The cloning and characterization of human MyD88: a member of an IL-1 receptor related family

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Abstract Murine MyD88, an RNA with homology both to the interleukin-1 receptor signaling domain and to 'death-domains', is rapidly upregulated during differentiation of the myeloleukemic cell line M1. We have cloned the human homologue of murine MyD88 and re-evaluated the murine sequence. The open reading frame for both species encodes a 296 amino acid protein, which for murine MyD88 is 53 amino acids longer than originally published. Human MyD88 cDNA is encoded by 5 exons, and maps to chromosome 3p21.3-p22 by fluorescence in situ hybridization (FISH). Overexpression of the death domain region leads to transcriptional activation of the IL-8 promoter.

Key words: Interleukin 1 receptor; Death-domain; Chromosome 3p

1. Introduction

Several proteins are known to share sequence homology with the type I interleukin 1 receptor (IL-1R) [1]. This homology either extends throughout the entire open reading frame, as is the case in T1/ST2 [2], IL-1Rrp1 [3] and IL-1RAcP [4], or alternatively is localized to the IL-1R cytoplasmic segment and the comparable domain of the homologue: rsc786 [5], Toll [6,7], 18-Wheeler [8], and MyD88 [9]. Signal transduction experiments have been performed using chimeras in which the cytoplasmic region of either T1/ST2 or IL-1Rrp1 has been fused to the extracellular and transmembrane regions of the type-I IL-1R, demonstrating that the T1/ST2 and IL-1Rrp1 share functional as well as sequence homology with the IL-1R [1,3].

MyD88 was identified as a myeloid differentiation primary response gene activated in murine M1 myeloid precursors following IL-6 induced terminal differentiation [9]. It differs from the other IL-1R related proteins in that the deduced protein sequence predicts a soluble cytoplasmically expressed protein. Here we describe the cloning of the human homologue of MyD88 and provide a preliminary characterization of this IL-1R related protein.

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Abbreviations: IL-1, interleukin 1; IL-6, interleukin 6; IL-1R, type I interleukin 1 receptor; IL-1Rrp1, IL-1 receptor related protein 1; IL-1RAcP, IL-1 receptor accessory protein; rsc786, randomly sequenced cDNA 786; PCR, polymerase chain reaction; TNF, tumor necrosis factor

The nucleotide sequences reported in this paper have been submitted to the Genbank/EMBL Data Bank with accession numbers U84408 and U84409.

2. Materials and methods

2.1. Northern blot analysis

RNA samples (2.5 μg total RNA, or 0.5 μg poly(A)$^-$ for IMTLH) were electrophoresed, transferred to nylon membranes and hybridized as previously described [10] to antisense riboprobes made from the murine MyD88 cDNA. Evenness of loading was monitored by staining the filters with methylene blue prior to hybridization.

2.2. Exon mapping

The intron positions within the coding region of human MyD88 were determined by PCR amplification of human genomic DNA using sets of sense and antisense oligonucleotides based on the cDNA sequence. The resultant products, where differing in size from that obtained using cDNA as a template, were cloned into pBluescript (Stratagene) and sequenced.

2.3. Fluorescence in situ hybridization (FISH mapping)

Chromosomal mapping was performed on metaphase spreads from normal human lymphocytes prepared according to the methods of Fan et al. [11]. Genomic DNA comprising the majority of the coding region and the 4 introns was labeled with biotin-16-dUTP (Boehringer Mannheim) by nick translation. Fluorescence in situ hybridization and detection of immunofluorescence were performed according to the technique of Pinkel et al. [12] with minor modifications [13]. The chromosome preparations were stained with both diamidino-2-phenylindole and propidium iodide (Oncor) [11] and observed with a Zeiss Axiophot fluorescence microscope. Hybridization of the 1.83 kb genomic probe to human chromosomes revealed specific labeling on chromosome 3 at sub-band 3p21.3-p22.

2.4. MyD88 expression constructs

Mammalian expression constructs of human MyD88 were prepared in the plasmid pDC304, a variant of pDC302 [14], and encoded either the full length (residues 1–296, MAAGG...ALSLP), N-terminal half (residues 1–151, MAAGG...ITTLD), or C-terminal half (residues 135–296, DSSVP...ALSLP) of MyD88. The C-terminal construct contained an artificially introduced start methionine.

