NOTE  Immunology

Molecular Clonings and Sequences of Djungarian (Phodopus sungorus) and Chinese (Cricetulus griseus) Hamster Interferon-Gammab

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(Received 20 March 2003/Accepted 22 July 2003)

ABSTRACT. Djungarian (Phodopus sungorus) and Chinese (Cricetulus griseus) hamster IFN-γ genes were cloned and sequenced. The Djungarian and Chinese hamster genes were both 525bp nucleotides, resulting in 174 amino acids in full length with a predicted molecular weight (MW) of 19,560 dal and 19,775 dal, respectively. The first 23 amino terminal amino acids consisted of a hydrophobic signal sequence when cleaved, which would result in a mature 151 amino acid polypeptide with a predicted MW of 17,115 dal in the Djungarian hamster IFN-γ and 17,255 dal in the Chinese hamster one.

KEY WORDS: Chinese hamster, Djungarian hamster, Interferon-gamma.

Interferon-gamma (IFN-γ) was first identified in mitogen-activated lymphocyte supernatants as a distinctive antiviral activity [13]. IFN-γ can be produced either by CD4+ T cells in response to an antigen present in the MHC class II molecules or by cytotoxic T lymphocytes after recognition of an antigen associated with MHC class I [3]. In addition, NK cells also elaborate IFN-γ after exposure to TNF-α and microbial products [3]. IFN-γ which plays a major role in the generation and regulation of the immune response is the earliest detectable cytokine at the site of immunization with protein antigens and one of the Th1-specific cytokines that promote Th1 response and inhibit Th2 response [2].

Several species of hamster have been reported to be susceptible to some parasites [1, 4, 10, 11]. For example, Syrian hamsters (Mesocricetus auratus) are susceptible to Babesia microti [1] and Leishmania donovani [4], Chinese hamsters (Cricetulus griseus) susceptible to Acanthamoeba keratitis [11], and Djungarian hamsters (Phodopus sungorus) susceptible to Neospora caninum [10]. In particular, the Syrian hamster is highly susceptible to many intracellular parasites and has been used as an experimental animal for the isolation of a number of human pathogens [1]. The reason for this high susceptibility is unknown, and because of a lack of reagents, substantive molecular immunological studies of these models of infectious diseases have not been undertaken. It will be important that IFN-γ as a marker of Th1-specific cell growth is cloned and its gene is sequenced for monitoring the hamster’s immune response when infected with some pathogenic protozoa. In this study, we cloned the molecules and determined the nucleotide sequences of Djungarian and Chinese hamster IFN-γ, and compared them with those of other animals.

Ten-week-old Djungarian and Chinese hamsters were infected intraperitoneally with Babesia microti AJ strain (107/head). In 4 weeks after infection, these hamsters were necropsised, and their spleens were extracted. Their 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 and 50 µg/ml gentamicin (Wako, Japan) in a 5% CO2 atmosphere at 37°C in the presence of 10 µg/ml concanavalin A (Sigma, U.S.A.) for 24 hr prior to isolation of the RNA. Total RNAs were extracted with TRIZOL reagent (Gibco-BRL), and mRNAs were purified with an Oligotex-dT30 mRNA purification kit (Takara, Japan). First-strand cDNA synthesis was completed with a First-Strand cDNA Synthesis Kit (Amersham Biosciences Corp., U.S.A.). The primers for amplification of IFN-γ genes were designed from regions of homology found among the corresponding published mouse, guinea pig, rat, woodchuck, canine, bovine, caprine and human cDNA sequences. The sequences of the primers used to amplify IFN-γ cDNA are as follows: 5′ untranslated region (UTR), ATCAGYTRASTCCTTGGACC; 3′ UTR, CATCACA-GAAAAGTTGCTATC. Fragments of cDNA comprising the complete coding region for IFN-γ were amplified by polymerase chain reaction (PCR). The amplification products were cloned by ligation into the pCR2.1 plasmid (Stratagene, U.S.A.) and transformed into competent Escherichia coli INVαF’ cells according to the manufacturer’s instructions.

