Early Replication of Friend Leukaemia Viruses in Spleen Macrophages

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SUMMARY

The ability of spleen macrophages to support Friend leukaemia virus replication was studied by testing for infectivity in adherent cells obtained from mice at various times after infection. Virus-releasing macrophages appeared early after infection and reached a high proportion. The released virus was probably synthesized de novo since macrophage infectivity was strongly reduced by treatments which affected cellular viability or synthetic activities and electron microscopy showed C-type particles budding from macrophages.

Friend leukaemia virus complex (FLV) causes erythroleukaemia in adult mice of susceptible strains and, accordingly, its main target cell with respect to transformation has been identified as an erythroid precursor (Friend et al. 1971; Tambourin & Wendling, 1975; Golde et al. 1979). However, FLV can replicate in and/or affect the activity of other cell types (for review, see Tambourin et al. 1979). Thus, for example, the lymphatic leukaemia component (LLV) or FLV has been shown to grow in lymphocytes of the B lineage and this property has been invoked to explain the immunodepressive properties of FLV (Cerny et al. 1976). The observation that a functional deficit of macrophages (Mφ) contributes to the reduced antibody responsiveness of FLV-infected spleens (Bendinelli et al. 1975; Specter et al. 1978) prompted us to investigate the ability of FLV to replicate in spleen Mφ. The results presented here show that these cells not only effectively support the replication of FLV, but do so very soon after infection.

Inbred BALB/c mice, aged 8 to 10 weeks, were injected intravenously (i.v.) with \(4 \times 10^3\) (S+L−) focus forming units (f.f.u.) of the entire FLV complex or with an equal dose of the Rowson-Parr isolate of LLV (RP-LLV). Both virus preparations, obtained as previously described (Bendinelli & Nardini, 1973), are NB-tropic and free of lactic dehydrogenase virus. The strain of FLV is of the anaemia-inducing type; i.v. inoculation of 250 f.f.u. is sufficient to induce splenomegalies of over 1 g at 3 weeks post-infection (p.i.). At days 1, 3, 5, 8, 10 and 15 p.i., groups of three to four mice were killed and their pooled spleens used to prepare single cell suspensions in McCoy 5a medium supplemented with 10% heat-inactivated foetal calf serum. The cells were then washed five times in 10 vol. of medium and separated into Mφ-enriched and Mφ-depleted subpopulations by the adherence method of Mosier (1967) modified as follows: \(2 \times 10^7\) viable spleen cells were incubated at 37 °C in 5% CO₂ for 1 h in Falcon plastic dishes (6 cm diam.) and then gently agitated. The cells resuspended by this treatment were transferred to fresh dishes, further freed of adherent cells by two cycles of adherence and used as the Mφ-depleted population. The cells which remained adherent to the dishes after the first cycle of separation were thoroughly washed by pipetting fresh medium over the entire surface of the plate. Cells displaced by this treatment were discarded and the washing procedure repeated twice. The plates were then incubated for the time required to purify the non-adherent cells (approx. 2 h) and were washed with fresh medium every 30 min. As judged by morphology, resistance to detachment by trypsin, immune phagocytosis of erythrocytes (sheep red cells opsonized with
rabbit antiserum, 2 h incubation) and uptake of latex beads (0.8 μm, Dow Chemical Co., Midland, Mich. U.S.A.; 2 × 10⁹/ml, 2 h incubation), no less than 97% of the adherent cells were macrophages and this proportion was not reduced by infection during the period of observation. These firmly adherent cells were suspended with the help of a rubber policeman (the yield per plate was between 2 × 10⁵ and 3 × 10⁵) and used as the Mφ-enriched population. A sample of the original unmanipulated suspension was kept in the cold during the period of separation and used as the non-separated population.

The three cell populations (Mφ-depleted, Mφ-enriched, non-separated) were then tested for virus release by the infectious centre (IC) assay on D56 S+L- cells (Bassin et al. 1971), a technique which has been calculated to detect cells shedding as few as 1 f.f.u. of virus in 40 h of cultivation (Chesebro et al. 1978). The cells were washed five times in 100 vol. of cold medium, checked for viability by trypan blue exclusion (viability ranged between 80 and 95%), and then diluted and seeded, at numbers ranging from 10⁷ to 10⁸ viable cells per dish, into Falcon dishes (6 cm diam.) plated the day before with 1-6 × 10⁵ D56 S+L-cells. The number of IC per 10⁶ viable cells seeded was calculated by averaging those dishes which at 4 days after seeding had developed between 10 and 200 foci. As a sensitivity control, each experiment included dishes infected with 100 f.f.u. of FLV. As a further control, the supernatants of the last cell washing were tested for focus formation at the dilution used for the preparation of the highest cell seed, but were consistently negative.

