Abstract Inducible costimulator (ICOS) is a novel receptor belonging to the same family as CD28 and CTLA4, which regulate T-lymphocyte activation in the immune response. The genes for these molecules are located adjacent to each other on Chromosome 2q33. Many autoimmune diseases have been found to be genetically linked to or associated with genetic markers near the CTLA4 gene. However, as all three genes are closely linked and have related functions, it is possible that the findings could be explained by variation in CD28 or ICOS. Few data on genetic variation in the ICOS gene are available. We sequenced the ICOS gene in 13 healthy unrelated individuals and found eight single nucleotide polymorphisms. One was located in the first intron, and the others in the untranslated region of the last exon. The allele frequencies and linkage disequilibrium were determined from a population sample of 63 Finnish individuals. The results show that the ICOS gene is polymorphic, but no variation in the coding sequence was detected, implying that the genetic linkage of this gene region to autoimmune diseases may not result from structural variation in the ICOS molecule. These polymorphisms, however, should be useful in genetic studies of this candidate gene.

Keywords ICOS · Polymorphism · T lymphocytes · Costimulation · Autoimmune diseases

Introduction

Activation of T lymphocytes requires an antigen-specific signal through the MHC-antigen complex and T-cell receptor, together with a costimulatory signal through B7 molecules and CD28 (Lenschow et al. 1996). CTLA4 (cytotoxic T-lymphocyte-associated antigen 4) is an important negative regulator of the immune response, competing with CD28 for the same B7 ligands (Walunas et al. 1994). Recently, a third member of this family of co-stimulatory molecules was found. Inducible costimulator (ICOS) has significant amino acid homology with CD28 and CTLA4 (Hutloff et al. 1999), and binds to GL50 (also known as B7-H2), a molecule related to the B7-1 and B7-2 ligands of CD28 and CTLA4 (Ling et al. 2000). Unlike the constitutively expressed CD28, ICOS is induced on the T-cell surface upon cell activation. The role of costimulation by ICOS in the immune response remains to be elucidated. In addition to promoting the Th2 phenotype and antibody responses, a critical role in the differentiation and effector function of Th1 cell type has also been suggested (Sperling and Bluestone 2001).

The ICOS gene has been mapped to Chromosome 2q33, adjacent to the genes for CD28 and CTLA4 (Coyle et al. 2000), and contains 5 exons and 4 introns, spanning about 20 kb (Ihara et al. 2001). This gene region has been linked to and associated with several autoimmune type of diseases, including, for example, type 1 diabetes, autoimmune thyroid diseases, multiple sclerosis, and coeliac disease (Kristiansen et al. 2000). Although all of these genes are plausible functional candidates in autoimmune diseases, the studies so far have focused mostly on the known CTLA4 polymorphisms. Except for the recently reported two microsatellites in the fourth intron (Ihara et al. 2001), no data on ICOS polymorphisms are available. In the present study we systematically screened the ICOS gene for genetic variation and identified eight novel single nucleotide polymorphisms. The allele frequencies and linkage disequilibrium (LD) between the polymorphisms were determined in a larger sample.
Materials and methods

Subjects

Citrate anticoagulated blood samples were obtained from 13 healthy Finnish volunteers who were unrelated. DNA samples of 50 unrelated Finnish cadaver organ donors were genotyped for the polymorphisms.

Cell preparation and T-cell activation

Peripheral blood mononuclear cells were isolated by Ficoll-Paque (Amersham Pharmacia Biotech AB, Sweden) gradient centrifugation. The cells were stimulated for 24 h with anti-hCD28 mAb (1 µg/ml) and with plate-bound anti-hCD3 mAb (R&D Systems, USA).

Isolation of RNA and cDNA synthesis

Total RNA was isolated from activated cells using RNAzol B-solution (Wak-Chemie Medical, Germany) according to the manufacturer’s directions. Complementary DNA was prepared using OmniScript RT-PCR kit (Qiagen, Germany), following the manufacturer’s instructions. ICOS cDNA was PCR-amplified with ICOS-specific primers A and B (Fig. 1). Following an initial DNA polymerase activation step for 15 min at 95 °C, cDNA was amplified by 35 cycles of 94 °C for 1 min, 61 °C for 1 min and 72 °C for 2.5 min, followed by a final elongation at 72 °C for 10 min. The PCR reaction, in a final volume of 50 µl, consisted of 1 µg of cDNA, 10×PCR buffer with 15 mM MgCl₂ (Qiagen), 25 pmol each of ICOS-specific primers A and B (Fig. 1), 0.2 mM dNTPs (Amersham Pharmacia Biotech Inc., N.J., USA), and 1.25 units of HotStarTaq DNA polymerase (Qiagen). The ICOS cDNA was purified with QIAquick PCR Purification Kit (Qiagen) according to the manufacturer’s instructions.

Sequencing

At the time of the study, genomic reference sequences were available in public databases for exon 1 (clone AQ680857) and the 3’ end of the last exon (clone AC009965). These regions, together with 250 bp of intron 1, were sequenced from genomic DNA (Fig. 1). The remaining regions of the exons were sequenced from a 2.3-kb fragment of cDNA in six partially overlapping fragments, using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.) and ABI PRISM 310 sequencer (Applied Biosystems). Some samples were sequenced by the SEQLAB Sequence Laboratories in Göttingen, Germany.