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Developmental regulation of gene expression for the MPTPδ isoforms in the central nervous system and the immune system

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Abstract MPTPδ is a murine transmembrane protein tyrosine phosphatase which has three isoforms, types A–C, differing in the structure of the extracellular regions. In this study, we examined MPTPδ isoform expression in the brain and the immune system at discrete developmental or differentiation stages. RT-PCR analysis demonstrated that another isoform, type D, is transcribed from the MPTPδ gene. In the brain, only type D was expressed until postnatal day 7 (P7), but after P14, all four isoforms were detected. In contrast, the spleen, thymus and all the hematopoietic cell lines examined express only types B and C isoforms. An in situ hybridization study showed that MPTPδ mRNA is diffusely expressed throughout the spleen, but its expression in the thymus is restricted to the medullary region.

Key words: Protein tyrosine phosphatase; Cell differentiation; Isoform

1. Introduction

Protein tyrosine phosphorylation plays a central role in the regulation of normal cell activation, proliferation and differentiation [1–3]. The phosphorylation state of tyrosine residues on cellular proteins is strictly controlled by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) under physiological conditions. While a number of PTKs, identified as growth factor receptors and oncogene products, are well characterized, the understanding of structure and function of PTPs has been very limited. In 1988, it was demonstrated that the cytoplasmic region of CD45, a transmembrane molecule on the hematopoietic cells, has a sequence similar to a cytoplasmic PTP (PTP1B) [4] and has intrinsic PTP activity [5]. Since then, more than 40 PTPs have been cloned from various species and tissues using either cross-hybridization or a polymerase chain reaction (PCR) technique based on the sequence similarity of the consensus sequences in the PTP domains [6,7]. They are classified into two groups: soluble-form PTPs with a single PTP domain, and transmembrane PTPs (also referred to as receptor-type PTPs; RPTPs), almost all of which contain two PTP domains in the cytoplasmic regions.

Based on the structural diversity of the extracellular region, RPTPs have been subdivided into four groups by Fischer et al. [8]. Among them, type II RPTPs are characterized by the presence of varying numbers of immunoglobulin (Ig)-like and fibronectin type III repeat (FN-III)-like domains in the extracellular regions and include LAR [9,10], PTPδ [11,12], PTPκ [13], PTPμ [14], PTPσ (NE-3) [15,16] and *Drosophila* DPTP [10]. Since these structural motifs are also utilized by cell adhesion molecules such as the neural cell adhesion molecule (N-CAM), it has been postulated that type II RPTPs might be involved in cell adhesion. Indeed, PTPκ and PTPμ were shown to mediate homophilic cell adhesion when expressed in insect cells using recombinant baculovirus expression systems [17–19]. However, physiological ligands of type II RPTPs and how the binding of ligands influences the PTP activity still remain to be clarified.

It is well known that several PTKs, such as the src-family of PTKs, are abundantly expressed in the central nervous systemm [20–23] and the immune system (reviewed in [3]). The receptors for nerve growth factor and several neurotropic factors have intrinsic PTK activity [24–27]. In the immune system, soluble-form PTKs have been shown to be associated with antigen receptors (reviewed in [3]) and growth factor receptors [28]. All these observations suggest that protein phosphorylation is involved in the signal transduction for cell activation and proliferation. In addition, tyrosine phosphorylation was demonstrated to be modulated during the development of the central nervous system [29]. To analyze the mechanisms of regulation during differentiation from the perspective of PTPs, we have recently cloned and characterized a mouse type II RPTP, named MPTPδ. The MPTPδ gene is expressed in the specialized regions of the brain and in B cells, which shows a high degree of sequence similarity to LAR and PTPσ, and generates at least three isoforms differing in the extracellular region possibly by an alternative splicing mechanism [12]. Type A is composed of one Ig-like and four FN-III-like domains; the type B, one Ig-like and eight FN-III-like domains; and the type C, three Ig-like and eight FN-III-like domains [12] (Fig. 1).

To elucidate the functional roles of MPTPδ in the central nervous system and the immune system, we tried to examine the expression of MPTPδ at discrete developmental or maturational stages by reverse transcription (RT)-PCR using isoform-specific primers, and to determine the localization of the MPTPδ gene expression within the lymphoid organs by in situ hybridization. The results herein demonstrated that the MPTPδ gene generates another isoform, the extracellular region of which is composed of three Ig-like and four FN-III-like domains, that the spleen, thymus and all the hematopoietic cell lines tested express types B and C MPTPδ isoforms, and that in the brain, the expression of MPTPδ isoforms is developmentally regulated. Furthermore, MPTPδ mRNA is mainly expressed in the medulla of the thymus and only sparsely in the cortex. All these results suggest that MPTPδ may be involved in the regulation of differentiation and maturation of the brain and lymphocytes.

