Tissue-Specific Expression of Splice Variants of Human *IL-4* and *IL-6* Gene mRNA

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The spectrum of alternatively spliced *IL-4* and *IL-6* gene mRNA was studied in peripheral blood mononuclears from healthy donors and in human fetal tissues. It was found that the expression of alternatively spliced *IL-4* and *IL-6* gene mRNA in fetal tissues is tissue specific and that hemopoiesis- and immunopoiesis-related tissues differ by the amount of IL-4 and IL-4δ2 mRNA. An mRNA variant IL-4alt3 carrying partial exon 3 deletion was for the first time identified in human mononuclear cells.

Key Words: *cytokines; alternative splicing; tissue specificity*

Alternative splicing (AS) is one of the most important posttranscription events. Translation of alternatively sliced mRNA yields proteins with considerably different and sometimes even opposite properties. AS is traditionally considered as a rare event observed in only 5% human genes, but numerous bioinformation studies showed another picture: AS variants were detected in up to 95% human genes [6]. Many modern biomolecular studies are aimed at explanation of the usefulness of AS for the organism at certain stages of ontogeny and evolution. Many studies proved that AS is a phenomenon typical of transcripts of cytokine, their receptors, growth factors and chemokines; it results in the formation of tissue-specific isoforms of different localizations (membrane-bound, secretory, and intracellular) and functions [1]. Our previous study revealed no tissue specificity of the expression of alternatively spliced IL-4 and IL-6 gene mRNA in mouse tissues, including embryonic tissues [7]. Here we studied the spectrum of alternatively spliced mRNA of these genes in mononuclears of healthy donors and in human fetal tissues.

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MATERIALS AND METHODS

Human fetal tissues (abortion material) were obtained after abortion induced on gestation week 20-24 by social indications. Fetal tissues were tested for the absence of prevailed invections (type 2 herpesvirus infection, hepatitides B and C, cytomegalovirus, HIV, and tests for Hbs-Ag and RW). All experiments on fetal tissues were approved by local etical committee; signed informed consent was obtained from all patients. All donors signed informed consent for sampling biological material and received necessary explanations concerning the purposes of the study. Peripheral blood mononuclear cells (PB MNC) were isolated and cultured using routine methods [2].

RNA isolation, synthesis of cDNA, and PCR were carried out as described previously [6]. Deoxyribooligonucleotide primers were synthesized at Institute of Chemical Biology and Fundamental Medicine, Siberian Division of the Russian Academy of Sciences; their sequences are presented in Table 1.

Expression of IL-4 and IL-4δ2 in the thymus, liver, and spleen of 24-week fetuses, *i.e.* in tissues directly participating in hemopoiesis and immunopoiesis, was evaluated by real-time PCR on a ICyclerIQ system (Bio-Rad Laboratories) using SYBR Green I intercalating fluorescent dye. All samples were ampli-

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TABLE 1. Sequences of Primers of Reverse-Transcription PCR

mRNA	Code	Sequence	Fragment length, b.p.
hIL-6	6hum1	5'-cgaaagagaagctctatctccc-3'	711
	6hum2	5'-tgcccattaacaacaacaatc-3'	
hIL-4	MFIL-4up	5'-gaagatgcatatgcacaagtgcgatatcacctacc-3'	390
	MFIL-4rew	5'-gaaggatcctcagctcgaacactttgaatatttc-3'	
	HIL-4e2	5'-cgagttgaccgtaacagacat-3'	IL-4 - 239
	HIL-4e1/3	5'-cagagcagaagaacacaaatg-3'	IL-4δ2 - 213
	HIL-4R	5'-tggcttccttcacaggacagg-3'	
RPS26	m350	5'-gctgcggcctccactatg-3'	136
	m223	5'-agagaaggaacaatggtcgtgc-3'	

fied simultaneously (two points for each cDNA sample); analysis was repeated twice. cDNA aliquot was standardized by measuring the content of cDNA for human ribosome protein S26.

DNA hydrolysis was performed in 1× buffer corresponding to the chosen restriction endonuclease according to enzyme manufacturer's recommendations. The result was evaluated after electrophoresis of the reaction products in 6% PAAG. PCR products were sequenced by the method of Senger [5] on an ABI Prism 310 Genetic Analyzer using a BigDye Terminator Cycle Sequencing kit according to manufacturer's recommendations.

RESULTS

IL-6 is known to be structurally and functionally similar to growth factors. It is important for self-maintenance of stem cells and tissue differentiation; therefore, the presence and predominance of some isoforms in different tissues during embryogenesis can play a role in tissue formations. In light of this we used a system of flanking primers (6hum1, 6hum2) enabling detection of all possible alternatively spliced *IL-6* mRNA and studied the expression of this gene in PB MNC from healthy donors and in human fetal tissues.

