ジャンガリアン(Phodopus sungorus),チャイニーズ(Cricetulus griseus),およびシリアン(Mesocricetus auraus)ハムスターのインターロイキン10遺伝子のクローニングと塩基配列の決定

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Molecular Cloning and Sequences of Interleukin-10 in the Djungarian (*Phodopus sungorus*), Chinese (*Cricetulus griseus*), and Syrian (*Mesocricetus auratus*) Hamster

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ABSTRACT. Interleukin 10 (IL-10) genes of Djungarian, Chinese, and Syrian hamsters were cloned. The clones of IL-10 consisted of 537 bp nucleotides and 178 amino acids in full length, and the nucleotide and amino acid sequences exhibited a high degree of homology with those of the mouse and human. Since the number and position of signal sequences, N-glycosylations and cysteine sites in the IL-10 amino acid sequences of the hamsters were the same as those of the mouse, we suggest that the IL-10 molecular structures of the hamster are closer to that of the mouse than human.

KEY WORDS: cytokine, hamster, interleukin 10.

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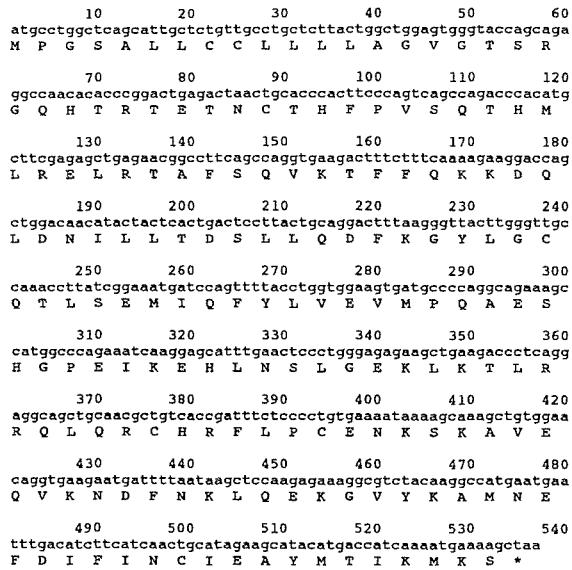
Several species of hamsters have been reported to be susceptible to some parasites. For example, the Djungarian hamster (*Phodopus sungorus*) is susceptible to *Neospora caninum* [11], the Chinese hamster (*Cricetulus griseus*) is susceptible to *Acanthamoeba keratitis* [12], and the Syrian hamster (*Mesocricetus auratus*) is susceptible to *Babesia microti* and *Leishmania donovani* [1, 3, 5]. In addition, Djungarian hamsters have high incidence of neoplasia, are susceptible to carcinogens, and are easily infected by oncogenic viruses, such as Rous sarcoma virus, human adenovirus-12, and simian virus 40 [10]. The reasons for the high susceptibilities are unknown, and molecular immunological studies of them have not been undertaken because of a lack of substantive reagents.

Interleukin-10 (IL-10) was identified as cytokine synthesis inhibitory factor (CSIF) of Th1-specific cytokines such as interferon-gamma (IFN-γ) [2]. The major biological activities of IL-10 include inhibition of proliferation of Th1 cells and of monocyte/macrophage-dependent T cells, activation of B cells and mast cells, and potentiation of immunoglobulin [7]. In contrast to IFN-γ which promotes Th1 cells, IL-10 is one of the Th2-specific cytokines that promote Th2 immuno-response [8]. Both mouse IL-10 (mIL-10) and human IL-10 (hIL-10) are expressed as non-covalent homodimers, but it has not yet been confirmed whether or not the mIL-10 and/or hIL-10 monomer has biological activity [7]. Cloning of IL-10 as a marker of Th2-specific cell growth and its gene sequences would be an important development for monitoring the hamster's immune response to pathogens.

