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Frequency and antigenicity of type C retrovirus-like particles in human placentas

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Summary. Seventeen placentas from normal Japanese women, obtained from 15 full term gestations and at 2 earlier gestational periods, were observed by electron microscopy for the presence of type C retrovirus-like particles. Immature free and budding particles were found in 16 placentas including those obtained pre-term. Free virus-like particles were seen in the intercellular spaces and within the lysosomes of syncytiotrophoblasts of the chorionic villi. Forms budding from syncytiotrophoblasts were found in 3 cases. Coincident data with the electron microscopic observations were obtained by immunohistological methods. Specific positive staining with antisera against heterogeneic whole murine leukaemia virus were seen within the cytoplasm of trophoblastic cells both in and along the basal lamina.

Key words: Type C retrovirus-like particles – Electron and immunohistological microscopy – Human placentas

Introduction

Type C retroviruses are present in a naturally integrated form in the germ line of a wide range of animal species, including mice, chickens, and primates, and are associated with the onset of leukaemias and lymphomas in these animals (Stephenson 1980). In addition, they are related to autoimmune diseases as a target antigen (Yoshiki et al. 1974). In human tissues, recent studies have detected type C retrovirus-like particles in some leukaemias and lymphomas, by electron microscopy and biological methods (Poiesz et al. 1980; Posner et al. 1981; Reitz et al. 1981; Rho et al. 1981; Poiesz et al. 1981; Kalyanaraman et al. 1981; Hinuma et al. 1981; Robert-Guroff et al. 1981). It has also been reported that some component proteins of type C retrovirus have been located in the glomeruli of the kidneys

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in human systemic lupus erythematosus (SLE) (Mellors and Mellors 1976). These findings may suggest that the genome of type C retrovirus, as in animals, is integrated into the gene of human cells. However, until now, type C retrovirus-like particles have been found only in small numbers of normal human placentas (Kalter et al. 1973; Imamura et al. 1976; Dirksen and Levy 1977).

In the present work, type C retrovirus-like particles were detected in 16 of 17 normal placentas of Japanese women by electron microscopy. In addition, Positive staining was seen immuno-microscopically in the cytoplasm of trophoblasts and the basal lamina of chorionic villi in the 17 placentas, using antisera against the whole murine leukemia virus. These data suggest that type C retroviruses exist in all placentas.

Materials and methods

Human placentas and electron microscopic observation

Of 17 normal placentas of Japanese women, 14 were obtained at term after spontaneous delivery. The remaining three placentas were obtained, respectively, at 22 weeks by therapeutic abortion, at 41 weeks after Caesarean section, and at 10 weeks by hysterectomy. Small pieces of tissue were removed from various portions of the placentas immediately following delivery and were then washed and immediately fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4). Specimens were postfixed in 1% osmic acid, dehydrated in graded ethanol and propylene oxide, embedded in Epon 812, and sectioned on an MT-1 Porter Blum ultramicrotome. Five to seven Epon blocks were sectioned for each placenta. Then these thin sections were double-stained with uranyl acetate and lead citrate, and were examined with a JEM-100C electron microscope at 80 KV.

Viruses and antisera

Mouse fibroblast cell lines (SC-1) infected by murine leukaemia virus (MuLV), ecotropic (Friend) virus or amphotropic virus, were obtained from Dr. Ishimoto (Institute for Virus Research, Kyoto University) and were cultured in our laboratory. Purified viruses were obtained from a 1.16 density zone by a 15–60% sucrose density gradient of the cultured fluids. Disruption of viruses was performed employing the Tween-80 ether treatment as described by O'Connor et al. (1965). Purified viruses (from 500 ml of cultured fluids) were suspended in 20 ml of Tris-NaCl buffer containing 2 mg/ml Tween-80, following which an equal volume of ethyl ether was added. After mixing for 1 h at 4° C, the aqueous phase separated by centrifugation at 1,000 G was dialized against Tris-NaCl buffer. The aqueous phase contained viral nucleoids, membranes and soluble proteins and was subcutaneously immunized against rabbits, emulsified in an equal volume of complete Freund's adjuvant. The second and third immunizations were carried out at two weekly intervals, emulsified in an equal volume of incomplete Freund's adjuvant. The rabbit antisera against all the component of Friend-MuLV (referred to as anti-whole Friend virus) or amphotropic MuLV (referred to as anti-whole amphotropic virus) were intensively absorbed with a 1:1 packed volume of normal human liver homogenates and sonicated uninfected SC-1 cells in order to remove antisera against the normal components of the cultured cells. The fluorescent microscopic reactivity of these absorbed rabbit antisera against each virus-infected SC-1 cell was 512-fold in dilution.

