The 47-kD Protein Increased in Neutrophil Actin Dysfunction With 47- and 89-kD Protein Abnormalities Is Lymphocyte-Specific Protein

By T. Howard, Y. Li, M. Torres, A. Guerrero, and T. Coates

A male child born of related parents suffered recurrent infections because of neutrophil actin dysfunction with increased amounts of a 47-kD protein and decreased amounts of an 89-kD protein (NAD 47/89). The patient and family members were studied to define the nature of the abnormal proteins and to examine their role in the functional defects of neutrophil actin dysfunction (NAD) 47/89 polymorphonuclear neutrophils (PMNs). NAD 47/89 PMNs are defective in motility, microfilamentous cytoskeletal structure, and formyl peptide-induced actin polymerization and express increased amounts of a 47-kD protein and decreased amounts of an 89-kD protein intermediate abnormality in amount of 47-kD and 89-kD proteins in PMNs from parents and a female sibling suggest the disease is an autosomal recessive disorder. Immunoblots with monoclonal antibody (MoAb1) and polyclonal antibody raised to 47-kD protein showed the 89-kD protein is antigenically distinct from the 47-kD protein and the 89-kD protein is not gelsolin. 125I-actin binding to one-dimensional (1D) and 2D gels of PMN proteins from NAD 47/89 proband, family members, and controls showed the 47-kD protein binds actin, is acidic (pI = 4.5 to 4.7), is recognized by the MoAb1, exists on 2-D gels as three distinct actin binding species (MWapp 52 kD, 47-kD, and 44-kD), and is present in control PMNs in lesser amount than in PMNs of NAD 47/89 proband or parents. Immunofluorescence demonstration of the 47-kD actin binding protein on MoAb1 matrix yielded a multicomponent complex with proteins of MWapp 180 kD, 71 kD, 47 kD and actin. Cloning, sequencing, and expression of a 1.58-kb cDNA selected for MoAb1 reactivity from a HL60 expression library and microsequence of native PMNs, 47-kD actin binding protein showed the overexpressed 47-kD protein is lymphocyte-specific protein 1 (LSP1), which is known actin binding protein. The results show LSP1 is expressed in PMNs and suggest overexpression of LSP1 is related to the motility and cytoskeletal abnormalities in NAD 47/89 PMNs. ©1994 by The American Society of Hematology.

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Submitted January 1, 1993; accepted September 1, 1993.

Supported by National Institutes of Health Grants No. AI25214 to T.H. and AI23547 to T.C. T.H. is an Established Investigator of the American Heart Association.

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0006-4971/94/8301-002553/00/0


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which is an F-actin binding protein not previously identified in PMNs. The studies yield new insights into the biochemical basis of NAD 47/89 and suggest a role for LSP1 in regulating actin dynamics in PMNs.

MATERIALS AND METHODS

Materials. The following materials were used: All standard chemicals (Sigma Chemical Co, St Louis, MO); Balb/C mice (Charles River Labs, Boston, MA); tissue culture plates (Costar, Cambridge, MA); HAT medium (ICN-Flow, Irvine, CA); nitrocellulose paper (Schleicher and Schuell, Keene, NH); CNBR-activated Sepharose 4B, Sephadex G-150, and G-25 (Pharmacia, Piscataway, NJ); 125I-labeled antibodies and Extractigel (Pierce, Rockford, IL); protein A (Sigma, St Louis, MO).

PMN preparation. All cells were purified endotoxin free. For studies of normal PMNs, cells were purified endotoxin free (<10 pg/ml LPS) on Percoll gradients in autologous plasma as modified by Howard from 1 to 6 units of whole citrate anticoagulated blood. All cells were treated with 5 mmol/L diisopropylfluorophosphate (DFP) to inhibit proteolysis. Gel electrophoresis and immunobLOTS. One-dimensional (1-D) denaturing 5% to 15% gradient and 7.5% polyacrylamide gel electrophoresis (PAGE) in sodium dodecyl sulfate (SDS) buffers were done as described. The PMN protein was solubilized 1:1 in 0.125 mol/L TRIS HCl pH 6.8, 4% SDS, 20% glycerol, 10% mercaptoethanol and sonicated. For immunobLOTS, the proteins were transferred to nitrocellulose (NTC) paper and blotted as described. NTC paper was blocked with nonfat dry milk as described. To assure that the 47-kD protein was not the actin concentration was maintained at or below 1 mmol/L. Laying to the high salt concentration of the incubation and wash buffers. For some experiments, the 125I-actin binding to proteins transferred to NTC was assayed and the NTC blocked as described. Also, variable periods of the autoradiography were necessary clearly to emphasize actin binding proteins of variable abundance. Radiograph scanning showed excess cold actin blocked >80% of the 125I-actin binding to 47-kD protein in control or NAD 47/89 PMNs.

