Acta Biochimica Polonica

Vol. 49 No. 2/2002

427–431

QUARTERLY

Communication

Molecular cloning and sequencing of partial cDNA of tumor necrosis factor and p75 tumor necrosis factor receptor of Syrian golden hamster (*Mesocricetus auratus*) with the use of universal primers★✪

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Received: 02 January, 2002; revised: 2 April, 2002; accepted: 23 May, 2002

Key words: hamster, TNF, p75 TNF-R, PCR primers

In this study we cloned and analysed partial cDNA of tumor necrosis factor (TNF) and p75 TNF-R receptor of Syrian golden hamster (*Mesocricetus auratus*). We obtained a 382-bp sequence of TNF and a 148-bp sequence coding for p75 TNF-R. The primers used for the cloning were designed on the basis of inter-species homology, thus presumably can be used for cloning and analysis of TNF and p75 TNF-R genes of other mammals.

Tumor necrosis factor (TNF, cachectin) is a cytokine with a wide variety of functions. It regulates immune and inflammatory reaction, induces cell death and differentiation, and affects also cellular metabolism of lipids and amino acids. TNF is secreted mainly by activated macrophages, although other normal and tumor cells are also able to produce TNF (Aggarwal & Natarajan, 1996). TNF is involved in pathogenesis of many infectious, au-

★Part of this work was presented at 6th Meeting of Young Immunologists' Section of the Polish Society for Experimental and Clinical Immunology, Gdańsk 2000, Poland.

✪This work was partially financed by grant No. 6 P207 12106 from the State Committee for Scientific Research (KBN, Poland) and core fund DS/B000-4-0072 of the Intercollegiate Faculty of Biotechnology, Gdańsk, Poland.

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Abbreviations: p75 TNF-R, TNF receptor p75; TNF, tumor necrosis factor, cachectin; I, deoxyinosine.

toimmune and neoplastic diseases (Aggarwal & Natarajan, 1996; Männel *et al.*, 1993; Fiers, 1991). In order to induce cellular response TNF should bind to specific cell-surface receptors of which two species have been identified: low-affinity p55 TNF-R and high-affinity p75 TNF-R. Both receptors are present on most nucleated mammalian cells and their number depends on the tissue (De Kossodo *et al.*, 1994; Tartaglia & Goeddel, 1992). In the present study we cloned and analysed partial cDNAs of TNF and p75 TNF-R of Syrian golden hamster (*Mesocricetus auratus*), an animal species useful for study on cancer, infectious diseases and other (Melby *et al.*,1998; Slominski & Paus, 1993).

MATERIALS AND METHODS

Male Syrian hamsters were used as a source of hamster tissues. Cells from spleen were isolated and cultured with *Escherichia coli* lipopolysaccharide (1 μg/ml) and then total RNA was isolated using TRI REAGENT^{TM} (Molecular Research Center, Canada). First strand cDNA was synthesized by reverse transcription (RT) of 1 μg of total RNA with 15 U of AMV-RT (Promega Corp, U.S.A.). Primers for polymerase chain reaction (PCR) were originally designed on the basis of a homology search within the cDNA sequences of p75 TNF-R and TNF genes from various species using the program OLIGO 4.1 (National Biosciences, U.S.A.). The sequences of the primers selected were: for the p75 TNF-R gene – 5′ TCC AGC AGC AGC TCC CTA GA 3′ and 5′ ACG TTC ACG ATG CAG GTG AC 3′, while for the TNF gene – 5′ AGC ACI GAA AGC ATG ATC C 3′ and 5′ GAA GAG AAC CTG GGA GTA G 3′. PCR products of 420 bp for TNF and 188 bp for p75 TNF-R were isolated from agarose gel by electroelution and cloned into pGEM-T Easy vector (Promega Corp., U.S.A.) and transformed into *E. coli* JM109. Plasmid DNA was isolated using alkaline lysis and screened using PCR. Two independent PCR cloning experiments for each gene were performed using products obtained from different RT reactions. One clone from each cloning was chosen for sequencing, which was performed on both DNA strands. The template plasmid DNA for sequencing was prepared by using DNA Miniprep Plus kit (A&A Biotechnology, Poland), while the sequencing was performed on a Perkin-Elmer ABI PRISM 310 sequencer.