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Abbreviations: PTP, protein tyrosine phosphatase; PTK, protein tyrosine kinase; Ig, immunoglobulin; FN-III, fibronectin type III repeat; RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

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2. Materials and methods

2.1. Cell lines and culture conditions

The following cell lines were used in this study: pro-B cell line NFS-70; pre-B cell lines NFS-5, NFS-25, and 70Z/3; surface Ig-positive B cell lines WEHI-231, I-29, WEHI-279, BAL-17, CH1, X16C8.5, and A-20; myeloma cell line P3U1; antibody-producing hybridoma 3-3.1; T cell lines EL-4 and RLδ1; and macrophage lines P388D.1 and J774. All of the cell lines were cultured in RPMI1640 (Nikken Bio Medical Laboratory, Kyoto, Japan) supplemented with 10% heat-inactivated fetal bovine serum, 2-mercaptoethanol (50 μM), streptomycin (100 μg/ml) and penicillin (100 U/ml) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

2.2. Mice

Brain, thymus and spleen from adult female and time-pregnant female SJL mice and their litters were used. Successful copulation was determined by the presence of a vaginal plug and recorded as embryonic day 0 (E0). The day of birth was recorded as postnatal day 1 (P1).

2.3. RT-PCR

Total cellular RNA was prepared using the single-step acid guanidium thiocyanate method. For the first strand cDNA synthesis, 10 μg of total RNA was heated at 70°C for 5 min, then 40 pmol of specific 3′-antisense primer (5′-AGCACTGTTCCCACGATTGGT-3′) or oligo (dT) primer, dNTP (10 mM), reverse transcription buffer and human placental ribonuclease inhibitor (Toyobo, Osaka, Japan) were added together with 200 U of Moloney murine leukemia virus reverse transcriptase (BRL, Gaithersburg, MD) in a final volume of 20 μl. The reaction mixture was incubated at 42°C for 2 h. The synthesized cDNA was directly used as a template for subsequent PCR amplification. The following four sets of primers specific for each domain of MPTPδ were used (see Fig. 2): for one Ig-like domain (types A and B), 5′-CAGACCATACTTGTACGG-3′ (sense, primer 1), and 5′-CTGGGCTATTGCTTCAAT-3′ (antisense, primer 2); for three Ig-like domains (types C and D), 5′-GAGACACCCCCCAGGTTT-3′ (sense, primer 8), and 5′-CGGATTACCGCTGGCTGC-3′ (antisense, primer 9); for four FN-III-like domains (types A and D), 5′-TTCCGTCTGTCTGCAACG-3′ (sense, primer 3) and 5′-ATCCACTTCTTCCACCAT-3′ (antisense, primer 4); for eight FN-III-like domains (types B and C), 5′-TCGGATACTACCAAGTAC-3′ (sense, primer 6) and 5′-GCCTTCTGAGTG GAGGTT-3′ (antisense, primer 7). To detect the expression of each MPTPδ isoform, the following combinations of primers were used (Fig. 2): for type A, primers 1 and 4; for type B, primers 1 and 7; for type C, primers 8 and 7; for type D, primers 8 and 4. For the internal control, oligo (dT)-primed cDNA was amplified with glyceraldehyde 3-phosphate dehydrogenase (GAPDH)-specific primers (Clonetech, Palo Alto, CA). The PCR products were subsequently electrophoresed on 1% agarose gels, transferred onto a Nytran membrane (Schleicher & Schuell, Dassel, Germany), and hybridized to ^{32}P-labelled probes. The following cDNA fragments were amplified by PCR using previously isolated cDNA clones as templates and used as probes for each domain and isoform (Fig. 2): for one Ig-like domain and types A and B, a 432-bp fragment amplified with primers 1 and 2; for three Ig-like domains and types C and D, a 399-bp fragment amplified with primers 8 and 9; for four FN-III-like domains and types A and D, a 216-bp fragment amplified with primers 3 and 4; and for eight FN-III-like domains and types B and C, a 756-bp fragment amplified with primers 6 and 7.

