Transcriptional Control of Endogenous Virus Genes in Murine Lymphocytes

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SUMMARY

The expression of endogenous retrovirus in murine lymphocytes is under genetic control and also depends on the differentiation state of the lymphocytes. We have used a cDNA probe complementary to induced virus RNA to quantify transcription of virus sequences in lymphocytes from mitogen-stimulated lymphocytes of the AKR, 129/J and Balb/c mice. Balb/c lymphocytes show the clearest case for induction of new virus sequences in response to stimulation. All strains including 129/J show expression of virus sequences in unstimulated control lymphocytes. The data indicate that mitogen induction of endogenous retrovirus is regulated at the transcriptional level.

Germ line DNA of all mice seems to contain endogenous retrovirus sequences (Chattopadhyay et al., 1974, 1979), but different mouse strains vary in their spontaneous expression of virus proteins. Untreated spleen cells of inbred strains have been shown by radioimmune assay (Strand et al., 1974) to range in spontaneous expression between the extremely high producers of virus protein (AKR strain) to those apparently negative for expression (129/J strain). Mitogenic induction of xenotropic endogenous virus from spleen cells follows a similar pattern (Schumann & Moroni, 1977). AKR strain lymphocytes, already high in ecotropic virus production, are stimulated by certain mitogens to release xenotropic virus also. Fresh spleens of Balb/c mice, nearly negative for infectious virus, show a marked increase in xenotropic virus production after mitogenic stimulation. The 129/J strain is always negative for infectious virus. These facts indicate genetic regulation of virus expression.

To investigate the molecular mechanism of mitogen-stimulated virus induction we have examined virus RNA levels in stimulated lymphocytes from these different strains by cDNA/RNA hybridization. We prepared a virus-specific $^3$H-cDNA probe to Balb/c xenotropic virus originally induced from spleen cells by mitogen and passaged in mink CCL-64 cells (Monckton & Moroni, 1980). Sucrose density-gradient-purified virus was added (approx. 1 mg protein/ml) to a mixture of (final concentrations) 50 mM-tris pH 8; 60 mM-NaCl, 6 mM-magnesium acetate, 20 mM-dithiothreitol; 0·1 mM each dTTP, dATP, dGTP, 20 μg/ml actinomycin D; $^3$H-dCTP (0·33 mM, 15·5 Ci/mmol); Nonidet P-40 from 0·01 to 0·015%; and 1 mg/ml calf thymus primers (Taylor et al., 1976). After 16 to 18 h incubation at 37 °C, SDS was added to 1% and the template RNA was hydrolysed by adding NaOH to 0·3 m and further incubation for 2 h. The cDNA was chromatographed over G50 Sephadex and precipitated with 2 vol. ethanol. This probe was used throughout these studies to measure virus-specific RNA expression in different strains and tissue.

As a first test of the probe it was hybridized to RNA from infected and uninfected mink cells. To avoid contamination with cellular DNA, RNA was extracted from cells that had been lysed in high salt buffer (50 mM-MgCl$_2$, 0·5 mM-NaCl, 2 mM-CaCl$_2$, 0·01 m-tris pH 7·4; Penman, 1966) by 0·5% SDS and digested with 100 μg/ml DNase I (RNase free). After extracting with a phenol–chloroform–isoamyl alcohol mixture (50:48:2, by vol.) the aqueous phase was precipitated with ethanol, resuspended in a digestion buffer (10 mM-NaCl, 10 mM-MgCl$_2$, 2·5 mM-CaCl$_2$, 50 mM-tris pH 7·4) and digested with 20 μg/ml DNase I.
for 10 min at room temperature. High mol. wt. RNA, collected in the void volume of a G50 Sephadex column, was free of DNA and had an $A_{260/280}$ profile greater than 2. The hybridization mixtures contained 0.41 M-phosphate buffer, 0.2% SDS, serial dilutions of RNA, plus $^{3}$H-cDNA. After incubation for 100 h at 67 °C the hybrids were assayed by S1 nuclease (Sigma) digestion in an S1 buffer (0.03 M-sodium acetate pH 4.5; 0.4 M-ZnSO$_4$; 0.25 M-KCl; 10 μg/ml denatured calf thymus DNA; Fan & Baltimore, 1973). When calculated as a Cr₀t value (Britten & Smith, 1976), the various hybridizations had maximum values between $8 \times 10^3$ and $20 \times 10^3$ mol s/l.

The results of hybridizations between virus cDNA and cellular RNA from mink cells replicating the virus and parental uninfected cells are shown in Fig. 1 (a). The plateau value, 70%, is consistent with literature values for this type of hybridization (Callahan et al., 1975; Cabradilla et al., 1976). Background hybridization was consistently 8%, representing 3% more than the S1-resistant fraction of cDNA assayed in the absence of RNA. This implies that the probe was specific for virus sequences and had no homology with RNA from cells of the host line in which the virus was replicating.

The cDNA probe was then hybridized to virus RNA purified from infected cell culture supernatants by sucrose density-gradient centrifugation and phenol extraction as described. Although the maximum hybridization assayed was again approx. 70%, it was not a plateau value (Fig. 1b, inset). This intercept suggests that 100% of the probe was capable of hybridizing to virus RNA, although the hybridization curve indicates it was asymmetric or redundant in its copying of a portion of the genome. The specificity of the probe to virus sequences, however, makes it a reasonable tool to investigate the expression of induced virus RNA in mitogen-stimulated lymphocytes.