The assay revealed the presence of *IL-6, IL-6δ2,* and *IL-6δ2δ4* mRNA (Fig. 1), which agrees with previously described spectrum of alternatively spliced forms [4]. In fetal tissues, the same spectrum of alternatively spliced IL-6 mRNA was revealed as in human BP MNC (Table 2), but these variants were differently distributed in different tissues.

The spectrum of alternatively spliced IL-4 mRNA in fetal tissues was studied using a system of flanking primers (MFIL-4up, MFIL-4rew). In our experiments, two variants of *IL-4* gene mRNA were detected, IL-4 and IL-4δ2, analogous to variants found in PB MNC from healthy donors. The results (Table 2) illustrate tissue-specificity of mRNA splicing of *IL-4* gene in fetal tissues, similarly to that observed for *IL-6*.

In fetal tissues involved in hemopoiesis and immunopoiesis (thymus, liver, and spleen), the ratio of IL-4 and IL-4δ2 mRNA was quantitatively evaluated by reverse-transcription PCR with real-time fluorescent detection of the results (Fig. 2). The data suggests that the content of full-length IL-4 mRNA in the thymus is higher by 1-2 orders of magnitude than in the liver and spleen. Quantitative analysis of IL-4δ2 mRNA showed that its content in the liver 2-5-fold surpassed that in the spleen and thymus. According to published data, an opposite situation is observed in

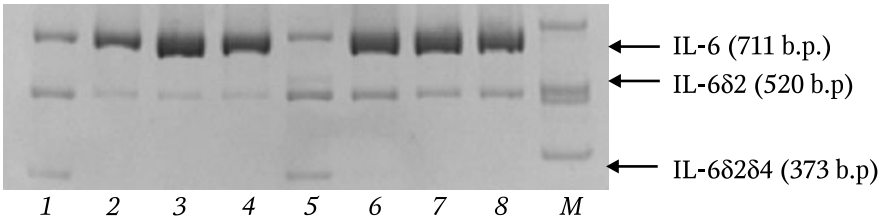

Fig. 1. Electrophoresis of PCR products with primers 6hum1/6hum2 on templates from native and cultured PB MNC.

1-4: donor A, *5-8*: donor B. *M*: molecular weight marker PblKS(-)/HaeIII (bands corresponding to 767, 458, 434, 267 b.p. are seen). *1* and *5*: native MNC; *2* and *6*: MNC after culturing; *3* and *7*: MNC cultured with concanavalin A (10 μg/ml); *4* and *8*: MNC cultured with IL-18 (40 ng/ml).

TABLE 2. Presence of Specific mRNA Sequences in Fetal Human Tissues

Tissue	IL-6	IL-6δ2	IL-6δ2,4	IL-4	IL-4δ2
Pancreas	+	-	-	+	+
Brain	+	-	-	+/-	+
Skin	-	+	+	-	-
Kidney	+	+	-	-	+/-
Lung	+	+	-	+/-	-
Liver	-	+	+	+	+
Spleen	+	+	-	+	+
Thymus	+	+	-	+	+
Thyroid gland	+	+	-	+	+
Adrenal gland	+	+	-	-	-
Skeletal muscle	+/-	+/-	-	-	+/-
Myocardium	+	+	-	+/-	-
Ovary	-	+	+	+/-	+/-
Testis	+	+	-	-	-

Note. Results for 2-4 tissue samples are presented. "+": presence of PCR product in all samples: "–": absence of specific PCR product; "+\–": presence of PCR producs in only some samples.

thymus cells of adult humans: weak expression of IL-4 mRNA and enhanced expression of IL-4δ2 mRNA [3].

It is known that production of cytokines and their isoforms in cell culture can be modulated by cytokines and growth factors. Our experiments revealed no appreciable changes in IL-4/IL-4δ2 mRNA after addition of various cytokines (Fig. 3). At the same time, an additional smaller DNA fragment was detected in MNC of some donors cultured without stimulants and in the presence of IL-18 (Fig. 3). Restriction analysis and direct sequencing revealed partial exon 3 deletion in its nucleotide sequence. Since this splice variant was not described for human cells, we designated it as IL-4alt3. Analysis of cells from the same donors repeated after 2 months detected the fragment corresponding IL-4alt3 only in cells treated with IL-18; stimulation with concanavalin A abolished the expression of this splice variant.