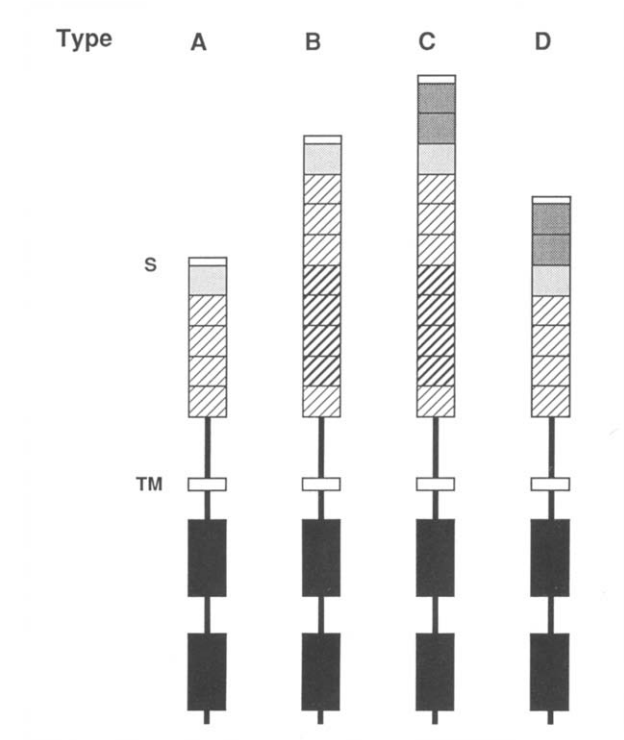

Fig. 1. Schematic representation of the structures of the MPTPδ isoforms. Open boxes indicate the putative signal peptides (S) and the transmembrane peptides (TM). Stippled and hatched boxes indicate the Ig-like and FN-III-like domains, respectively. In addition, densely stippled and hatched boxes indicate the first and the second insertions as described in [12], respectively. Solid boxes indicate PTP domains.

2.4. In situ hybridization

In situ hybridization was carried out according to the previously described protocol [12] except for the use of digoxigenin (DIG)-11-UTP (Boehringer-Mannheim, Mannheim, Germany) for cRNA labelling. The cRNA probe corresponding to a part of the MPTPδ extracellular region reacted with all the isoforms. A sense RNA probe was used as the control. After hybridization and washing, the slides were incubated with alkaline phosphatase-labelled anti-DIG antibody (Boehringer-Mannheim, Mannheim, Germany) overnight and hybridized signals were visualized with Nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl-phosphate.

3. Results and discussion

Since the MPTPδ gene is abundantly expressed in the adult mouse brain [12], we first examined the expression of MPTPδ isoforms in the brain at discrete developmental stages by RT-PCR. In the brain from E16 to P7, only the 399-bp fragment with primers 8 and 9 (for three Ig-like domains) and the 216-bp fragment with primers 3 and 4 (for four FN-III-like domains) were detected (Fig. 3). This suggests that the extracellular portion of MPTPδ expressed in the brain during this period appears to be composed of three Ig-like and four FN-III-like domains. To confirm that this MPTPδ isoform is in fact transcribed, RT-PCR was performed using primers 8 and 4. As shown in Fig. 4, only the 1,866-bp fragment was detected in the brain from E16 to adult. Since we could not identify the isoform having three Ig-like and four FN-III-like domains in the extracellular region by the cDNA cloning [12], we regarded it as another isoform and termed type D (Fig. 1). In the brain after P14, however, the PCR fragments that corresponded to the one and three Ig-like and the four and eight FN-III-like domains could be detected (Fig. 3). To identify the MPTPδ isoforms expressed in the brain after P14, we used isoform-specific combinations of primers (Fig. 2) for RT-PCR analysis and demonstrated that all four isoforms are detected (Fig. 4). These results indicate that the brain expresses all four isoforms, types A–D, after P14. Since exon/intron organization of MPTPδ gene has not been characterized, it is formally possible that the primers used in this study might amplify the DNA fragments of corresponding size from the contaminated genomic DNA. However, this possibility seems to be unlikely because no DNA fragments were detected when total cellular RNA from each sample was

