Cellular Myosin Heavy Chain in Human Leukocytes: Isolation of 5' cDNA Clones, Characterization of the Protein, Chromosomal Localization, and Upregulation During Myeloid Differentiation

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We have isolated 5' cDNA clones encoding a member of the cellular myosin heavy chain gene family from human leukocytes. The predicted amino acid sequence shows 93% identity to a chicken cellular myosin heavy chain, 76% to chicken smooth muscle, and 40% to human sarcomeric myosin heavy chain. The mRNA is expressed as a 7.4- to 7.9-kb doublet in many nonmuscle cells, and is upregulated in myeloid cell lines on induction from a proliferating to a differentiated state. Antisera raised against a peptide made from the predicted amino acid sequence specifically reacts with a 224-kD polypeptide in leucocyte cell lines, and the protein is also upregulated during the induction of monocytic and granulocytic differentiation in these cells. The gene for this cellular myosin heavy chain maps to chromosome 22, bands q12.3-q13.1, demonstrating that it is not located in the previously described sarcomeric gene clusters on chromosomes 14 and 17. This cellular myosin heavy chain may be a major contractile protein responsible for movement in myeloid cell lines because no mRNA for sarcomeric myosin heavy chain is detected in these cells.

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ACTOMYOSIN COMPLEXES have been implicated as the generators of force producing muscle contraction, cell locomotion, karyokinesis, cytokinesis, phagocytosis, secretion, capping, and changes in cell shape.1-3 Amino acid sequencing and cDNA cloning studies have defined several broad classes of myosin heavy chain families, including (1) sarcomeric myosin heavy chains, which can be subdivided into skeletal and cardiac; (2) smooth muscle myosin heavy chains; (3) myosin I heavy chains; and (4) cellular (also described as "nonmuscle") myosin heavy chains. Members within each family have a high degree of amino acid similarity across species lines, but a significantly lesser degree between families, even within the same species.4-9

Previous studies recognized that although cellular and muscle myosin heavy chains have similar morphologic and biochemical properties,6-11 these chains have distinct peptide cleavage maps12,13 and antigenic properties.11,14 Characterization of cellular myosin heavy chains purified from different cell types showed differences in these chains, and biochemical studies suggested that several types were present in some cells.12

Purified myosin heavy chains from brain tissue,12 lymphocytes,13 leucocytes,11,15,16 and platelets17-19 are 200- to 220-kD proteins that appear as two globular heads attached to a fibrous rodlike tail in electron micrographs. Each globular head domain binds to adenosine triphosphate (ATP), actin, and a pair of myosin light chains, as is the case with smooth muscle and sarcomeric myosin heavy chains. The tail domains of cellular myosin heavy chains form a helical coiled-coil structures through which the two molecules associate.1 Despite the known differences of the muscle and cellular isoforms, purified proteins mixed in vitro are still able to form hybrid bipolar filaments.18

Recently, the nucleic acid sequences of several cellular myosin heavy chains have been reported,18,20,22 and comparisons of these genes to other previously isolated sarcomeric and smooth muscle genes clarify earlier biochemical studies on cellular myosin heavy chains. In particular, Saez et al22 reported the cDNA sequence of the 3' end of a member of the human cellular myosin heavy chain family. We report here the isolation of 5' cDNA clones for this gene, the localization of this gene to human chromosome 22q12.3-q13.1, and the identification of the protein using antipeptide antibodies. Human cellular myosin heavy chain mRNA and protein levels are increased during the differentiation of myeloid cell lines, suggesting a role for this protein in the motile processes of these phagocytic cells.

MATERIALS AND METHODS

Isolation and characterization of cDNA and genomic clones. cDNA libraries used in these studies include a human peripheral blood mononuclear cell A g11 library (approximately 50% monocytes and 50% lymphocytes)23 and a random-prime 7-day Me2SO4-induced (granulocytic) HL-60 library (kindly provided by Dr Stuart Orkin, Children's Hospital, Boston, MA). cDNA libraries were screened as described with antisera to the surface antigen CD11b24 or with cDNA probes25 labeled with [α-32P]dCTP by the random primer method.26 Nucleotide sequence was determined using the dideoxy chain termination method23 and specific oligonucleotide primers synthesized on an Applied Biosystems 381A DNA synthesizer (Applied Biosystems, Foster City, CA).

