Early Expression of Human J Chain and \( \mu \) Chain Gene in the Fetal Liver

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Key words: joining chain/\( \mu \) chain/PCR assay/ ontogeny/fetal liver/secretory IgA

ABSTRACT. The expression of J chain and \( \mu \) chain genes has been investigated during human fetal ontogeny by the polymerase chain reaction performed on liver sections. With this technique, we have been able to detect expression of J chain and \( \mu \) chain in a single 6-10-\( \mu \)m-thick formalin-fixed paraffin-embedded section of fetal liver tissue. J chain expression reached a maximum at 16 weeks of gestation but was clearly detectable in the liver at the 6th week. Although not detectable at the 6th week, \( \mu \) chain mRNA became readily detectable at 7 weeks of gestation. These results indicate that J chain expression precedes that of \( \mu \) chain by at least one week. Therefore, our results imply that J chain is the first immunoglobulin-related polypeptide expressed during the embryogenesis and differentiation of B cells in the fetal liver.

J chain is a 15 kD polypeptide that may participate in the intracellular polymerization of IgM or IgA (1, 2). The regulation of its expression in B cells has been studied by several laboratories in an effort to understand developmental gene activation in these cells (1). The exact point in the development of human or mouse B cells at which J chain synthesis is initiated remains controversial (1, 2). In human systems, J chain expression has been studied by immunohistochemical techniques in lymphoid cells at various stages of their differentiation. J chain was found not only in polymeric immunoglobulin-synthesizing cells, but also in IgG-, IgD-, and heavy or light chain-containing cells (2-6). However, with more sensitive techniques, J chain was also found at earlier stages of B cell differentiation, prior to immunoglobulin synthesis (7-11). The latter findings are at variance with the results obtained in chicken (12) and mouse cells (1, 13-15), where the J chain gene seems to be activated during B cell differentiation to pentamer IgM-secreting cells (1, 16). The finding that J chain expression precedes the expression of immunoglobulin chains in human lymphoid cell lines requires further clarification as to the exact stage of its expression and its function in the differentiation of normal B cells. To identify the point at which J chain and \( \mu \) chain gene expression is initiated, we have used the polymerase chain reaction (PCR) technique (17) to determine the presence of J and \( \mu \) chain mRNA in human fetal tissues. In previous studies, total RNA has been extracted from formalin-fixed paraffin-embedded tissue for Northern blot analysis (18), and portions of myosin heavy chain and leukemia-specific mRNA sequences have been amplified by PCR on total RNA extracted from fresh tissues (19), cell lines (20) and crude cells (21). Therefore, we have adapted the PCR to formalin-fixed paraffin-embedded tissue for the detection of J chain, thus making it possible to use a single thin slice of embedded tissue as the amplification target.

MATERIALS AND METHODS

Cell lines. Human cell lines Daudi and Raji that express J and \( \mu \) chains (22) were obtained from the American Type Culture Collection (Rockville, MD). The K562 chronic myelogenous leukemia cell line (23) was used as the J chain-negative control.

Paraffin sections. Buffered 10% formalin-fixed and paraffin-embedded hepatic tissues were obtained from 16 aborted embryos with fetal age from the 6th to 28th week of gestation. Informed consent for study of fetal tissues was obtained prior to surgery. Gestational age was determined by crown-rump length, fetal foot length, fetal palm length and morphological features of hand and foot surfaces (24, 25). Subsequent sections were stained with hematoxylin and eosin to confirm the identity of the tissue to be analyzed.

Immunofluorescence method. After ethanol fixation, sections were deparaffinized and incubated with tetramethylrhodamine isothiocyanate (TRITC)-conjugated rabbit anti-human J chain antibody or with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human \( \mu \) chain antibody.
human J chain developed and characterized in this laboratory (3, 5, 8, 11, 22, 26) (diluted 1:50), or with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human μ chain (heavy chain-specific) (diluted 1:10) (Dako Corp., Carpinteria, CA) for 30 min in a humidified chamber at room temperature. After incubation, all sections were mounted with buffered glycerol-PBS and examined with a fluorescence microscope equipped with filters for optimal discrimination of TRITC and FITC fluorescence.