Immunonaffinity chromatography. MoAb1 purified from mouse ascites on protein A matrix was linked to CNBr-activated Sepharose 4B beads. The PMNs from 3 to 6 units of blood were homogenized by Dounce (1,500 strokes) into homogenizing buffer (40 mmol/L KCl, 10 mmol/L EDTA, 6 mmol/L DFP, 1 mmol/L ATP, 40 mmol/L imidazole pH 7.15) and dialyzed into G-buffer (0.3 mmol/L CaCl2, 0.1 mmol/L EDTA, 0.5 mmol/L ATP, 1 mmol/L NaNO3, 0.5 mmol/L mercaptoethanol, 10 mmol/L TRIS Cl pH 8.0). The dialyzed homogenate was fractionated on the MoAb1 column, (capacity greater than all soluble 47-kD protein from 10 units PMNs) in 10 mmol/L sodium phosphate buffer pH 8.0. The column was eluted with 100 mmol/L triethylamine buffer pH 11.5 in dropwise fractions and immediately corrected to pH 7.0 with addition of 1 volume of phosphate buffer pH 6.8 to 4 volumes eluate. Eluate fractions positive for 47-kD protein were determined by MoAb1 immunoblot, pooled, and concentrated to yield a multi-molecular complex. To assure that the 47-kD protein was not the heavy chain of IgG, eluate fractions were immunoblotted with secondary antibody only and no immunoreactive species were identified.

To obtain the purified 47-kD actin binding protein the solubilized PMNs in homogenizing buffer were treated with 2% SDS and heated to 45°C for 10 minutes. Then the SDS was removed by extensive dialysis followed by phase fractionation on an Extractigel column. The Extractigel column was equilibrated with extracted PMNs. Secondary screens selected anti-47-kD—positive clones that yielded greater OD for immunoreactive 47-kD in the PMNs of mother and father when immunoblotted on the same NTC sheet. Quantitative immunoblots with one IgG1 antibody, MoAb1, report quantitatively greater amounts of 47 kD protein in proband's (greater than four times control) and parent's PMNs (greater than two times control) than control PMNs (see Fig 2). In the absence of proteolysis, MoAb1 yields only one immunoreactive band on 1-D SDS-PAGE. Subsequently, on 2-D gels (7% PAGE second dimension) MoAb1 identified three peptides (see Fig 6). When the three peptides were seen on 2-D gels, the original MoAb1 clones were again subcloned. All subclones recognized the same three distinct peptides on 2-D gels. These peptides therefore likely reflect distinct proteolytic fragments of 47-kD protein or distinct post-translational modifications. Regardless of explanation, the peptides are not explained by mixture of several anti-47-kD antibodies to different 47-kD proteins.

Preparation of 125I-actin and actin gel overlay. Rabbit skeletal muscle actin was prepared and column purified on Sephadex G-150 to remove denatured actin. Ac was radiolabeled with 125I-iododextrins, the labeled actin was eluted from a G-25 column, dia- lyzed into low-ionic strength buffer, and the protein concentration determined. The method yielded 84% recovery of actin with specific activity of 10 to 30 mCi/mg protein. The 125I-actin overlay was as described for binding protein in gels with minor modifications; that is, the actin concentration was maintained at or below 1 mmol/L. Laying to the high salt concentration of the incubation and wash buffers. For some experiments, the 125I-actin binding to proteins transferred to NTC was assayed and the NTC blocked as described. Also, variable periods of the autoradiography were necessary clearly to emphasize actin binding proteins of variable abundance. Radiograph scanning showed excess cold actin blocked >80% of the 125I-actin binding to 47-kD protein in control or NAD 47/89 PMNs.
LSPI IS OVEREXPRESSED IN NAD 47/89

