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Monoclonal antibodies for clinical trials of Duchenne muscular dystrophy therapy

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Abstract

Most pathogenic mutations in Duchenne and Becker muscular dystrophies involve deletion of single or multiple exons from the dystrophin gene, so exon-specific monoclonal antibodies (mAbs) can be used to distinguish normal and mutant dystrophin proteins. In Duchenne therapy trials, mAbs can be used to identify or rule out dystrophin-positive "revertant" fibres, which have an internally-deleted dystrophin protein and which occur naturally in some Duchenne patients. Using phage-displayed peptide libraries, we now describe the new mapping of the binding sites of five dystrophin mAbs to a few amino-acids within single exons. The phage display method also confirmed previous mapping of MANEX1A (exon 1) and MANDRA1 (exon 77) by other methods. Of the 79 dystrophin exons, mAbs are now available against single exons 1, 6, 8, 12, 13, 14, 17, 21, 26, 28, 38, 41, 43, 44, 45, 46, 47, 50, 51, 58, 59, 62, 63, 75 and 77. Many have been used in clinical trials, as well as for diagnosis and studies of dystrophin isoforms.

Keywords: Duchenne muscular dystrophy; Dystrophin; Monoclonal antibody; Epitope mapping; Clinical trial; Exon-skipping; Therapy; Phage display

1. Introduction

One of the long-term aims of the MDA Monoclonal Antibody Resource (www.glennmorris.org.uk/mabs.htm) has been to produce monoclonal antibodies (mAbs) against dystrophin that recognise specific amino-acid sequences spread throughout its 79 exons and 3684 amino-acids.

We have paid particular attention to mAbs against the exons that are commonly deleted in Duchenne muscular dystrophy; these can be used to characterise mutant Becker dystrophins at the protein level in muscle biopsies [1]. The antibodies have also been used to characterise some of the short forms of dystrophin, including Dp71 [2–4], Dp140 [5] and Dp260 [6], and to identify nNOS-binding regions of dystrophin [7]. Exon-specific mAbs can also be used to distinguish tissue-specific isoforms of full-length dystrophin. Thus, our MANEX1 mAbs were mapped to the first three amino-acids of the muscle-specific isoform of dystrophin (first exon encodes LWWEEVEDCY) and so will not recognize either the brain isoform (first exon encodes ED) or the isoform in cardiac Purkinje cells (first exon encodes SEVSSD) [8]. They have also been used to investigate the nature and origin of revertant fibres in Duchenne patients [9] and mdx mice [10]. More recently, they have found a major application in monitoring the success of clinical trials of various experimental treatments for Duchenne MD. The dystrophin in revertant fibres always has missing exons, whereas dystrophin supplied in gene or cell therapy trials is usually full-length and will contain the exons deleted by the Duchenne mutation. Dystrophin-positive fibres in an early myoblast therapy trial were shown to be due to revertant fibres using exon-specific mAbs [11] and most subsequent trials have used this method to demonstrate

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successful dystrophin replacement [12,13]. In an early study, our exon-specific mAbs were used to distinguish revertant fibres in a Duchenne patient from dystrophin-positive fibres that might have arisen from stem cells in donor bone marrow transplants given several years earlier [14]. The exon-specific mAbs have also been used in trials of gene therapy [15], antisense oligo-based [16], morpholino-based [17,18] and stop codon readthrough [19,20] therapies. MANDYS106 has been particularly popular for "exon-skipping" approaches which aim to convert severe Duchenne patients into milder, Becker-like patients [16–18].

These applications require mAbs that work well for both immunolocalization and western blotting. Synthetic peptide immunogens do not work well for either globular regions or the triple-helical rod regions of dystrophin, although we had some success with peptides from the four non-helical linkers, or "hinges", in the dystrophin rod [21]. For this reason, we have produced most dystrophin mAbs by using large recombinant fragments as immunogens and mapping the epitopes subsequently to single exons or groups of exons.