RNA isolation from paraffin sections. A single 6–10 μm section cut from the paraffin block was placed in an Eppendorf tube. The average surface area of the tissue was 0.4 cm², though smaller fragments were also used successfully. The sections were homogenized in 1 ml of buffer (100 mM NaCl, 50 mM Tris-HCl pH 7.6, 20 mM EDTA, 10 mg/ml ATA). SDS was added to achieve a 1% final concentration and Proteinase K to make 0.5 mg/ml; the mixtures were incubated for 16 h at 48°C. Phenol extraction was performed 3 times. LiCl₂ was added to make a 2 M final concentration, and the mixture was incubated for 16 h at 0°C. RNA was obtained by centrifugation at 15,000 x g for 20 min. Precipitates were dissolved in 10 mM Tris-HCl (pH 7.6) with 1 mM EDTA and 10 mg/ml ATA. mRNA was obtained from RNA using Oligotex dT 30 (Nihon Roche, Tokyo, Japan).

Oligonucleotides used for amplification. Based on the published DNA sequences of Max and Korsmeyer (9), oligonucleotides for J chain from exon 4 of the human J chain gene was synthesized on a DNA synthesizer (Model 380; Applied Biosystems, Inc., Foster City, CA). To detect the μ chain and β-actin, Cμ4 (27, 28) and β actin (29) oligonucleotides were synthesized, respectively. The sequences of primers and probes, and size of PCR products are listed in Table 1.

Amplification method and detection of human J chain and μ chain mRNAs. cDNA was synthesized using murine leukemia virus reverse transcriptase according to Ferre and Garduno (21). Individual samples were added to the reverse transcription reaction buffer to make a final volume of 20 μl (1× reverse transcription buffer, 2 units of RNase inhibitor (Takara, Kyoto, Japan), 1.0 mM each of dNTP's, 10 pM of the 3' PCR primer, 200 units of cloned MuLV-1 reverse transcriptase (BRL, Gaithersburg, MDJ). The reaction mixture was incubated at 37°C for 45 min, cooled on ice, and then diluted with 80 μl of PCR buffer (50 mM KCl, 50 mM Tris-HCl, 2.5 mM MgCl₂ and 100 mg/ml BSA, pH 8.4), followed by addition of 40 pM of the 3' PCR primer, 50 pM of the 5' PCR primer and 2.5 unit of Taq polymerase (Cetus, Emeryville, CA). To prevent evaporation 150 μl of mineral oil was added. The reaction was started by heat-denaturation of the RNA-cDNA hybrid for 20 sec at 95°C, annealing the primers for 15 sec at 55°C, and then extending the primers for 1 min at 72°C. The reaction cycle was repeated 40 times by using a DNA-thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). After the final cycle, the temperature was held at 72°C for 10 min to allow reannealing of the amplified products, and then the mixture was chilled.

For each sample, a 10 μl aliquot of the amplified DNA reaction mixture was fractionated by 1.7% agarose gel-electrophoresis, and after staining with ethidium bromide, the amplified product was visualized by UV fluorescence.

Southern blotting. PCR products electrophoresed in 1.7% agarose gel were transferred to a nylon membrane and then analyzed by the Southern blot method with a 32P-labeled oligonucleotide probe. The membrane-transferred PCR products were washed twice with 2× SSPE [1× SSPE: 0.18 M NaCl, 10 mM NaH₂PO₄, 1 mM EDTA (pH 7.4), 0.1% SDS] at room temperature.

After prehybridization (50% formamide, 5XSSC, 0.01 M Na₂PO₄, 0.5% non-fat dry milk, 0.1% SDS), the membrane was hybridized with the 32P-labeled internal probe at 42°C for 3 h. The filter was washed three times with 6× SSPE and then subjected to autoradiography. The intensity of the signal was determined by densitometric tracing.