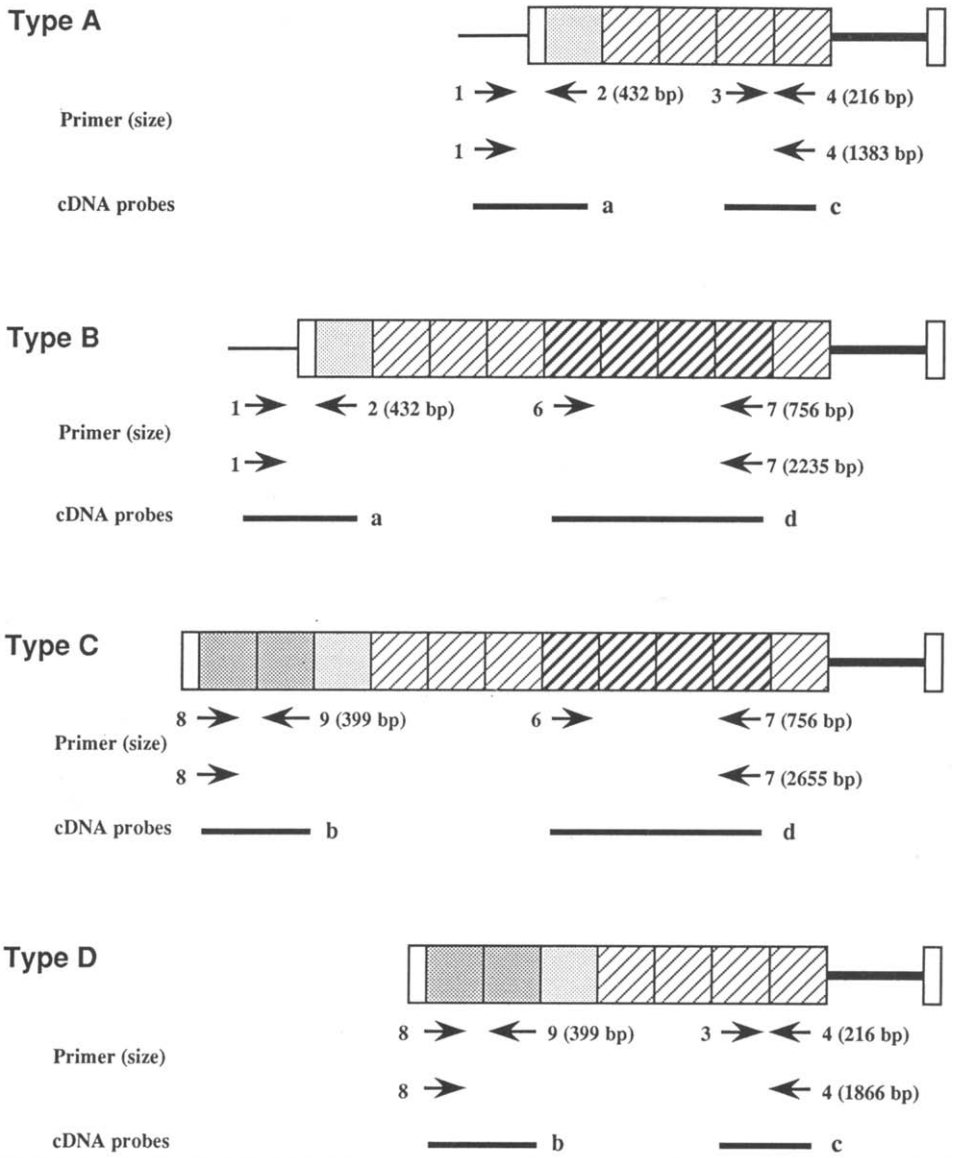

Fig. 2. Schematic representations of primers used in this study and expected PCR products for each MPTPδ isoform and each domain. All boxes and a thick line indicate the protein-encoding region and thin lines indicate the 5′-untranslated region. Stippled and hatched boxes indicate the Ig-like and FN-III-like domains, respectively. Open boxes at the 5′- and 3′-ends indicate the putative signal peptides and TM, respectively. The cDNA fragments which were generated by PCR using previously isolated cDNA clones as templates were used for probes to detect isoform-specific or domain-specific PCR products and are expressed as thick lines: probe a, for types A and B MPTPδ isoforms and one Ig-like domains; probe b, for types C and D isoforms and three Ig-like domains; probe c, for types A and D isoforms and four FN-III- like domains; and probe d, for types B and C isoforms and eight FN-III-like domains.

directly used as a template for PCR amplification (data not shown).

