Cloning and expression of cDNA for chemokine receptor 9 from Olive flounder *Paralichthys olivaceus*

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Cysteine-cysteine chemokine receptor 9 (CCR9) homologue cDNA was isolated from olive flounder leukocyte cDNA library. Olive flounder CCR9 homologue consisted of 1709 bp encoding 367amino acid residues. When compared with other known CCR peptide sequences, the most conserved region of the olive flounder CCR9 peptide is the seven transmembranes. A phylogenetic analysis based on the deduced amino acid sequence showed the homologous relationship between the olive flounder CCR9 sequence and that of Mouse CCR9. The olive flounder CCR9 gene was predominantly expressed in the Peripheral blood leukocytes (PBLs), kidney, spleen, and gills.

Key words: cDNA, Olive flounder, CCR9, Transmembrane

Introduction

Chemokines are cytokines with particular combinations of cysteine residues at the amino terminus, and with molecular masses of 5-14 kDa. Like other cytokines, they are secreted by various cell types and are multi-functional. In mammals, they serve not only as chemo-attractants in leukocyte migration during inflammation, but also influence integrin activation, angiogenesis, hematopoiesis and homing of lymphocytes in lymphoid tissues (Coillie *et al.*, 1999; Fernandez *et al.*, 2002). To date, more than 50 chemokines have been described. The amino terminal cysteine residues occur in several forms: CXC (α type), CC (β type), C (γ type) and CX3C (δ type) (Vaddi *et al.*, 1997; Baggiolini, 1998; Coillie *et al.*, 1999).

Chemokines mediate their effects through G protein coupled 7 transmembrane domain receptors, which are currently divided into 4 families based on the type of chemokine that they bind; they are CXCR1 to CXCR5, CCR1 to CCR10, XCR1, or CX3CR1. Recent data have identified several chemokines that are constitutively expressed in lymphoid and extralymphoid tissues, indicating that these chemokines might have homeostatic function by regulating lymphocyte trafficking to or within lymphoid organs and in peripheral tissues (Vicari *et al.*, 1997; Willimann *et al.*, 1998; Butcher *et al.*, 1999; Campbell *et al.*, 1999; Morales *et al.*, 1999; Campbell and Butcher, 2000; Ansel *et al.*, 2000; Kunkel *et al.*, 2000; Papadakis *et al.*, 2000; Wurbel *et al.*, 2000).

Chemokine receptor 9 (CCR9) is the only known receptor for the chemokine ligand 25, also called thymus-expressed chemokine (Youn *et al.*, 1999; Zaballos *et al.*, 1999; Norment *et al.*, 2000). CCR9expressing cells migrate toward chemokine ligand 25, and both molecules colocalize in the thymic cortex and in the small intestinal mucosa (Youn *et*

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al., 1999; Zaballos et al., 1999; Kunkel et al., 2000; Norment et al., 2000; Papadakis et al., 2000; Wurbel et al., 2000). Up-regulation of CCR9 can be induced by pre-TCR signaling (Norment et al., 2000), retinoic acid (Iwata et al., 2004), and dendritic cells from Peyer's patches and mesenteric lymph nodes (Lindbom et al., 2003; Mora et al., 2003). Thus, mucosal CCR9 induction may play an important role in the maintenance of CCR9 on gut T cells. Profound down-regulation of CCR9 by TCR cross-linking was shown in persistently activated Th1 cells generated from umbilical cord CD4+ T cells stimulated with IL-2 and IL-12 together with antibodies to IL-4 (Zabel et al., 1999). Interestingly, this effect was reversible because CCR9 reappeared after removal of the stimulus. Thus, the level of CCR9 expression on T cells might be modulated by the degree of T-cell activation.

In the present study, we cloned and characterized a novel chemokine chemokine cDNA in Olive flounder, examined its phylogenetic relationship with other chemokine receptors, and detection of its mRNA transcripts in various tissues using RT-PCR.

Materials and Methods

Cloning and sequencing of olive flounder CCR9 cDNA

CCR9 cDNA was identified from the analysis of

expressed sequence tags (ESTs) of olive flounder kidney stimulated with a Con A/PMA cDNA library. Kidney was taken from a single olive flounder and stimulated with Con A/PMA as previously described by Nam *et al.* (2003). cDNA clone was sequenced using ThermoSequenase (Amersham) with M13 forward and M13 reverse primers (Table 1) and an automated DNA sequencer LC4200 (Li-Cor). Phylogeny was inferred using the PHYLIP program (ver. 3.5), and by distance analysis using the neighbor joining method. The values supporting each node are derived from 1000 re-samplings.