Fig 1. The 47-kD and 89-kD proteins in PMNs of NAD 47/89 proband and family members. 1-D 5% to 16% SDS-PAGE of total PMN proteins from the NAD 47/89 proband (P, P), mother (M), father (F), siblings (S1, S2), and simultaneously collected controls (C1, C2, C3) run at different times. Protein loads are C1, P = 80 μg; C1, P = 40 μg; C2, F, M, C3, S1, and S2 = 40 μg, respectively. Note the relative decrease in an 89-kD protein (> ) and increase in 47-kD protein (*) in P, F, M, and S2 but not in S1. (See reference 8 for quantification.)

RESULTS

As originally shown,27 analysis of PMN proteins from the NAD 47/89 proband revealed a marked increase in a 47-kD protein (4.5- 5.0-fold control) and decreased 89-kD protein (<0.1-fold control) on gel scans of Coomassie blue-stained gels (Fig 1). Here, the study is extended further by analysis of PMNs from family members. Family studies demonstrated quantitative abnormalities in 47-kD and 89-kD proteins in PMNs of both parents and one sibling that were intermediate to those of controls and the NAD 47/89 proband (Fig 1) as previously reported.27 In the sibling, an intermediate increase in 47-kD protein was the only abnormality. The amount of 47-kD protein in a second sibling was normal. This result paired with previously reported functional studies on the parents27 suggested the NAD 47/89 disorder is inherited. To assist analysis of the abnormal proteins, a monoclonal antibody, MoAb1, was prepared by immunization of mice with the 47-kD protein band excised from the 1-D SDS-PAGE of paternal PMNs. Details of the antibody preparation are described in the Materials and Methods section. Paternal PMNs were used because the patient received an allogeneic bone marrow transplant and no further PMNs were available.27 On quantitative 1-D immunoblots of proband, parents, and control PMNs, the MoAb1, monoclonal
The decreased 89-kD protein is not gelsolin. Quantitative abnormalities in 47-kD and 89-kD proteins associated with defective actin polymerization in NAD 47/89 PMNs suggested either or both proteins may contribute to the defect by interacting with actin. Therefore, we determined whether the 47-kD and 89-kD proteins were actin binding proteins. Gelsolin is a 90-kD actin binding protein that inhibits actin polymerization in vitro, is present in PMNs, and regulates chemotactic peptide-induced actin polymerization in PMNs. Several lines of evidence indicated the 89-kD protein, which is decreased in NAD 47/89 PMNs, is not gelsolin. First, immunoblots of PMNs from the patient and parents yielded a positive reaction with a previously characterized antigelisolin antibody (not shown). Second, quantitative immunoblots of PMNs from control, father, and mother with antigelisolin showed that the quantity of immunoreactive gelsolin was similar in all PMNs (father, 3.2 ± 1.4; mother, 4.4 ± 1.5; control, 3.5 ± 0.9 ng gelsolin/µg whole PMN protein mean ± SD N = 6). Finally, on immunoblots of proteins separated by 2-D gel electrophoresis, the pl of gelsolin in paternal, maternal, and control PMNs was similar and identical to the reported pl (not shown). Therefore, the 89-kD protein, which is decreased in the PMNs of the proband and his father, is not gelsolin.

The 47-kD and 89-kD proteins are unique proteins. The reciprocal decrease in the 89-kD protein and increase in the 47-kD protein suggested the 47-kD protein could be a peptide fragment produced by proteolysis of the 89-kD protein. Alternatively, the two proteins could be structurally unique proteins. To determine whether the two proteins are unique, mouse and rabbit polyclonal antibodies were raised to the 47-kD protein band excised from 1-D SDS-PAGE of paternal PMNs. As shown in Fig 3, the polyclonal anti-47-kD antibody recognized only proteins of approximate molecular mass (Mr app) ≤ 47 kD in father’s and family’s (not shown) PMNs. There was no apparent reactivity at 43 kD, which is the molecular weight of actin. Furthermore, the polyclonal anti-47 kD did not recognize muscle actin (data not shown), which therefore suggested the 47kD protein in the abnormal PMNs was not an actin with an altered molecular weight. Proteins of Mr app < 47 kD also reacted with the polyclonal anti-47 kD and probably represent proteolytic fragments of the 47-kD protein. In addition, no polyclonal antibody reactivity to an 89-kD protein was observed; thus showing the 47-kD and 89-kD protein are antigenically distinct proteins.
NAD 47/89 PMNs, is not gelsolin. Further studies are needed to define the nature of the 89-kD protein.

