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Serum Somatomedin C, Bioassayable Growth-Promoting Activity (Thymidine Activity), and Transferrin in Human Fetuses: *In Utero* Study

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ABSTRACT. Serum somatomedin C, thymidine uptake stimulating activity, and transferrin were measured in fetal blood collected by ultrasound-guided puncture of umbilical vessels *in utero* during prenatal assessment for mother-to-fetus transmissible infections. Serum somatomedin C and transferrin were measured by immunoassay. Thymidine activity was measured by assay of [^{3}H]thymidine incorporation into lectin-activated human lymphocytes. Studies were conducted in 48 healthy fetuses at gestational ages 21–28 wk. From 21–24 to 25–28 wk, serum somatomedin C significantly increased from 0.05 ± 0.06 to 0.24 ± 0.03 U/ml, while thymidine activity significantly decreased from 1.41 ± 0.15 to 0.95 ± 0.06 U/ml. Transferrin levels did not change. These data suggest that the humoral control of fetal growth at midpregnancy involves mechanisms other than direct regulation by somatomedin. (*Pediatr Res* 20: 71–73, 1986)

Abbreviations

Sm C, serum somatomedin
TA, thymidine activity
IGF, insulin-like growth factor

The role of growth factors in fetal development is not clear. Available information indicates that the levels of somatomedin/IGF I and II in human fetal blood are low (1–3) while it has been reported that another factor measured as fetal brain radioreceptor assayable somatomedin is in high quantity (4). In previous studies we characterized a method to measure the growth-promoting activity of human serum by assessing the incorporation of tritiated thymidine into lectin-activated human lymphocytes (5, 6) and applied it to newborns (7). The availability of normal fetal blood collected *in utero* for purpose of prenatal diagnosis of infection provided the opportunity to extend the study to fetuses. Herein we report our results in fetal blood, in association with measurement of Sm C and transferrin.

MATERIALS AND METHODS

The serum samples used in this study were obtained by direct puncture of the umbilical vessels *in utero*. Intrauterine cord blood sampling was performed to determine whether maternal rubella or toxoplasmic infection had involved the fetus. The ultrasound-guided technique and its results in the prenatal diagnosis of mother to fetus transmissible infections have been published elsewhere by Daffos *et al.* (8, 9). Two ml of fetal blood were collected. The size of the red blood cells was determined immediately in a Coulter counter to ensure that no maternal blood was mixed with the fetal blood. After clotting at room temperature, an aliquot of serum was kept frozen at −20° C for later study. For this work we used only sera from noncontaminated fetuses. Follow-up showed that they were normal at birth. We were thus able to collect samples from 48 fetuses at 21–28 wk of pregnancy. The fetal age was assessed from both the first day of the last menses and from ultrasound measurement of cranial biparietal diameter.

Serum growth-promoting activity was measured by assessing TA into human lectin-activated lymphocytes, as detailed in several previous publications from this laboratory (5, 6, 10, 11). Each serum was studied in triplicate at six dilutions, 0.03–1.25%. The value of TA was determined by reference to a standard considered to be 1 U/ml, made of a pool of sera from eight normal adult males. Burn's slope ratio assay was used for this calculation. Finney's "g" test was used to compare the results. The coefficient of variation within assays was 5–10%. The interassay variation was 10–15%.

Sm C was measured by radioimmunoassay using the currently available kit from Immuno Nuclear Corporation (Stillwater, MN) after separation of somatomedin from carrier protein on a Sep-Pack column. As standard we used the same pool of sera which was used in the TA assay, assuming a value of 1 U/ml. The sensitivity is 0.05 U/ml. The intraassay and interassay variations are less than 5%. Transferrin was measured using the Mancini's technique of radial immunodiffusion (12) with NOR-Partigen plates (Behring, Germany). Statistical comparison of mean values were done using the Student's *t* test.

This work was approved by the local ethics committee. Puncture of umbilical cord was performed for diagnostic purposes after informed consent from the mother. The amount of serum used for the present investigation was less than 0.5 ml for each individual fetus.

RESULTS

The mean ± SEM levels of TA, Sm C, and transferrin are given in Table 1. Values at 21–24 wk of pregnancy are compared to those found at 25–28 weeks. We observed a significant decrease of TA with gestational age ($p < 0.001$) which contrasted with a significant increase of Sm C ($p < 0.001$). Fetal serum transferrin levels did not change significantly with gestational age.