The determined nucleotide and deduced amino acid sequences, and multiple sequence alignments were analyzed by GENETYX ver. 8.0 (SDC Software Development).

Tissue distribution of olive flounder CCR9 gene transcript

Total RNA (50 ng) from the brain, heart, intestine, kidney, liver and spleen were reverse-transcribed into cDNA using an AMV Reverse Transcriptase First-Strand cDNA synthesis kit (Life sciences). PCR was performed on the resulting cDNA using the CCR9 RT-F / CCR9 RT-R specific primer set (Table 1). β -actin was amplified as a control using the Beta-actin-F and Beta-actin-R primers (Katagiri *et al.*, 1997). The PCR mixtures were denatured at 94°C for 2 min and then subjected to 25 cycles of 94°C for 30 s, 57°C for 30 s and 72°C

Table 1. The oligonucleotide primer sets used in the present study.

Primer name	Sequence of oligonucleotide primer
M13 forward	5'-CACGACGTTGTAAAACGAC-3'
M13 reverse	5'-GGATAACAATTTCACACAGG-3'
CCR9 RT-F	5'-GACTGAAGACCATGTCAGATGTG-3'
CCR9 RT-R	5'-GTTGACTCTTGTTGACCAATGGC-3'
Beta actin-F	5'-TTTCCCTCCATTGTTGGTCG-3'
Beta actin-R	5'-GCGACTCTCAGCTCGTTGTA-3'

for 1 min. The products were visualized by separation on a 1.5% agarose gel.

Results

Cloning and sequencing of olive flounder CCR9 cDNA

The full-length cDNA, designated as OFCCR9, had significant identity to the mammalian CC chemokine receptor group. The OFCCR9 cDNA has a length of 1709 bp, and contains an ORF of 1,104 bp encoding 367 amino acid residues. The 3' UTR contained a polyadenylation signal (AUUAAA) and a polyadenylation site (Fig. 1).

In the phylogenetic analysis, olive flounder CCR9 is grouped with mouse CCR9, and closely related with mouse and human CCR7 (Fig. 2). This grouping was well supported by bootstrapping.

Alignment of the deduced amino acid sequences of OFCCR9 and other vertebrate chemokines shows that the sequence conserves five cysteine residues at positions 31, 113, 163, 192 and 283 (Fig. 3). OFCCR9 has also seven hydrophobic regions at positions 45-67, 80-102, 124-143, 164-186, 206-228, 249-271 and 299-318 that are representing the seven transmembrane domains (TM), which are typical G-protein-coupled receptors (Fig. 3).

Tissue distribution of olive flounder CCR9 gene transcript

Expression of the OFCCR9 gene in the tissues of olive flounder was detected by RT-PCR. Olive

