfected with the helper virus produced the helper and SFFV also, indicating that these mutations did not affect the replication of the exogenously infecting virus.

From NRK cells persistently infected with the helper component of Friend leukaemia virus, Kabat et al. (1980) isolated various cellular mutants by means of immunoselection. Viral polypeptide expression was defective in several respects. The defects appeared to reside in cellular genes and not in viral genes (Kabat et al., 1980; Fitting et al., 1981). This may indicate that cellular factor(s) are involved in processing of the proteins of murine leukaemia virus (MuLV). In order to confirm these previous data, we performed similar experiments with the Friend leukaemia cell line 745a which produces both the helper virus and the spleen focus-forming virus (SFFV) (Friend et al., 1971). We obtained different types of mutants which may have defects in viral genes. The information will be detailed here.

Antiserum against ecotropic Friend leukaemia virus helper (anti-ecotropic virus serum) was obtained by injecting undisrupted FN2 virions into rabbits intravenously. The anti-ecotropic virus serum preferentially neutralized ecotropic virus, and killed only the ecotropic MuLV-producer cells in the presence of complement (Yoshikura & Odaka, 1982). Antiserum to xenotropic NZB-IU-1 (Hartley & Rowe, 1976) has been described previously (Yoshida & Yoshikura, 1981). The antiserum reduced the titre of SFFV by 10- to 1015-fold at a dilution that did not affect the titre of ecotropic helper virus (J. Yokota et al., unpublished data). In this respect, the antibody resembled SFFV-specific antibody reported by Anand et al. (1981a). Antiserum against rabbit SIRC cells (anti-SIRC) prepared in rabbits was used as the control serum. Molecular species precipitated by these antisera were defined by co-electrophoresis with those precipitated by monospecific goat antisera, anti-gp70, anti-p15(E) and anti-p30, supplied by the National Cancer Institute, U.S.A. The anti-xenotropic virus serum precipitated gPr90 env, gp70, p15(E), gp55, p58 and Pr68gag from 745a cell lysates, whereas anti-ecotropic virus serum precipitated gPr90 env, gp70, p15(E), Pr68gag and p30, but not gp55. The 58K protein precipitated by the anti-xenotropic virus serum was not consistently observed, but it was probably the protein suggested to be a more processed form of gp55 which appears on the cell surface (Ruta & Kabat, 1980). In the following experiments, we used the antisera prepared by ourselves, since the

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Fig. 1. Immunoselection of cellular mutants resistant to anti-ecotropic virus antiserum. (a, b) Immunoselection performed by culturing 745a (a) or cloned 745a-C1 cells (b) at a concentration of 5 x 10⁶ cells/ml in 200 μl of the antiserum serially diluted twofold plus 8 μl of normal rabbit serum as a source of complement. The cells surviving the highest partially toxic dose (arrows) were repeatedly immunoselected. •, No apparent mortality of the cells; ○, partial mortality of the cells; ●, complete cell killing. (c, d) After 9 cycles of treatment of 745a cells (c) and 6 cycles of treatment of 745a-C1 cells (d) the surviving cells were cloned and examined for their susceptibility to anti-ecotropic virus serum (1/40 antiserum dilution for 745a-derived cells, and 1/32 for 745a-C1-derived cells). □, Clone FR-6 derived from 745a; ●, clone FNR-422 derived from 745a-C1; other clones were indicated by circles. (e, f) Complement-dependent cytotoxic test of 745a parent (○), 745a after 5 cycles of immunoselection (△) and FR-6 (□) (e) and that of 745a-C1 (●) and FNR-22 (■) (f). Antibodies used were anti-ecotropic virus serum (A) and anti-xenotropic virus serum (B).

quantity of the monospecific antisera was limited and also because the monospecific anti-gp70 precipitated both gp70 and gp55, whereas our anti-ecotropic virus serum discriminated between these two molecules.