For fluorometric in situ chromosomal hybridization, a genomic cosmid clone (CL411) was isolated from a human placental library (provided by Dr Chris Lau, University of California, San Francisco) using L4 as a probe under high stringency conditions.27
**Cells lines and culture.** The human myeloid lines HL-60 (American Type Culture Collection [ATCC] no. CCL240) and U937 (ATCC no. CRL1593) were propagated in RPMI 1640 supplemented with 10% fetal calf serum (Hyclone, Logan, UT). For Northern blot analysis of monocytic differentiation, cells were cultured at $5 \times 10^5$/mL and induced with $3 \times 10^{-8}$ mol/L phorbol 12-myristate 13-acetate (TPA) (Sigma, St Louis, MO) for 24 hours or with 1,25-dihydroxy vitamin D$_3$ (vitamin D$_3$) for 72 hours; alternatively, HL-60 cells were induced toward a granulocytic phenotype with 1.1% Me$_2$SO, for 72 hours. Uninduced and induced cells were analyzed for growth, morphology after Wright-Giemsa staining, and surface expression of the monocytic marker CD11b, and harvested for Northern or Western blot analysis as described below.

**Northern blot analysis.** RNA expression was analyzed using Northern blots as previously described, with minor modifications. Ten micrograms of total RNA was isolated from the indicated cells by the guanidium isothiocyanate method, separated on 1% formaldehyde/agarose gels, transferred to nylon membranes (Biotrans; ICN, Irvine, CA), fixed by an ultraviolet cross-linking apparatus (Stratagene, La Jolla, CA), and hybridized sequentially to random primed [$\alpha$-$^{32}$P]dCTP-labeled probes. The blots were washed at a final stringency of 68°C in 0.2X standard sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS) (1X SSC is 150 mmol/L NaCl, 15 mmol/L sodium citrate, pH 7.0) and exposed at -80°C with a Dupont Cronex Lightning Plus intensifying screen (Dupont, Wilmington, DE) for the indicated times. The probes used sequentially were clone L4 for cellular myosin heavy chain, a 3.6-kb EcoRI human embryonic skeletal muscle myosin heavy chain cDNA, and a 6.6-kb human 28S ribosomal RNA probe (kindly provided by Dr Asterios Tsiftsoglou, University of Thessaloniki, Greece). Equivalent amounts of RNA were loaded in each lane as assessed by visual inspection of the ethidium bromide stained gel and hybridization of the 28S probe (except as noted in the legend to Fig 2). The blot was stripped of probe between hybridizations by washing in 50% formamide, 10 mmol/L NaPO$_4$, pH 7.2 for 1 hour at 68°C, followed by 2X SSC, 0.1% SDS washes at room temperature with shaking.

**Synthesis of peptides and generation of antipeptide antisera.** KM1, a 23-residue peptide representing amino acids 52 through 74 of the cellular myosin heavy chain sequence predicted from the cDNA clones, and KM2 (amino acids 354 through 372) were synthesized using 9-fluorenyl methyloxycarbonyl (FMOC) solid-phase chemistry on a Rapid Amide Multiple Peptide Synthesis System (RAMPS; New England Nuclear, Bedford, MA) manual synthesizer, coupled to keyhole limpet hemocyanin (KLH), and injected into rabbits. Animals were boosted every 6 weeks and bled 10 days later. Production of specific antisera was monitored by enzyme-linked immunosorbent assay (ELISA) of peptide-coated microtiter wells.

