Abstract  The human interleukin-4 (IL-4) gene produces an exon 2-lacking alternative splice variant, termed IL-4δ2, and described as a naturally occurring antagonist of IL-4-driven activity. We report the isolation of an IL-4δ2 cDNA from chimpanzee (Pan troglodytes) bone marrow samples and cynomolgus macaque (Macaca fascicularis) activated peripheral lymph node cells. The complete IL-4 cDNA sequence from chimpanzee is also provided for the first time. The phylogenetic analysis of several known IL-4 sequences revealed a highly conserved structure of coding regions among primates, suggesting that alternative IL-4 transcript splicing may be a process shared by other simian and potentially pro-simian species as well. Extension of the study to other mammalian species led us to the assumption that generation of IL-4 splice variants may be common to primates, lagomorphs (rabbit), and rodents of the sciuridae family (woodchuck), but is unlikely to occur in mice and rats (muridae), for which IL-4 splice variants have indeed never been described. Potential implications of alternatively spliced cytokine products with possible antagonistic or competitive inhibitory function, for the choice of suitable animal models of IL-4-regulated immune processes, are discussed. This study also indicates the importance of considering alternative splicing when defining cytokine bioassays, most particularly in the present context of transcriptomics, involving the generalization of sequence-based detection methods such as quantitative reverse transcription PCR.

Keywords  Interleukin-4 · Splice variant · Non-human primates · Animal model · Quantitative polymerase chain reaction

Introduction

Interleukin-4 (IL-4), alternatively described as B-cell, T-cell, and mast cell-growth stimulating factor (Farrar et al. 1983; Howard et al. 1982; Pure et al. 1983), is a key immunoregulatory cytokine playing a pivotal role in defining the Th2 polarization of immune responses. Secreted predominantly by activated T cells (Howard and Paul 1982), but also by B cells, mast cells (Brown et al. 1987), and basophils (MacGlashan Jr. et al. 1994; Paul 1991; Seder et al. 1991), IL-4 controls a broad spectrum of cellular functions. Its pleiotropic effects include co-stimulation of activated B cells, with increased expression of IgM, CD23, MHC class II, and IL-4 receptor (IL-4-R) on their surface (Defrance et al. 1987; Hudak et al. 1987; Renz et al. 1991; Roehm et al. 1984; Rousset et al. 1988; Shields et al. 1989), induction of immunoglobulin class-switch recombination in B cells from IgM to IgG4 and IgE (Lundgren et al. 1989), maintenance of T-cell proliferation (Mitchell et al. 1989; Severinson et al. 1987; Yokota et al. 1986) and their differentiation from the Tp0 toward the Th2 phenotype (Fernandez-Botran et al. 1986; Mosmann et al. 1986; Romagnani 1991); finally, IL-4 is also known to inhibit IL-2-induced interferon-γ production in natural killer cells, as well as IL-2-mediated cytotoxicity in LAK cells (Spits et al. 1988). Given its widespread effects, IL-4 appears to be involved in the
pathophysiological mechanisms of various diseases and immune affections, such as allergy (Barata et al. 1998; Robinson et al. 1993; Wierenga et al. 1990; Ying et al. 1994), certain autoimmune diseases (Miossec et al. 1994; Sakkas et al. 1999), and chronic infection associated with human leprosy and schistosomiasis (Yamamura et al. 1991; Zwingenberger et al. 1991).

The human IL-4 gene spans about 10 kb, and consists of four exons (Arai et al. 1989) encoding a 900-base mRNA (Yokota et al. 1988). An alternative splice variant of human IL-4, lacking exon 2 and termed IL-4δ2, has been described (Sorg et al. 1993). Originally identified in in vitro phytohemaglutinin-stimulated peripheral blood mononuclear cells, IL-4δ2 mRNA was also shown to be expressed in vivo in peripheral T cells (Alms et al. 1996) and lymphoid tissue (Klein et al. 1996), and was more recently detected in nasal and endobronchial biopsy specimens of asthmatic subjects (Atamas et al. 1996; Glare et al. 1999).

Skipping of exon 2 occurs without disruption of the IL-4 reading frame (Sorg et al. 1993). Although deletion of the 16-amino acid exon 2 product is thought to affect one of the two postulated binding sites of the cytokine to its receptors (Kruse et al. 1993; Powers et al. 1992), the binding ability of IL-4δ2 splice variant to IL-4-R molecules appears to be retained, albeit somewhat decreased compared with IL-4, thus making it a potential competitive inhibitor of IL-4/IL-4-R interaction. Several in vitro functional studies have provided evidence for a role of the δ2 isoform as a naturally occurring differential antagonist of IL-4 activity. Notably, IL-4δ2 was shown to inhibit IL-4-stimulated T-cell proliferation (Atamas et al. 1996), to block IgE synthesis in B cells, and to decrease CD23 expression on their surface. IL-4δ2 also antagonizes the inhibitory action of IL-4 on lипopolysaccharide-induced COX-2 expression and prostaglandin E2 secretion in monocytes (Arinobu et al. 1999).

