Bovine Lymphosarcoma: Processing of Bovine Leukaemia Virus-coded Proteins

By R. Z. Mamoun, T. Astier, B. Guillemain* and J. F. Duplan

Institut National de la Santé et de la Recherche Médicale Unité 117, 229 cours de l'Argonne, 33076 Bordeaux Cedex, France

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Summary

This report describes pulse-chase experiments performed with cells infected with tumour-derived bovine leukaemia virus (BLV) and using purified γ-globulins directed against BLV structural proteins, namely gp51, p24, p15 and p12. A gpr72 was found to be the precursor of the gp51 with a gpr70 intermediate. The p12 was shown to be derived from a pr40 with numerous intermediates (pr35, pr22, pr16 and pr14). The p24 and p15 originate from a pr40. Both the pr42 and pr40 are derived from a common pr70. A P45, a P52 and a P27 were also detected. Because these three proteins were found to accumulate progressively in cells and because they were not observed to be processed, they might play a physiological role in infected cells.

The bovine leukaemia virus (BLV) is known to be the aetiological agent of 'leukosis enzootica bovis' (Miller et al., 1969; Kawakami et al., 1970). This virus was further shown to be exogenous (Kettmann et al., 1978). In view of the fact that in cattle, natural BLV infection is restricted to B lymphocytes (Paul et al., 1977; Kenyon & Piper, 1977; Kettmann et al., 1978), we established a number of cell lines from bovine lymphoid tumours according to a previously published procedure (Mamoun et al., 1981). One of these cell lines (LB59Ly) produced a BLV isolate which was used to infect normal bovine embryo spleen cells (BESP) in which it replicated. Using ovine cells (FLK-BLV) infected with the American isolate of the BLV, Ghysdael et al. (1978) showed the presence of pr145, pr70 and pr45 polyproteins containing the p24 antigenicity and of a gpr72 protein containing the gp51 antigenicity. In a previous report (Mamoun et al., 1983) we described the virus-coded proteins present in cells of tumour origin or in cells infected in vitro with different BLV isolates including that from FLK-BLV cells. In addition to the above described proteins, three proteins were detected. One of them, a P42 protein with p24 and p15 antigenicities, was detected in every isolate, although FLK-BLV ovine cells infected by and producing a virus which is not of tumour origin (Van Der Maaten & Miller, 1976) contained only a trace amount of this protein. The two others, P52 and P27, were present in both FLK-BLV and in cell lines infected with tumour-derived BLV isolates; these two proteins contained antigenic determinants associated with the gag structural proteins p24, p15 and p12. No evidence of an 'onc-protein' was obtained. These findings raised the question of the physiological role of these polyproteins and especially of that of the P42 and P27. For this we performed pulse-chase experiments aimed at the understanding of the eventual precursor-product relationships between the above-mentioned proteins, and at deciding if they are transient or final products of BLV-induced metabolism. Moreover, this technique opens up possibilities of revealing new proteins with a short half-life. The cells (BESP-LB59) used in these experiments are BESP cells chronically infected with BLV from LB59Ly cells.

The biogenesis of the p24 was investigated by means of immunoprecipitations of BESP-LB59 cell extracts with rabbit γ-globulin anti-BLV-p24 as shown in Fig. 1(a); a P70 and a P45 were synthesized simultaneously within less than 5 min; other new proteins (P42, P37 and P27) appeared after 20 min of incubation. P52 and the p24 were detected after 35 min and at the end
Fig. 1. Processing of the BLV p24 (a), p15 (b), p12 (c) and gp51 (d) proteins in BESP-LB59 cells. Cells in culture (T75) were labelled with $^{14}$C-labelled amino acids (45 mCi/milliatom of carbon) at 0-1 mCi/ml for 5 min in amino acid-free culture medium. They were shifted to unlabelled medium with the complete set of amino acids for 0 to 185 min (total duration of the experiment: 5 to 190 min). Labelled cells were lysed at the times (min) shown, immunoprecipitated and analysed by SDS-polyacrylamide gel electrophoresis (Mamoun et al., 1983). The specific rabbit (anti-p24 or anti-p12) or bovine (anti-p15 or anti-gp51) $\gamma$-globulins were purified from sera of rabbits immunized with Triton X-100-disrupted FLK-BLV virus or from sera of referral cases of bovine enzootic lymphosarcomas by immunoaffinity chromatography on purified gp51-, p24-, p15- or p12-linked Sepharose (Mamoun et al., 1983).
of 1 h all the above-mentioned proteins were still present. During this period, the amount of the P45 remained stable. Finally, after an extended incubation time (3 h), the same protein pattern was still observed but the amount of P70 and P42 had decreased, whereas that of P27 and p24 had increased. A protein with a mol. wt. exceeding 100K was observed in these experiments, but lacked specificity, because it was also encountered when cells not infected with BLV (BESP) were immunoprecipitated by the same rabbit anti-BLV-p24 γ-globulins (data not shown).

The biogenesis of the p15 was investigated in the same manner with the same extracts but with bovine anti-BLV-p15 γ-globulin. The same pattern of proteins with the same kinetics was observed as for the biogenesis of the p24. Like p24, p15 appeared only after 35 min incubation and accumulated thereafter (Fig. 1b). All these proteins are BLV-coded, as they were absent in extracts of uninfected BESP cells treated with the same bovine γ-globulins (data not shown). This result indicates that the use of bovine instead of rabbit γ-globulins greatly improves the specificity of the technique.

