Molecular cloning of the CD3ε subunit of the T-cell receptor/CD3 complex in dog

Richard A. Nash, Uwe Scherf, and Rainer Storb

Clinical Research Division, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104, USA

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The T-cell receptor (Tcr)/CD3 complex of T lymphocytes consists of either a Tcr α/β or Tcr γ/δ heterodimer coexpressed at the cell surface with the invariant subunits of CD3 labeled γ, δ, ε, ζ, and η (Samuelson et al. 1985; Baniyash et al. 1988). Recognition by the T-cell receptor of an antigen presented in a major histocompatibility complex (MHC)-restricted fashion results in T cell activation (Weiss et al. 1986). There is some suggestion that the CD3 portion of the complex mediates signal transduction as perturbation of the Tcr/CD3 complex by monoclonal antibodies to CD3 can mimic the effects of antigen and activate T cells (Imboden and Stobo, 1985). The CD3ε subunit on human T cells is an M, 20000 non-glycosylated peptide that is essential for the cell surface expression of the Tcr/CD3 complex. The contribution of the CD3ε peptide chain to the function of the complex is currently unclear as deletion of the cytoplasmic domain does not prevent T cell activation (Transy et al. 1989a).

The CD3ε subunit is the most immunogenic part of the CD3 portion of the complex, so most anti-human CD3 monoclonal antibodies (mAb) are directed toward these epitopes (Transy et al. 1989b). mAbs directed against CD3ε epitopes can either inhibit or trigger signal transduction leading to T cell activation. In clinical trials, CD3-specific mAbs have been shown to offer a significant new approach to immunosuppressive therapy (Todd and Brogden 1989). After in vivo administration, there is depletion of peripheral T cells as well as modulation of the cell surface Tcr/CD3 complex. Modulated cells reversibly lose the expression of the Tcr/CD3 complex and become functionally incompetent. To further the in vivo studies of the properties of CD3 in the dog, which has been a valuable animal model for solid organ and marrow transplantation, the cDNA for the CD3ε subunit was cloned. The authors are willing to share the cDNA clone described in this report.

To obtain a T-lymphocyte enriched population, peripheral blood and splenic mononuclear cells were isolated from a Ficoll-Hypaque gradient. They were then cultured for 48 h in RPMI 40 supplemented with 5% fetal calf serum (FCS) in the presence of 1% phytohemagglutinin (PHA) and 5% pokeweed nitrogen. RNA was extracted on a cesium chloride gradient (Maniatis et al. 1982). A cDNA library in a Lambda Zap cloning vector (Stratagene, La Jolla, CA) was constructed and packaged (Short et al. 1988). Over 1 000 000 plaques from the library were screened using an amplified gene product [polymerase chain reaction (PCR)] of the more highly conserved transmembrane and cytoplasmic region from the mouse CD3ε cDNA as a probe. Probes were radiolabeled with 32p deoxycytidine triphosphate using random hexamers (Feinberg and Vogelstein 1983). Hybridization was performed in a buffer containing 35% (V/V) formamide at 37°C overnight. Nylon filters were washed at room temperature in 2× saline sodium phosphate-EDTA (SSPE), 0.5% sodium deoxycholate (SDS) and then 0.5× SSPE for 20 min each. Plasmids [pBluescript SK (–)] containing the canine CD3ε inserts were subcloned from the Lambda Zap phage by an in vivo excision process in the presence of helper virus (Short et al. 1988). Clones were sequenced in pBluescript SK (–) using the dideoxynucleotide method (Sanger et al. 1977) as described in the Sequenase (US Biochemicals, Cleveland, OH) protocol for double-stranded DNA. All pBluescript SK(–) cDNA clones were initially sequenced from the flanking T3 and T7 primers. Further specific primers for the insert were constructed to enable sequencing of both strands. Sequence analysis was done with Genepro Version 4.2 software (Riverside Scientific Enterprises, Bainbridge Island, WA).

Three unique clones were isolated from the dog cDNA library of 3×10⁶ plaque forming units and further characterized by sequencing. The open-reading frame contained 606 base pairs (bp) from the start codon.
“ATG” to the stop codon “TGA” (Fig. 1). The nucleotide sequence homology of the coding region between dog and both human and mouse is 68% and 65% respectively. The 5' untranslated sequence is 239 bp in length. The polyadenylation signal (AATAAA) is 558 bp 3' to the termination codon. In the predicted amino acid sequence of the dog CD3e subunit there is a 22 amino acid stretch of typical sequences so that the mature peptide would have an aspartic acid residue at the first position of the amino terminus. The mature peptide of mouse and human also have an aspartic acid in the first position. There are four cysteines in the extracellular portion of the mature peptide at positions 54, 96, 114, and 117 which are conserved in all three species. The presence of the negatively charged aspartic acid residue in the transmembrane may help stabilize interactions in the Tcr/CD3 complex and is conserved in the same position of the canine CD3e peptide, as I noted in human and mouse CD3e peptides. As in other species, the amino acid sequence of canine CD3e subunit would predict that it is non-glycosylated because of the absence of the target asparagine residues in proper association with serine or threonine (Asn-X-Ser or Asn-X-Thr).

A comparison of predicted amino acid sequences of CD3e subunits from dog, human, and mouse is summarized (Fig. 2). Overall homology of one species form of the CD3e subunit to the other two species is 58% each. A hydrophobic region typical of the transmembrane portion of a cell surface peptide chain like the CD3e subunit is highly conserved and is similar to the transmembrane region of γ and δ subunits of CD3. The hydrophilic cytoplasmic tail is also highly conserved between species but quite different than the other CD3 subunits -γ and δ. Conservation of sequence of the cytoplasmic tail over an significant phylogenetic distance suggests a strong selective pressure which could be some critical function especially in the signal transduction process. The N-terminal extracellular domain of the mature peptide is less conserved than the transmembrane or cytoplasmic domains. The function of the extracellular domain is currently unclear. The significant difference of the CD3e extracellular domains from the three species that have been described would suggest difficulty in developing cross-reacting antibodies between species.

The CD3ε gene has been described as part of the immunoglobulin (Ig) superfamilly (Gold et al. 1987). It has been shown that members of this family have evolved from a primordial gene encoding about 100 amino acids (Hill et al. 1966). In the Ig-like region of the human sequence of CD3ε there is a nonapeptide that is almost identical to the repetitive sequences and, in this region, they are shorter than human by the size of one repeat (Fig. 2). The absence of repetitive sequences in mouse and dog sequences minimize alignment.