745a cells were subjected to 9 cycles of treatment with anti-ecotropic virus serum plus complement in the manner described previously (Yoshikura & Odaka, 1982). Surviving cells
Fig. 2. (a, b) Polyacrylamide gel electrophoresis of viral proteins in 745a cells (lanes A) and FR-6 cells (lanes B). Cells at a concentration of 10^6 cells/ml were labelled with 100 μCi/ml D-[3H]glucosamine (a) or 50 μCi/ml L-[35S]methionine (b) for 6 h. The cell lysates with the same radioactivity were immunoprecipitated with anti-xenotropic virus serum (lanes 1), anti-ecotropic virus serum (lanes 2) or anti-SIRC serum (lanes 3). The immunoprecipitates and also whole cell lysates without immunoprecipitation (lanes 4) were electrophoresed. (c, d) Polyacrylamide gel electrophoresis of viral proteins in parental clone 745a-C1 and three anti-ecotropic virus serum-resistant mutants derived from the clone. Cells were labelled with 50 μCi/ml L-[35S]methionine for 6 h. The cell lysates were precipitated with anti-xenotropic virus serum (c) or anti-ecotropic virus serum (d) except lanes 1 which were immunoprecipitated with anti-SIRC (negative control). Lanes 1 and 2, 745a-C1; lanes 3, FNR4-22; lanes 4, FNR-7; lanes 5, FNR-18.

were selected at each cycle. During the cycles of selection, sensitivity to the antiserum gradually decreased, and after 5 cycles of treatment the cytotoxic curve was displaced leftward by eightfold dilution; no downward displacement was observed. After 9 cycles, the culture became completely resistant; surviving cells were cloned in methyl cellulose. Twenty randomly isolated clones were tested for sensitivity in the cytotoxic test. Three clones showed significantly reduced response to anti-ecotropic virus serum, whereas others were entirely sensitive (Fig. 1 a, c). Of the three resistant clones, FR-6 cell line, which showed the lowest response to anti-ecotropic virus serum, was further examined. Although it was resistant to anti-ecotropic virus serum, it was sensitive to anti-xenotropic virus serum (Fig. 1 e). The kinetics of cell growth and erythroid
differentiation of 745a and of FR-6 cells were similar. FR-6 cells were virus non-producers as indicated by negative XC plaque formation and negative reverse transcriptase activity (data not shown). In order to detect which species of viral proteins were defective in FR-6 cells, FR-6 and 745a cells (1 × 10^6 cells/ml) were labelled with either d-[3H]glucosamine or L-[35S]methionine for 6 h. The cell lysates were precipitated with anti-ecotropic virus serum or with anti-xenotropic virus serum, and electrophoresed (Fig. 2a, b). In FR-6, gp70, p15(E) and the 58K protein (p58 in Fig. 2b) were absent and gPr90^env accumulated relative to the corresponding
protein in 745a cells. Probably, processing of \( gPr90^{env} \) to gp70 and p15(E) (Naso et al., 1976) was blocked in FR-6.

We repeated the immunoselection using 745a-C1, which was cloned immediately before the start of immunoselection. After 6 cycles of immunoselection with anti-ectotropic virus serum, the culture became almost entirely resistant to the antibody; among 30 clones obtained, seven were resistant (Fig. 1b, d, f). The cells of these clones were labelled with \( \text{L-[35S]} \)methionine in the same manner as before. The lysates were precipitated with anti-ectotropic virus serum or with anti-xenotropic virus serum, and electrophoresed (Fig. 2c, d). All of them were devoid of \( gPr90^{env} \), gp70 and p15(E), although retaining gp55 and Pr68\(^{ag}\) (three of them are shown in Fig. 2c, d). These resistant clones were distinct from FR-6 which retained \( gPr90^{env} \). No FR-6-type mutant was obtained from 745a-C1.

The unsuccessful detection of some of the \( \text{env-related} \) products in the above mutants could have been due to different turnover rate of the molecules in the mutants relative to the parental 745a line. In order to test this possibility, the cells were pulse-labelled for 30 min, and then chased for 2 h. The cell lysates and the culture fluids were analysed both after the pulse and after the chase. We detected gp70 only in the cell lysates and the culture fluid of the parental line after a 2 h chase, and not in those of the mutants either after the pulse or after the chase (Fig. 3). This suggests that these two types of mutants were actually devoid of gp70. In FR-6, the band of \( gPr90^{env} \) became more intense after a 2 h chase, whereas in the parental line, it tended to become less intense after the chase, indicating a block in the processing of \( gPr90^{env} \) in FR-6 mutant. In the 745a-C1-derived clone, \( gPr90^{env} \) was not detected at any stage. In this experiment, a molecule of about 80K was detected in all of the cell lysates after a 30 min pulse. A protein of the same molecular weight appeared in the culture fluid after a 2 h chase. The 80K protein was precipitated by anti-xenotropic virus serum slightly better than by anti-ectotropic virus serum, while gp70 was precipitated better by anti-ectotropic virus serum. The 80K protein and gp70 are probably unrelated. Our FR-6 mutant resembled the anti-gp70-resistant mutant called H-4 isolated from NRK cells which had been originally infected with helper virus (Kabat et al., 1980). The mutant cell was devoid of gp70 and p15(E), and the defect was reported to reside in the cellular gene (Fitting et al., 1981).