The 47-kD protein is an actin binding protein. To determine whether the 47-kD protein is an actin binding protein, the ability of PMN proteins to bind actin was examined on 125I-actin overlays as previously described. As shown in Fig 4A several proteins in control’s and father’s PMNs bind 125I-actin, including proteins of (molecular weight) 90 kD, 47 kD, and 25 kD. The 90-kD protein is gelsolin. A 47-kD actin binding protein was present in control PMNs and in PMNs from proband and family. The intensity of the 47-kD actin binding activity was greater in PMNs from family members with increased 47-kD protein than in control PMNs (Fig 4B). The intensity of actin binding activity generally correlated with the protein staining for 47-kD actin on gel. Specifically, 47-kD actin binding activity was greater than control in proband, paternal, maternal, and one sibling PMNs, and larger amounts of 47-kD actin binding activity and protein were observed in the PMNs of the proband and father than those in the mother or sibling with increased 47-kD protein. Binding affinity of the 47-kD actin binding protein was similar in control and abnormal cells as evidenced by similarities in the amount of 125I-actin bound over a range of 125I-actin concentrations (0.1, 0.5, 1.0, 2.0, 10 nmol/L 125I-actin) (not shown). The 125I-actin binding activity of the 47-kD protein was saturable at \( \leq 1 \) nmol/L actin in family and control PMNs and was displaced by >20-fold excess of cold actin. The results show a 47-kD actin binding protein is present in normal PMNs and is increased in NAD 47/89 PMNs. Therefore, depending on the nature of its interaction with actin, the 47-kD actin binding protein could contribute to the cytoskeletal abnormalities in NAD 47/89 PMNs.

Partial characterization of the 47-kD actin binding protein. To further characterize the 47-kD actin binding protein, PMN proteins from father, mother, and control PMNs were separated on 2-D gel electrophoresis and analyzed by MoAb1 immunoblots and 125I-actin overlay. Three peptides (47 to 54 kD) of increased intensity were apparent in the parent’s PMNs when compared with controls (Fig 5, first row) and were recognized by MoAb1 (Fig 5, second row). The three peptides were not explained by impurity of the AB1 clone (see Material and Methods section) but could be either proteolytic fragments of a protein, post-translational modifications of a single protein or a family of proteins with a shared epitope. The pl of the peptides was similar to the pl of the protein(s) in control PMNs. Concurrent 125I-actin gel overlays showed increased 125I-actin binding to 47- to 54-kD proteins of pl 4.5 to 4.7 (Fig 5, third row), which coincided with the regions of MoAb1 immunoreactivity. The MoAb1 immunoreaction was greater with maternal and paternal PMNs than control PMNs (Fig 5, second row). Therefore, the 47-kD protein recognized by MoAb1 is an actin binding protein that is increased in NAD 47/89 and the pl of the increased 47-kD actin binding protein in NAD 47/89 PMNs and normal PMNs is similar.

The 47-kD actin binding protein forms a multiheteratric complex with actin. Because the 47-kD actin binding pro-
tein is present in normal PMNs and the patient received a bone marrow transplant, normal PMNs were used to purify small quantities of the 47 kD actin binding protein by MoAb1 immunoaffinity chromatography (see Materials and Methods section for details). Briefly, mechanical homogenates of control PMNs were dialyzed against an F-actin depolymerizing buffer and the homogenate was passed over an MoAb1 immunoaffinity column. The column was then eluted and the fractions containing the 47-kD actin binding protein were pooled and concentrated.