RESULTS AND DISCUSSION

The obtained 382 bp sequence of hamster TNF (GenBank accession number AF315292) and 148 bp of hamster p75 TNF-R (GenBank accession number AF315291) are shown in Fig. 1. The partial cDNA of TNF contains a sequence coding for 126 amino acids including most of the leader sequence and almost half of the sequence of the mature protein. This sequence demonstrates 89.3% homology to the appropriate part of murine cDNA, 87.7% to rat cDNA, 93.2% to *Peromyscus leucopus* cDNA, and 83% to human cDNA. Those values are in accord with published data pointing to high homology of TNF various species (Wedlock *et al.*, 1996; Pennica *et al.*, 1985). A comparison of the reported sequence with that published by Melby and co-workers (1998) indicates differences in four bases (see Fig. 1): guanine (in our sequence) instead of adenine in position 39 and cytosine instead of guanine in positions 103, 196 and 250. All of them affect the deduced amino-acid sequence. In the case of three of those differences (in positions 103, 196 and 250), the identified cytosine residues in our cDNA clones are homologous to the cytosine residues of the known TNF sequences from other species (Fransen *et al.*, 1985; Estler *et al.*, 1992; Crew & Filipowsky, 1992; Wang *et al.*, 1985). Although in both known TNF sequences of members of the family *Muridae* adenine was identified in position 39 (Fransen *et al.*, 1985; Estler *et al.*, 1992), we postulate the presence of guanine in the ham-

A

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  1   gcgacgtggagctggcagaggaggcgctccccaaaaaggcgtggggcccc
 51   cagaactccagccggtgcctatgcctcagcctcttctccttcctgcttgt
101   ggcaggggccacaatcctcttctgcctgctgaacttcggggtgatcggcc
151   cccaaagggaagagaagttccccaaccctatcatcggctccatggcccag
201   acactcacactcagatcatcttctcaaaattcgaacgacaagcctgtggc
251   ccacgttgtagcaaaccaccaagtggaggagcagctggagtggctgagcc
301   atcgtgccaatgccctcctggccaacggcatgtctctcaaagacaaccag
351   ctggtgataccagcagacgggctgtacctggt
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B

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1     gagctcagccagtgctggggacaggagggcgcccctgaggagccagcccc
51    aggccacagtcacggaggaagcccaagggtctcaggaggcctgtgcaggc
101   tccaggagttcagattcctcccacggcagccatgggacccatgtcaat
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Figure 1. Nucleotide sequence of partial cDNA of Syrian hamster TNF (A) and p75 TNF-R (B).

Shaded are the nucleotides differing from those present in the hamster TNF sequence published by Melby *et al.* (1998). Note that both sequences are given without sequences complementary to those of PCR primers. This explains the length difference between PCR products and the sequences shown.

ster gene since guanine is also present in TNF sequences of other close relatives of hamster *Cavia porcellus* (*Cavidae)* and: *Peromyscus leucopus* (*Sigmonodontidae)* (Crew & Filipowsky, 1992). Of note is that *P. leucopus* has the highest overall homology to hamster within the TNF gene. In agreement with Melby, we found nine nucleotides unique for hamster as well as a unique 6-nucleotide deletion, which starts in position 176.

The homology of deduced amino-acid sequences (see Fig. 2), similarly to the nucleotide sequence, is high. The highest level of identity with hamster shows the sequence of *P. leucopus* (95.2%), while the murine, rat, guinea pig and human sequences show 89.7%, 88%, 80.2% and 73.8% of identity with the hamster sequence, respectively. Both hamster amino-acid sequences — described by Melby (Melby *et al.*, 1998) and obtained in this work — possess features characteristic for TNF — conserved β-sheets and a strongly hydrophobic 22-amino-acid membrane fragment of leader sequence (Wedlock *et al.*, 1996). The four amino-acid differences observed between our sequence and that published by Melby most probably do not affect the function of TNF. Our results indicate the existence of structurally similar amino acids (in three cases alanines instead of glycines) and a substitution of alanine for threonine in the region where no significant homology between distant species is found. It is also possible that the differences observed may represent genetic variations between cohorts of hamsters used for our and Melby's analyses.