TCCATGGATGCCTTCAAGAACATCACAGGATTCACCACGAAGCACCGTCCTTGTTCACCACTGATGACTACTACGAGGACCAGGACCAGGACCGG 18 M D A F K N I T G F T K E P S L F T D Y Y E D Q D L 29 ATGTGTGACCGGGCACCAGTGCGGACTTCAGGCGGTTTGTTT
M D A F K N I T G F T T K E P S L F T T D D Y Y E D Q D L 29 ATGTGTGACCGGGCACCAGTGCGGACTTTCAGGCGTTGTTTGAGCCGCCGCTGTTCTGGATGATCACCCTGGATGGCGGAGCTGGGAAC 27 M C D R A P V R T F R R L F E P P L F W M I T L D G G A G N 59 CTGGCTGTGGTGTGGATCTACCTGAACTTCCGGCGGCGACTGAAGACCATGTCAGATGTGTACCTGCTGAACCTGGCAGTGGCCGATCTG 36 L A V V W I Y L N F R R R L K T M S D V Y L L N L A V A D L 89 CTGTTCCTGGTCACACTGCCGCTGTGGGCGGCCGAGGCATCACACGGCTGGAATTTTGGTTTTGCCTCTGTAAGATCAACTCCGCCCTC 45 L F L V T L P L W A A E AS H G W N F G F A L C K I N S A L 119 TACAAGGTGAACCTGTTCAGCAGCATGCTACTGCTCACATGCTACACGGCTGGTGTGTGCTATGTCCAGTCACACGGGCACAA 54 Y K V N L F S S M L L L T C I S V D R Y V V I V Q S T R A Q 149 AACTCACAGCTTGAGCAGCGCGTCGCAAGTGCTGCTGGTGGTGGTGGTGGGGGGTGTGGCGCCTGCTG
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$\begin{array}{c} ctggctgtggtgtggatctacctgaacttccggcggcgactgaagaccatgatagaccatgtacgatgtgtacctgctggagtggcgatcgat$
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$\begin{array}{c} CTGTTCCTGGTCACACTGCCGCTGTGGGCGCGCGAGGCATCACACGGCTGGAATTTTGGTTTTGCCTCTGTAAGATCAACTCCGCCCTC & 45 \\ \underline{L} & F & \underline{V} & \mathbf{T} & \underline{P} & \underline{L} & \underline{P} & \underline{L} & \underline{N} & \underline{A} & \underline{A} & \underline{E} & \underline{AS} & \mathbf{H} & \mathbf{G} & \mathbf{W} & \mathbf{N} & F & \mathbf{G} & F & \mathbf{A} & \underline{L} & \mathbf{C} & \mathbf{K} & \mathbf{I} & \mathbf{N} & \mathbf{S} & \mathbf{A} & \underline{L} \\ \hline TACAAGGTGAACCTGTTCAGCAGCATGCTACTGCTCACATGCATCAGTGTTGACCGCTATGTTGTCATTGTCCAGTCCACCAGGGCACAA & 54 \\ \mathbf{Y} & \mathbf{K} & \mathbf{V} & \mathbf{N} & \underline{L} & \underline{F} & \mathbf{S} & \mathbf{N} & \mathbf{L} & \underline{L} & \underline{L} & \mathbf{T} & \mathbf{C} & \mathbf{I} & \mathbf{N} & \mathbf{N} & \mathbf{I} & \mathbf{V} \\ AACTCACAGCTTGAGCGCCGTCGCTACAGTTGCTTGGTGTGTGT$
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TACAAGGTGAACCTGTTCAGCAGCATGCTACTGCTCACATGCATCAGTGTTGACCGCTATGTTGTCATTGTCCAGTCCACCAGGGCACAA54YKVNLFSMLLTCISVVVVQSTRAQ144AACTCACAGCTTGAGCGCCGTGCGCTACAGTTGCTTGGTGTGGTGGTGGGGGGTGTGGCTGCTGGCCGCTGCT
Y K V N L F S S M L L L T C I S V D R Y V I V Q S T R A Q 144 AACTCACAGCTTGAGCGCCGTCGCTACAGTTGCTTGGTGTGTGGGGGGTGTGGGCTGCTGGCCGCTGCT
AACTCACAGCTTGAGCGCCGTCGCTACAGTTGCTTGGTGTGGTGTGGGGGGTGTGGCTGCTGCTGCTG
N S Q L E R R R Y S C L V C <u>V G V W L L A L L A T P E L V</u> 179 TITGCCACCATCGTTAACGTGGACTCTCAGCAGTACTGCAGGATGGTGGTGCTCCCCACTACCTGGGCAACTGCACCAAGATCGTGGTGCTG <u>F A T I V N V</u> D S Q Q Y C R M V F P T Y L G N C T K <u>I V V L</u> 209 TCATTACTGGTGAGCATGGGGTTCTTCTTACCTTCATCATCATGGCATTTTGCTACAGCGTTATTGTTGCCAAGCTGCTCAATACCCGA 81 <u>S L L V S M G F F L P F I I M A F C Y</u> S V I V A K L L N T R 239 AACTTCCAAAAGCACAAGGCCATGCGCGCTCATATTGGCTGTGGTGGTGGTGGTGGTGCTCGTCAATGGCGGTGACTGGTG N F Q K H K A M R <u>V I L A V V V V F V M S Q L P Y N G V L V</u> 269 ATGGAGGTTACGCAGGCCTCCAACATGACTATGACGGACTGCGCGGGAGGGGGAGGGTGCAAGGCTTCGACATGTTGGGACAGGTACTAAAGAGTCTG 99 <u>M E V T Q A S N M T M T D C E E V K R F D M L G Q V L K S L</u> 299