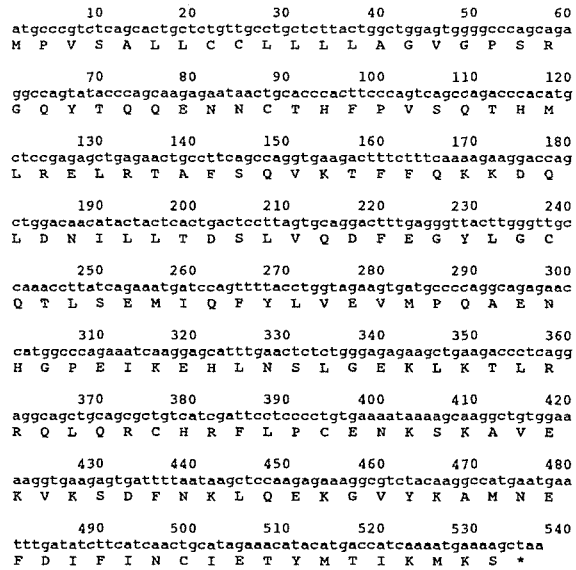
In this study, we cloned the IL-10 molecules of Djungarian, Chinese, and Syrian hamsters, determined the nucleotide, predicted the amino acid sequences and compared them with mIL-10 and hIL-10.

Two each of Djungarian, Chinese, and Syrian hamsters at 10 weeks old were infected intraperitonealy with *B. microti* AJ strain (1×10^7/head) to stimulate splenocytes of hamsters. Five weeks post-infection, these hamsters were sacrificed, and their spleens were extracted. The spleen cells were isolated by passage of the organs through a wire screen mesh, and the cells were cultured in SFM medium (Gibco-BRL, U.S.A.) containing 10% heat-inactivated fetal calf serum (HARLAN SERA-LAB, England) and 50 μg/ml gentamicin (Wako, Japan) in the presence of 10 μg/ml concanavalin A (Sigma, U.S.A.) in a 5% CO_2 atmosphere at 37°C for 24 hr prior to isolation of the RNAs. Total RNAs were extracted with TRIZOL reagent (Gibco-BRL), and mRNAs were purified with an Oligotex-dT30 <Super> mRNA purification kit (Takara, Japan). First-strand cDNA synthesis was completed with a cDNA PCR Library Kit (Takara). The primers for amplification of IL-10 genes were designed from homologous regions found among the corresponding published mouse, rat, and human cDNA sequences. The sequences of the primers used to amplify IL-10 cDNA were as follows; 5' untranslated region (UTR) for Djungarian hamster IL-10 (dIL-10), CCTTGCAGAAAACAGAGCTCCA; 5' UTR for Chinese hamster IL-10 (cIL-10), CCTTGCAGAAGACAGAGCTCCA; 5' UTR for Syrian hamster IL-10 (sIL-10), CCAGTCAGCCAGACCCAC; 3' UTR for IL-10 of all three breeds of hamster, CTGATCTAGACCTGCAGGCTC (RA primer enclosed in cDNA PCR Library Kit). Fragments of cDNA comprising the complete coding region for each IL-10 were amplified by polymerase chain reaction (PCR). Amplifications were performed in a thermal cycler (TP240, Takara) for 35 cycles at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 60 sec. The amplification products were cloned by ligation into the pT7 Blue-T plasmid (Novagen, U.S.A.) and transformed into competent *Escherichia coli* INVαF' cells according to the manufacture's instructions.

The DNA insert was sequenced with vector-specific primers and an automated, fluorescent DNA sequencer (SQ-5,500E; Hitachi, Japan). The resulting sequences were identified by a search of the National Center for Biotechnology Information (NCBI) databases for homologous sequences using the basic local alignment search tool



(A)



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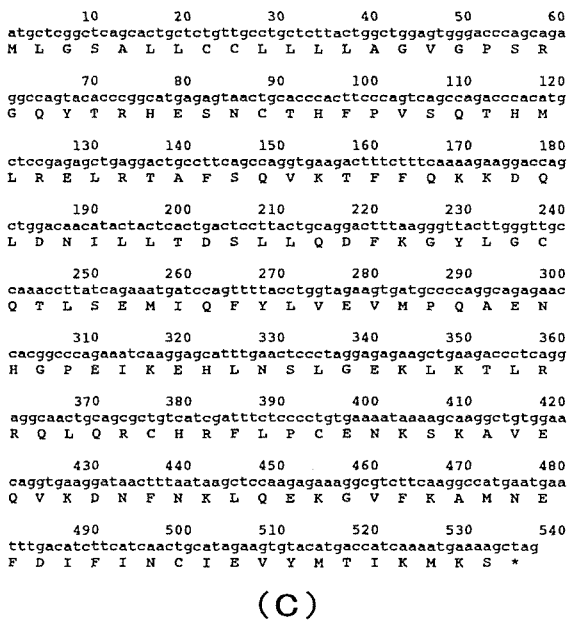

(C)

Fig. 1. Nucleotide and predicted amino acid sequences of Djungarian, Chinese, and Syrian hamster IL-10. (A) Djungarian hamster IL-10. (B) Chinese hamster IL-10. (C) Syrian hamster IL-10.