The sequence of hamster p75 TNF-R has not been published yet. The obtained 148-nucleotide fragment of hamster p75 TNF receptor (see Fig. 1B) covers part of the cytoplasmic domain of the receptor and codes for 49 amino acids. Homology of this sequence to the appropriate part of the murine cDNA is 83.8%, while to the human sequence — 75%. These results correspond to literature data describing the highest homology between species in the cytoplasmic domain of the receptor — 73% between human and mouse, while homology of the whole nucleotide sequence is 62% (Tartaglia & Goeddel, 1992). A comparison of human and mouse p75 TNF-R to the reported hamster amino-acid sequence (see Fig. 3) allows distinguishing two 11–13 amino-acid regions of high homology located at the ends of

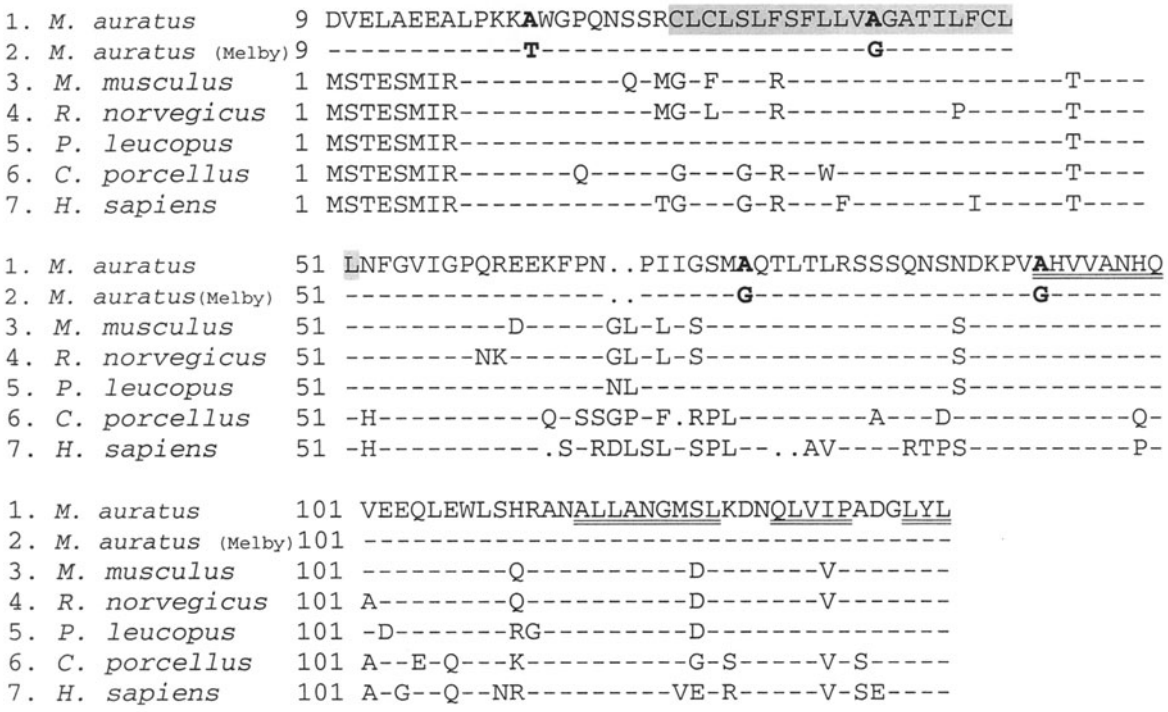

Figure 2. Comparison of amino-acid sequence of the obtained fragment of hamster TNF with the previously published hamster sequence and with the sequences of other species.

Amino acids different from those present in the hamster sequence are indicated, while the identical amino acids are replaced by "–". Dots are in place of deletions, doubly underlined are amino acids building β-sheets, shaded is the transmembrane part of the protein, and amino acids different from the hamster sequence published by Melby are bolded. GenBank accession numbers used in sequence comparison: 2. Af046215 (Melby *et al.*, 1998), 3. x02611 (Fransen *et al.*, 1985), 4. x66539 (Estler *et al.*, 1992), 5. m59233 (Crew & Filipowsky, 1992), 6. u39839 (Yuan, H.T., Kelly, F.J. and Bingle, C.D, 1996, data published only in GeneBank), 7. m10988 (Wang *et al.*, 1985).

the cloned sequence and a region of lower homology located in the middle of the presented sequence. This region contains 8 amino acids unique for hamster.