2.5. IL-8 promoter activation assay

COS7 cells (1×10^5 cells per well in a 12-well tissue culture plate) were cotransfected with 500 ng of the appropriate test DNA and 1500 ng of the pIL8p reporter plasmid ([1] and T.P.B., unpublished data). This reporter consists of nucleotides −130 to +44 of the human IL-8 promoter fused to the IL-2Rα chain coding sequence. 2 days post-transfection the cells were washed twice with binding medium containing 5% (w/v) non-fat dry milk (5% MBM) and blocked with 2 ml 5% MBM at 37°C for 30 min. Cells were then incubated at room temperature for 60–90 min with 1.5 ml/well of 5% MBM containing 1 μg/ml of the mouse anti-IL-2Rα antibody 2A3 [15] with gentle rocking. Cells were washed once with 5% MBM and incubated with 1 ml/well of 5% MBM containing 1:100 dilution of ^{125}I-goat anti-

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mouse IgG (Sigma) for 60 min at room temperature. Wells were washed four times with 5% MBM and twice with PBS. Wells were stripped by the addition of 0.5 M NaOH, and total counts were determined.

3. Results

Northern blot analysis using an antisense riboprobe revealed that MyD88 mRNA is expressed in a variety of human and murine tissues albeit at varying levels (Fig. 1). Murine cells and tissues appear to contain a single RNA species at ~2.2 kb. The apparent size of the murine mRNA is at odds with that previously reported [9]. In the myeloleukemic cell line M1, this message appears upregulated in response to IL-6. Human cell lines and tissues contain two hybridizing RNA species (~1.6 and 3 kb). The ratio of each species varies between cell types.

To isolate cDNA clones of human MyD88, we screened a λ gt10 cDNA library prepared from the KB epidermoid carcinoma cell line, using a single stranded DNA probe derived from the murine sequence. Sequence analysis of the cDNA clones revealed considerable homology of the partial human sequence with the proposed 5′ UTR of the published murine sequence. In order to determine the correct initiating methionine of the human and murine mRNAs, we performed 5′ RACE (rapid amplification of cDNA ends) on total RNA isolated from human KB cells and from IL-6 stimulated murine M1 cells (Fig. 2).

This analysis extended the open reading frame of murine MyD88 another 159 nucleotides (53 amino acids) upstream of that indicated in the original publication [9], to a methionine which is also conserved in human MyD88 mRNA. In the human sequence, an additional in-frame ATG is present in a proportion of the mature mRNA transcripts identified by the 5′ RACE technique (Fig. 2); this methionine is not conserved in murine MyD88 mRNA. It is unknown whether this additional ATG is utilized in vivo.

The homologous regions of murine and human MyD88 cDNA, beginning at the shared methionine, predict a protein of 296 amino acids (Fig. 3). No signal peptide or transmembrane regions are predicted, indicating that both proteins are soluble cytoplasmic proteins, with a theoretical molecular mass of 33.2 kDa. Human MyD88 shares 81% overall amino acid identity with murine MyD88, and the two proteins are of greatest identity (93%) in the C-terminal half of the protein.

As previously determined from murine MyD88 [1,16,17], human MyD88 contains an ~150 amino acid C-terminal region which shows homology to the type I IL-1R cytoplasmic domain (21% identity/51% similarity). In addition, the N-terminal ~100 amino acids contains a region that has similarity to the 'death-domain' of FAS and TNF p55 receptor cytoplasmic regions [18]. The gene encoding human MyD88 was mapped to chromosome 3p21.3-p22 (data not shown; see Section 2).

The positions of introns within the human MyD88 locus were mapped by PCR amplification of human genomic DNA using oligonucleotides derived from the human cDNA sequence. The coding region of human MyD88 contains 4 introns (Fig. 3). The location of the second intron, near the start of the region homologous to the IL-1R cytoplasmic domain, occurs at a position similar to that of an intron in the human IL-1 receptor [19].