TITGCCACCATCGTTAACGTGGACTCTCAGCAGTACTGCAGGATGGTGTTCCCCACTTACCTGGGCAACTGCACCAAGATCGTGGTGCTG 72 F A I V N V D S Q Q Y C R W F P T Y L G N C T K I V V L 20 TCATTACTGGTGAGCATGGGGGTTCTTCTTACCCTTCATCATCATGGCATTTGCTACAGCGTTATTGTTGCCAAGCTGCTCAATACCCGA 81 S L V S M Q F F I M A F C Y S V I V A K L N T R 233 AACTTCCAAAAGCACAAGGCCATGCGGCGCTCATATTGGCTGTGGTGGTGGTGGTGGTGGTGGTGGTCGCCAGGTACTGCGCGGCGCGCGC
F A I V N V D S Q Q Y C R V F P T Y L G N C T K I V V L 201 TCATTACTGGTGAGCATGGGGGTTCTTCTTACCCTTCATCATCATGGCATTTGGCAAGGGGTATTGTGTGCCAAGCGGCGCTCAATACCCGA 81 S L V S M G F F P F I M A F C Y S V I V A K L N T R 233 AACTTCCAAAAGGCACAAGGCACTGCGGCGCGCTCATATTGGCGGTGGTGGGTG
TCATTACTGGTGAGCATGGGGTTCTTCTTACCCTTCATCATCATGGCATTTGCTACAGCGTTATTGTTGCCAAGCTGCTCATATACCCGA 81 S L V S M G F F P F I M A F C Y S V I V A K L N T R 233 AACTTCCAAAAGCACAAGGCCATGCGCGCGCTCATATTGGCTGTGGTGGTGGTGGTGGTGGTGGTGGTCGTCATGTCCGACCAGGCGCCTCCAACATGGGTGTACTGGGC 90 N F Q K H K A M R V I L A V V V F V M S Q L P Y Q 263 N F Q K H K A M R V V V V V Y Y Q 263 N F Q K H K A M V V V V V Y Y Y Y Q 263 263 263 263 263 264 294 294
<u>SLLVSMGFFLPFIIMAFCY</u> SVIVAKLLNTR 233 AACTTCCAAAAGGCACAAGGCCATGCGCGCGCATATTGGCTGGGGGGGG
AACTTCCAAAAGCACAAGGCCATGCGCGCTCATATTGGCTGTGGTGGTGGTGGTCGTCATGTCCCAGCTGCCTTACAATGGTGTACTGGTC 90 N F Q K H K A M R V I L A V V V F V M S Q L P Y N G V L V 269 ATGGAGGTTACGCAGGCCTCCAACATGACTATGACGGACTGCGAGGAGGGGGAGGGGTGAAGCGTTCGACATGTGGGACAGGTACTAAAGAGTCTG 99 M E V T Q A S N M T M T D C E E V K R F D M L G Q V L K S L 299
N F Q K H K A M R <u>V I L A V V V V F V M S Q L P Y N G V L V</u> 269 ATGGAGGGTTACGCAGGCCTCCAACATGACTATGACGGACTGCGAGGAGGGGGGGAGGGGTGAAGGCGTTCGACATGTGGGACAGGTACTAAAGAGTCTG <u>M</u> E V T Q A S N M T M T D C E E V K R F D M L G Q V L K S <u>L</u> 299
ATGGAGGTTACGCAGGCCTCCAACATGACTATGACGGACTGCGAGGAGGGGGAGAGGGTTCGACATGTTGGGACAGGTACTAAAGAGTCTG 99 <u>M</u> E V T Q A S N M T M T D C E E V K R F D M L G Q V L K S <u>L</u> 299
<u>M</u> EVTQASNMTMTDCEEVKRFDMLGQVLKS <u>L</u> 299
GCTTACATGCACGCCTGCCTCAACCCCTTCCTCTATGCATTTGTGGGCGTGCGT
<u>AYMHACLNPFLYAFVGV</u> RFRHDVLQLLHCS329
AGCAGCCGCTGCATGCCATTGGTCAACAAGAGTCAACTGGGTCAGACCAACAGGAGTCCGCTTAGCTCAACTCGAGTCTCTGTGATGTCA 117
S S R C M P L V N K S Q L G Q T N R S P L S S T R V S V M S 359
GACAGTGACACCTCACAGGCACTGTCACTGTAACAACACACCACTCAATTTTACACTTGAGCTGACACAGACATCAATCTCATCAATGTCTCA 126
DSDTSQALSL* 369
TCAACTTCATCACTCCATCATTTAATTGAGTTTCACTTCAAACTATTTTAAATAATTGAATTTGTTGTCTTTCTAGGACATTTCCAGGA 135
AAAAAAGTGATGTGTGGCTTCCTTGGGTCATGAAACGTCTGTTTTCTCCCATATTGACTGTGTGCATCTTTAACAGGAAGTGAATCTC 144
AAAATACAAGTGAACAAACAAAACTCATGTGTTACTGTCAGAGCTGCTAACAGATGACAGATGCTCATATGTAAATAGTTTTGCTGGATG 153
TTTTGGTCTATGAAGATGTAGAGATTGTACATGTGAGCATGACAGGAGGAAGTTGATCAGTCACACTGGTTTCTTCACTTCCTCCCCACA 162
TCCTGTTTATGTTTTCACTCTGTTCTGTAAAACATCTCAATGTTTGTATTAAATTTGGATTCACATGTTTTTATTCAAAAAAAA