2. Materials and methods

2.1. Epitope mapping

Epitope mapping using phage-displayed random peptide libraries in filamentous phage was performed as previously described [22] using a modification of an earlier method [23]. Monoclonal antibody mixtures were diluted 1:50 with Tris-buffered saline (TBS) and immobilised onto sterile 35 mm Petri dishes coated directly with 1 ml of 1:200 dilution of rabbit-anti-[mouse Ig] in TBS (DAKOpatts, Denmark). Biopanning was performed using a 15-mer peptide library in phage f88–4, maintained in the K91Kan strain of *Escherichia coli* and generously supplied by Smith (University of Missouri). Any remaining binding sites on the dishes were blocked using 4% BSA in sterile TBS. A sample of the phage library (10^{13} virions) was pre-incubated in dishes coated with the rabbit anti-mouse antibodies alone to ensure any binding was specific for the target mAbs. Following the first round of biopanning, the bound phage were eluted and amplified by infection of K91Kan *E. coli* cells. Two rounds of biopanning were performed. Individual colonies of the phage-infected cells after the second round were grown on nitrocellulose membrane (BA85) and screened by western blotting to reveal positive clones. Positive clones were subjected to western blotting with individual mAbs from the mixture used for biopanning. After blocking non-specific sites with 5% skimmed milk protein in TBS, membranes were incubated with mAb supernatant (1/100 dilution in TBS). Antibody-reacting clones were visualized following development with biotinylated horse anti-mouse Ig in a

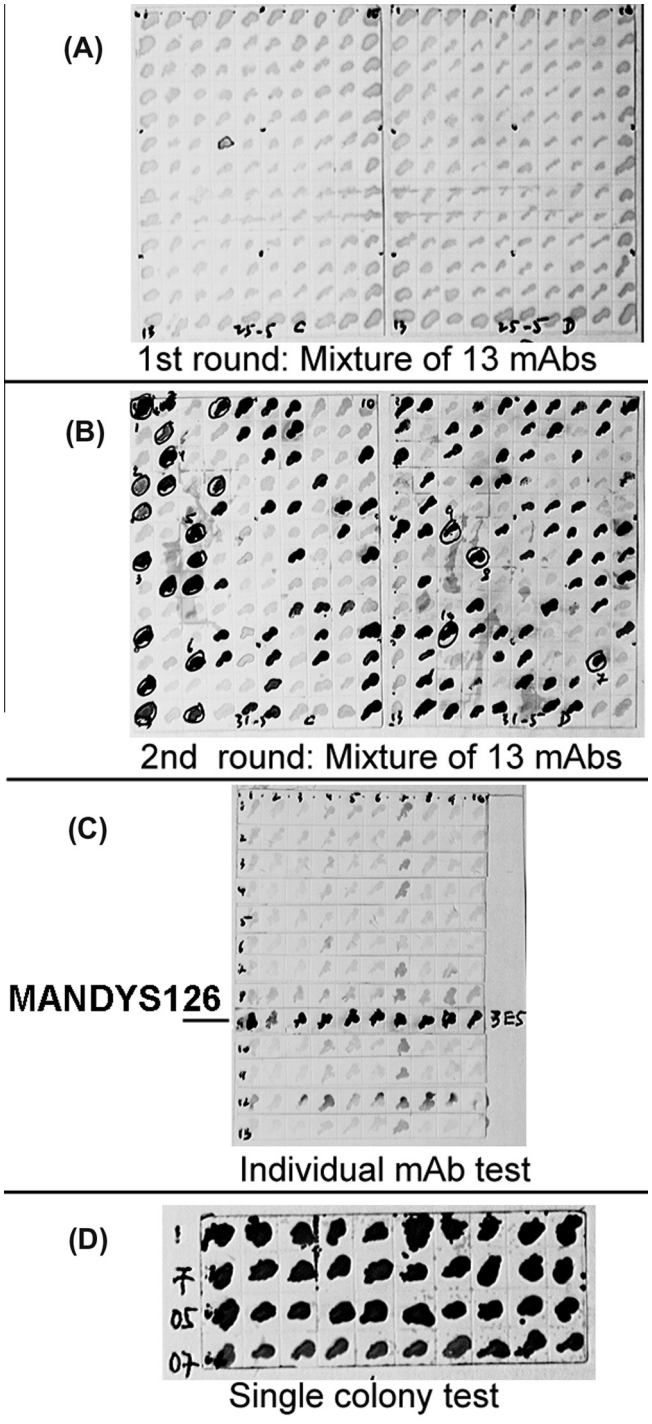

Fig. 1. Use of MANDYS126 to isolate phage expressing surface peptides which mimic its epitope. The four steps shown are described in the results section. (A) There are few mAb-positive clones after the first round of biopanning with a mixture of 13 mAbs. (B) After two rounds of biopanning, several *E. coli*. colonies reacted with the mAb mixture and ten of these (circled in B) were selected. (C) The ten colonies were streaked onto 13 horizontal strips and each strip was incubated with a single mAb from the mixture of 13. Only MANDYS126 gave a strong reaction. (D) Four of the ten colonies were cloned again and 10 clones were tested for mAb reaction – this was repeated until all 10 clones were positive to ensure that the phage contains a single sequence only. One colony from each of these clonings was amplified for isolation and sequencing of phage DNA.