# Ontogenetic Features of the Expression of mRNA Isoforms for Leukemia-Inhibitory Factor in Human Fetal Tissues and Mononuclear Cells

**V. A. Sadovskaya, S. V. Sennikov, A. A. Ostanin, G. V. Seledtsova, A. N. Silkov, and V. A. Kozlov**

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 2, pp. 85-88, 2009
Original article submitted May 6, 2008

The expression of leukemia-inhibitory factor mRNA in human fetal tissues and mononuclear cells was studied during ontogeny. The expression of mRNA isoforms for leukemia-inhibitory factor was tissue-specific at the stage of prenatal development. The transition from antenatal and neonatal development to the postnatal period was accompanied by a decrease in the expression of mRNA isoforms for leukemia-inhibitory factor in mononuclear cells.

**Key Words:** *leukemia inhibitory factor; alternative promoter; gene expression; ontogeny*

Leukemia-inhibitory factor (LIF) is a polyfunctional cytokine. This cytokine is expressed and produced by cells of various tissues, including the heart, liver, endometrium, brain, pituitary, lungs, and thymus [5,6,9]. Studying the expression of LIF in mammals showed that transcription of *lif* gene from alternative promoters yields 3 products (LIF-D, LIF-M, and LIF-T). These transcriptases contain alternative first exons and identical second and third exons [7]. Further experiments showed that LIF protein isoforms are characterized by different location in cells. LIF-M and LIF-T isoforms are matrix-associated and intracellular cytokines. Protein compartmentalization correlates with high, intermediate, and low extracellular LIF activity during the expression of hLIF-D, hLIF-M, and hLIF-T, respectively [11].

The expression of matrix RNA (mRNA) for extracellular and intracellular isoforms is characterized by the independent regulation [4,11]. These isoforms have different biological activity, which is mediated by various signal pathways [11].

Here we studied the expression of LIF mRNA isoforms in fetal tissues (20-22 weeks gestation) and human blood mononuclear cells (MNC) during prenatal, neonatal, and postnatal ontogeny.

## MATERIALS AND METHODS

Fetal tissues and blood ($n$=12) from conventionally healthy women (mean age 26±3 years) were used in the experiments. Pregnancy was terminated for the so-called "social indications" (mean gestation period 20-22 weeks). Medical abortion was induced by intraamniotic administration of 25 mg Enzaprost. The absence of intrauterine infection in fetuses (herpes, hepatitides B and C, cytomegalovirus, *etc*.) was confirmed by bacteriological and histological tests of the abortion material and afterbirth.

We also examined samples from pregnant women with spontaneous labor. The umbilical vein of healthy mature infants ($n$=13, 5-7 ml) was taken after omphalotomy (placenta in the uterus, *in utero*). The umbilical vein was drained. Blood samples

Institute of Clinical Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

0007-4888/09/1474-0521 

were put in a sterile tube with 0.5 M EDTA. All studies with fetal tissues and fetal/umbilical blood were approved by the Local Ethics Committee. All women signed the informed consent form.

MNC from fetal/umbilical blood samples and peripheral blood of conventionally healthy donors ($n$=15) were routinely isolated by centrifugation in a Ficoll-Verografin density gradient (ρ=1.082 g/liter) [1]. Total RNA was extracted with phenol [2]. RNA content was measured by electrophoresis in 1% agarose gel (Chemapol) in Tris-acetate buffer.

For the reaction of reverse transcription, 1 μg total RNA was denatured in the presence of 0.5 μg universal primer oligo(dT)16 at 72°C for 5 min and put on ice. The reaction mixture consisted of 1 μg total RNA, 0.5 μg oligo(dT)16, 500 μM each dNTP (Sigma), and 20 U M-MuLV reverse transcriptase (Biosan). The reaction buffer for reverse transcriptase contained 20 mM $MgCl_2$, 20 mM Tris-HCl (pH 8.3 at 25°C), 100 mM KCl, and 1 mM dithiothreitol. The reaction (volume 20 μl) was conducted at 37°C for 1.5 h. The enzyme was denatured at 95°C for 5 min.

cDNA samples were subjected to PCR with 3 pairs of primers specific for transcript sequences of LIF-D, LIF-M, and LIF-T. Nested PCR with the obtained products was performed to increase reaction sensitivity and specificity. Amplification products were analyzed in 2% agarose gel stained with ethidium bromide.

