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A-Type Particles in Placentas of Four Mouse Strains (38719)

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We have recently reported the presence of C-type viruses in placentas of primates and other animal species (1–3). These findings have now been confirmed by several investigators (4, 5). In addition, others have described virus-like particles in nonprimate fetal germinal tissues, namely: guinea pig oogonia, oocytes (6), and testes (7). These virus-like particles are similar in morphology to viruses reported in normal (8, 9) and leukemic guinea pigs (8, 10), and in leukemic (11) and nonleukemic mice (11–15). In this study, we describe the presence of intracisternal A-type viral particles within the cytotrophoblastic layer of the placental labyrinth of several mouse strains.

Materials and Methods. Placental tissues, obtained 15–17 days postcoitus from at least three different mice of each of the four mouse strains (i.e., AKR, Balb/c, C3H, and DBA/2N), were fixed for 1 hr at room temperature in 3% glutaraldehyde with 0.1 *M* Sorenson's phosphate buffer (pH 7.2). Tissues were postfixed for 1 hr at 4° in Dalton's chrome–osmium (16). Dehydration in ethanol and propylene oxide was followed by embedding in Epon 812 (17). Thin sections were cut on an LKB UM I or UM II ultramicrotome and were stained with saturated uranyl acetate and lead citrate (18). Sections were examined with either an AEI EM6B or Hitachi HU 11 C electron microscope.

Magnification scales of electron microscopes used in this study were calibrated using a carbon grating replica and method supplied by Ernest F. Fullam, Inc. (Schenectady, New York).

Results. A-type particles were observed within dilated endoplasmic reticulum of mouse placental labyrinth cytotrophoblasts (Fig. 1). These structures were not observed within the two trophoblastic layers adjacent to the fetal endothelium. Particles had a 35- to 45-nm electron-lucent core surrounded by an electron-dense ring (Fig. 2). The ring was encircled by a space of low electron density which was limited by an outer membrane coat. The particles measured 85–95 nm and averaged 90 nm over-all diameter. Extracellular particles and small particles (50 nm) with electron-dense centers were not observed.

Intracisternal A-type particles were found in labyrinthine cytotrophoblasts of all mouse strains examined; however, there was a marked variation noted in numbers of particles among the four strains. AKR, Balb/c, and C3H mice contained approximately equivalent numbers of A-type particles, while DBA/2N mice were found to have a greater number of viruses. It was not uncommon to find two to six particles clustered in cytotrophoblasts of DBA/2N mice; but only occasional particles were observed in cytotrophoblasts of AKR, Balb/c, and C3H mice.

Viruses were not confined to the cytotrophoblastic layer of the placental labyrinth, but were found also in small cytotrophoblastic elements within the junctional zone of all mouse strains (Fig. 3). This zone is located between the labyrinth and the decidua basalis. These junctional zone A-type particles were found in roughly equivalent numbers among strains. Particles in the cytotrophoblastic elements were much the same size as those in labyrinthine cytotrophoblasts (Fig. 4). No extracellular particles were observed.

Discussion. The presence of virus particles in placental tissues has been described. Trophoblasts from baboons (1) and humans (2) have been reported to contain apparent endogenous C-type particles. Similar findings in the rhesus monkey (3, 19), cat (20), and other animals (3) have been reported. Even though viruses have been observed in placentas of other animals, A-type parti-