Thus, we demonstrated identical spectra of splice variants for *IL-6* and *IL-4* gene mRNA in human PB MNC and fetal tissues, but the distribution of splice variants of cytokine genes during embryogenesis is tissue specific. For instance, the expression levels of both IL-4 and IL-4δ2 mRNA in the liver, spleen and thymus differ from those in PB MNC. Addition of recombinant cytokines to cultured human PB MNC did not significantly change IL-4/IL-4δ2 mRNA ra-

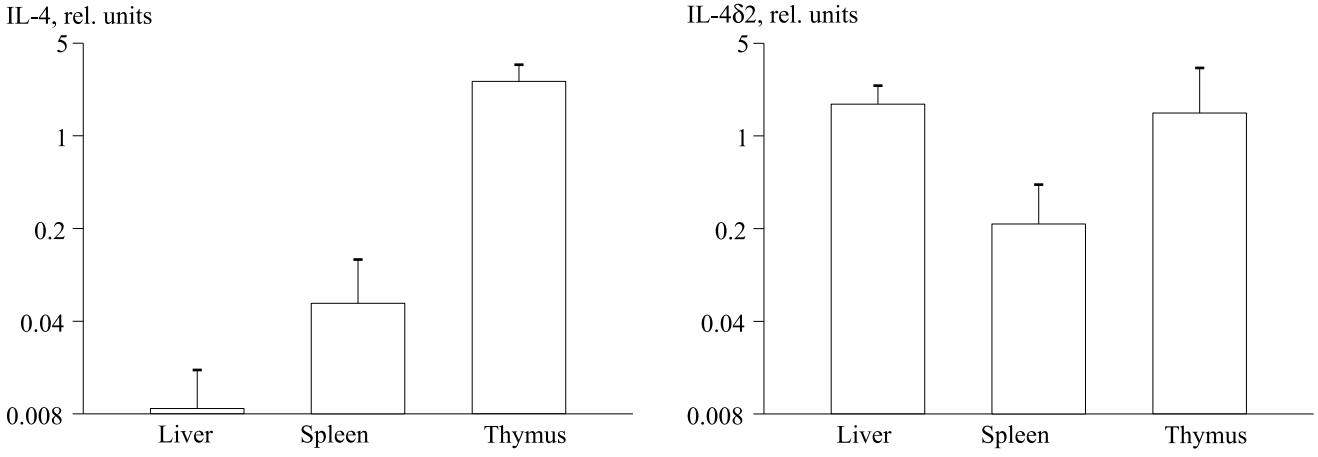

Fig. 2. Relative content of IL-4 and IL-4δ2 mRNA in human fetal tissues.

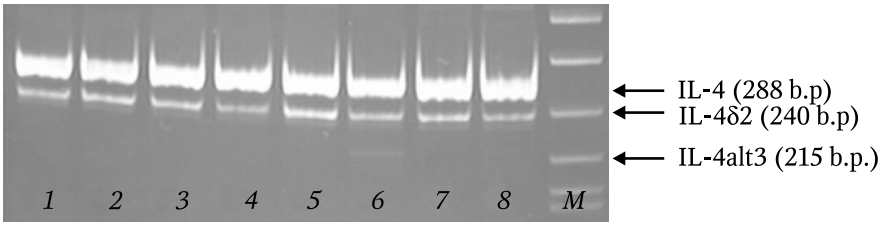

Fig. 3. Electrophoresis of amplification products with primers AIL-4U/AIL-4R on cDNA from PB MNC cultured in the presence of recombinant cytokines: *1*) without cytokines; *2*) IL-2; *3*) IL-4; *4*) IL-4δ2; *5*) IL-10; *6*) IL-18; *7*) IFN-γ; *8*) erythropoietin. *M*: molecular weight marker pUC19/MspI (bands corresponding to 404, 331, 242, 147, and 111 b.p. are seen).

tio, while IL-18 modulates the spectrum of expressed splice variants and induces the appearance of a new variant, IL-4alt3.

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REFERENCES

1. S. V. Sennikov, A. N. Silkov, and V. A. Kozlov, *Med. Immunol*., No. 3, 389-400 (2001).
2. A. N. Silkov, V. A. Gavrilenko, V. V. Denisova, *et al., Byull. Eksp. Biol. Med*., **143**, No. 1, 78-80 (2007).
3. S. P. Atamas, J. Choi, V. V. Yurovsky, and B. White, *J. Immunol.*, **156**, No. 2, 435-441 (1996).
4. M. P. Bihl, K. Heinimann, J. J. Rüdiger, *et al., Am. J. Respir. Cell Mol. Biol.,* **27**, No. 1, 48-56 (2002).
5. F. Sanger, S. Niclein, and A. R. Coulson, *Proc. Natl. Acad. Sci. USA*., **74**, 5463-5467 (1977).
6. E. T. Wang, R. Sandberg, S. Luo, *et al., Nature*,. **456**, 470-476 (2008).
7. O. P. Yatsenko, M. L. Filipenko, E. A. Khrapov, *et al., Cytokine,* **28**, Nos. 4-5, 190-196 (2004).