## RESULTS

The expression of LIF mRNA isoforms was studied in human fetal tissues. Total RNA samples were isolated from the liver ($n$=7), spleen ($n$=5), thymus ($n$=6), ovaries ($n$=5), testes ($n$=4), kidney ($n$=5), brain ($n$=7), adrenal glands ($n$=3), thyroid gland ($n$=1), lungs ($n$=1), and tooth germs ($n$=3).

The study of amplification products in agarose gel showed that the expression of LIF mRNA isoforms is heterogeneous and occurs not in all tissues (Table 1).

The intensity of hemopoiesis in the liver is high at the 20th-22nd week of intrauterine development. LIF is one of the main regulators of this process [4]. The soluble isoform produced in the liver probably plays a role in the regulation of proliferation and differentiation of hemopoietic stem cells. These cells migrate into organs playing the major role in hemopoiesis and immunopoiesis. Differences in the expression of LIF mRNA are probably related to the development and proliferation of immunocompetent cells in the spleen and thymus and various effects of LIF isoforms in these organs.

LIF expression was undetectable in renal tissues, which is consistent with the results of previous experiments on mice [8].

LIF plays an important role in the hypothalamic—pituitary—adrenal system [11], which probably contributes to activation of LIF expression in the adrenal glands. The presence of mRNA isoforms for LIF-D and LIF-M reflects the polymorphic regulation of this system by LIF.

The isoform of LIF-M mRNA is strongly expressed in the brain tissue. It probably results from the fact that LIF-M has a major regulatory role in the development and function of the nervous system during this period [4,8].

**TABLE 1.** Occurrence Frequency of Alternative LIF Transcripts in Fetal Tissues at 20-22 Weeks Gestation

| Fetal tissue | LIF isoform | | |
|---|---|---|---|
| | hLIF-D, soluble | hLIF-M, matrix-associated | hLIF-T, intracellular |
| Liver | 7/7 | 3/7 | 0/7 |
| Thymus | 1/6 | 0/6 | 0/6 |
| Spleen | 0/5 | 2/5 | 0/5 |
| Ovaries | 1/5 | 1/5 | 0/5 |
| Testes | 1/4 | 1/4 | 0/4 |
| Kidney | 0/5 | 0/5 | 0/5 |
| Adrenal glands | 3/3 | 2/3 | 0/3 |
| Brain | 2/7 | 5/7 | 0/7 |
| Tooth germs | 2/3 | 1/3 | 0/3 |
| Thyroid gland | 0/1 | 0/1 | 0/1 |
| Lungs | 0/1 | 0/1 | 0/1 |

**Note.** Here and in Table 2: numerator, number of positive samples; denominator, total number of samples.

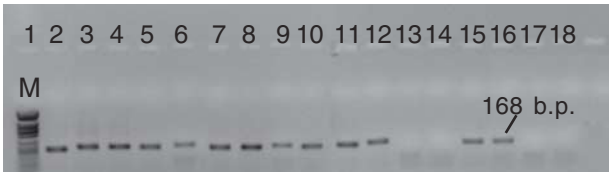


**Fig. 1.** Electrophoretogram of nested PCR for LIF-M mRNA in samples of peripheral blood MNC and fetal tissues at 20-22 weeks gestation. *M*: DNA molecular weight marker, pUC19/MspI. Arrow: amplification product, 168 b.p. Fetal MNC (*1*); fetal MNC (*2*); liver (*3*); testes (*4*); brain tissues (*5*); adrenal glands (*6*); ovaries (*7*); brain tissues (*8*); brain tissues (*9*); liver (*10*); tooth germs (*11*); tooth germs (*12*); adrenal glands (*13*); liver (*14*); liver (*15*); adrenal glands (*16*); thymus (*17*); control (*18*).