(BLAST). Sequence comparisons were conducted with the Genetyx computer system (Software Development Co., Ltd., Japan). The sequences and predicted amino acids of dIL-10, cIL-10, and sIL-10 were compared with mIL-10 and hIL-10. The GenBank accession numbers used for the sequence comparisons were mIL-10, NM010548 and hIL-10, NM000572.

Nucleotide and predicted amino acid sequences of IL-10 of the Djungarian, Chinese, and Syrian hamsters are shown in Fig. 1. They were 537 bp nucleotides and 178 amino acids in full length with a predicted molecular weight (MW) of 20,494 dal, 20,587 dal, and 20,593 dal, respectively. The first 18 amino acids from the amino terminal consisted of a hydrophobic signal sequence as determined by analysis with Genetyx software (Software Development Co., Ltd., Japan). When the signal sequences were cleaved, the results were a mature 160 amino acid polypeptide with a predicted MW of 18,779 dal for dIL-10, 18,834 dal for cIL-10, and 18,867 dal for sIL-10.

The homologies of nucleotide and predicted amino acid sequences of IL-10 among Djungarian, Chinese and Syrian hamsters, mouse and human are shown in Table 1. The nucleotide and predicted amino acid sequence homologies of dIL-10: cIL-10 were 94.4% : 93.3%, cIL-10: sIL-10 were

Table 1. Homologies of nucleotide and predicted amino acid sequences among Djungarian, Chinese and Syrian hamsters, mouse, and human IL-10

		Homology of nucleotide sequence (%)				
		PS[a]	CG[b]	MA[c]	MM[d]	HS[e]
Homology of aa[f] (%)	PS	–	94.4	94.6	89.9	81.8
	CG	93.3	–	95.3	90.7	81.4
	MA	94.4	93.3	–	89.9	81.9
	MM	87.1	86.0	85.4	–	81.0
	HS	74.7	73.6	73.6	73.0	–

a) PS: Djungarian hamster (*Phodopus sungorus*).
b) CG: Chinese hamster (*Cricetulus griseus*).
c) MA: Syrian hamster (*Mesocricetus auratus*).
d) MM: Mouse (*Mus musculus*).
e) HS: Human (*Homo sapiens*).
f) aa: Amino acid sequence.

95.3% : 93.3%, and dIL-10: sIL-10 were 94.6% : 94.4%, respectively. The homologies of nucleotide and predicted amino acid of IL-10 between the hamsters and mouse were 89.9–90.7% and 85.4–87.1%, respectively, while those between IL-10 of hamsters and human were 81.4–81.9% and 73.6–74.7%, respectively. The IL-10 cDNA clones exhibit a high degree of nucleotide sequence homology between mouse and human IL-10 (>80%) throughout their entire length; the only significant difference from mIL-10 was the insertion of a human *Alu* repetitive sequence element in the 3'-UTR of the hIL-10 cDNA clone [7]. The *Alu* repetitive sequence in the 3'-UTR regions of IL-10 nucleotide sequences of the three hamsters was not inserted (data not shown).

Predicted amino acid sequences of IL-10 of Djungarian, Chinese and Syrian hamsters, mouse, and human are shown in Fig. 2. Mature hIL-10 contains 4 cysteine residues and one N-glycoslylated site, and mature mIL-10 contains 5 cysteine residues and two N-glycosylated sites [6, 13]. IL-10 of each of the three hamsters contained 5 cysteine residues and two N-glycosylated sites, the same as mIL-10. Mouse IL-10 has one N-glycosyation site near its N-terminus that is lacking in hIL-10. This glycosylation is heterogeneous, resulting in a mixture of 17, 19, and 21 kDa species [6, 9]. However, N-glycosylation is not required for any biological activity because recombinant mIL-10 expressed in *E. coli* retains all its known biological activities [7].