It has been reported that although N-CAM has five Ig-like domains, only the two most amino-terminal Ig-like domains are involved in the homophilic binding [30], that the migration of neurons in developing rat cerebellum is linked to the expression of another Ig superfamily of the cell adhesion molecule, Ng-CAM [31,32], and that in *Drosophila*, three molecules of the Ig superfamily, namely fasciclin II, fasciclin III, and neuroglican, are expressed not only during neurogenesis but also after the nervous system is completely established [33]. Thus, it may be that MPTPδ with three Ig-like domains are required for the migration of neurons or the synapse formation during neurogenesis. Given that neurogenesis is almost completed by P14, it is reasonable to assume that strong binding with three Ig-like domains is not required after P14 except for the sites where the neural architecture should be firmly maintained, and isoforms with one Ig-like domain are possibly generated by alternative splicing. However, the precise physiological meaning of the MPTPδ isoform alteration during neuronal development remains to be clarified.

Next, we tried to identify the MPTPδ isoforms expressed in the immune system. In the previous study using Northern blot analysis [12], it was shown that although MPTPδ mRNA is not detected in the lymphoid organs, three pre-B cell lines and one surface-IgG-positive B cell line express MPTPδ mRNA. As shown in Fig. 5, RT-PCR analysis demonstrated that one and three Ig-like and eight FN-III-like domains are detected in the

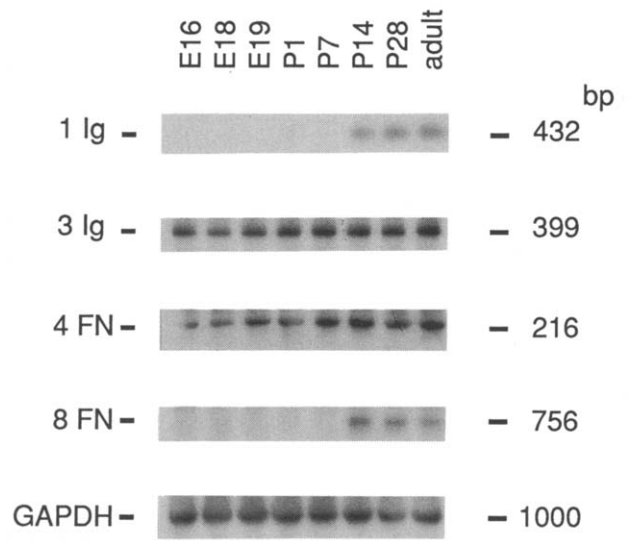

Fig. 3. Expression of MPTPδ extracellular domains in the brain examined by RT-PCR analysis. The first strand cDNA was synthesized from 10 μg of total cellular RNA with MPTPδ-specific antisense primer and subjected to PCR with each domain specific primers. To estimate the amount of mRNA and the efficiency of the first strand cDNA synthesis, we used GAPDH-specific primers for the amplification of oligo d(T)-primed cDNA. PCR products were electrophoresed on 1% agarose gels, transferred to Nytran membranes and probed with ^{32}P-labelled cDNA probe specific for each domain. The size of PCR products for one Ig-like, three Ig-like, four FN-III-like and eight FN-III-like domains were 432 bp, 399 bp, 216 bp, and 756 bp, respectively. Brain RNAs from E16, E18, E19, P1, P7, P14, P28 and adult mice were used. The size of each PCR product is indicated on the right.