Alternative splicing of IL-4 has not, to date, been reported in rodents – notably mice and rats – laboratory animals that are widely used as models in immunological and medical research. The aim of the present study was to investigate the possible origins and functional implications of alternative IL-4 message splicing in evolution, using both experimental as well as in silico – computer-based – research tools. Within this context, we sought a potential δ2 variant in non-human primates. IL-4δ2 transcripts were identified in bone marrow samplings from the chimpanzee as well as in peripheral lymph node cells from the cynomolgus macaque. Their complete cDNA sequences are reported in this paper, along with the previously undescribed IL-4 cDNA from chimpanzee. To our knowledge, this is the first report of an alternative IL-4 splice variant in non-human primates. Human and simian sequences are compared with those from other mammalian species within the context of a phylogenetic analysis of IL-4 message evolution. Implications of the present data for the study of type 2 cytokine-driven immunological processes, notably with respect to different cellular and animal models as well as detection methods used, are discussed.

Materials and methods

Cell separation and stimulation

Chimpanzee bone marrow mononuclear cells, cynomolgus macaque peripheral lymph node cells, and human peripheral blood lymphocytes were isolated by density separation over Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden), as described elsewhere (Tai et al. 2000). Cells from cynomolgus were stimulated for 24 h with 5 ng/ml phorbol myristate acetate and 5 µg/ml ionomycin (Sigma-Aldrich, Munich, Germany) in RPMI 1640 culture medium (Invitrogen, Carlsbad, Calif., USA) supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine (Glutamax, Invitrogen), and antibiotic/antimycotic agent (penicillin 10 units/ml, streptomycin 10 µg/ml, and amphotericin B 25 µg/ml for a x100 solution, Invitrogen). Aliquots of ~10^7 cells were collected by centrifugation, and pellets stored at −80°C in sodium dodecyl sulfate-containing lysis buffer (1 ml per sample, MicroFastTrack kit, Invitrogen) until further treatment.

mRNA isolation

mRNA extraction from activated simian peripheral lymph node cells was performed using the MicroFastTrack mRNA Isolation kit (Invitrogen), according to manufacturer’s instructions. Briefly, polyA-tailed mRNA was isolated from cell lysate samples via capture onto oligo-dT-cellulose, several wash steps, and final elution using supplied buffers. Purified mRNA was concentrated by ethanol precipitation in the presence of AcONH4 and using glycogen as a carrier, resuspended in nuclelease-free water and stored at −80°C until use.

RT-PCR detection of IL-4δ2 mRNA

δ2 transcripts were detected via real-time fluorescent PCR, using the LightCycler (Roche) technology. One-step RT-PCR was performed with the LightCycler RNA Master SybrGreen I kit (Roche Diagnostics, Meylan, France), according to manufacturer’s instructions, with Mn(OAc)2 brought to 3.25 mM final concentration and using 100 ng mRNA as starting material. PCR amplification was carried out with previously described human IL-4δ2-specific primers (Seah and Rook 1999): 1–3 exon junction-spanning forward primer: 5'-CAGAGCAGAAGA/ACACAACTG-3', exon 4 reverse primer: 5'-CGTCTTTAGCCTTTCCAAG-3', at a final concentration of 0.5 µM each. The following program conditions were applied: RT for 20 min at 61°C (slope 20°C/s), denaturation for 30 s at 95°C (slope 20°C/s), and 50 cycles of amplification. The cycling profile consisted of 1 s denaturation at 95°C, 8 s annealing at 65°C, with a second target temperature of 58°C (step size 0.5, step delay 1 cycle), and elongation for 12 s at 72°C. Slope was 20°C/s for the first two cycling segments, and 2°C/s for the last. Signal was acquired at 81°C, in order to exclude from quantification potential products of non-specific amplification. After completion of the last cycle, specificity of amplification was controlled via generation of a melting curve consisting of the following steps: initial denaturation at 95°C for 5 s, cooling to 65°C for 15 s and subsequent slow-heating, at a rate of 0.1°C/s, to reach a maximal temperature of 95°C, with continuous fluorescence acquisition. Serial dilutions of human IL-4δ2 RNA were assayed in parallel to serve as positive controls for RT-PCR-based splice variant detection, and were used to generate a standard curve for δ2 quantitation. Purified δ2 RNA was obtained via in vitro transcription, using the RibopMAX T7 large scale RNA synthesis kit (Promega, Charbonnières, France) with PCR-generated, T7 promoter-containing linear DNA templates. Quantitative and qualitative processing of generated data was achieved using the LightCycler Data Analysis software package.

Simian IL-4δ2 cDNA cloning

mRNA samples (100 ng) were reverse transcribed into single-strand cDNA with the Enhanced Avian RT First Strand Synthesis