Type-specific Binding Antibody to Baboon Endogenous Virus (M7) Reverse Transcriptase

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SUMMARY

A type-specific binding antibody to the baboon endogenous virus, M7, reverse transcriptase was developed and characterized using a double antibody immunoprecipitation assay. This assay allows the analysis of non-enzyme neutralizing binding antibody, as well as the detection of early antibody production before high titre enzyme neutralizing antibodies appear. The antibody described is unique among hyperimmune antisera to DNA polymerases in that it recognizes only type-specific determinants on the enzyme molecule. Analysis of the enzymes of several BaEV isolates indicated a grouping of those from *Papio cynocephalus*, *P. anubis* and the HL23V_b isolate. The RD-II 4 enzyme was in a separate class, and the *P. papio* and *P. hamadryas* DNA polymerases were distinguished from all the other BaEV enzymes but not from each other.

Immunological studies using antisera prepared against the enzymes of the gibbon ape leukaemia viruses (GaLV) and the woolly monkey (simian) sarcoma virus (SSV) have shown the enzymes of this group of primate type-C retroviruses to be highly related to each other as well as to a DNA polymerase with properties of reverse transcriptase present in some human leukaemic cells (Todaro & Gallo, 1973; Gallagher *et al.* 1974; Gallo *et al.* 1975; Mondal *et al.* 1975) and also in a case of a preleukaemic disorder (Chandra *et al.* 1975; Chandra & Steel, 1977). High titre antisera against the DNA polymerases of the baboon endogenous viruses, a separate group of primate type-C retroviruses, have not been available and similar investigations of immunological relationships have used antisera prepared against reverse transcriptase of the related feline endogenous virus, RD-114. That RD-114 and BaEV are closely related has been adequately demonstrated by biological studies, nucleic acid hybridizations and serological analyses (Hellman *et al.* 1974; Sherr *et al.* 1974; Benveniste *et al.* 1974a, b; Sherr & Todaro, 1974). Nevertheless, because endogenous virus components in human cells might be more closely related to BaEV than to RD-114, antisera to BaEV proteins would be valuable.

We have previously shown that a double antibody immunoprecipitation (DAI) assay detected a highly specific antibody to cellular DNA polymerase γ; this antibody was incapable of inhibiting enzyme activity and would have been missed had sera been screened only with an enzyme neutralization assay (Robert-Guroff & Gallo, 1977). In applying the DAI assay to antisera prepared against BaEV reverse transcriptase, a high titre binding antibody with minimal ability to neutralize homologous enzyme activity and possessing only type-specificity, was detected.

Rauscher murine leukaemia virus (RLV), SSV and BaEV, M7 (Benveniste *et al.* 1974b), were obtained through the courtesy of Dr Jack Gruber, Office of Program Resources and Logistics, NCI. The baboon type-C virus, BILN (Goldberg *et al.* 1974), isolated from the inguinal lymph node of a lymphomatosus baboon, and the feline endogenous virus, RD-114, were purchased from Electro-Nucleonics Laboratories, Inc., Bethesda, Md. The BaEV
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Table I. Comparison of enzyme neutralization and DAI assays for monitoring development of antibody to reverse transcriptase*

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<th>DAI assay</th>
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<td>Number of inoculations</td>
<td>Antibody titre</td>
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<td>(µg IgG for 50% neutralization)</td>
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<td>Anti-M7 reverse transcriptase</td>
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<td>Anti-SSV reverse transcriptase</td>
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* Titrations of varying concentrations of immune IgG virus M7 or SSV reverse transcriptases were carried out by neutralization and DAI assays. Values for the extent of inhibition and extent of binding of M7 reverse transcriptase are reported for assays using 41 µg and 1-7 to 2-5 µg of immune IgG respectively. Values of the extent of inhibition and extent of binding of SSV reverse transcriptase are reported for assays using 33-5 µg and 3 µg of immune IgG respectively.
† Not determined.

isolate from normal lung of *Papio papio*, PP-1, (Todaro et al. 1976) was kindly supplied by Dr George Todaro. GaLV, BaEV grown in either canine foetal thymus cells (BK-CT) or in NC37 cells (BaEV/NC37), and HL23V_bab, the baboon component of the reported virus isolate from human cells (Teich et al. 1975; Chan et al. 1976; Okabe et al. 1976; Reitz et al. 1976), were obtained from Pfizer, Inc., Maywood, N. J.