Immunofluorescence

Indirect immunofluorescence (IF) was used for the location of viral antigens in the unfixed placental tissues. Small pieces of the placental tissues were frozen with hexane in dry-iced

acetone (−70° C) and sectioned at 2 μ in thickness, using a Leitz Histokryotom. These thin sections on slide glasses were washed twice in phosphate buffered-saline (PBS) and incubated with the rabbit antisera against the viruses for 45 min at room temperature. After washing twice, incubation with fluorescent conjugated goat anti-rabbit IgG antisera (Cappel Laboratories, Cochranville, Pa, USA) was carried out for 30 min at room temperature. After another washing, one drop of 50% glyceline-PBS solution was placed on the tissue. The specific fluorescent staining was observed with a Nikon Fluophot microscope and photographed by using Kodak Ektachrome film (ASA 400).

Peroxidase staining

The horseradish peroxidase-anti/horseradish peroxidase (PAP) method was performed as has been described by Sternberger et al. (1970), for the location of viral antigen in the placentas. Briefly, the thin sections of frozed placenta tissues were processed through first antibody incubation as for IF, after fixation with acetone for 10 min. These thin sections were washed twice in PBS and incubated with sheep anti-rabbit IgG (Cappel Lab., USA) for 45 min at room temperature. They were then washed, twice and incubated with PAP solution (Cappel Lab., USA) for 30 min at room temperature. The thin sections were then washed twice in PBS and the location of peroxidase was revealed by freshly prepared 3-3′ diaminobenzidine substrate for 5 min at room temperature.

Results

Electron microscopic observation

Of the 17 normal placentas, type C retrovirus-like particles were found in 16 (Table 1). In cases 10, 11 and 14, virus-like particles were seen in each Epon block examined. In case 14, they were seen as in a group (Fig. 1).

Table 1. Ultrastructural finding of type C retrovirus-like particles in placentas of normal women

Placenta case	Gestation period (weeks)	Nature of delivery[a]	Type C virus-like particles[b]
PLA 1	42	SD	++
PLA 2	41	SD	+
PLA 3	41	SD	++
PLA 4	22	TA	+(budding+)
PLA 5	40	SD	−
PLA 6	39	SD	++
PLA 7	40	SD	+(budding+)
PLA 8	40	SD	+
PLA 9	40	SD	+
PLA 10	40	SD	+++
PLA 11	40	SD	+++
PLA 12	41	CS	+
PLA 13	39	SD	++
PLA 14	41	SD	+++(budding+)
PLA 15	40	SD	++
PLA 16	10	OP	+
PLA 17	39	SD	++

[a] SD = spontaneous delivery; TA = therapeutic abortion; CS = Caesarean section; OP = hysterectomy

[b] +++ = readily found; ++ = less readily found; + = found after exhaustive searching; − = not found