Fig. 1. Nucleotide and deduced amino acid sequences of the olive flounder CCR9 cDNA. The putative seven transmembranes are underlined, and the polyadenylation signal is shown in bold print.



Fig. 2. Phylogenetic tree of amino acid sequences of olive flounder CCR9. The positions of the olive flounder CCR9 was estimated by the neighbor joining method of clustering in the PHYLIP program. Sequences were obtained from DDBJ/EMBL/GenBank.

flounder CCR9 gene transcripts was expressed predominantly in the PBLs, head kidney, trunk kidney, spleen, and gills, but not in liver, brain, and muscle after 25 cycles of PCR (Fig. 4).

Discussion

Chemokine receptor 9 (CCR9) is the only known

receptor for the chemokine ligand 25, also called thymus-expressed chemokine (Norment *et al.*, 2000; Youn *et al.*, 1999; Zaballos *et al.*, 1999). CCR9-expressing cells migrate toward chemokine ligand 25, and both molecules colocalize in the thymic cortex and in the small intestinal mucosa (Youn *et al.*, 1999; Zaballos *et al.*, 1999; Kunkel *et al.*, 2000 Norment *et al.*, 2000; Papadakis *et al.*,

Flounder CCR9	1:MDAFKNITGFTTKEPSLFTTDDYYEDQDLMCDRAPVRTFR	40
Mouse CCR9	1:MMPTELTSLIPGMFDDFSYDSTASTDDYMNLNFSSFFCKKNNVRQFA	47
Human CCR7	1:MDLGKPMKSVLVVALLVIFQVCLCQDEVTDDYIGDNTTVDY-TL-FESL-CSKKDVRNFK	57
Mouse CCR7	1:MDPGKPRKNVLVVALLVIFQVCFCQDEVTDDYIGENTTVDY-TL-YESV-CFKKDVRNFK	57
	* . ** *	
F1		100
Flounder CCR9	41. KLPBPPLFWMIILDUGAUNLAVVWIIHNFKKKLKIMSDVILLNLAVADLLFLVILFLWAA	100
Mouse CCK9	48: SHFIIPPLYWLVFIVGILGNSLVILLVYW-YCTRVKIMIDMFLLNLAIADLLFLAILPFWAI	106
Human CCR7	58: AWFIPIMYSTICFVGLLGNGLVVLTYI-YFKRLKTMTDTYLLNLAVADILFLLTLPFWAY	116
Mouse CCR7	58: AWFIIPLMYSVICFVGLLGNGLVILTYI-YFKRLKTMTDIYLLNLAVADILFLLILPFWAY	116
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Flounder CCR9	101: EASHGWNFGFALCKINSALYKVNLFSSMLLLTCISVDRYVVIVQSTRAQNSQLERRRYSC	160
Mouse CCR9	107: AAAGQWMFQTFMCKVVNSMYKMNFYSCVLLIMCISVDRYIAIVQAMKAQVWRQKRLLYSK	166
Human CCR7	117: SAAKSWVFGVHFCKLIFAIYKMSFFSGMLLLLCISIDRYVAIVQAVSAHRHRARVLLISK	176
Mouse CCR7	117: SEAKSWIFGVYLCKGIFGIYKLSFFSGMLLLLCISIDRYVAIVQAVSRHRHRARVLLISK	176
Flounder CCR9	161 - I VOVCVWI I ALLI ATPEL VEATIVNUDSOOVCOMVEDTVI CNCTRTVVI SI I VSMCEE	218
Mouso CCPO	167. WYOTTIWWAANI CTDETI VSOVSCESCIAT-CTWVDEDENAVI VSAVI TI VVISEV OT	210
Human CCP7	177 T COVCTWIT ATVI STDELL VSDI ODGSEGANDOSI I TEUVEARTITIOVAONVICEI	224
Mouro CCP7	177. LOOVOTWILATVLSTFELLISDLWKSSEEWANKOSLI TENVEATITTOVAOWVECEI	200
mouse cck/	111.LOUVUINMLALFLOITELLIOULQKAOGEDILKCOLVSAQVEALIIIQVAQMVFGFL	233