The biogenesis of the p12 was studied under the same assay conditions but using rabbit anti-BLV-p12 γ-globulin. The same proteins (P70 and P45) as above were observed for the first 10 min of incubation. After 20 min of incubation, P37 and P27 appeared but P42 did not. Fifteen min later, a P52 appeared and there was an accumulation of a P27. At 60 min, numerous new proteins were recorded, namely P40, P35, P22, P16, P14 and p12, whereas the P70 tended to disappear. After 3 h incubation, the intermediates had disappeared and only the final cellular products remained: the apparent concentration of P52, P45 and P27 were the same as after 1 h of incubation, whereas the amount of the P70 and of the p12 were drastically diminished. Because of the short half-life of the p12 and of the constant level of P45, one may conclude that the p12 does not originate from the P45 (Fig. 1c). In addition to these proteins, we noticed the presence of high molecular weight products exceeding 70K that are not related to BLV infection because they were also precipitated by the same antiserum in uninfected BESP cells (data not shown).

For the biogenesis of the gp51 we used bovine γ-globulin anti-BLV-gp51. We were able to show (Fig. 1d) the labelling of a P72 within 5 min. After 20 min the radioactivity had shifted to a P70 product and then to the gp51 and the gp35, the amount of these two proteins being maximal at 60 min and drastically decreasing within the following 2 h. This kinetics indicates that the gp51 originates from a gpr72 with a gpr70 intermediate. The gp51 might be recognized by the bovine γ-globulins either on the glycosylated or on the peptide portions, and, because the gp35 could be recognized only on the same glycosylated part, it is not known whether the two glycoproteins have a common peptide antigenicity and furthermore whether they share the same precursor(s). Using control BESP cells and the same bovine γ-globulins no non-specific protein was precipitated (data not shown).

The processing of BLV-coded proteins in LB59Ly cells was investigated in the same type of experiment using the same rabbit or bovine γ-globulins. The processing of the p24, p15, p12 and gp51 was found to be strictly identical to that observed for BESP-LB59 cells, i.e. the same precursors and intermediates with identical kinetics (data not shown).

The present results combined with those previously reported (Ghysdael et al., 1978; Astier et al., 1978; Mamoun et al., 1983) are schematically presented in Fig. 2.

From the integrated viral DNA, two messenger RNAs are synthesized in cultured cells, a 35S and a 24S mRNA (Astier et al., 1978; Mamoun et al., 1982). By analogy to other mammalian retroviruses (for review, see Stephenson, 1980) one can assume that the 24S molecule is the messenger of the env proteins whereas, as shown by Ghysdael et al. (1978) the 35S molecule would generate the gag-pol precursors.

With respect to the biogenesis of the envelope glycoprotein gp51, our data confirm the existence of a gpr72 precursor (Ghysdael et al., 1978) in addition to a gpr70 intermediate. The short cellular half-life of the gp51 suggests that it is mostly shed as a virion structural protein. Because the gp51 could be recognized by spontaneous immune sera either on the glycosylated moiety (Portetelle et al., 1980) or on the peptide fraction (Bruck et al., 1982), and because the gp35 could be recognized solely on the glycosylated part, it is not known whether the two glycoproteins have a common peptide antigenicity; furthermore, the exact origin of the gp35 remains to be elucidated.
The diagram presented in Fig. 2 also illustrates the processing of the BLV gag proteins. Using anti-p24 or anti-p15 \( \gamma \)-globulins, we could show that these two BLV structural proteins are derived from a common pr42 precursor which itself originates from a pr70 (originally described by Ghysdael et al., 1978). Interestingly enough, this scheme seems to be different from that observed with the American BLV strain (FLK-BLV) in which the pr42 was only present in trace amounts (Mamoun et al., 1983). Using anti-p12 \( \gamma \)-globulins, we could show that this BLV structural protein had the same pr70 precursor but originates via a different pathway, namely pr40, pr35, pr22, pr16 and pr14.

Three BLV-induced cellular proteins, i.e. P45, P52, P27 containing the p24, p15 and p12 antigenicities seem to play a physiological role in view of their presence even after 3 h incubation in every BLV-producing cell. The P45 is synthesized in less than 5 min and is not a precursor of viral structural proteins, for it is not processed at a time at which the p12 itself and the intermediates of the p24, p15 and p12 have already disappeared. The P52 product is observed after 35 min incubation. The P27 has some interesting characteristics: it is a final product and despite its low mol. wt. it shares the three antigenicities of the p24, p15 and p12. The same antigenicities were again observed in a 37K protein, which could originate either from the pr70 or from the P45 and seems to be an intermediate of the P27.

The above results indicate that the p24 and the p15 originate from a pr42 precursor while the p12 originates from a pr40; according to A. Burny (personal communication) the p12 is localized at the -COOH end of the gag gene. Thus, in the pr70, the pr40 must be localized at the -COOH end terminus. Therefore, one can assume the existence of a 28K polypeptide in between the pr42 and the p12. The exact position of the pl19 in the BLV genome has not been determined and could be localized either to the left or to the right (in the 28K polypeptide) of the p24. Nevertheless, if the gag gene order described for other retroviruses (for review, see Stephenson, 1980) is also true for BLV, the 'b' gene would be the 'phosphorylated' pl19 (Mamoun et al., 1983), the 'c' gene would be the 'major protein' p24, the 'd' gene would be the 'basic' p12, and the p15 would thus be the 'a' gene (Fig. 2).
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REFERENCES


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