RESULTS

Sensitivity and specificity of the PCR assay. To evaluate the sensitivity and specificity of the assay, a dilution experiment was conducted. Total RNA from

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<th>Table 1. Sequence of Primers and Probe.</th>
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<td>Primers &amp; probes</td>
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</tr>
<tr>
<td>J chain:</td>
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<td>IS4-1</td>
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<td>IS4-2</td>
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<td>IS4-3</td>
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Fig. 1. Sensitivity of reverse-transcriptase PCR assay. Lanes: 1, Daudi RNA; 2–7, dilutions of Daudi RNA by 1–6 orders of magnitude, respectively; 8, Daudi RNA without reverse transcriptase. One μg of Daudi total RNA was used as the initial sample (lanes 1 and 8); consecutive 1:9 dilutions were made with K562 RNA at 10 μg/ml as diluent.

Fig. 2a. PCR analysis of human J chain mRNA. A 10 μl aliquot from 100 μl of a amplified product was fractionated by agarose gel electrophoresis, and visualized by ethidium bromide staining under UV light. Samples in lanes: the marker is Hae III digest of φX174 (lane 1). The signal of β-actin was detected in all samples (lane 2). Human J chain-positive cell controls, Daudi (10 μg) (lane 3) and Raji (10 μg) (lane 4); human J chain-negative cell controls, K562 (10 μg) (lane 5) and K562 without reverse transcriptase (10 μg) (lane 6); samples from fetal liver: 6th week (lane 7), 7th week (lane 8), 8th week (lane 9), 9th week (lane 10), 10th week (lane 11), 16th week (lane 12), 20th week (lane 13), 24th week (lane 14), 26th week (lane 15) and 28th (lane 16).

Fig. 2b. Southern blot analysis of PCR products for human J chain mRNA. Samples in lanes: human J chain-positive cell controls, Daudi (lane 1) and Raji (lane 2); human J chain-negative cell controls, K562 (lane 3) and K562 without reverse transcriptase (lane 4); samples from fetal liver: 6th week (lane 5), 7th week (lane 6), 8th week (lane 7), 9th week (lane 8), 10th week (lane 9), 16th week (lane 10), 20th week (lane 11), 24th week (lane 12), 26th week (lane 13), 28th (lane 14) and β-actin (lane 15).

Daudi cells was diluted stepwise from 1 to 6 orders of magnitude using 10 μg of K562 total RNA per ml as the diluent. The amplified products were analyzed by gel electrophoresis and visualized under UV light by ethidium bromide fluorescence. A 10^{-5} dilution of 1 μg of RNA from the J chain-positive Daudi cell line still pro-
vided an easily detectable signal in this assay (Fig. 1). One μg of RNA is roughly equivalent to the amount contained in the cytoplasm of 10⁵ Daudi cells (10 pg of cytoplasmic RNA per cell). Thus, a 10⁻⁵ dilution represents RNA from about one Daudi cell. Because only 1/10th volume of the amplified product was used for analysis, the positive signal represents the amplified product of less than one cell equivalent. This result demonstrates that detection is feasible even when the expression of J chain and μ chain occurs in very few cells.

To prove that the assay would detect RNA rather than contaminating DNA, we also amplified samples without reverse transcriptase. Lack of a band on lane 8 (Fig. 1) indicated that the RNA was not contaminated with genomic DNA.

**Expression of J chain and μ chain.** In the fetal liver, we found a significant reactivity for J chain expression at the 6th gestational week (Fig. 2a and b), while expres-

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**Fig. 3.** Southern blot analysis of PCR products for human μ chain mRNA. Samples in lanes: human μ chain-positive cell controls, Daudi (10 μg) (lane 1); human J chain-negative cell controls, K562 without reverse transcriptase (10 μg) (lane 2); samples from fetal liver: 6th week (lane 3), 7th week (lane 4), 8th week (lane 5), 9th week (lane 6), 10th week (lane 7), 16th week (lane 8), 20th week (lane 9), 24th week (lane 10), 26th week (lane 11) and 28th week (lane 12).