Four protein bands (MW = 180 kD, 71 kD, 47 kD, 43 kD) consistently (n = 12) eluted from the immunoaffinity column (Fig 6A). The 43-kD band comigrated with actin and reacted with antiactin antibody (data not shown). The 47-kD band reacted with MoAb1. Coelution of actin with the 47-kD protein recognized by MoAb1 further indicates that the 47-kD protein is an actin binding protein. The actin binding activity of the 47-kD protein was confirmed by 125I-actin overlays of the eluate (Fig 6B). In all preparations, the 47-kD protein appeared to be the major protein on Coomassie blue-stained gels. On 12 different complex preparations, the 47-kD protein to actin ratio was always >1:1 as
determined by scans of coomassie blue stained gels. Therefore, in PMN homogenates, the 47-kD actin binding protein exists in a multimolecular complex with actin and two other proteins.

Identification of the 47-kD actin binding protein as LSP1.

To identify the PMN 47-kD actin binding protein, the protein was purified to homogeneity, a cDNA was cloned from an expression library, and native PMN 47-kD actin binding protein was microsequenced. The 47-kD actin binding protein was purified from the complex by MoAb1 immunoaffinity chromatography after denaturation by heating in 2% SDS. The Mr(app) of the 47-kD actin binding protein was 47 kD on 5% to 16% gradient and 52 to 54 kD on 7.5% SDS-PAGE, which is a behavior shared by other proteins. For this reason, the protein will be referred to as 47 to 54 kD. Column eluates contained only a 47- to 54-kD protein and proteolytic fragments (Fig 6C) that react with MoAb1 (not shown). The 47- to 54-kD band was used to prepare polyclonal anti-47- to 54-kD antibody and for microsequencing.

A XZAP cDNA expression library from dimethyl sulfoxide (DMSO) induced HL60 promyelocytic cells was screened with polyclonal anti-47- to 54-kD and MoAb1. Of nine positive clones, one clone, C13, contained a 1.58-kb cDNA and reacted with both antibodies. The nucleotide sequence of C13 revealed an open reading frame of 1020 bases encoding a predicted polypeptide of 339 amino acids and was 97% identical to the human LSP1 sequence in GENETYCAL bank Fig 7A). Transient expression of the cDNA in MoAb1 negative, CV-1 monkey kidney cells \(^{31}\) converted >50% of cells to MoAb1 positive. MoAb1 immunoblots of transfected kidney cell lysates showed a single 47- to 54-kD protein and in vitro transcription/translation \(^{32,35}\) of C13 yielded a single 47- to 54-kD protein that immunoprecipitated with MoAb1 (Fig 8A,B).

Finally, to confirm MoAb1 recognition of LSP1 in PMNs, MoAb1 purified, native PMN 47- to 54-kD protein was microsequenced. Native 47- to 54-kD protein was N-terminally blocked. \(^{36}\) V8 protease digests of the protein yielded a 20-kD fragment with N-terminal sequence (Gly-Thr-Ile-Glu-Gln) identical to residues 183 to 187 in the predicted amino acid sequence of human LSP1 (hLSP1) \(^{26}\) (Fig 7B). Therefore, the PMN actin binding protein (Mr(app) 47 kD on 5% to 16% and 52 to 54 kD on 7.5% SDS-PAGE) recognized by MoAb1 and overexpressed in NAD 47/89 PMN is LSP1, a protein which coprecipitates with the IgM receptor of lymphocytes, \(^{37}\) associates with detergent insoluble fraction of lymphocytes, \(^{38}\) and binds F-actin. \(^{39}\)

DISCUSSION

This report describes an inquiry into the nature of and relationship between 47-kD and 89-kD proteins present in abnormal amount in PMNs with defective cellular motility, cytoskeletal structure, and in situ actin polymerization. \(^{40}\) The disorder is called neutrophil actin dysfunction with 47-kD and 89-kD protein abnormalities (NAD 47/89). The results show (1) the 89-kD and 47-kD proteins are antigenically distinct proteins; (2) the 89-kD protein is not gelsolin; \(^{41}\) (3) the 47-kD protein is an actin binding protein recognized by MoAb1 and overexpressed in NAD 47/89 PMNs; and (4) the PMN 47-kD actin binding protein is LSP1, which is an actin binding/regulatory protein previously identified only in lymphocytes. \(^{42}\) The results suggest overexpression of LSP1 may contribute to the defects in motility, cytoskeletal structure, and actin dynamics of NAD 47/89 PMNs.