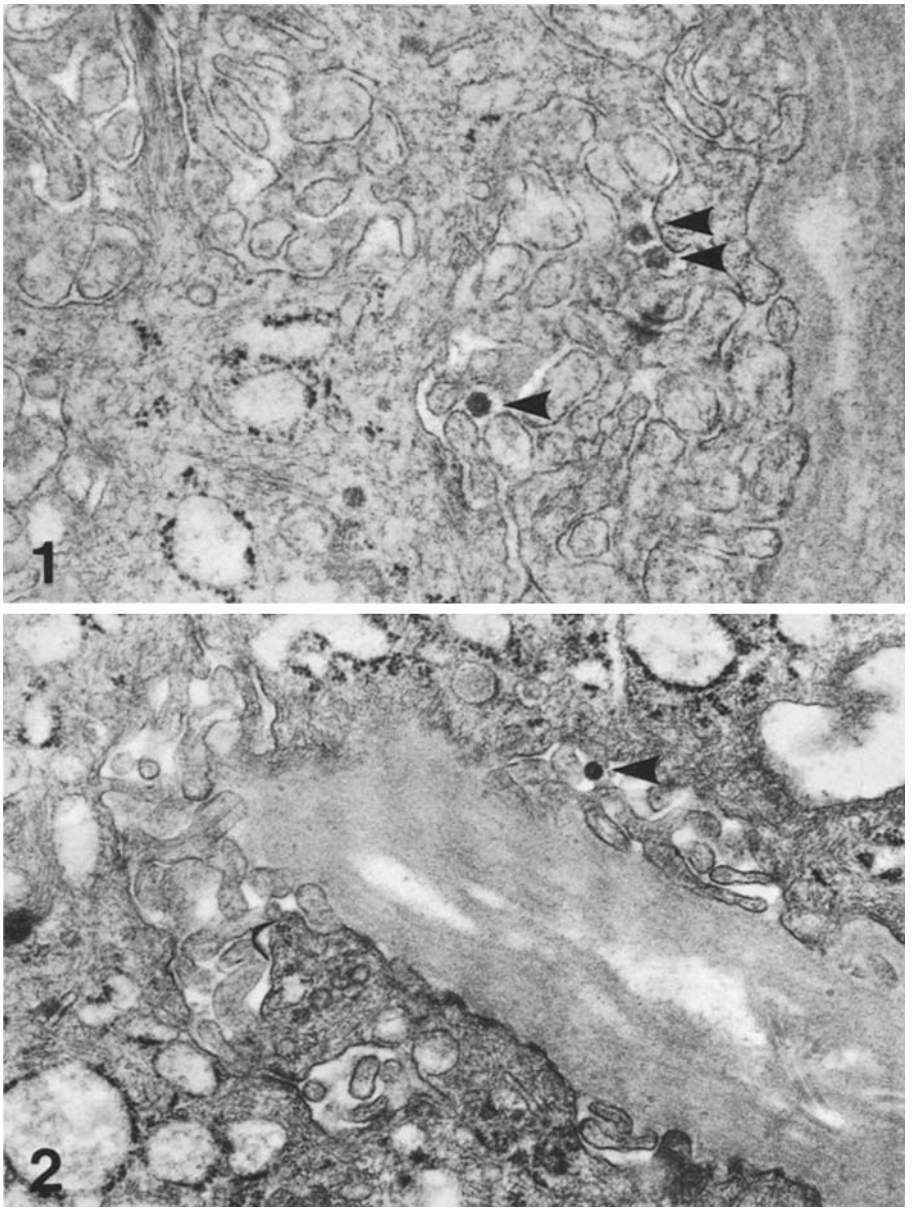
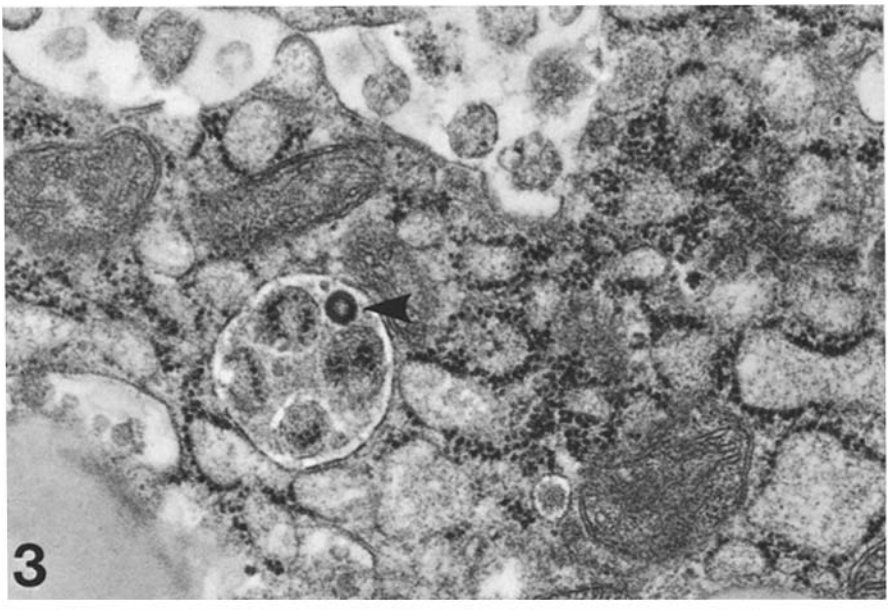
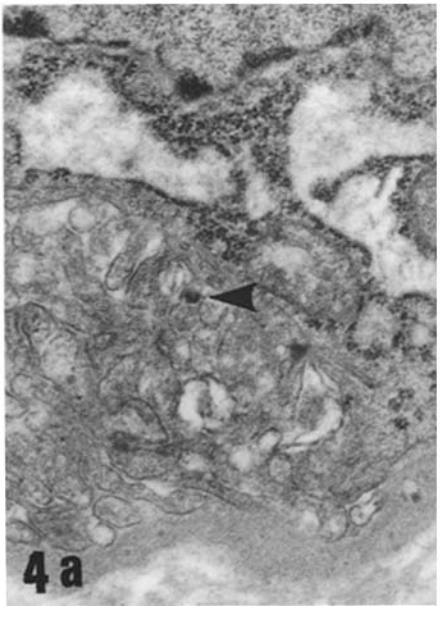