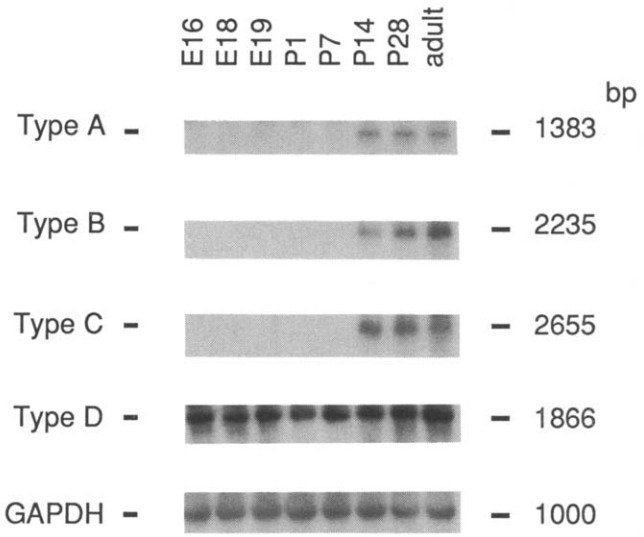

Fig. 4. Expression of MPTPδ isoforms in the brain examined by RT-PCR analysis. The same cDNA as described in Fig. 3 was subjected to PCR reaction with the following sets of primers: for type A MPTPδ isoform, primers 1 and 4; for type B isoform, primers 1 and 7; for type C isoform, primers 8 and 7; and for type D isoform, primers 8 and 4 (Fig. 2). PCR products were electrophoresed, transferred to Nytran membranes and probed with the ^{32}P-labelled cDNA probe specific for each isoform. Probes a and b (Fig. 2) were used in this study to detect types A and B and types C and D, respectively. The same results were obtained when probes c and d (Fig. 2) were used to detect types A and D and types B and C, respectively. The size of each PCR product is indicated on the right.

thymus and the spleen from P1 to adult, indicating that B and C isoforms are expressed. The level of the MPTPδ expression was almost identical throughout the maturational stages. Similarly, all the hematopoietic cell lines tested appeared to express both B and C MPTPδ isoforms (Fig. 6). A slightly larger fragment was found in the blots of the eight FN-III-like domains (lanes 11–16) but it was confirmed by sequence analysis to be an artificial PCR product due to cross-amplification. As in the case of the brain, it was confirmed by use of the isoform-specific combinations of primers (Fig. 2) that both types B and C MPTPδ isoforms are expressed in the lymphoid organs and hematopoietic cell lines (data not shown). Thus, the MPTPδ isoforms with eight FN-III-like domains are preferentially expressed in the immune system. The reason for the absence of isoforms with four FN-III-like domains is not known.

Since the thymus and the spleen were shown to express MPTPδ mRNA by the RT-PCR study (Fig. 5), localization of the MPTPδ expression in these tissues was examined by in situ hybridization using cRNA probes. The results demonstrated that MPTPδ signals are diffusely identified in the spleen with slightly heavier labelling in the follicular regions (Fig. 7A). In the thymus, the MPTPδ expression was detected mainly in the medullary region and only sparsely in the cortex (Fig. 7C). No specific hybridization was observed in the sections hybridized with a sense cRNA probe (Fig. 7B and D). Since T cells are known to migrate from the cortex to the medulla during maturation, it is possible that MPTPδ constitutes a critical regulatory element for T cell maturation and differentiation.

In summary, we examined the expression pattern of the MPTPδ isoforms in the central nervous system and the immune system. The results demonstrated that in the brain, the isoform expression seems to be regulated in a developmental stage-specific manner. Although an MPTPδ isoform change was not detected during the course of lymphocyte differentiation, the restricted expression of MPTPδ within the thymus suggests the possible involvement of MPTPδ in T cell maturation. Thus, MPTPδ may serve as a valuable indicator for the analysis of

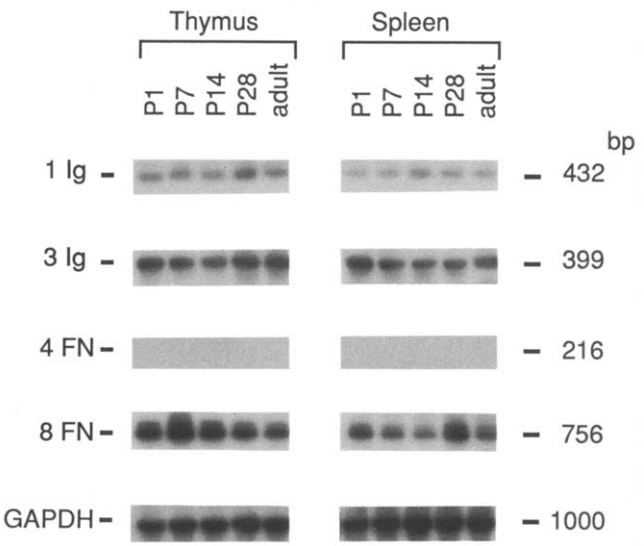

Fig. 5. Expression of MPTPδ extracellular domains in the thymus and the spleen examined by RT-PCR analysis. RNAs from thymus and spleen of P1, P7, P14, P28 and adult mice were used for RT-PCR. The size of each PCR product is indicated on the right.