As primers used in this work were designed on the basis of homology between species, the primer pairs permit amplification of TNF and p75 TNF-R DNA not only of hamster but also of other species (see Fig. 4). Thus, the primers originally developed in this study, could be applicable to DNA amplification from species, the sequences of TNF and p75 TNF-R of which genes are known as well as unknown. Besides, the primers and the cDNAs obtained in this

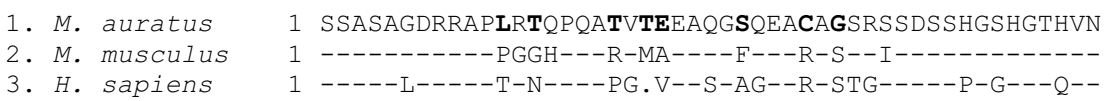

Figure 3. Alignment of hamster, mouse and human amino-acid sequences of p75 TNF-R.

Amino acids different from those present in the hamster sequence are shown, the identical amino acids were replaced by "–". Amino acids unique for the hamster sequence are bolded. The sequences used for comparison were 2. m59378 (Goodwin *et al.*, 1991), 3. m55994 (Kohno *et al.*, 1990), according to their GenBank accession numbers.

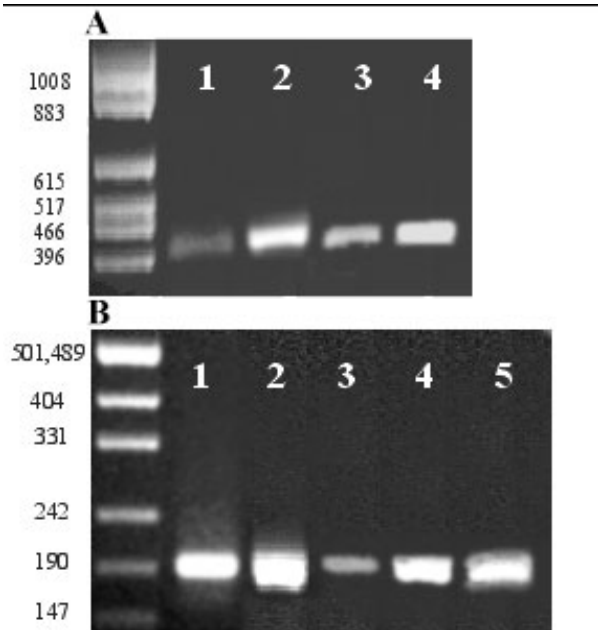

Figure 4. The ability of the originally designed primer pairs to amplify DNA of TNF (A) and p75 TNF-R (B) from various mammalian species (1-hamster, 2-mouse, 3-rat, 4-human, 5-rabbit).

PCR products (TNF – 420 bp for hamster and 426 bp for other species; p75 TNF-R – 188 bp) and molecular weight markers: pKO3/*Hin*fI and pUC19/*Msp*I were separated on 1% agarose gels and stained with ethidium bromide. Reaction mixture contained (conditions for p75 TNF-R amplification different from those for TNF are in parentheses): 1 x Taq DNA polymerase buffer, 2.5 mM $MgCl_2$, 2 (1) U Taq DNA polymerase (Promega Corp., U.S.A.), 50 (100) pmol each of forward and reverse primers, 200 μM dNTPs (Promega Corp., U.S.A.) and redistilled water to total volume 50 μl. PCR parameters were as follows (values for p75 TNF-R are given in parentheses): initial denaturation at 95° for 4 min; 33 cycles of: 95° for 30 s (1 min), 54.6°C for 30 s (58.8°C for 1 min), 72°C for 1 min (2 min); final extension at 72°C for 5 min (7 min).

work can serve as specific molecular probes in studies on the biology of TNF and its receptors in hamster models of infectious and neoplastic diseases.

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