Fig. 1. A group of type C retrovirus-like particles (*arrows*) with a dense outer layer and a less dense inner component are seen in the intercellular spaces of syncytiotrophoblasts. (Case 14, × 40,000)

Fig. 2. Type C retrovirus-like particle (*arrow*) is seen in the space between a syncytiotrophoblast and the basal lamina. (Case 9, × 26,000)





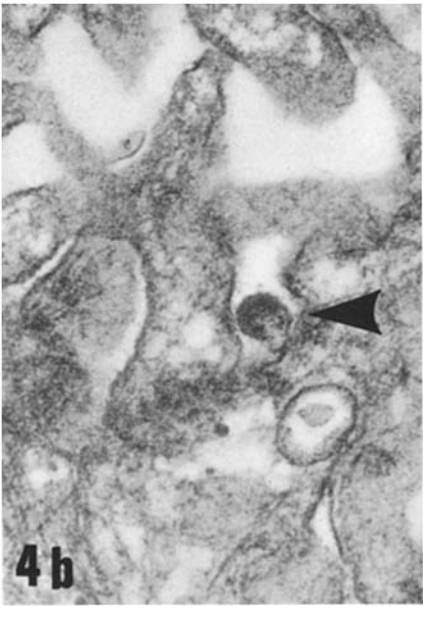


Fig. 3. Type C retrovirus-like particle (*arrow*) is seen within the lysosome in the cytoplasm of a syncytiotrophoblast. (Case 16, ×42,000)

Fig. 4a, b. Budding forms of type C virus-like particles are seen at basal portion of the syncytiotrophoblasts. (**a**, Case 14, ×26,000; **b**, Case 4, ×66,000)