**Western blot analysis of cellular myosin heavy chain protein.** Uninduced and TPA induced HL-60 and U937 cells were lysed in lysis buffer (1X Hanks' balanced salt solution, 0.5% NP-40, 2 mmol/L phenyl methyl sulfonyl fluoride [PMSF], 3 mmol/L...
EDTA, 1 μmol/L pepstatin A, and 1 μmol/L leupeptin), separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 6% gels, and electrophoretically transferred to nitrocellulose membranes. The blots were incubated with preimmune or immune antisera for 3 hours at room temperature, followed by a 1:2,000 dilution of alkaline phosphatase-conjugated goat antirabbit IgG (Biorad, Richmond, CA). Blots were developed using the NBT/BCIP Protoblot System (Pormega, Madison, WI). Each lane contained protein from 1 × 10^5 cells. For blocking experiments, 500 μg of the indicated peptide was added to the first incubation step.

In situ chromosomal hybridization. Human metaphase cells were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. Radiolabeled L4 probe was prepared by nick translation of the entire plasmid with all four 3H-labeled deoxynucleoside triphosphates to a specific activity of 8 × 10^6 to 1 × 10^7 dpm/μg. In situ hybridization was performed as described previously. Metaphase cells were hybridized at 0.5, 1.0, and 5.0 ng of probe per milliliter of hybridization mixture. Autoradiographs were exposed for 11 days.

RESULTS

Isolation of 5’ cDNA clones for human cellular myosin heavy chain. The original cellular myosin heavy chain cDNA clone, termed L4, was isolated during the screening of a human peripheral blood mononuclear cell expression library with rabbit antisera raised against the monocyte surface antigen CD11b (see Fig 1). Because this cDNA hybridized to a large 7.4- to 7.9-kb mRNA doublet that appeared to be upregulated in HL-60 cells during the course of myeloid differentiation (Fig 2A), we proceeded to sequence the entire cDNA. The L434 and LT-1 cDNA clones were isolated using cDNA probes. Figure 1 depicts the three cDNA clones for cellular myosin heavy chain that were isolated, as well as their relationship to the previously published 3’ sequence of this gene. No differences were detected among the overlapping sequences of L4, L434, and LT-1.

Expression of human cellular myosin heavy chain mRNA in myeloid cells increases during the course of myeloid differentiation. We have performed studies of the expression of this gene in different cell types using the 2.6-kb insert from the L4 cDNA clone as a probe. In leukocyte cell lines, an mRNA doublet of approximately 7.4 to 7.9 kb is detected (Fig 2A, lane 2). Both bands of the mRNA doublet are strongly upregulated when the myeloid HL-60 cell line is induced to differentiate toward monocyctic cells with the phorbol ester TPA or with vitamin D3, or toward granulocytic cells with MeSO4 (Fig 2A, lanes 2 through 5). An identical 7.4- to 7.9-kb mRNA doublet is expressed in normal peripheral blood mononuclear cells, several T- and B-cell lines, and normal bone marrow (data not shown). A probe for the human embryonic skeletal myosin heavy chain that detects a single 6-kb band in fetal and adult skeletal and adult left atrial and ventricular cardiac tissue did not produce any detectable signal in HL-60 (nonmuscle) cells, but did light up the expected 6-kb mRNA species in cardiac muscle cells (Fig 2B). Interestingly, our cellular myosin heavy chain probe detects an mRNA doublet of identical size in cardiac tissue that is distinct from the 6-kb mRNA species detected using the sarcomeric myosin heavy chain probe (Fig 2A, lane 1).

