Group Specific Antigen and RNA-Directed DNA Polymerase Activity in Normal Baboon Placenta (37568)

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The observation of C-type RNA particles in placental tissue derived from normal baboons (*Papio cynocephalus*) and humans was recently described (1, 2). While morphological demonstration of mature and budding particles is highly suggestive, it is essential to demonstrate that these particles also have biological characteristics normally attributed to the C-type RNA tumor viruses of lower animals, namely RNA-directed DNA polymerase and group specific (gs) antigen. This report provides this type of biological characterization.

Materials and Methods. Placental tissue obtained by cesarean section from baboons at the end of the first trimester was used for enzymatic and antigenic studies. For the electron microscopic studies, these as well as tissues derived at later gestational stages were used. Within 0.5 hr after surgery, sections of placental tissue were either frozen and stored at -70° for subsequent biological characterization or fixed for electron microscopy.

All gs antigen complement-fixation assays were performed on 10% (w/v) placental extracts in phosphate-buffered saline at 1:2, 1:4 and 1:8 dilution, with 1:20, 1:80 dilution of antisera prepared in goats against RD-114 and Mason-Pfizer monkey virus (M-PMV) and in rats against murine sarcoma virus (MSV). Controls for the complement-fixation test consisted of test sera, test and known antigen, as well as normal rat and goat sera.

Placental tissue was homogenized for enzyme assay in 1 vol of cold 0.85% NaCl, equal in microliters to the number of milligrams of tissues, 2 vol of a solution containing 20 m*M* Tris–HCl (pH 8), 10% Triton X-100, 8 m*M* magnesium acetate, 4 m*M* dithiothreitol, 1.0 *M* KCl and 10% glycerol. A 100,000*g* supernatant was run on a 10–30% glycerol gradient. Gradient fractions were assayed for RNA-directed DNA polymerase using a poly rA·oligo dT template by published procedures (3).

Results and Discussion. Results of the complement-fixation assays are presented in Table I and those of the DNA polymerase assays in Table II. These tissues had complement-fixation activity with all sera tested, suggesting a common interspecies specific antigen. Similarly, three of the four placentas tested had relatively high RNA-directed DNA polymerase activity, whereas one had somewhat lower enzyme activity. Interestingly, the RNA-directed DNA polymerase peak sedimented similarly to that of estrogen-stimulated mouse uterine tissue (3). In both cases, the enzyme appeared to have a slightly higher molecular weight than that of Rauscher murine leukemia virus (RLV) polymerase. Highly significant was the fact that whereas the mouse uterine RNA-directed DNA polymerase was inhibited by specific antisera prepared against murine C-type virus (RLV) polymerase (4), the baboon DNA polymerase was not inhibited by highly specific antisera prepared against RLV, Wooley monkey, M-PMV and Gibbon virus polymerases. These antisera have been reported, on the basis of concentration, not to show cross reactivity (5).

C-Type viruses have been recovered from

TABLE I. Group Specific Antigen Activity of Baboon Placental Tissue.

Animal no.	gs	Dilution of anti-MSV pool 2×1235 1:20	1:80	gs	Dilution of of anti-RD-114 pool 2S781 1:20	1:80	gs	Dilution of anti-M-PMV pool 2S718 1:20	1:80
0549	+[a]	8[b]	8	+	8	8	+	8	8
0589	+	8	8	+	4	4	+	8	2
2598	+	8	8	+	8	8	+	8	8
N171	+	8	8	+	8	8	+	8	8

[a] Positive for gs antigen.
[b] Reciprocal of antigen dilution.

a number of animal species and are suggested as the cause of naturally occurring cancers in these animals. The situation concerning man is not as clearly defined, although these particles have been noted by others (6). Our data indicate that the placenta, a tissue of normal physiological functions, contain C-type particles having an enzyme and an antigenic activity generally associated with cell cultures or tumor tissue containing morphologically similar particles. Expression of these viral components has been observed in embryonic tissue (7), in hormonally treated target tissue (4, 8) and late in life when the incidence of cancer increases (7).

It is most interesting that these particles were found in the placenta, a tissue possessing a cellular component (syncytial trophoblast), capable of invading and wandering throughout the host. Further, while the trophoblast cells are antigenic, they are not rejected by the host. We therefore have a situation whereby a normal tissue interposed between the mother and offspring contains particles similar to those associated with cancer development in other host systems.

TABLE II. RNA-Directed DNA Polymerase Activity in Baboon Placental Tissue.

Animal no.	rA • dT peak height[a] (cpm)[b]
0549	3300
0589	525
2598	3400
N171	1450

[a] 100 μl suspension of a 25% homogenate on a 4 ml 10–30% glycerol gradient.

[b] Assay mixtures contained 50 m*M* Tris–HCl (pH 8.2, measured at 37°), 0.01% Triton X-100, 0.1 m*M* manganese acetate, 2 m*M* dithiothreitol, 75 μM [^{3}H] TTP (1.0 Ci/mmole), 6 μg/ml $[rA]_n$, and 10 μg/ml $(dT)_{12-18}$. The poly- and oligonucleotides were mixed before being added to the reaction mixture. 50 μl reactions were carried out for 1 hr at 37°, and 40 μl samples were taken for measurement of acid-precipitable counts.

Obviously these results do not directly favor either the oncogene (7) or protovirus (9) theories of carcinogenesis. The presence of these particles in primate placental tissue may permit a study of their source and their relationship to the host animal. The observation of such particles in placental tissue derived from a 27-day-old pregnancy indicates their early presence. It is assumed that they are derived from the blastocyst, but additional studies are under way to trace the source of these C-type particles.

Summary. C-Type particles were observed in normal baboon placentas at various stages of gestation. This tissue was found to have interspecies specific antigens of the C-type RNA tumor viruses and RNA-directed DNA polymerase activity that could not be inhibited by antisera prepared against the previously recognized reverse transcriptases of several RNA tumor viruses.

This study was funded in part by Contract NIH-NCI-E-71-2348 from the Special Virus Cancer Program, NCI; Grant RR00361 from the U.S. Public Health Service and WHO Grant Z2/181/27. The laboratory at the Southwest Foundation for Research and Education serves as the WHO Regional Reference Center for Simian Viruses.

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Received April 13, 1973. P.S.E.B.M., 1973, Vol. 144.