In order to know whether the block of processing of \( gPr90^{env} \) in FR-6 is produced by a cellular mutation or a viral one, the cells were superinfected with ecotropic helper virus FN2N7. If the mutation is cellular as suggested, infecting virus will not be produced from the cells after superinfection, because the \( gPr90^{env} \) of exogenously infecting MuLV will also be blocked in the cells. In fact, FR-6 cells were successfully superinfected with FN2N7 and produced virions as detected by the recovered infectivity in the culture fluid. The released virus was exogenous, because FN2N7 MuLV was the NRK-adapted helper virus in the Friend leukaemia virus stock, and this virus plates equally well on both NRK and mouse cells (Yoshikura & Yoshida, 1978). The recovered virus retained this character (virions released from 745a parent plated only poorly even in mouse D3hl cells) (Table 1). At the same time, the cells became sensitive to complement-dependent cytotoxicity mediated by anti-ectotropic virus serum which killed only the ecotropic MuLV producers (data not shown). Probably, the viral genome in FR-6 cells, especially the \( \text{env-related} \) gene, was mutated to produce inefficient \( gPr90^{env} \) cleavage.

### Table 1. Virus production from 745a cells and from uninfected and superinfected FR-6 cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>Infectious centre (p.f.u./10^3 cells)</th>
<th>Titre (p.f.u./ml) of supernatant when titrated on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D3hl</td>
</tr>
<tr>
<td>745a</td>
<td></td>
<td>3 × 10^2</td>
</tr>
<tr>
<td>FR-6</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>FR-6/FN2N7*</td>
<td></td>
<td>7 × 10^1</td>
</tr>
</tbody>
</table>

* FR-6 cells which grow in suspension were co-cultured for 10 days with FN2N7-infected NRK cells which grow in monolayer. Floating FR-6 cells were freed of FN2N7-infected NRK cells and subcultured for 2 or 3 passages to remove the NRK cells.
The 745a-Cl-derived resistant clones were also successfully superinfected with FN2N7 MuLV, and produced SFFV and the helper virus in the culture fluid (data not shown). They also appear to have defects in the viral gene. The non-producer lines obtained by Anand et al. (1981b) appear to have a defect in the expression of the 5' portion of the helper viral genome, and non-producer lines derived from a Rauscher virus-transformed erythroid cell line could be superinfected with Friend, Moloney and Gross viruses (Mol et al., 1982). However, the genetic defects in our mutants appear to be different from these non-producers. The ease with which we obtained the mutants may indicate a high spontaneous mutation rate in the viral genome in these cells. Actually, spontaneous occurrence of non-producer cells in virus-producing Friend leukaemia cells has been reported by others (Collins & Chesbro, 1980; Anand et al., 1981b).

An interesting feature of the immunoselection experiments was that the cytotoxic curve was gradually displaced leftward instead of downward during the 9 cycles of selection. If the process was only selection of a pre-existing mutant or a mutant which appeared during the selection, the curve should have been displaced downward. In addition, even after the culture as a whole became almost completely resistant to the antibody, many sensitive virus-producer cells were present as shown in Fig. 1. As FR-6 was stably resistant to the antibody for more than 6 months, the clone could be a true mutant, but many other cells in the population which resisted the antibody after immunoselection could have been the ones whose target molecules were simply masked in the presence of antibody as in antigenic modulation of FLC-745 cells (Genovesi et al., 1977) or TL cells (Old et al., 1968). We recently observed that the virus non-producers obtained above formed large aggregates spontaneously when mixed with virus-producing parental cells and the virus producers in such aggregates appeared to resist complement-dependent cytotoxicity. This phenomenon may partly explain the above paradoxical phenomenon.

In spite of our efforts, we failed to obtain clones that were significantly resistant to the anti-xenotropic virus serum. As anti-xenotropic virus serum in the presence of complement killed all the mouse haematopoietic cells tested so far as reported by Morse et al. (1979) and Yoshikura & Odaka (1982), some of the xenotropic virus-related antigens may be expressed on all mouse haematopoietic cells, and the presence of these antigens could be essential for cell survival.

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REFERENCES


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