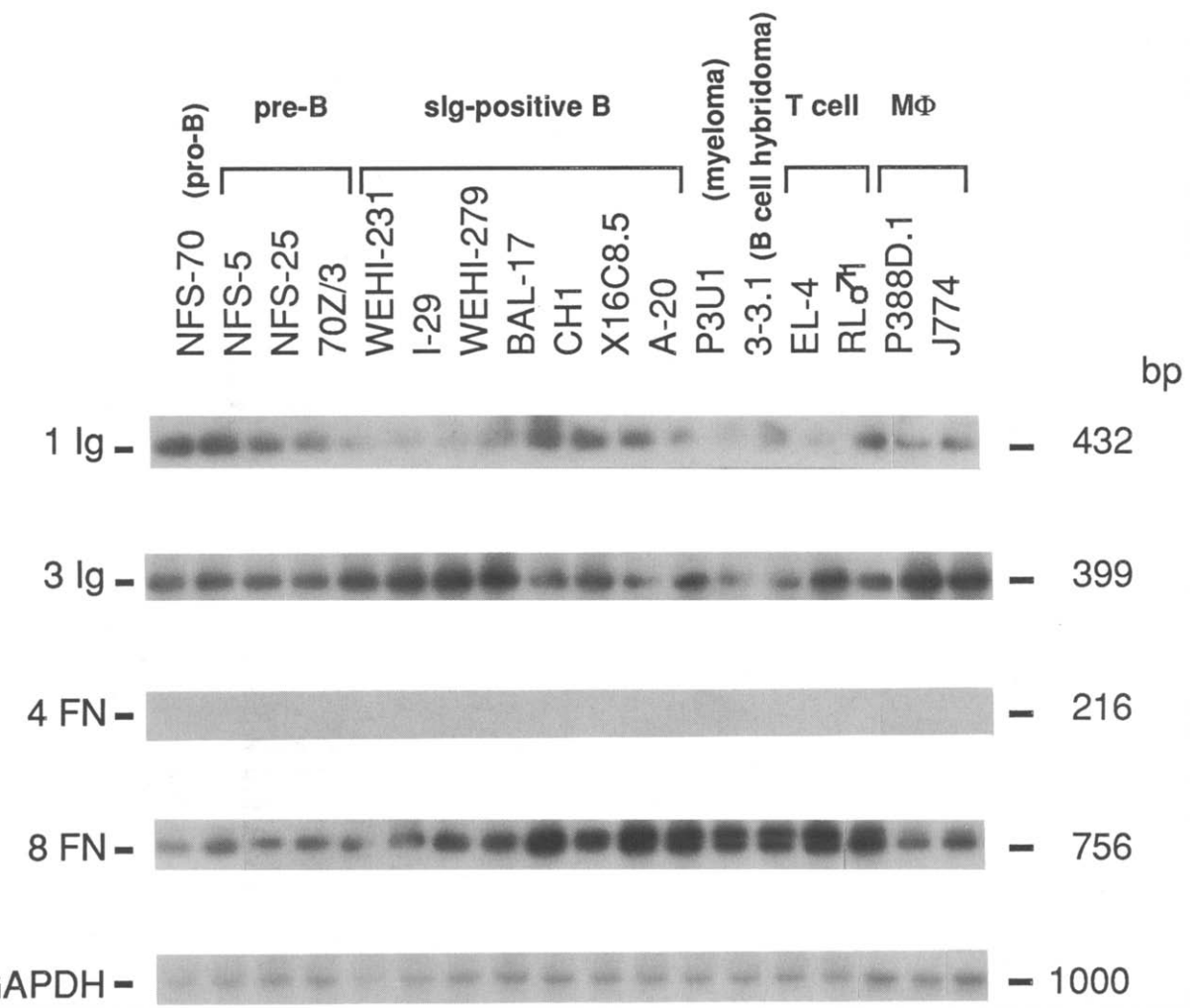

Fig. 6. Expression of MPTPδ extracellular domains in the hematopoietic cell lines examined by RT-PCR analysis. RNAs from the following cell lines were used for RT-PCR: pro-B cell line NFS-70; pre-B cell lines NFS-5, NFS-25 and 70Z/3; surface Ig-positive B cell lines WEHI-231, I-29, WEHI-279, BAL-17, CH1, X16C8.5 and A-20; myeloma cell line P3U1; antibody-producing hybridoma 3-3.1; T cell lines EL-4 and RLδ1; and macrophage lines P388D.1 and J774. The size of each PCR product is indicated on the right.

the regulatory mechanisms of differentiation and maturation in the central nervous system and in the immune system. To firmly establish the physiological roles of MPTPδ, it will be necessary to analyze the MPTPδ isoform expression at the protein level, to identify the ligand(s) for each isoform and to understand the regulation of PTP activity upon ligand binding. Studies to this end are currently in progress.