The individual results of TA and Sm C measurements are shown in Figure 1. A negative correlation exists between TA and gestational age: $y = 4.24 - 0.125\ x$, $r = -0.76$, $p < 0.01$. It contrasts with the positive correlation found between Sm C and

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Table 1. *Mean ± SEM values of bioassayable TA, radioimmunoassayable SM C and transferrin in fetuses aged 21–24 and 25–28 wk*

Measurements	21–24 wk	25–28 wk
TA (U/ml)	1.41 ± 0.15	0.95 ± 0.06
	(n = 24)	$p < 0.001$ (n = 24)
Sm C (U/ml)	0.05 ± 0.06	0.24 ± 0.03
	(n = 12)	$p < 0.001$ (n = 8)
Transferrin (g/liter)	1.09 ± 0.05	1.00 ± 0.06
	(n = 24)	NS (n = 24)

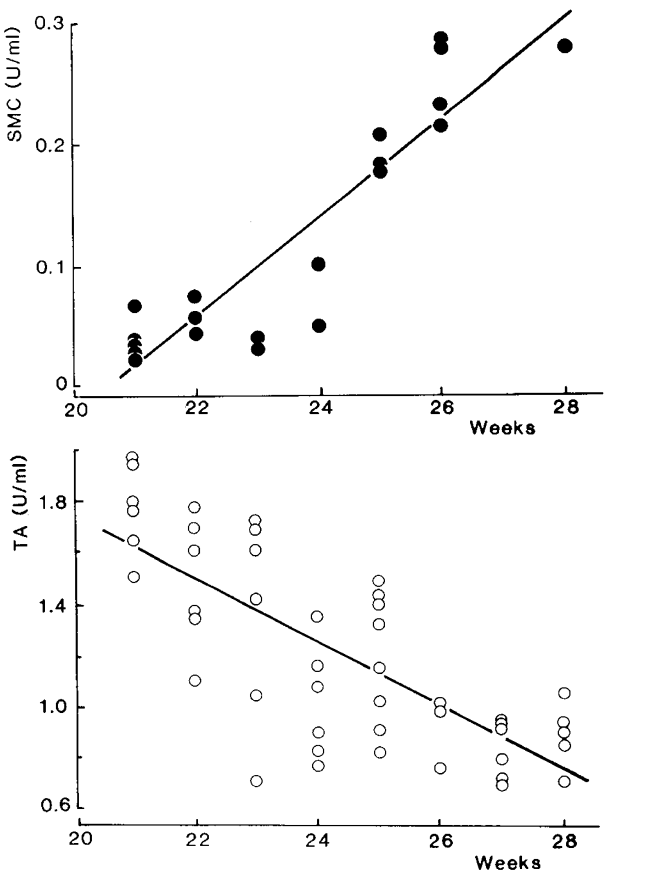

Fig. 1. Correlation between gestational age and 1) SMC ●, 2) TA ○ in fetuses aged21–28 wk.

the length of gestation: $y = 0.04 \times -0.82$, $r = 0.91$, $p < 0.001$. The small amount of fetal serum available was not sufficient to measure both TA and Sm C in the same sample so that it was not possible to search for correlation between the two measurements in individual fetuses.

DISCUSSION

Few data are available regarding somatomedin and related growth factors in fetal blood. Most have been obtained by animal studies. D'Ercole *et al.* (1) found that Sm C in fetal pig serum, measured by radioreceptor assay using placental membranes, is about 25% of the adult level and is constant throughout fetal life. These authors first suggested that the low fetal levels of Sm C may reflect low levels of the serum Sm-binding protein rather than an absolute deficiency of biologically active somatomedin. In the fetal rat, Daughaday *et al.* (13) demonstrated that, after preliminary acid-ethanol extraction allowing separation from carrier proteins, radioimmunoassayable IGF I is relatively elevated during the last days of gestation, then falls after birth, while radioreceptor assayable IGF II and multiplication-stimulating activity reach high levels in the late fetal and early postnatal days. These authors suggest that IGF II may have important growth-promoting significance in rat fetal and neonatal life.

In human fetuses it was shown that circulating Sm C exists predominantly in a low molecular weight form of 40,000 daltons instead of the 150,000 dalton form characteristic of adult serum (2) and that specific Sm C receptors of the placental membranes, identified from as early as 6 wk of gestation, demonstrated no apparent structural changes through the course of pregnancy (14).