The feline leukaemia virus (FeLV) reverse transcriptase, purified by sequential DEAE-cellulose, phosphocellulose, poly(C)-agarose and hydroxyapatite chromatographies, was provided by Dr W. Carl Saxinger in our laboratory. All other virus reverse transcriptases were purified through DEAE-cellulose, phosphocellulose and poly(U)-Sepharose as previously described (Robert-Guroff & Gallo, 1977). Standard assay systems for cellular and virus DNA polymerases have been described (Robert-Guroff et al. 1977), as have the enzyme activity neutralization and DAI assays (Robert-Guroff & Gallo, 1977). Antibodies to M7, SSV and RD-114 reverse transcriptases and to goat anti-rabbit IgG were prepared in rabbits by a previously described protocol (Robert-Guroff & Gallo, 1977). The IgG fraction of all antisera was used in immunological assays, and was purified as described (Robert-Guroff & Gallo, 1977) and additionally centrifuged at 30000 g for 15 min following dialysis against 0-1 m-tris-HCl, pH 8. Protein determinations on perchloric acid precipitated samples were carried out by the method of Lowry et al. (1951).

We have observed that the DAI assay is useful for the detection of high titre antibodies before enzyme neutralizing antibodies appear. Table I shows the development of antibody to M7 reverse transcriptase and compares enzyme inhibition, measured by the neutralization assay, to the extent of binding and titre of the same antisera, measured by the DAI assay. While neutralizing antibody appeared following the 4th inoculation, it was very weak and of limited usefulness. On the other hand, the binding antibody which also appeared following the 4th inoculation, had a high titre, consequently, characterization of the antiserum was carried out using the DAI assay and IgG prepared from serum following the 4th
Fig. 1. Comparison of specificities of Anti-M7 and Anti-RD-114 reverse transcriptases. Titration of antibodies were carried out with each of the indicated DNA polymerases at a fixed concentration. (a), (b) specificity of anti-M7 reverse transcriptase determined by DAI assay. (c), (d) specificity of anti-RD-114 reverse transcriptase determined by the neutralization assay. The upper panels (a and c) show titrations against DNA polymerases representative of the infectious primate type-C virus group: ○ SSV, ● GaLV; the BaEV-RD-114 group: ● M7, ○ RD-114; and lower mammalian retroviruses: ● RLV, ■ FeLV. The lower panels (b) and (d) show titrations against additional DNA polymerases of the BaEV-RE-114 group:

inoculation. That the early detection of binding antibody is a general phenomenon and not simply a result obtained with a particular rabbit is also illustrated by results obtained with antisera produced against SSV reverse transcriptase (Table 1). High titre neutralizing antibody to SSV reverse transcriptase was detected only in sera obtained following the 8th inoculation. However, only five inoculations were necessary to produce a high titre antiserum capable of binding 90% of the homologous enzyme activity. The antibody titres shown in Table 1 illustrate the greater sensitivity of the DAI assay compared to the neutralization assay. Very small amounts of IgG can easily detect homologous enzyme. The titres of the sera obtained throughout the inoculation periods point out that 'binding antibody' is produced quickly and then its titre remains fairly constant. The apparent loss of titre and extent of binding of serum obtained after the 5th inoculation in the anti-M7 system is due to obtaining serum before peak titre was reached.

The remarkable specificity of the binding antibody to M7 reverse transcriptase is shown in Fig. 1. M7 reverse transcriptase is the only mammalian retrovirus DNA polymerase recognized by the antiserum except that of RD-114 (Fig. 1a) which is bound 50% but only at an immune IgG concentration four times greater than the amount necessary to bind 90% of the homologous enzyme. Other enzymes tested, but not shown, which were also not
recognized by the antiserum include the reverse transcriptases of the rat leukaemia virus, the avian myeloblastosis virus, the Mason-Pfizer monkey virus and the normal human cellular DNA polymerases, $\alpha$, $\beta$, and $\gamma$.