The DNA insert was sequenced with vector-specific primers and an automated, fluorescent DNA sequencer (SQ5500E; Hitachi, Japan). The resulting sequences were identified by a search of the NCBI databases for homologous sequences that used BLAST. Sequence comparisons were conducted with the Genetyx computer system (Software Development Co., Ltd., Japan), which makes optical alignment. The sequence and predicted amino acid sequence of Djungarian and Chinese hamster IFN-γ were compared with those of the Syrian hamster, gerbil, mouse, rat, woodchuck and human ones. The GeneBank accession numbers used in the sequence comparison were as follows: Syrian hamster IFN-γ, AF034482; gerbil IFN-γ, L37782; mouse IFN-γ, M28995;...
rat IFN-γ, X02325; woodchuck IFN-γ, Y14138, and human IFN-γ, M29383.

Nucleotide and predicted amino acid sequences of Djungarian and Chinese hamster IFN-γs are shown in Fig. 1. Those of Djungarian and Chinese hamster one were both 525 bp nucleotides and 174 amino acids in full length with a predicted molecular weight (MW) of 17,115 dal and 17,255 dal, respectively. The first 23 amino terminal amino acids consisted of a hydrophobic signal sequence, when cleaved, which would result in a mature 151 amino acid polypeptide with a predicted MW of 17,115 dal in Djungarian hamster IFN-γ and 17,255 dal in the Chinese hamster IFN-γ. The nucleotide and predicted amino acid sequence homologies of Djungarian and Chinese hamster IFN-γs are 90.9% and 87.9%, respectively. The nucleotide and predicted amino acid sequence homologies of Djungarian hamster and Syrian hamster, gerbil, mouse, rat, woodchuck and human IFN-γs are 94.1%: 90.2%, 77.9%: 67.2%, 74.4% and 56.7%, 76.5%: 59.2%, 71.3%: 53.9%, and 68.1%: 50.9%, respectively. Both sequence homologies of Chinese hamster and Syrian hamster, gerbil, mouse, rat, woodchuck, and human IFN-γs are 91.4%: 87.9%, 77.5%: 65.5%, 73.2%: 54.1%, 74.9%: 57.3%, 69.7%: 54.7%, and 68.1%: 50.9%, respectively. As for the nucleotide and amino acid sequence of each IFN-γ among three kinds of hamsters, they were conserved well.

Amino acids sequences of several animals' (Djungarian, Chinese, Syrian hamsters, gerbil, mouse, rat, woodchuck, and human) IFN-γs are shown in Fig. 2. The C-terminus which contains a polycationic 128–131 KRKR region in mature IFN-γ is conserved in all these species. The unique KRKR polycationic tail at the 3' end of the C-terminal α-helix is required for biological activity [9]. Removal of this region leads to a complete loss of activity of the respective mouse protein [7]. These hamster and gerbil sequences or woodchuck and human one has an additional 17 or 9 amino acids at the C-terminus, respectively, compared with the mouse and rat sequences [7]. Removal of the C-terminal 9 amino acids from the human IFN-γ protein was found to significantly enhance its antiviral activity [7], so it is thought that these additional residues sterically block the proximal residues from a strong interaction with the IFN-γ receptor. The biological activity may increase when the 17 amino acid additional residues in 3 hamster and gerbil IFN-γs are removed from the C-terminal. Therefore, they can contribute to the hamster's susceptibility to intracellular pathogens. His111 of the human protein lies within the C-terminal region and it is supposed to bind directly to the receptor [12]. Point mutation of this position leaves the protein inactive [8] but this position is not conserved in any of the animal species (see Fig. 2). The N-terminal part (1–31) and C-terminal part (95–133) of mouse IFN-γs are involved in receptor binding [5]. The N-terminal region is not better conserved among the different species than other regions of the molecule, supporting the assumption that this region is responsible for the species specificity [6]. Among IFN-γs of the three kinds of hamsters, both amino acid sequences of the N-terminal part (1–31) and the C-terminal part (95–133) are well conserved together in comparison with that of any one of the animals. Therefore, as for these IFN-γs, it is expected that binding to their receptors and biological activities resemble each other.

The molecular cloning and sequences of these cytokines will facilitate investigation of their antiparasitological role.

| Fig. 1. | Nucleotide and predicted amino acid sequences of Djungarian and Chinese hamster IFN-γ. | (A) Djungarian hamster IFN-γ | (B) Chinese hamster IFN-γ |
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in a natural animal system for several kinds of parasitic infection.

REFERENCES