Fig. 1 (a) shows the results obtained with FLV-infected spleen cells. At all times tested, the Mφ-enriched population exhibited a number of IC markedly higher than the non-separated population. The latter population, in turn, was slightly but constantly more active than the Mφ-depleted one. For example, at the time peak numbers of virus-releasing cells were observed in the spleen (day 15 p.i.), over 30% of the Mφ-enriched cells behaved as IC, compared with 9% of the non-separated cells and 6% of the Mφ-depleted cells. In addition, at the time IC were first detected, only the Mφ-enriched cells were positive. This occurred on day 3 p.i. which is also the earliest time infectious FLV is detectable in spleen extracts (Bardinelli & Nardini, 1973). Fig. 1 (b) depicts the results obtained with RP-LLV-infected
spleen cells. In keeping with the lower titres attained in vivo by RP-LLV compared to those reached following FLV inoculation (Bendinelli & Nardini, 1973), IC-giving cells were generally fewer than in FLV-infected spleen cells, but the general pattern was the same. It is important to notice that virus-releasing cells were again first detectable (day 3 p.i.) in the Mφ-enriched population.

In recent studies adherent peritoneal cells infected in vitro with FLV were found to give rise to numbers of IC higher than could be accounted for by productively infected Mφ, unless trypsinized to remove non-Mφ contaminants and residual free virus (Marcelletti & Furmanski, 1979). In the present experiments the following evidence confirmed that IC developed by adherent spleen cells were, indeed, due to Mφ shedding de novo synthesized virus and not to contaminating cells or virus nor to release of phagocytized or passively absorbed virus.

(i) The introduction of trypsinization (0.25%, applied for 5 min at 37 °C to the dishes used to prepare the adherent cells and followed by two washes) as a further step of Mφ purification, or pre-incubating the adherent cells with mouse anti-FLV serum (20 min at 4 °C followed by three washes) to neutralize extracellular virus, did not affect, or reduced only very marginally, the number of IC produced.

(ii) Pre-incubation of the adherent cells with silica (Dorentrop, lot DQ12, particle size < 5 μm, provided by Dr A. C. Allison; 50 mg/ml, 1 h at 37 °C followed by six washes), an agent selectively toxic for Mφ (Allison et al. 1966), reduced IC production by 60% or more.

(iii) Several treatments known to damage cell viability or synthetic activities decreased IC formation by adherent cells to a substantial though variable extent: freezing and thawing (three cycles) decreased IC formation by 80%, u.v.-irradiation (1000 ergs/mm²) by 87%, and pre-incubation with pactamycin (10⁻⁴ M for 30 min at 37 °C followed by three washes) by 65%.

(iv) Electron microscopy showed that adherent cells were virtually all Mφ and that they were budding type-C virus particles (Fig. 2).

Since the S⁺L⁻ test detects LLV only (Bassinet al. 1971), to assess whether Mφ were also producing the erythroleukaemia component, 5-day FLV-infected Mφ-enriched cells were injected i.v. at two different concentrations into adult BALB/c mice. Three weeks later the recipients had developed typical erythroleukaemia and their spleens weighed more than those of mice given equal numbers of Mφ-depleted or non-separated cells, thus showing that the erythroleukaemia component also replicates very effectively in splenic Mφ. In keeping with this finding, long-term cultures of spleen Mφ obtained from infected mice and treated at intervals with trypsin to remove contaminating cells continued to release both FLV components in the supernatant (data not shown).

Previous investigations had shown that Mφ obtained from the peritoneal cavity of infected mice may release FLV, though no attempts were made to ascertain whether they actually synthesize virus (Odaka & Köhler, 1965; Levy & Wheelock, 1975; Marcelletti & Furmanski, 1978, 1979). In such studies, the proportion of peritoneal Mφ giving IC at no time exceeded 0.6%, even when the cells were assayed late in the course of infection or following exudate elicitation. In the present study, productively infected Mφ in the spleen reached much higher proportions and appeared very early after virus inoculation. Thus the sensitivity of the spleen to FLV appears to be expressed also at the level of the Mφ. It has been suggested that only Mφ precursors and Mφ which are in the process of DNA synthesis can be productively infected by FLV (Marcelletti & Furmanski, 1979). We are now investigating the possibility that the elevated susceptibility to FLV of Mφ in the spleen is due to the presence in this organ of numerous Mφ precursors (Yokochi et al. 1979).

In this study, spleen Mφ were also found to produce IC earlier and in higher numbers than unseparated or non-adherent spleen cells. The available data do not allow one to
Fig. 2. (a and b) Electron micrographs of adherence-purified macrophages obtained from the spleen of FLV-infected mice. Note budding (BV) and extracellular (EV) C-type particles and ingested latex beads (L).
distinguish with certainty whether this finding reflects a preferential replication of FLV in Mφ or is simply due to selective loss of certain cell types (virus-transformed erythroid cells, for example) during manipulations or a higher efficiency of Mφ in the IC assay as compared to other splenic cell types. However, the latter possibility is unlikely because Mφ were also more active than other spleen cells in inducing erythroleukaemia in vivo.

The significance of the present findings to the pathogenesis of FLV infection remains to be established. It is possible that the reduced ability of Mφ to cooperate with lymphoid cells in the induction of antibody responses which is characteristic from the very early stages of infection with FLV viruses (Bendinelli et al. 1979) is a consequence of the early virus replication in these cells. The fact that Mφ represent an important site of FLV replication may also contribute to explain the effects exerted by Mφ-active substances on FLV infection (for review, see Bendinelli et al. 1979).

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REFERENCES


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