The weak cross reaction with RD-114 reverse transcriptase was unexpected, in view of the known relationship of RD-114 and BaEV. Therefore, we considered that the anti-M7 serum might be able to distinguish between the various baboon endogenous virus isolates. Titration of the binding antibody with reverse transcriptases purified from isolates of the BaEV-RD-114 group are shown in Fig. 1(b). The antibody cannot distinguish the reverse transcriptase of the M7 baboon isolate (from *P. cynocephalus*) from that of HL23V$_{\text{Bab}}$. The enzymes of BILN (from *P. hamadryas*) and PP-1 (from *P. papio*) viruses are easily distinguished from that of M7. We have also tested reverse transcriptases purified from BK-CT and BaEV/NC37 preparations. The virus represented in both cell lines originated from cells of a baboon, probably *P. anubis* (R. Guy Smith, personal communication), raised at the Baylor College of Medicine (Todaro et al. 1974). The enzymes from both preparations were indistinguishable from the M7 reverse transcriptase as expected, since *P. anubis* is a subspecies of *P. cynocephalus*. We examined an antibody to RD-114 reverse transcriptase in order to determine if the antibody to M7-reverse transcriptase was unique, or if the RD-114 reagent could also detect some type-specific determinants. A comparison of the homologous cross reaction (Fig. 1d) with the results of titrations against other BaEV isolates suggests that some type specificity is present. Anti-RD-114 reverse transcriptase does inhibit the RD-114 enzyme to a greater extent than the BILN and PP-1 reverse transcriptases. However, if this antiserum thus recognizes type-specific determinants of the BaEV reverse transcriptases, the utility of this recognition is limited, because the same antiserum also reacts with the group- and interspecies-specific determinants of mammalian retrovirus reverse transcriptases (Fig. 1c). Although it can distinguish the enzymes of infectious primate type-C viruses (represented by SSV) from the BaEV group, it cross reacts significantly with RLV and to a lesser extent with FeLV reverse transcriptases. On the other hand, the M7 antiserum is very specific for the BaEV (Fig. 1a). It seems to possess no group or interspecies specificities. These results showing type-specificity of the binding antibody to M7 reverse transcriptase were not due to protein concentration differences. Enzymes were tested over a range of protein concentrations and in all cases, immune IgG was shown to be in excess.

The binding antibody to BaEV reverse transcriptase is unique among antisera to retrovirus DNA polymerases because it does not recognize the group- and interspecies-specific determinants of reverse transcriptase. The only previous observations of type-specific determinants on the reverse transcriptase molecule were obtained by means of radioimmunoassay (Krakower et al. 1977; Krakower & Aaronson, 1978); type-specificity could not be detected by immunological assays dependent on enzyme activity. Whether the strong type-specificity of the anti-M7 reverse transcriptase resulted from the nature of the immunizing antigen, the response of the particular rabbit, or a special property unique to the M7 DNA polymerase is not known. The particular rabbit is probably not responsible because we have prepared a second antiserum to baboon reverse transcriptase and preliminary results indicate that it also has type-specificity. Additionally, the type-specific determinant on the enzyme molecule must be near the active site of the enzyme, as analysis of the low titre neutralizing antibody suggests that it also detects type-specific determinants. However, complete titration curves must be completed before this result is firmly established.

The type-specific anti-M7 reverse transcriptase should prove useful in identifying the species of origin of BaEV-related retroviruses. Our studies have shown that the M7 and HL23V$_{\text{Bab}}$ reverse transcriptases are indistinguishable from each other, yet different from
the BILN enzyme, confirming the observations of Okabe et al. (1976) obtained by nucleic acid hybridization. Our results also agree with those of Todaro et al. (1976) showing the relatedness of the BaEV isolates from P. cynocephalus and P. anubis and the lack of relationship of these to both the P. hamadryas and P. papio isolates. The data represented here do not suggest a clear distinction between the enzymes of these latter isolates as would be expected based on previous results obtained by nucleic acid hybridization (Todaro et al. 1976), but titration with greater amounts of immune IgG might show such a distinction.

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Laboratory of Tumor Cell Biology
National Cancer Institute
Bethesda, Md. 20014, U.S.A.

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