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Flounder CCR9	219: LPF1IMAFCYSVIVAKLLNTRNFQKHKAMRVILAVVVVFVMSQLPYNGVLVMEVTQASNM	278
Mouse CCR9	225: LPFMVMAFCYTTTTHTLVQAKKSSKHKAL NVTTTVLTVFTMSQFPVNSTLVVQAVDAYAM	284
Human CCR7	234: VPLLAMSECYLVTTRTLLQARNEERNKATKVTTAVVVVFTVFQLPVNGVVLAQTVANENT	293
Mouse CCR7	234: VPMLAMSFCYLTTTRTLLQARNFERNKATKVTTAVVVVFTVFQLPVNGVVLAQTVANFNT	293
	* * *** ** ** .***. ***.	200
Flounder CCR9	279: TMTDCEEVKRFDMLGQVLKSLAYMHACLNPFLYAFVGVRFRHDVLQLLHCSSSRCMPLVN	338
Mouse CCR9	285:FISNCTISTNIDICFQVTQTIAFFHSCLNPVLYVFVGERFRRDLVKTLKNLGCISQAQWV	344
Human CCR7	294: TSSTCELSKQLNIAYDVTYSLACVRCCVNPFLYAFIGVKFRNDLFKLFKDLGCLSQEQLR	353
Mouse CCR7	294: TNSSCETSKQLNIAYDVTYSLASVRCCVNPFLYAFIGVKFRSDLFKLFKDLGCLSQERLR	353
	· · *. · · *. · *. · ** **. **. ** **. *** *. · · · ·	
Flounder CCR9	339: KSQLGQTNRSPLSSTRVSVMSDSDTSQALSL	369
Mouse CCR9	345:SFTRREGSLKLSSMLLETTSGALSL	369
Human CCR7	354: OWSSCRHIRRSSMSVFAFTTTTFSP	378
Mouse CCR7	354 : HWSSCRHVRNASVSMFAFTTTTFSP	378
Nouse cont		0.0

Fig. 3. Comparison of the derived amino acid sequence of oliveflounder, human, and mouse, CCRs. Asterisks indicate identical amino acid residues, and dashes indicate gaps introduced for maximal alignment. Cysteine residues are shown in bold, and the transmembrane regions are boxed.

2000;; Wurbel et al., 2000).

In the phylogenetic analysis (Fig. 2), olive flounder CCR9 is grouped with mouse CCR9 (Wurbel *et al.*, 2000). This grouping was well supported by bootstrapping. The phylogenetic analysis indicated that known human, cow, and mouse CCRs are more closely related than the olive flounder. This result is possibly due to differences between mammalian and non-mammalian species. Based on the conservation of five cysteine residues and seven transmembrane regions of the molecule, we predict that the function of olive flounder CCR9 is similar to that in mammals.

The mRNA expression of CCR was detected in leukocytes, kidney, spleen, intestine, liver, and gill of olive flounder. The CCR9 gene was predomi-



Fig 4. Detection of olive flounder CCR9 mRNA levels from various tissues of healthy olive flounder by RT-PCR. M indicates a 100-bp ladder marker.

nantly expressed in several tissues that contained lymphocytes (PBLs, head kidney, trunk kidney, spleen, and gill) (Fig. 4), thus suggesting that this gene plays important roles in the immune system.

Further studies of recombinant protein of olive flounder CCR9 to understand the roles of this gene should be performed to get a better understanding of the fish immune system.

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