To test the expression of virus-specific sequences under defined conditions, spleens were removed from three strains of mice (from Bomholdgard, Denmark) and cultured in RPMI supplemented with 8% foetal bovine serum, penicillin (100 IU/ml; Calbiochem), and streptomycin (100 μg/ml; Calbiochem) for 3 days at 37 °C. Spleen cells from adult (6- to 10-week-old) AKR mice, which normally express high levels of ecotropic virus, were characterized for their response to mitogen stimulation [16 μg/ml lipopolysaccharide (LPS) from Escherichia coli; Difco]. The virus cDNA was hybridized to whole cell RNA extracted as previously described from control and LPS-stimulated cultures. Ecotropic virus expression in the control cultures was seen (Fig. 2a) due to the extensive homology between eco- and xenotropic viruses (Callahan et al., 1974). Mitogen stimulation resulted in a three- to fourfold
increase in virus RNA concentration as can be seen in the steeper slope of the double-reciprocal plot (Fig. 2a, inset). Increases in the representation of xenotropic virus sequences were expected since this virus is recovered from stimulated but not control cultures (data not shown). These differences are difficult to distinguish by hybridization as the background of ecotropic virus represents 10 times the amount of virus RNA found in the xenotropic virus-producing fibroblasts (Fig. 1a). Thus, LPS treatment of AKR strain lymphocytes may result in transcription of xenotropic-specific sequences, as much as 20% of the probe, but these sequences are difficult to measure above the ecotropic sequences expressed at all times.

The 129/J mice represent the converse of virus expression in AKR mice. Ecotropic sequences are thought to be lacking altogether in the cell genome although it may contain the amphotropic virus genome (Chattopadhyay et al., 1979). Since infectious virus cannot be recovered from lymphocyte cultures of this strain after mitogenic stimulation, we asked whether a portion of the virus genome was expressed in response to mitogen stimulation. Virus cDNA was hybridized to whole cell RNA extracted from 129/J strain spleen lymphocytes untreated or treated with LPS and bromodeoxyuridine (BrdUrd, 5 μg/ml). BrdUrd has previously been shown to amplify virus induction by mitogens (Moroni et al., 1975).
No difference in the hybridizations with control lymphocyte RNA and that of mitogen-stimulated cells was observed (Fig. 2b).

However, over RNA concentrations similar to those used from virus-producing fibroblasts, the hybridization exceeded background values. This surprising result indicated that some expression of virus sequences in spleen lymphocytes of the 129/J mouse strain did occur. To confirm that this expression was more than an unusual background, RNA from fresh thymus and brain of 129/J strain mice was extracted by removing the organ, freezing in liquid N₂, grinding to a powder, resuspending the powder in high salt buffer and extracting as before. For example, in Fig. 2(c) the hybridization of cDNA to brain cell RNA is compared to spleen and thymus lymphocyte RNA. While the hybridization with brain cell RNA never exceeded the 8% observed with uninfected mink fibroblasts, that with spleen lymphocytes reached at least 10% higher values. Fresh thymocytes also show transcriptions of virus sequences consistent with the known expression of the G₁vir retrovirus antigen on these cells (Stockert et al., 1971; Morse et al., 1979). The spleen cell population of 129/J mouse strain appears to express endogenous virus sequences not found in brain tissues of these mice.

We next tested Balb/c mice in which xenotropic virus production is mitogenically induced over very low backgrounds. Virus probe was hybridized to whole cell RNA extracted from control spleen cell cultures or those treated with LPS in the presence of BrdUrd as previously described (Fig. 2d). The hybridization with RNA from mitogen-stimulated lymphocytes reached a higher saturation plateau than that of the unstimulated control cell RNA as shown by the double-reciprocal plot (Fig. 2d, inset). Since these values were approx. 65% for stimulated and 24% for control cells, 40% more of the virus sequences represented by the probe are found in mitogen-stimulated lymphocytes compared to controls. As indicated by the parallel lines of the double-reciprocal plot, the newly expressed sequences are present at the concentration of those previously expressed in control cultures. This implies that mitogen induction of endogenous virus from Balb/c lymphocytes correlates with the expression of new virus sequences which are not found in unstimulated cells. We cannot, however, distinguish between de novo virus RNA synthesis and alterations in the rates of virus RNA metabolism. Whichever of these mechanisms is operative, it seems probable that endogenous virus is induced from Balb/c mice by a change at the RNA level rather than by altered translation or maturation of virus proteins.

The expression in all mice strains of some virus sequences was an unexpected finding, particularly in the 129/J strain. Support for our findings with 129/J lymphocytes came from the recent demonstration that cultured 129/J lymphocytes show specific retrovirus antigens by immunofluorescence (J. Jongstra & C. Moroni, unpublished results).

The specific and regulated expression of some virus sequences in lymphocytes raises the question whether the corresponding protein products are involved in some lymphocyte function. Proteins translated from these sequences may represent the gp70 virus envelope protein found on normal spleen cells of nearly all mice strains (Elder et al., 1977). We have hypothesized (Moroni et al., 1980) that these surface-exposed virus proteins or antigenically similar proteins may play a role in immune cell function of the mouse. Further work is in progress to test this hypothesis.

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SHORT COMMUNICATIONS

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