The effects of MyD88 over-expression were examined in COS7 cells transiently transfected with a mammalian expression construct containing either full-length, N-terminal (residues 1–151) or C-terminal (residues 135–296) human MyD88 (Fig. 4). Cells were co-transfected with a reporter construct containing the IL-2Rα gene under the transcriptional control of the human IL-8 promoter [1]. This region contains the binding sites for the transcription factors AP-1, NFIL6 and NFκB, and therefore surface expression of IL-2Rα, which can be measured by a radiolabelled antibody directed to the receptor, is presumably proportional to the level of activation of some or all of these transcription factors.

Over-expression of full length MyD88 produced a dramatic (12-fold) increase on the level of transcription from the IL-8

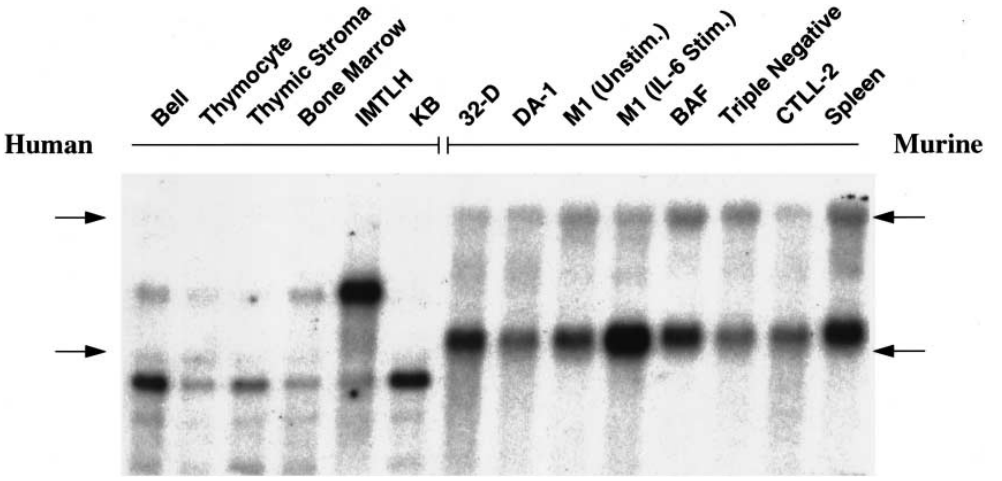

Fig. 1. RNA analysis of MyD88 expression. RNA from different human or murine sources was probed with an antisense riboprobe from the murine MyD88 cDNA. The arrows represent the positions of the 28S and 18S rRNA. Human RNA sources were: Bell (B-cell line), thymocyte (primary cells), thymic stroma (primary cells), bone marrow (primary cells), IMTLH (bone marrow stroma cell line), and KB (epidermoid carcinoma cell line). Murine RNA sources were: 32-D (early myeloid cell line), DA-1 (myeloid leukemic cell line), M1 (myeloleukemic cell line) prepared from untreated cells, or from cells treated with 10 ng/ml human IL-6 for 24 h, BAF (pre-B-cell line), triple negative ($Thy1^+$ $CD3^-$ $CD4^-$ $CD8^-$ fetal liver cells), CTLL (T-cell line), spleen (total primary splenocytes). The blot shows low level non-specific hybridization of murine riboprobe to murine 28S rRNA.