Chromosome localization shows a unique gene distinct from previously described members of the muscle myosin heavy chain families. The localization of the cellular myosin heavy chain gene was performed using somatic cell hybrid analysis and in situ hybridization. Hybridization of the L4 probe to Southern blots of human x rodent somatic cell hybrids showed a perfect concordance between human chromosome 22 and myosin heavy chain coding sequences (data not shown). There was a significant discordance (17% to 68%) with all of the other human chromosomes. To precisely localize the human cellular myosin heavy chain gene using an independent method, we hybridized the L4 cDNA probe to normal metaphase chromosomes. This resulted in specific labeling of chromosome 22. Of 100 metaphase cells examined from this hybridization, 33 (33%) were labeled on region q1 of one or both chromosome 22 homologues. The distribution of labeled sites on this chromosome is illustrated in Fig 3; of 219 labeled sites
observed, 46 (21%) were located on this chromosome. These sites were clustered at bands q11.2-q13, and this cluster represented 19.6% (43 of 219) of all labeled sites (cumulative probability for the Poisson distribution is <.005). The majority of the labeled sites were located at 22q13. A smaller, yet significant cluster of labeled sites was observed on the long arm of chromosome 19 (q19, 12 labeled sites, 5.5%, P < .005). Similar results were obtained in three additional hybridization experiments using this probe. In addition, hybridization of a biotin-labeled cosmid clone CL411 detected with fluorescein-conjugated avidin resulted in specific labeling only of chromosome 22, bands 22q12.3-q13.1 (data not shown). Together with the results of the analysis of somatic cell hybrids, these data indicate

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**Fig 4.** DNA and predicted amino acid sequence of cellular myosin. The consensus nucleotide sequence of our three cDNA clones is presented in 5' to 3' orientation with the predicted amino acid sequence in single letter code below each line. Nucleotides are numbered at the right of each line, and resulting differences in predicted amino acid sequence in parentheses below our amino acid sequence. These sequence data are available from EMBL (Heidelberg, Germany)/GenBank (Los Alamos, NM)/DDBJ (Mishima, Japan) under the accession number M38372. (Fig 4 continued on following page.)
that the human cellular myosin heavy chain gene is localized to chromosome 22, at band q12.3-q13.1.

DNA sequence analysis of cellular myosin heavy chain clones. We have performed extensive DNA sequence analysis of the three overlapping cellular myosin heavy chain cDNA clones (Figs 1 and 4). Referring to the most 5' nucleotide of this stretch of 4,152 bp as bp 1, there is a single continuous open reading frame (ORF) beginning at bp 140 and extending 4,012 bp to the end of the L4 and L434 clones. The sequence at the first methionine represents the start of this ORF is very similar to a consensus nucleotide of this stretch of DNA sequence analysis of cellular myosin heavy chain: the human cellular myosin heavy chain gene is localized to chromosome 14p12.2-q13.1. Comparative analysis of cellular myosin heavy chain DNA and protein sequence. The 3' 1,872 bp of our cDNA clones are 99.7% identical to the 5' 1,872 bp of the sequence obtained by Saez et al8 with only six base pair changes and no gaps in the sequence (Figs 1 and 4). These six base pair differences result in four amino acid differences in the predicted protein sequence; in three of the four cases, the amino acid predicted by our sequence is identical to that of the chicken homologue of this myosin heavy chain, and4 in the fourth case the predicted amino acid residue differs in each of the three sequences. The composite structure (Fig 1) is suggestive of a myosin II protein by virtue of its size, sequence similarities to other known myosin heavy chain II genes (see below), and secondary structure analysis,39 which predicts that the tail region from residue 837 on will form an α helical structure.

The predicted amino acid sequence of our human cellular myosin heavy chain has 93% identity to that of the chicken smooth muscle (gizzard) myosin heavy chain: and in one case the amino acid predicted by our sequence is identical to that of the chicken homologue of this myosin heavy chain, and4 in the fourth case the predicted amino acid residue differs in each of the three sequences. The composite structure (Fig 1) is suggestive of a myosin II protein by virtue of its size, sequence similarities to other known myosin heavy chain II genes (see below), and secondary structure analysis,39 which predicts that the tail region from residue 837 on will form an α helical structure.