The 47-kD protein overexpressed in NAD 47/89 PMNs is recognized by MoAb1, is an acidic protein (pI 4.5 to 4.7), presents as three peptides on 2-D gel, binds to actin, and forms a multimolecular complex with actin in PMNs. Evidence that the increased 47-kD protein in NAD 47/89 is an actin binding protein derives from \(^{125}\)I-actin gel overlays and MoAb1 immunofluorography separation of the 47-kD protein. PMN proteins separated on 1-D PAGE or 2-D gels and probed with \(^{125}\)I-actin show actin binding to a 47-kD protein that coincides with the 47-kD protein recognized by MoAb1. The 47-kD actin binding protein is present in NAD 47/89 homozygotes and heterozygotes in greater amount and has a pI identical to the protein in control PMNs. MoAb1 immunofluorography chromatography of PMN homogenates also suggest the 47-kD protein is an actin binding protein. MoAb1 matrix retains not only the 47-kD protein but also actin and proteins of MW 180 kD and 71 kD in a multimolecular complex. SDS treatment of the PMN homogenate before immunochromatographic separation results in the elution of only the 47-kD actin binding protein that, like LSP1, \(^{26}\) migrates at 47 kD on gradient and 52-
Fig 7. Comparison of nucleotide and amino acid sequence for HL60-derived C13 cDNA and reported sequence for human LSPl cDNA. The determined nucleotide sequence (A) and predicted amino acid sequence (B) for the C13 cDNA from PMNs (C13) aligned with the reported nucleotide sequence and predicted amino acid sequence for (hLSPl).* (A) Translational start site and stop codon is underlined. Nucleotide sequence identities are indicated by vertical bars. (B) The arrowhead (v) at 183 amino acids indicates the N-terminus of the 20-kD V8 peptide used for microsequencing, and the 5 amino acid sequence determined on native PMN protein is underlined. The dot (.) indicates uncertain amino acids from the nucleotide sequence.

Molecular cloning of a cDNA, which expresses protein recognized by anti-47-kD protein and sequencing of the native PMN 47-kD actin binding protein shows the 47-kD actin binding protein is LSPl or a closely related protein. LSPl is a recently described F-actin binding protein previously identified only in T- and B-lymphoid cells by immunoblot and mRNA analysis with a mouse cDNA and antimouse LSPl antibody. The mouse LSPl differs significantly from human LSPl in amino acid sequence. Neither LSPl mRNA or protein was identified in granulocytes or uninduced HL60. However, as shown here by microsequencing of V8 protease fragments of native PMN 47-kD protein, by cloning of an LSPl cDNA from an expression library of promyelocytic HL60 cells induced to granulocytic differentiation, and by MoAb 1 immunoreactivity in normal and NAD 47/89 PMNs, LSPl or a closely related protein is expressed in PMNs and the amount expressed in NAD 47/89 PMNs is increased. Furthermore another myeloid cell line, PLB985 also expresses LSPl as evidenced by 47- to 54-kD immunoreactivity with MoAb prepared against human PMN protein; however, uninduced HL60, monocytic cell lines, and peripheral blood monocytes and lymphocytes are negative for the protein (unpublished results, January 1993). Divergence of our results from previous observations could derive from species specificity of the immunogens used to raise monoclonal antibodies, unique specificity of

54-kD on 7.5% SDS-PAGE. Because the 47-kD protein binds actin and copurifies with actin, the result strongly suggests that the 47-kD protein directly interacts with actin and forms a multimolecular complex with 71-kD and 180-kD proteins similar to that reported for LSPl in lymphoid cells. The nature of the 71-kD and 180-kD proteins complexed with actin and the 47-kD protein in the complex is not known. Neither MoAb nor polyclonal anti-47- to 54-kD recognize the 71-kD or 180-kD protein indicating they are antigenically distinct from the 47-kD actin binding protein. The nature of the protein interactions in the multimolecular complex is not further defined and requires the identification of all unknown protein components.
MoAb1 for a PMN-specific but related protein, or cell type-specific post-translational modifications of LSP1.