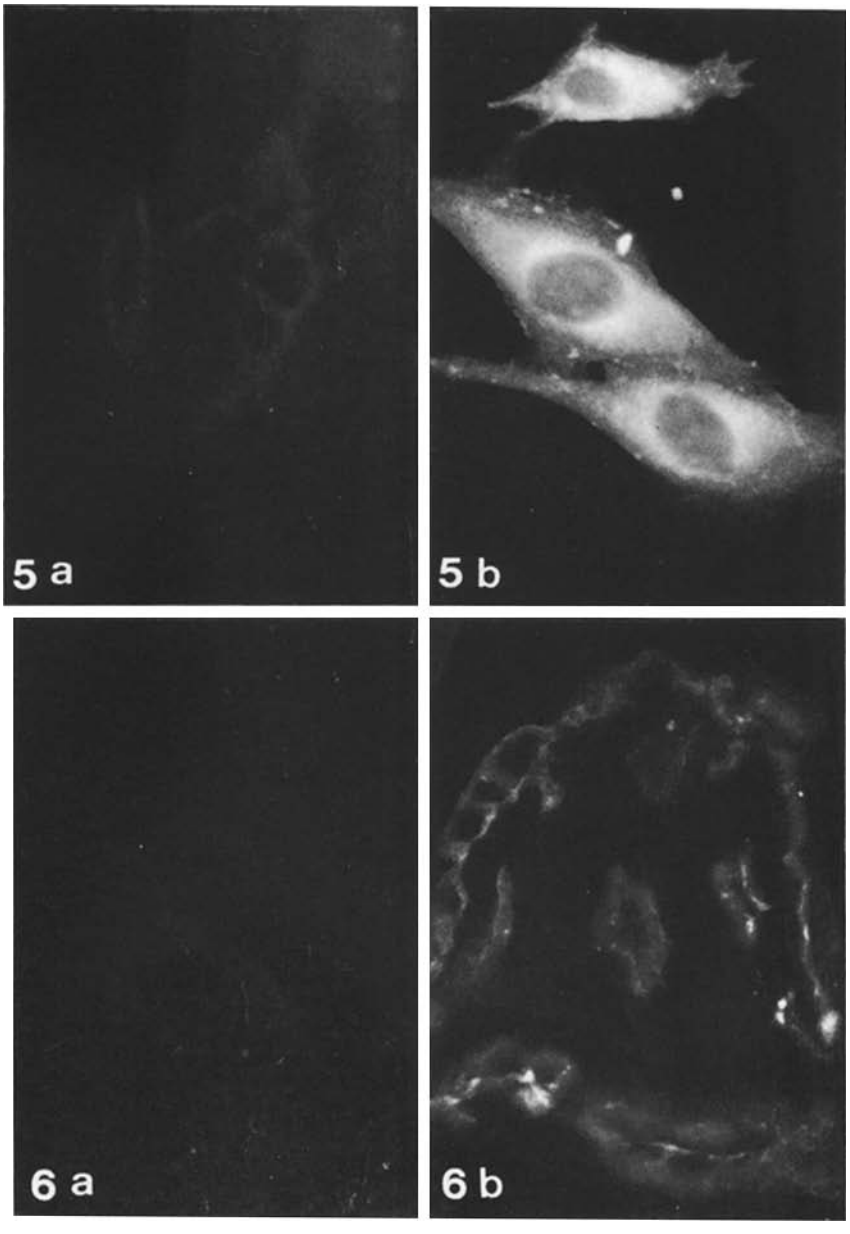


Fig. 5a, b. Immunofluorescence of SC-1 cells uninfected (**a**, × 160) or infected with amphotropic virus (**b**, × 400) using rabbit anti-whole amphotropic virus antiserum

Fig. 6a, b. Immunofluorescent demonstration of type C retroviral antigens in the human placenta using rabbit anti-whole amphotropic virus antiserum. (**a**, pre-immune serum, × 160; **b**, immune serum, × 320; Case 11)