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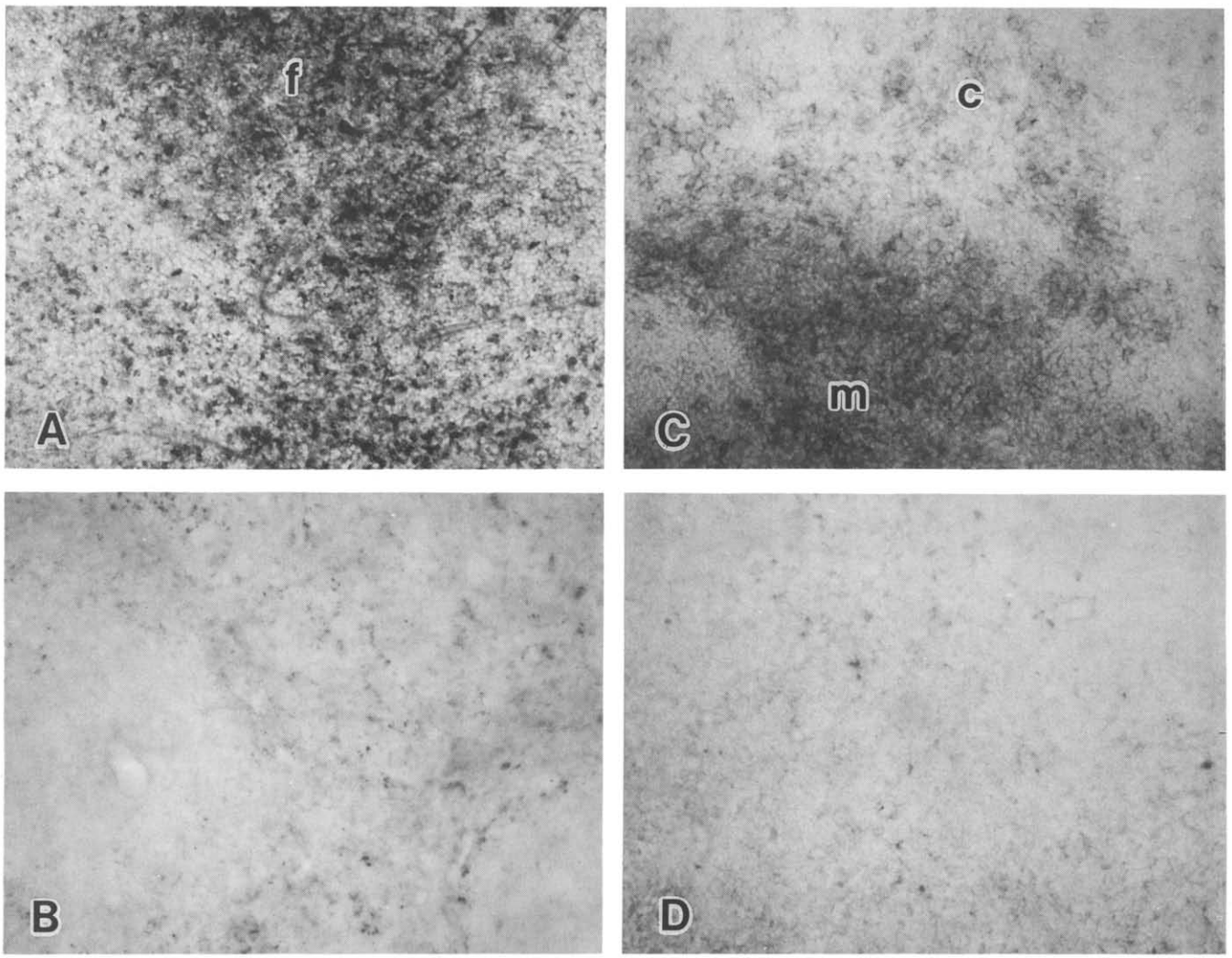

Fig. 7. In situ hybridization of the SJL mouse spleen (A and B) and thymus (C and D). Twenty μm-thick sections were hybridized with DIG-labelled antisense (A and C) and sense (B and D) MPTPδ cRNA probes. Note that the medulla of the thymus was heavily labelled (C). f, follicle; c, cortex; m, medulla.

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