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Antipeptide antibodies identify a 224-Kd polypeptide whose expression is upregulated during human myeloid differentiation...
To identify the protein encoded by this cDNA, we generated antipeptide antisera from the predicted amino acid sequence. Peptide KM1 (amino acids 52 through 74), which was used to generate a rabbit antiserum, contains only a 22% to 30% identity to the corresponding human embryonic skeletal and β cardiac myosin heavy chain sequences. As demonstrated in Fig 5A, KM1 antiserum specifically reacts with a 224-Kd protein in HL-60 cells induced toward monocytic differentiation, and this reaction can be blocked with the KM1 peptide and not the KM2 peptide, which was synthesized from a different part of the sequence (amino acids 354 through 372). The color reactions were deliberately allowed to overdevelop to look for nonspecific cross-reacting bands (lane 1) or possible degradation products (lanes 2 through 4). The apparent molecular weight of 224 Kd (Fig 5A) is similar to that of the translated skeletal myosin heavy chain cDNAs. When either of the myeloid lines HL-60 or U937 are induced toward monocytic differentiation, there is a significant increase of the amount of cellular myosin heavy chain protein per cell (Fig 5B).

**DISCUSSION**

In this work, we describe the isolation of the 5' 4.1-kb sequence of the human cellular myosin heavy chain cDNA and identification of the protein encoded by this gene. The results of somatic cell hybrid analysis and in situ chromosomal hybridizations indicate that the human cellular myosin heavy chain gene is localized to 22q12.3-q13.1, which is consistent with the previous localization of this gene (MYH9) to chromosome 22pter-22q13 using somatic cell hybrid analysis. The genes coding the eight other myosin heavy chains that have been localized are dispersed to four other human chromosomes. Human leukocyte myosin heavy chain thus appears to be a member of the cellular myosin family that has diverged in sequence and cell distribution from the muscle myosin heavy chain genes. Recently, Katsuragawa et al. isolated two partial cellular myosin cDNAs from chicken fibroblasts, one being homologous to the myosin sequence described in this report and the other being a very closely related myosin gene. Ketcham et al. have also reported the sequence of a Drosophila cellular myosin that is most likely an evolutionarily distant...
homologue or family member. It seems likely that other human cellular myosin genes will also be isolated; given the precedence of human skeletal and cardiac myosin heavy chain gene organization, a cluster of human cellular myosin genes may be found to reside on chromosome 22.

On Northern blot analysis, our cDNA identifies a doublet of 7.4 to 7.9 kb, which corresponds to the calculated mRNA sizes of the two cellular myosin polyadenylated species obtained by merging our consensus sequence with that of Saez et al. A cDNA probe for human embryonic skeletal myosin heavy chain, which cross-reacts on Northern blots with human cardiac mRNA, does not detect any species in HL-60 cells. Furthermore, our cellular myosin heavy chain probe detects a 7.4- to 7.9-kb doublet in human cardiac mRNA, which is easily distinguished from the 6-kb species identified with the human embryonic skeletal myosin heavy chain. These data suggest that none of the sarcomeric myosin heavy chains are expressed in leukocytes, and that an mRNA species very similar to cellular myosin heavy chain is expressed in cardiac muscle in agreement with previous reports.

Cellular myosin heavy chain increases significantly during the course of myeloid differentiation by both Northern blot analysis of mRNA (Fig 2A) and Western blot analysis of protein (Fig 2B). Under these conditions, cellular proliferation ceases and the cells appear macrophage-like as assessed by morphology and surface expression of CD11b. This suggests that this myosin is less likely to play a role in proliferation because the uninduced cells are rapidly dividing and the induced cells have ceased division, and more likely to be important in the function of fully differentiated leukocytes. An increase in the amount of cellular myosin after differentiation of a myeloid cell line was also seen by Sagara et al. Previous immunofluorescence studies using polyclonal antibodies generated against smooth muscle and macrophage myosin heavy chains have found rapid redistribution of myosin staining from a uniform cytoplasmic distribution in resting macrophages and polymorphonuclear leukocytes to the leading edge in stimulated cells and to the tips of pseudopods in phagocytosing cells, suggesting a role for cellular myosin in cell movement and phagocytosis.

Future experiments shall be directed at using anti-KM1 antiserum to determine whether cellular myosin heavy chain protein is produced in other cell types, as well as to localize it within leukocytes by immunofluorescence studies. We shall be particularly interested in its interaction with other cytoskeletal proteins in phagocytic cells as they undergo the processes of binding to and engulfing bacteria and other foreign invaders.

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