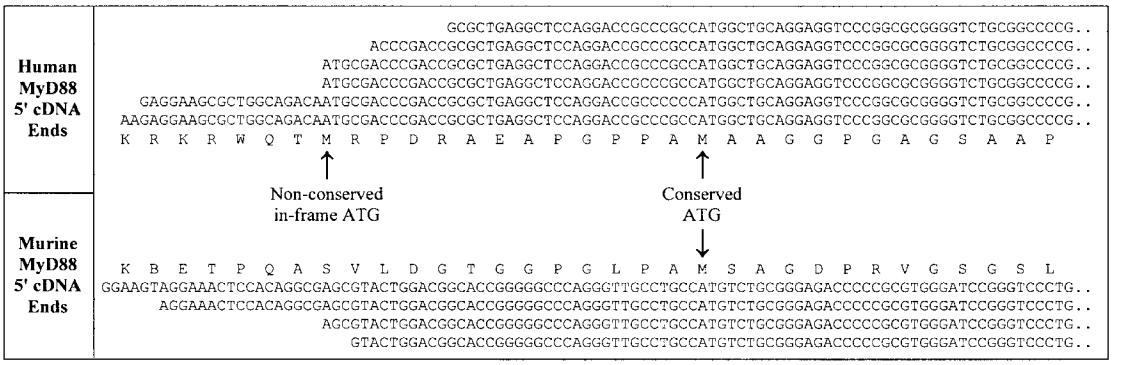

Fig. 2. Analysis of mRNA ends by 5′ RACE. The nucleotide sequences obtained by 5′ RACE (rapid identification of cDNA ends) performed on 1 μg total RNA from human KB cells, or IL-6 stimulated murine M1 cells, using a Clontech kit according to the manufacturer's protocol. The deduced protein sequence is also shown. The conserved ATG is assumed to encode the initiator methionine in the human mRNA.

promoter. The same magnitude of IL-8 promoter activation was produced in cells over-expressing the N-terminal domain of MyD88. In contrast, over-expression of the C-terminal domain produced only a modest increase in IL-8 promoter activation (3-fold). By comparison, COS7 cells transfected with empty vector, and then stimulated with 10 ng/ml human IL-1α, produce only a 7.5-fold increase in IL-8 promoter activation, by signaling through endogenous IL-1 receptors. A maximal response in this assay (obtained for example, by IL-1α stimulation of COS cells over-expressing transfected IL-1 receptors) is typically 20–30-fold stimulation (T.P.B., unpublished observations).

4. Discussion

We have cloned the human MyD88 transcript, and re-examined the murine MyD88 cDNA, and determined that the MyD88 protein is highly conserved in the two species as well as being 53 amino acids larger than originally reported. The new amino acids extend the region of homology to the death domains of the death-associated protein kinase (DAP-Kin), a member of the death-domain family typified by FAS and TNF p55 receptors. They do not, however, appear to code for a signal peptide and/or a transmembrane region. MyD88 remains unique among proteins homologous to the IL-1 receptor in being a cytoplasmic rather than a transmembrane protein.

Despite the presence of a death-domain in the N-terminal portion of MyD88, overexpression of full-length or partial MyD88 constructs in mammalian cells does not appear to induce apoptosis. In reporter assay experiments, however, over-expression of the full-length MyD88, or of the death-domain containing N-terminal region, does induce an up-regulation of transcription from the IL-8 promoter. This upregulation is specific, in that overexpression of other proteins in the same vector, including the C-terminal portion of MyD88, does not lead to an increase in IL-8 promoter driven transcription (this manuscript; T.P.B. and J.S., unpublished data). In studies of signal transduction by the TNF p55 and FAS receptors, death-domains have primarily been characterized in terms of mediating protein-protein interactions, rather than for their effects on transcription.

