Nucleotide sequence of ovine interleukin-1 beta

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The interleukins have been the subject of intense research interest because of their central role in the coordination of the processes of immune cell growth and differentiation, inflammation and responses to antigenic stimulation. Central to an understanding of these processes has been the cloning of the genes encoding these mediators. We have used PCR to obtain 3 overlapping clones which together constitute the ovine IL-1β c-DNA.

When ovine alveolar macrophages were stimulated with 100 ng/ml lipopolysaccharide from *Salmonella abortus equi* for 4 hours they produced IL-1 like activity as measured by a thymocyte co-proliferation assay. Total RNA was extracted from these cells and enriched for polyA$^+$ RNA by oligo dT-cellulose chromatography. cDNA synthesised from this RNA by the method of Gubler and Hoffman (1) was hybridised to primers chosen from sequences conserved in bovine (2) and human (3) IL-1β genes (nucleotide numbers 171−190 and antisense of 690−710 of the bovine gene). The cDNA was amplified by PCR to produce a central segment of the ovine gene. This segment was sequenced and from this sequence further primers were chosen for amplifying 5′ and 3′ regions of the gene. A similar cDNA population was tailed with dATP using terminal transferase, (so that both sense and antisense strands ends would have polyA tracts) and IL-1β molecules in it amplified using anchored PCR with a new primer (antisense of nucleotide numbers 297−314 of the ovine sequence) and oligo dT to generate the 5′ end. PCR with a further pair of primers (nucleotides 457−474 of the ovine sequence, and antisense of 995−1011 from the bovine 3′ untranslated region) was used to generate the 3′ end. (Anchored PCR using oligo dT did not give a full length product at the 3′ end). The 3 PCR products were cloned into pTZ18R expression vectors and several clones of each type sequenced using the dideoxy chain termination method with T7 DNA polymerase (Sequenase II, USB Ltd). The clones obtained encode an ovine IL-1β sequence consisting of 32 bp of 5′ leader sequence, an 801 bp coding sequence and 145 bp of 3′ untranslated sequence. Comparison of this sequence with bovine and human IL-1β shows similarities of 95.3% and 76.1% respectively at the nucleic acid level, or 84.9% and 57.5% at the protein level.

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