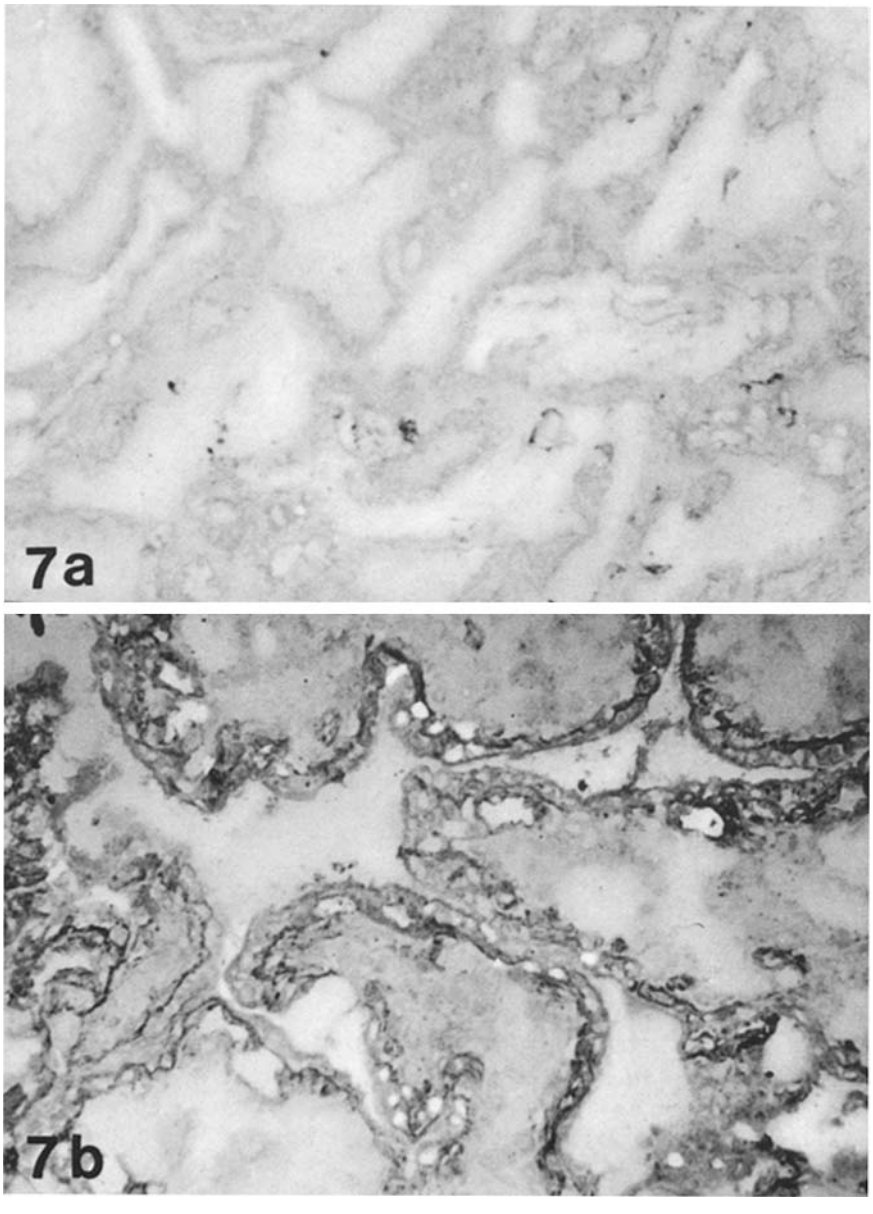


Fig. 7a, b. Immunoperoxidase staining in the human placenta using rabbit anti-whole amphotropic virus antiserum (**a**, pre-immune serum, ×160; **b**, immune serum, ×250; Case 11)

In cases 2, 8, 9, 12 and 16, extensive examination of 5–7 Epon blocks of the placenta tissues was required to obtain a positive finding. Type C retrovirus-like particles were seen freely in the intercellular spaces created by numerous microvilli of syncytiotrophoblasts and also in the space between a syncytiotrophoblast and the basal lamina of each chorionic villi (Figs. 1,

2). The outer diameter of these extracellular particles was approximately 100–130 nm. In case 10, at an earlier gestation period (10 weeks), the virus-like particles were not observed the intercellular spaces, but were found within the lysosomes in the cytoplasm of syncytiotrophoblasts (Fig. 3). These all showed the form of immature virus particles, possessing a nucleocapsid directly in contact with the viral envelope. Mature particles with a less dense outer layer and a dense core were not found in any of the placental tissues. Budding forms of the virus particles with an outer diameter of approximately 100–150 nm were seen in 3 cases from syncytiotrophoblastic cellular membranes (Fig. 4a, b). Virus-like particles were not seen at or on the surface border of the microvilli of syncytiotrophoblasts, nor were they found near the endothelial cells and fibroblasts located inside the basal lamina of the chorionic villi.

Immunofluorescent microscopy

Positive control studies were carried out using absorbed rabbit antisera against whole virus particles and a bright fluorescence was seen in the cytoplasm of the fixed SC-1 cultured cells infected by ecotropic or amphotropic viruses (Fig. 5b). A 1:8 dilution of the absorbed antisera was used for specific staining. No positive fluorescence was seen in the fixed SC-1 cells uninfected with the viruses (Fig. 5a).

In immunofluorescent studies on human placenta tissues, every case, including Case 5, showed weak linear specific staining with the 1:8 dilution of absorbed rabbit antisera. The cytoplasm of trophoblasts near the basal lamina and the basal lamina itself were positively stained (Fig. 6b). Normal human tissues, including the liver, spleen and kidney, were usually negative for this immunofluorescent study. Furthermore, the placental tissues were negatively stained with the pre-immune rabbit sera as the first antibody for the indirect immunofluorescent study (Fig. 6a).