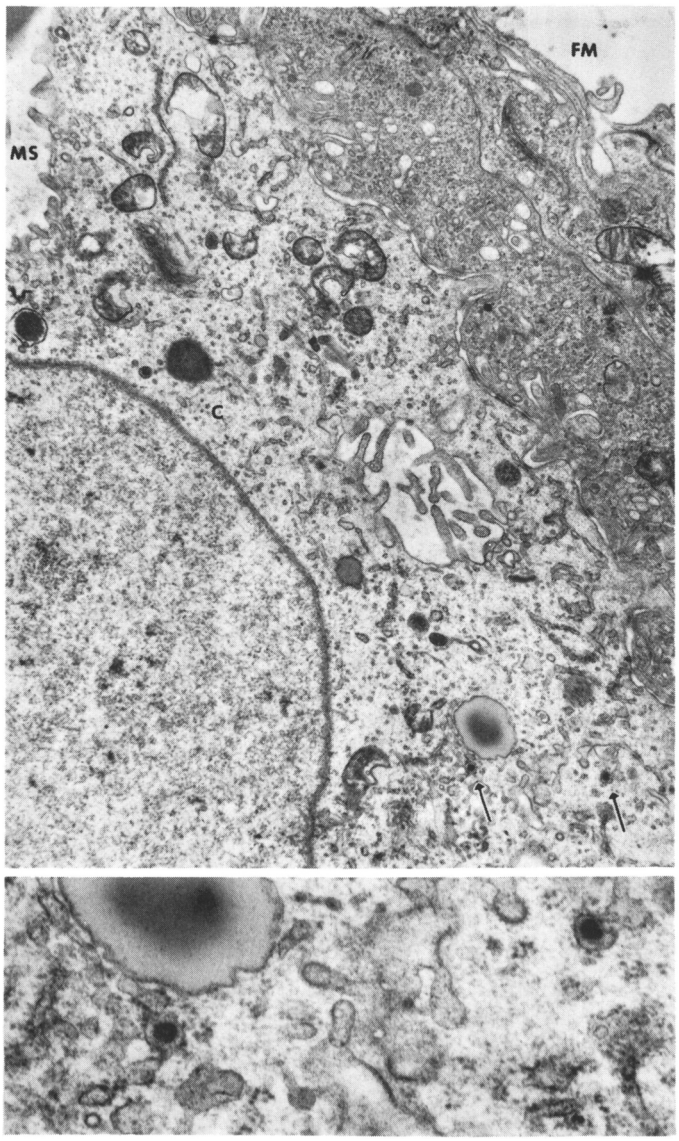

FIG. 1. Cytotrophoblast (C) of DBA/2N placental labyrinth. A-type particles are observed in dilated cisternae of rough endoplasmic reticulum (arrows). Maternal blood sinus (MS) and fetal mesenchyme (FM) are apparent. ×10,500. Insert depicts higher magnification of the viral particles. ×34,000.

cles have not been previously reported in mouse placental tissues (21).

Hormonal activation of C-type RNA tumor virus markers in mouse uterine tissues has been shown to be strain dependent (22). A-type particles have been observed in AK mice (leukemic and nonleukemic) and in C3Hf mice injected with mouse leukemia (Gross) virus, but none were seen in non-injected C3Hf mice (11). The numbers of A-type particles in blastocyst and egg cylinder embryos of eight mouse strains have been shown to vary (12). Noting a differential number of intracisternal A-type paricles in placentas of one of four "normal" mouse strains would, therefore, be somewhat expected.

Of four mouse strains studied, DBA/2N placentas contained the most viral particles; whereas, other studies have indicated that

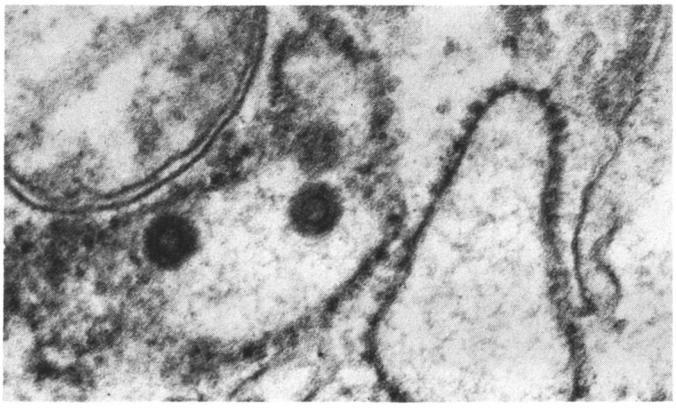

FIG. 2. Details of typical A-type particles of DBA/2N labyrinth cytotrophoblastic layer. ×83,000.

