Addendum

This is an addendum to the February 2000 article by Apostolopoulos et al., "Ly6d-L, a Cell Surface Ligand for Mouse Ly6d."

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Since our recent publication on the cloning of Ly6d-L (Apostolopoulos et al., 2000), Dr. Brendan Classon, Department of Pathology and Immunology, Monash Medical School, has brought to our attention the results of his search of the nucleic acid database with the Ly-6d-L DNA sequence, which should be brought to the attention of readers of *Immunity*. The results of Classon's search revealed a striking homology between the *antisense* of a consensus sequence of Ly6d-L and the hypothetical human homolog of the yeast GTPase activator protein, GYP-7 (accession number AL157464). We did not report this homology as the sequence was deposited after our manuscript was in press; however, the nucleotide sequence we reported has been verified from several clones and does not contain additional nucleotides used by Classon to arrive at the consensus sequence. Classon's assertion is that our published data must be incorrect and that the cDNA insert is transcribed in the reverse orientation and that mouse GYP-7 translated in our reported experiments. We can state that this is not the case for the following reasons:

(1) The expression cloning system used to isolate the cDNA (Aruffo and Seed, 1987; Seed and Aruffo 1987) allows the correct assignment of correct strand for translation, as transcription of the cDNA insert is driven by a strong promoter within the plasmid. The sequence reported in Apostolopoulos et al. (2000) is in the orientation reported as the original sequencing was performed using oligonucleotides with sequences flanking the 5′ cloning site, as well as the reverse sequence at the 3′ end. This identified the longest open reading frame commencing with a Met as reported.

(2) The Ly6d-L open reading frame was reengineered in the expression vector to include the FLAG tag sequence at either the amino-terminal or carboxyl-terminal end of the predicted protein. These inserts were sequenced in both directions, and both of these led to cell surface expression of a FLAG tagged protein on the surface of COS cells after transfection, as shown by cell surface staining (Figure 4) and immunoprecipitation from the cell surface following biotinylation (Figure 7). If the insert were being transcribed in the reverse orientation and then translated, the FLAG sequence would not be expressed.

How then can our data be reconciled with the information of the striking homology with the human homolog of GYP-7? While Classon suggested to us that unknown mechanisms produce antisense transcripts and translation of a truncated form of GYP-7, an intriguing possibility that merits further study is that two proteins are indeed translated from the one genomic segment of DNA: one strand gives rise to Ly6d-L and the other the mouse GYP-7 homolog. Such genes are known to occur in viruses, and there is now evidence for at least three eukaryotic genes being transcribed and translated in such a manner: c-erbAα/Rev-ErvAα (Lazar et al., 1989; Miyajima et al., 1989), Na/phosphate cotransporter (Huelseweh et al., 1998), and basic FGF (Kimelman and Kirschner, 1989; Li et al., 1996; Knee et al., 1997). While we have no direct evidence of such a mechanism existing for Ly6dL, the analysis of the genomic DNA, the production of antibodies to Ly6d-L, and RNA analysis using single-stranded cDNA should address this possibility.

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In searching the databases for significance, the only sequence we found worthy of note was with one EGF-like repeat of MOTCH, which we used for comparison (Reaume et al., 1992). The significance of this low homology is that known ligands of several other Ly-6 family members contain "EGF-like motifs" and this was discussed in our manuscript (Apostolopoulos et al., 2000). We considered a 33% amino acid identity over 57 residues for a 76 amino acid protein to merit some discussion; however, Dr. Classon is correct in pointing out to us that only three Cys residues of six are conserved and there is therfore only a weak sequence similarity between Ly6d-L and a single EGF domain of MOTCH—the mouse homolog of NOTCH. Whether Ly6d-L does indeed contain an EGF-like structure must await structural determination by X-ray diffraction or NMR.

Selected Reading

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