The N-terminus of mature mIL-10 was predicted to be Ser19 [6] but was later determined as Gln22 by radiochemical sequencing of mIL-10 [7]. The N-terminus of mature hIL-10 is known to be Ser19 as was predicted [7]. The N-terminus amino acid of mature IL-10 of the three kinds of hamsters was predicted to be Ser19 by computer analysis. However, the N-terminus amino acid of mature IL-10 of the hamsters might be Gln22 in nature, because IL-10 of the hamsters were more similar to mIL-10 than hIL-10 from the point of view of the number and position of N-glycosylations and cysteines.

In the amino acid sequence estimated from IL-10 genes of the hamsters, the position of the signal sequence, N-linked glycosylation and cysteine residues were the same as mIL-10. Thus this result suggests that secondary structures of IL-10 of the hamsters might be closer to the structure of mIL-10 than hIL-10. The predicted secondary structures of

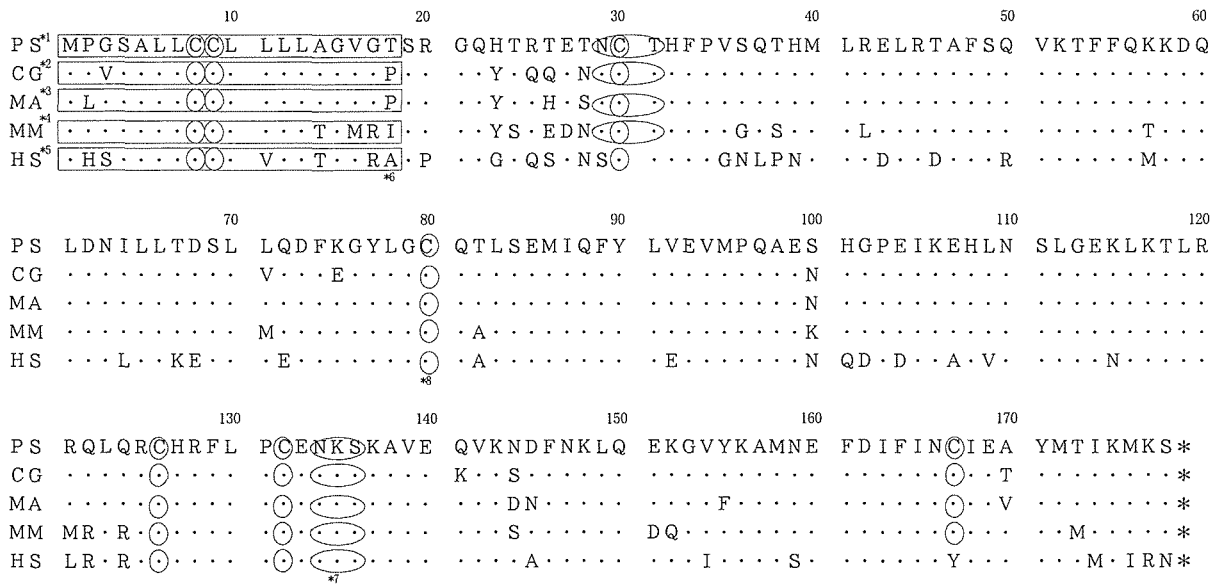

Fig. 2. Alignment of IL-10 amino acid sequences in the Djungarian, Chinese and Syrian hamster, mouse, and human. *1 PS: Djungarian hamster (*Phodopus sungorus*). *2 CG: Chinese hamster (*Cricetulus griseus*). *3 MA: Syrian hamster (*Mesocricetus auratus*). *4 MM: Mouse (*Mus musculus*). *5 HS: Human (*Homo sapiens*). *6 ▭: Signal peptide sequences. *7 ⬭: N-linked glycosylation site. *8 ©: Cysteine residue.

IFN-γ in the hamsters reported previously [4] were more similar to the structure of human IFN-γ than mouse IFN-γ. This difference in the sequence types between Th1 type cytokines such as IFN-γ, and Th2 type cytokines such as IL-10 might be associated with the difference of susceptibility to various pathogens. Interleukin-10 cloned from hamsters in this study will be usable as an immunological reagent for some infective experiments using hamsters.

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