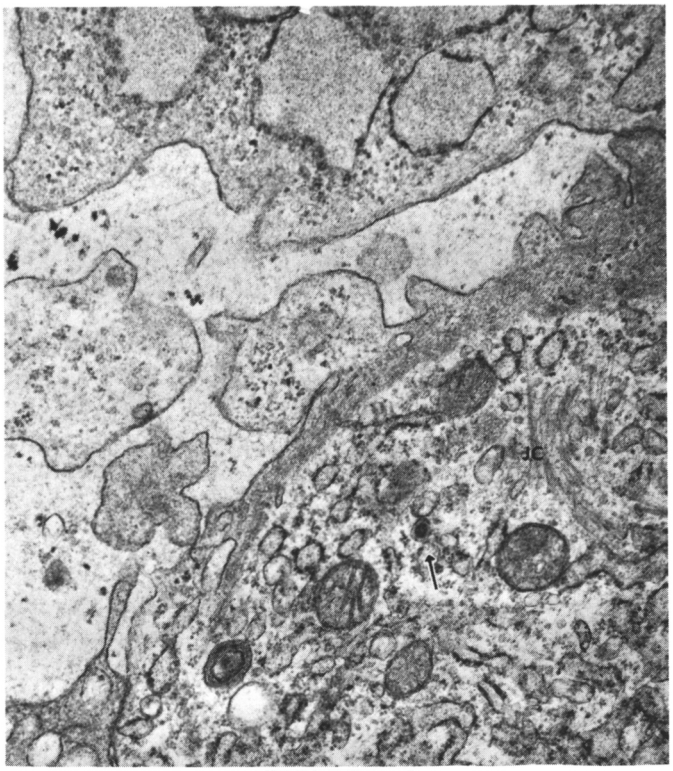

FIG. 3. AKR junctional zone cytotrophoblast (JC). One A-type particle is observed within the dilated cisternae of endoplasmic reticulum (arrow). ×20,000.

AKR embryos contained the most viral particles (12). Discrepancies may be due to differences in tissues. Perhaps A-type particle replication and maturation has been suppressed in some mouse strains (depending on the tissue) as has been previously suggested (12). With the limited evidence currently available concerning the prevalence of A-type particles in mouse embryos and placentas, speculation as to the reasons for strain variations and potential pathogenicity is unwarranted. Relative ease of observation or induction of C-type viruses in mouse tissues appears to have a well-documented relationship with high or low incidences of neoplasia (23). As yet, no such parallels can be drawn concerning the etiology of A-type viruses and their interactions, if any, with the C-type viruses.

The placental A-type particles reported

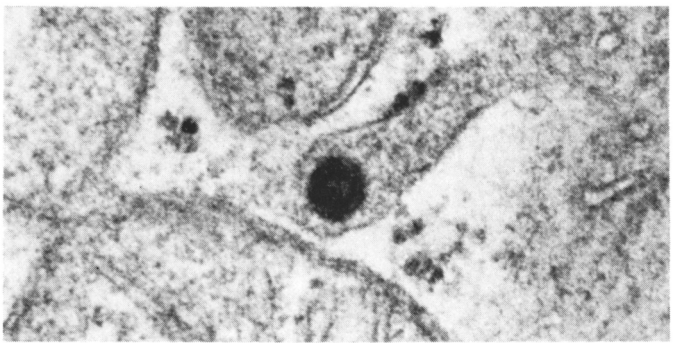

FIG. 4. Details of a Balb/c junctional zone A-type particle. ×87,000.

here (90 nm) are somewhat larger than "naturally" occurring A-type particles (70 nm) in CF-1 mouse ova and Balb/c, CF-1, Swiss Webster, and New Zealand Black embryos (13), and somewhat smaller than particles (100 nm) in mouse embryos of ICR/Ha, AKR, Balb/cf, C3H, GR/cam, C57Blf/He/Cam, RIII/Cam, Balb/c, and ICR/Ha × C57BL/6 strains (12). Particles in the 70- to 85-nm and 85- to 100-nm range have been reported in embryos of Swiss albino mice (14). Even though particles described herein differ in size from some previously reported A-type particles, they comply with the intracisternal A-type particle definition (24). Differences could be attributed to noncalibrated magnification scales on some electron microscopes, particle age, different preparatory technique, or to different mouse strains.

A-type particles have been found associated with the outer trophoblastic layer of mouse placenta and C-type particles have been found principally associated with the outer trophoblastic layer of baboon (1) and human (2) placentas. However, the outer trophoblastic layers of mouse and baboon (human) placentas are different. That is, the outer layer of mouse placenta is cytotrophoblastic and the outer layer of baboon (or human) placenta is syncytiotrophoblastic. The significance of this outer trophoblastic–viral association is unclear, as is the relationship, if any, of the two morphologically distinct viruses observed in these tissues. There have been conflicting views as to whether A-type particles represent immature C-type particles (8, 24). C-type viruses have been shown to be the causative agent of certain mouse leukemias and lymphomas (25). On the other hand, A-type particles have been shown to possess a 70S RNA (26) and a reverse transcriptase (27), but no biological activity has been demonstrated. In view of the foregoing, further investigation of these A-type particles would seem warranted in order to determine their relationship, if any, to other oncornavirus-like particles of "normal" mammalian tissues, and indeed to the viral etiology of cancer.

Summary. A-type particles have been detected by electron microscopy in placentas from four strains of mice: AKR, Balb/c, C3H, and DBA/2N. Complete structures were observed within rough endoplasmic reticulum cisternae of the placental labyrinth cytotrophoblasts. A marked prevalence of such particles was observed in the DBA/2N mice. Similar particles were also found in small cytotrophoblastic elements of the junctional zone (located between labyrinth and maternal decidua) of all mouse strains studied. The numbers of junctional zone particles in all four strains were roughly equivalent.

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