LIF plays an important role in the maintenance of proliferative activity of stem cells (SC). It was interesting to evaluate the presence of cytokine isoforms in tissues of tooth germs. Published data show that the degree of SC proliferation in these tissues is high during the prenatal development [3,10]. The expression of LIF isoform transcripts was high and polymorphic. Figure 1 illustrates the expression of LIF-M mRNA isoform.

The intracellular LIF-T mRNA isoform was undetectable in fetal tissue samples. Previous studies showed that LIF-T expression is very low in mouse fetal and adult tissues [4]. The intracellular isoform has a specific biological role. The synthesis of this isoform is induced only under certain conditions of regulation.

Our results show that the expression of LIF mRNA isoforms is selective and tissue-specific during the 20th-22nd week of intrauterine development. LIF is mainly expressed in tissues of the liver, brain, adrenal glands, and tooth germs. This period of ontogeny coincides with high-intensity hemopoiesis. LIF is involved in the regulation of these processes. Brain tissues are characterized by the development of the nervous tissues and proliferation and maturation of neurons. LIF plays a key role in these processes. These features probably contribute to high expression of LIF in the above mentioned tissues. The dominant isoform in the brain tissue differs from that in the liver tissue, which reflects various biological functions of LIF mRNA isoforms. The expression of LIF isoforms in the adrenal glands is related to their role in the development of the regulatory hypothalamic—pituitary—adrenal system. Probably, various isoforms are involved in different regulatory mechanisms of this system. LIF mRNA isoforms play a role in the maintenance of SC proliferation in tissues of tooth germs.

The expression of LIF mRNA isoforms in blood MNC was studied at various stages of ontogeny.

Blood samples from fetuses at 20-22 weeks gestation, umbilical blood from newborns, and venous blood from conventionally healthy donors were used in the experiments.

mRNA isoforms for LIF-D and LIF-M were found in all samples of blood MNC from fetuses at 20-22 weeks gestation, while mRNA isoform for LIF-T was revealed in only 1 sample. This period probably coincides with high-intensity growth and development of the organism. The blood contains circulating regulatory cells that express a variety of growth factors, including LIF. These cells differ by the expression of alternative transcripts, which is required for opposite regulation of the target cells. The expression of intracellular isoform in various tissues and cells probably depends on stimulatory signals from the microenvironment. Table 2 shows the expression of LIF mRNA in fetal MNC.

The expression of cytokine mRNA was studied in umbilical blood MNC. LIF expression was heterogeneous and differed in various newborns. The simultaneous presence of mRNA isoforms for LIF-D and LIF-M was revealed in only 3 of 13 newborns. Other newborns were characterized by the presence of only one of these isoforms or absence of LIF expression.

LIF transcripts were undetectable in peripheral blood MNC from 15 adult donors. It was probably associated with inhibition of histogenesis and decrease in the number of migrating cells expressing LIF.

Our findings illustrate the tissue-specific expression of LIF mRNA isoforms. At 20-22 weeks gestation, the expression of LIF mRNA isoforms in tissues of the liver, brain, adrenal glands, and tooth germs is much higher than in other tissues. Each

**TABLE 2.** Occurrence Frequency of Alternative LIF Transcripts in Blood MNC at Various Stages of Ontogeny

| LIF isoform | MNC from fetal blood at 20-22 weeks gestation (*n*=5) | MNC from newborn umbilical blood (*n*=13) | MNC from adult peripheral blood (*n*=15) |
|---|---|---|---|
| hLIF-D, soluble | 5/5 | 4/13 | 0/15 |
| hLIF-M, matrix-associated | 5/5 | 5/13 | 0/15 |
| hLIF-T, intracellular | 1/5 | 0/13 | 0/15 |

isoform is characterized by tissue-specific expression. The expression of alternative LIF transcripts in peripheral blood MNC progressively decreases during ontogeny. These changes reflect a decrease in stimulatory influences on the expression and number of LIF-producing circulating cells.

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