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**Fig. 4.** Levels of expression of J chain and μ chain in human fetal livers. The intensity of the reaction was quantitated using a densitometer. Peak areas are given as relative units.
sion of the $\mu$ chain was not detected in the same RNA samples (Fig. 3). Beginning at the 6th gestational week, the frequency of J chain expression increased and reached a plateau at the 16th week. Thus, the expression of $\mu$ chain at the 7th week lags behind that of J chain, but follows a similar rapid increase thereafter (Fig. 4). However, J chain-and $\mu$ chain-positive cells were not detected by the immunofluorescence method until the 16th gestational week (30).

DISCUSSION

It has been established that during B cell ontogeny, pre-B cells first express a cytoplasmic $\mu$ chain and then differentiate into surface IgM-positive B cells (31, 32). In humans, pre-B cells are found in the fetal liver as early as the 7th week of gestation and surface IgM-positive B lymphocytes appear approximately two weeks later, as determined by immunofluorescence techniques (33). Similarly, the appearance of cytoplasmic $\mu$ chain-positive cells precedes that of surface IgM-positive cells in mouse fetal liver (34).

As for the expression of J chain during the B cell differentiation pathway, McCune et al. (7) provided the first evidence for J chain expression at the pre-B cell stage using human lymphoblastoid cell lines and in vitro translation of mRNA, followed by immunoprecipitation, similarly, Hajdu et al. (8) found J chain synthesis in human leukemia cells and cell lines by radioimmunounoassay and immunoelectron microscopy, and suggested that J chain expression actually precedes $\mu$ chain synthesis during B lymphocyte maturation.

These results have been confirmed in a number of subsequent studies in which some, but not all, human pre-B leukemia cells and lymphoblastoid cell lines expressed intracellular J chain, but not $\mu$ chain, as revealed by immunoelectron microscopy, radioimmunounoassay of lysed cells, and Northern blot analysis (8, 9). In addition, J chain, but not Ig synthesis, has been induced in vitro in Epstein-Barr virus-transformed pre-B cells obtained from normal fetal bone marrow (10, 11). These transformed cells displayed morphological features of lymphoblasts and plasma cells, and contained high levels of intracellular J chain, but were Ig-negative and maintained the Ig genes either in the germ-line configuration or had undergone DJ or abortive VDJ rearrangement.

Our current results provide direct evidence for the presence of J chain mRNA in the absence of $\mu$ chain using PCR to avoid EBV-transformation. Furthermore, in this study we have shown that J chain mRNA is present at the 6th gestational week, and thus precedes the expression of $\mu$ chain mRNA by one week; this important point was not addressed in earlier studies. Previous investigations of J chain ontogeny have been performed in human and pig fetuses, and in chickens using immunohistochemical techniques withfluochrome labeled anti-J chain antibody (12, 30, 35). With this technique, J chain was detected in lymphoid cells which also displayed intracellular Ig. The apparent discrepancy with current results obtained in human fetuses is most likely due to differences in the sensitivity of the technique used for J chain detection. This possibility is further strengthened by the failure to detect J chain by immunofluorescence in human pre-B cells (3) which, however, are J chain-positive when alternative, highly sensitive techniques are used.

Although J chain is commonly found in mature cells engaged in the synthesis of polymeric IgA and IgM (1, 2), many recent studies indicate that it can be detected early not only in the ontogeny of human B cells, in EBV-transformed cells, and in malignant lymphoma cell lines and cells (7-9) but also in myeloma cells (36, 37). In such cells, J chain expression is frequently not linked to the expression of intracellular H and L chains, but is independently regulated. At present, the biological function of J chain in these cells remains unknown.

Acknowledgements. We would like to express our great thanks for Dr. Edward E. Max (Laboratory of Immunogenetics, National Institute of Health, USA) who generously provided human J chain DNA probe. We are grateful to Ms. Miwako Kamei for expert and dedicated technical assistance. This work was supported by a Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture (No. 03404052) and in part by US PHS grant AI-10854.

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*Received for publication, June 28, 1993 and in revised form, September 21, 1993*