An important point is that Sm C may be synthesized by multiple tissues. It was reported with cultured human fibroblasts (15) and demonstrated also in fetal mice (16). Recent data (17) have pointed out that the rate of DNA synthesis in cultured human fibroblasts and porcine smooth muscle cells *in vitro* is directly linked to their capacity to produce somatomedin-like peptides, further supporting that these peptides are involved in autocrine and paracrine growth regulation (18).

Other data in human fetuses were reported by Sara *et al.* (4), having collected cord blood of seven normal fetuses during fetoscopy performed between 16 and 22 wk of gestation for possible risk of congenital disease. Radioimmunoassayable somatomedin A was below detectable levels in six of seven fetuses, contrasting with high levels of what these authors named fetal brain radioreceptor-assayable Sm. The latter was measured by a radioreceptor assay using plasma membranes of human fetal brain as receptor; levels were approximately 4-fold higher in the fetuses studied than in cord blood of term newborns or in adult serum. The authors suggested that fetal blood contains a growth factor (5) not recognized by the radioimmunoassay of Sm; they referred to this growth factor(s) as "human embryonic somatomedin." However, these data have been revised by study of interactions of human cord serum with Sm receptors of placental membranes compared to those of human fetal brain, and the results of this new work suggest that the apparent increase of Sm seen with the fetal brain membranes radioreceptor assay is due to differences between adult and newborn Sm interaction with receptors rather than to a specific fetal form of Sm peptide (19).

In the human newborn, somatomedin levels are low when measured as sulfation bioactivity (20–23), radioreceptor activity (1, 24), and radioimmunoassay (3, 25–28). Moreover, preterm newborns have lower cord blood somatomedin values than full-term newborns (1, 21, 23, 29). Both insulin-like growth factors IGF I and IGF II are positively correlated with gestational age (3).

Bioassayable growth-promoting activity measured as TA also is low in cord blood and increases after birth, as demonstrated in earlier reports from our laboratory (10, 11, 30). TA is lower in cord blood of premature than of full-term newborns. Higher TA is found in blood obtained from neonatal vessels, either after clamping of the cord (7) or by capillary puncture immediately after birth (30), than in cord blood collected from the placental section. In term newborns whose birth weight was appropriate for gestational age, we found a serum TA of 1.50 ± 0.7 U/ml and a level of radioimmunoassayable Sm C of 0.52 ± 0.03 U/ml. In these newborns, individual values of TA and Sm C were positively correlated (7). Such a correlation was not studied in the present work, since the amount of serum available did not allow both measurements in the same fetuses.

The levels of Sm C measured by radioimmunoassay in the human fetuses in the present study rose from nearly undetectable values (0.05 ± 0.06 U/ml) at midpregnancy (21–24 wk) to 0.24 ± 0.03 U/ml at 25–28 wk. This latter value is about half of the 0.52 ± 0.03 U/ml level that we found in term newborns (7), in good agreement with the data of Bennett *et al.* (3) who measured IGF I and IGF II by radioimmunoassay in the cord blood of premature and term newborns and Sm C by radioreceptor assay in term newborns.

In sharp contrast, the serum TA decreased in the present study from 1.41 ±0.15 U/ml at midpregnancy to 0.95 ± 0.06 in 25- to 28-wk fetuses (Fig. 1); this latter value was lower than the 1.50 ± 0.07 U/ml previously found in the blood of term newborns (7). These differences suggest that factors other than somatomedins contribute to the stimulating effect of human serum upon thymidine incorporation into lectin-activated lymphocytes and they vary during fetal life.

Measurements of serum transferrin were included in the present study since no values for living fetuses *in utero* have been reported. No change in transferrin levels was observed from the fetal age 21–24 to 25–28 wk. The overall mean fetal level that we found (1.05 ± 0.05 g/liter) was significantly lower than that measured in our earlier study of term newborns (1.68 ± 0.14 g/liter) (7) and in another neonatal study (31).

In conclusion, this study of normal fetal blood confirms that the level of Sm C is very low at midpregnancy, a time where growth velocity is particularly high, and increases to term. On the other hand, the TA, high at midpregnancy, decreases. These data suggest that the humoral control of fetal growth may be related to mechanisms other than direct regulation by somatomedin.

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