Peroxidase staining for light microscopy

A 1:8 dilution of the absorbed antisera was used for high sensitivity staining. The location of positive staining in the chorionic villi was similar to that of fluorescent microscopy. Specific staining was seen in the basal lamina. Weak positive staining was also seen in the cytoplasm of trophoblasts (Fig. 7b). The placental tissues were negatively stained with the pre-immune rabbit sera as first antibody (Fig. 7a).

Discussion

In our electron microscopic study, we observed type C retrovirus-like particles in 16 of 17 (95%) normal placentas from Japanese women. This study supports previously reported results achieved in western countries (Kalter et al. 1973; Vernon et al. 1974; Imamura et al. 1976; Dirksen et al. 1977). Although the present finding is the highest in occurrence frequency when compared with previous reports, this does not necessarily mean that Japa-

nese women have a higher infection rate for the type C retrovirus. It is probable that immediate fixation of the placental tissues after delivery resulted in good preservation of the tissues and in a higher detection frequency of the virus-like particles with electron microscopy. Thus, we believe that the placental tissues from all pregnant women contain more or less the same amount of type C retrovirus-like particles.

In addition, we also detected virus-like particles in the placenta at a gestation period of 10 weeks. Such particles have not been found at such an early gestation period (Kalter et al. 1973; Schidlovsky et al. 1973; Vernon et al. 1974; Imamura et al. 1976). In order to achieve these results, however, an exhaustive search of thin sections from 10 Epon blocks of the placenta was required, and only two virus-like particles could be found within the lysosomes of syncytiotrophoblasts. No such virus-like particles could be found in the intercellular spaces in this case. It is not clear why, at such an early gestation period, such virus-like particles do not exist in the intercellular spaces of syncytiotrophoblasts. One possibility may be that lysosomal structures are abundant in syncytiotrophoblasts at an earlier gestation period, suggesting their higher phagocytic activity.

In the present immuno-histological microscopic study, the cytoplasm of trophoblasts and the associated basal lamina were positively stained with antisera against mouse leukemia virus. This finding suggests that the type C retrovirus-like particles observed by electron microscopy may possess cross-reactivity with other retroviral antigens in addition to those of humans. Furthermore, the appearance of the viral antigen within the cytoplasm of syncytiotrophoblasts suggests that the viral genome may be present and integrated into the gene of human cells.

Although type C retrovirus-like particles or antigens have not been detected in normal human tissues with the exception of the placenta, Mellors and Mellors have previously reported the location of type C retroviral antigens in the glomeruli of the kidney in human SLE patients (1976). These viral antigens are known to have cross-reactivity with some structural proteins of the known type C retroviruses of animals (Mellors and Mellors 1976). One of the present authers (Imamura et al. 1976) has reported that the groups of budding particles were frequently seen in the placentas from patients with SLE, indicating active replication of the virus. It is not clear why these virus-like particles and viral antigens are observed only in the placenta among human tissues. Their appearance in normal placentas might perhaps be the result of allogeneic stimuli by the fetal tissues, as has been described by others (Hirsch et al. 1972, 1978; Thiry et al. 1978).

More recently, it has been reported that some human leukaemias or lymphomas of T cells might be associated with a unique human type C retrovirus infection (Poiesz et al. 1980; Posner et al. 1981; Reitz et al. 1981; Rho et al. 1981; Poiesz et al. 1981; Kalyanarman et al. 1981; Hinuma et al. 1981; Robert-Guroff et al. 1981). Analysis of the proteins and nucleic acids of the isolates from cultured tumor cells has indicated that they were closely related to the type C retrovirus but were distinct from known animal type C retroviruses (Robert-Guroff et al. 1981; Poiesz et al. 1981; Kalyanaraman

et al. 1981; Rho et al. 1981; Reitz et al. 1981; Posner et al. 1981). No studies have as yet examined their antigenic cross-reactivity with the human placental virus. Here, it should be noted morphologically that only mature types were detected and that the budding form was unusual in cultured malignant T cells (Poesz et al. 1981; Hinuma et al. 1981), whereas the immature types with frequent budding forms were observed exclusively in human placentas. In human placentas, the retrovirus might be defective in the processing of cleavage from the large precursors of the viral proteins, suggesting that the viral genome has not been fully expressed, as has been described by Imamura and others (1976).

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