Sequence of rat interleukin 2 and anomalous binding of a mouse interleukin 2 cDNA probe to rat MHC class II-associated invariant chain mRNA

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Interleukin 2 (IL-2) is a lymphokine which is produced by T-helper cells following activation by antigenic or mitogenic stimulation; IL-2 is required for their subsequent proliferation and thus plays a key role in the initiation of immune responses (reviewed in Smith 1988). IL-2 has been cloned and sequenced in a number of species, but not in rat (Taniguchi et al. 1983, Yokota et al. 1985, Cerretti et al. 1986). We now report the isolation and sequence of a rat IL-2 cDNA clone and the characterization of a cross-reaction to rat class II major histocompatibility complex (MHC)-associated invariant chain mRNA observed when using a mouse IL-2 cDNA probe to analyze rat mRNA.

Rat spleen cells (2 x 10^6 cells/ml) were stimulated with concanavalin A (ConA) at 5 μg/ml in RPMI/2% fetal calf serum (FCS) for 3 days, washed and resuspended in fresh culture medium, and restimulated with ConA (5 μg/ml) plus phorbol myristate acetate (PMA; 10 ng/ml) for 3 h. A similar procedure induces high levels of IL-2 mRNA in human mononuclear cells (Granelli-Piperno et al. 1986). A cDNA library was constructed in the pATX vector (Barclay et al. 1987) and screened with a 406-bp Hind III-Acc I fragment from pcD-IL-2 (Yokota et al. 1985) that corresponds to the 3' portion of mouse IL-2. One full-length rat cDNA clone (pRIL-2.8) was sequenced completely (Fig. 1). The deduced amino acid sequence was very similar to that of IL-2 from other species (Fig. 2), except that pRIL-2.8 did not contain the unusual CAG repeating sequence that codes for a stretch of 12 consecutive glutamine residues that so far has been found only in mouse IL-2. It seems likely that this extra sequence has somehow been captured by the mouse gene in a recent event.

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the accession number M22899. Address correspondence and offprint requests to: A. N. Barclay.
Fig. 1. Sequence of the rat IL-2 cDNA clone. The initiation site codon, termination codon, and polyadenylation signal are shown in upper case letters. The final two adenosines are presumed, from their position, to be the beginning of the poly(A) tail. Plasmid cDNA inserts from clone pRIL-2.8 were subcloned into M13 and sequenced by the dideoxy chain termination method using T7 DNA polymerase (Pharmacia, Uppsala, Sweden). This sequence has been submitted to EMBL/GenBank datalibraries under accession number M22899.

Fig. 2. Comparison of rat IL-2 protein sequence. The rat protein sequence, deduced from the cDNA sequence above, is compared with sequences for mouse IL-2 (Yokota et al. 1985) and human IL-2 (Taniguchi et al. 1983). Residues identical in two or three species are boxed. Gaps introduced to maximize homology are indicated by a dash (−). The arrow indicates the signal sequence cleavage site.

Fig. 3a–c. Northern Blot analysis of lymphoblast RNA. A single Northern blot was prepared with RNA from the following sources: rat liver (lane 1); uninduced mouse EL-4 cells (lane 2); PMA-induced EL-4 cells (4 h PMA, lane 3; 8 h PMA, lane 4); GvH rat spleen (lane 5); and PHA/PMA-induced rat lymph node cells (lane 6). The blot was probed, stripped, and reprobed with the following probes: (a) A 5′-proximal Pst I-Hind III mouse IL-2 cDNA probe detecting mouse IL-2 mRNA from EL-4 cells (lanes 3 and 4) and rat invariant chain mRNA (lanes 5 and 6); (b) A 3′-proximal Hind III-Acc I mouse IL-2 cDNA probe which specifically detects IL-2 mRNA from the EL-4 cells; (c) A full-length rat invariant chain cDNA probe which detects rat invariant chain mRNA, but not the mouse IL-2 mRNA. Ten micrograms of total cellular RNA, or 5 µg of poly (A)+ RNA, were fractionated on a 6% formaldehyde, 1.2% agarose gel and transferred to GeneScreen Plus (New England Nuclear, Boston, Massachusetts). The probes were labeled by random hexanucleotide priming using [α-32P]dATP, and hybridizations were performed according to the manufacturer’s instructions. Following respective hybridizations, the filter was washed as follows: (a) 1× SSC/1% sodium dodecyl sulfate (SDS) at 60 °C; (b) 2× SSC/1% SDS at 65 °C; (c) 0.1× SSC/1% SDS at 65 °C.