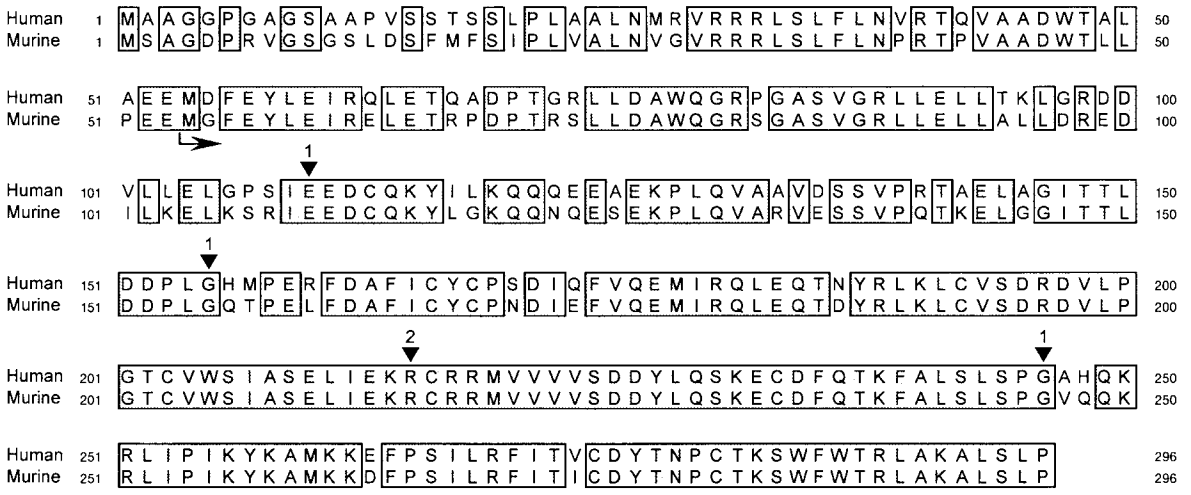

Fig. 3. Amino acid sequences of human (top) and murine (bottom) MyD88, as deduced from the cDNA clones. Identities are indicated by the boxed and shaded regions. The human sequence shown is translated from the ATG conserved in both murine and human forms. The sequence contains no predicted signal peptide or transmembrane regions. The published translational start of the murine protein is indicated by an arrow. The positions of the 4 introns within the coding region of the human sequence are indicated by filled triangles. The number above the triangle denotes the position within the codon that the intron occurs. The 'death-domain'-like region extends through residues 39–105; the IL-1R cytoplasmic domain homology extends through residues 155–296.

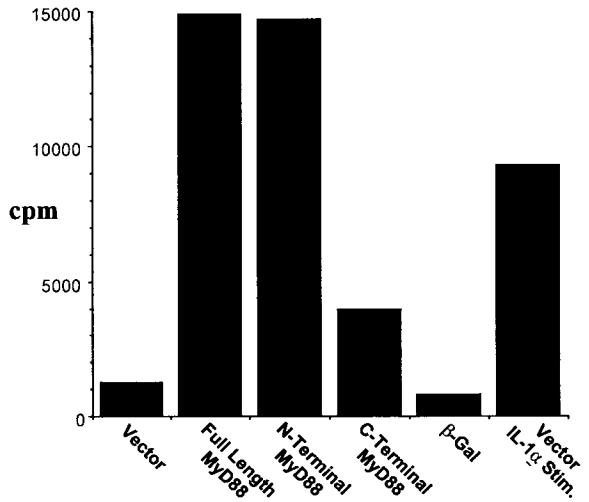

Fig. 4. Expression of human MyD88 induces transcription from the IL-8 promoter. COS7 cells were transfected with the indicated expression plasmids, together with a reporter plasmid containing the IL-8 promoter driving expression of the IL-2Rα chain cDNA. After 48 h, the level of surface IL-2Rα was determined by the binding of a murine anti-IL-2Rα antibody, followed by ^{125}I-goat anti-mouse IgG serum. The 'Vector/IL-1α Stim.' sample was incubated with 10 ng/ml human IL-1α for 16 h prior to performing the assay.

MyD88 was originally identified as a differentiation marker in murine myeloid precursors, and alteration in the expression level of murine MyD88 has been used as an indicator of terminal differentiation in these cells [20]. However, Northern blot analysis indicates much more widespread expression, not limited to myeloid cells. While the low level expression of MyD88 in unstimulated M1 cells may be due to the spontaneous differentiation that occurs in this cell line, the presence of MyD88 transcripts in other lymphoid and non-lymphoid lines warrants caution when associating differentiation state with the presence or absence of MyD88 message.

Many small cell lung carcinomas (SCLC) and non-SCLCs exhibit frequent allelic loss in the region of 3p21-p22 [21,22], indicating that this region may contain one or more tumor suppressor genes. Extensive investigation by many groups has further narrowed this region to approx. 1–2 Mb consisting of the region 3p21-p22 [23], 3p21.3-p22 [24], or 3p21.2-p21.3 [25]. The gene for human MyD88 maps to 3p21.3-p22, and is therefore a candidate tumor suppressor gene. However, analysis of a number of SCLC tumor lines showed that MyD88 was not homozygously deleted, although this does not rule out the possibility that transcription or translation of MyD88 was affected in these lines (G.S. and J.R.T., unpublished data).

By sequence homology, MyD88 appears to be a member of both the IL-1 receptor-like family and the death-domain family. Although the functional consequences of this homology have yet to be elucidated, it is tempting to speculate that the death domain may mediate association of MyD88 with another protein, perhaps a transmembrane receptor, that would then somehow regulate the signaling activity of the IL-1 receptor-like domain. If, for example, the death domain of MyD88 were to associate with the death domain of the TNF Receptor p55, then signaling by TNF might activate an IL-1 like response cascade. Further work will be required to test these hypotheses.

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