In our studies, native PMN LSP1 forms a multimolecular complex with actin and binds G-actin in \(^{125}\)I-actin overlays. Previous studies by Jongstra et al.\(^2\) also show the 52-kD recombinant LSP1 expressed in BW5714 lymphoid cells forms a multimolecular complex with actin that is similar to the complex observed in PMNs. In contrast, the recombinant LSP1 expressed in Escherichia coli tends to self-aggregate except in the presence of NP40, and in the presence of NP40, the E. coli–expressed LSP1 binds F-actin as assayed by sedimentation assays but not G-actin. The reason for the divergent results regarding the form of actin bound by recombinant LSP1 and native PMN LSP1 is unclear; however, the differences could reflect differences in the binding assays used. For example, other actin binding proteins like gelsolin can both cosediment with F-actin or bind G-actin on \(^{125}\)I-actin overlays.\(^6\) Alternatively, the results could result from exclusion of NP40 from our assays, the coexistence of phosphorylated and dephosphorylated forms of LSP1 in PMNs (unpublished results, July 1993) that differ in their ability to bind F- or G-actin, or the presence of other unique posttranslational LSP1 modifications that exist in native PMN LSP1 but not in the recombinant protein expressed in E. coli. Further studies are required to distinguish between these possibilities and to determine the exact mechanisms whereby LSP1 overexpression may cause abnormal microfilamentous cytoskeletal structure and defective actin polymerization in NAD 47/89 PMNs.

The concurrent quantitative increase in LSP1 and decrease in the 89-kD protein in NAD 47/89 raises interesting questions concerning the relationship between the two proteins and their contribution to the motility and cytoskeletal defects. The inverse changes in quantity of LSP1 and the 89-kD proteins observed in proband’s and multiple NAD 47/89 family members’ PMNs suggest that some structural or functional relationship exists between the two proteins; however, the genetic mechanism for the abnormal expression of the two proteins remains obscure. Our studies exclude one possibility; that is, a mutant 89-kD gene encodes an abnormal and proteolytically sensitive 89-kD protein that is cleaved to yield a 47-kD fragment. This possibility is

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**Fig 8.** Expression of the C13 cDNA in vitro and in eukaryotic cells. (A) MoAb1 immunoblot of protein extracted from \(4 \times 10^6\) vaccinia T7 polymerase infected CV-1 monkey kidney cells transfected with pBluescript SK vector alone (lane 1) or vector with the C13 cDNA insert under control of T7 promoter (lane 2) as described.\(^5\) The gel is 7.5% acrylamide. (B) Autoradiographs of 7.5% SDS-PAGE gels of total \(^{35}\)S-methionine–labeled protein (lane 2) translated in vitro from mRNA generated from C13 cDNA by the T3 promoter of pBluescript SK as described.\(^6\) The protein immunooabsorbed by MoAb1–coated Sepharose beads (lane 3) and postabsorption supernatant (lane 4); the protein immunooabsorbed by control antigelsolin-coated beads (lane 5) and control protein, chloramphenicol acetyltransferase, expressed from CAT mRNA (lane 1). Note total absorption by MoAb1 beads of 47- to 54-kD peptides.
unlikely, because the two proteins are antigenically distinct and LSP-1, which is recognized by MoAb1 and with similar pl, is present in normal PMNs. Studies that further characterize the 89-kD protein and elucidate the function of LSP1 in PMNs are necessary to further understanding of NAD 47/89 and the relationship of the proteins to functional defects in NAD 47/89 PMNs. In summary, the studies show the 47-kD actin binding protein that is increased in NAD 47/89 is LSP1, which is an actin binding protein present in normal PMNs. The data suggest overexpression of LSP1 may contribute to the defective actin polymerization, cytoskeletal structure, and motility of NAD 47/89 PMNs.

ACKNOWLEDGMENT

The authors appreciate the gift of the induced HL60 cDNA expression library from William Nauseef, MD, the valuable discussions and the cloning assistance of Dr William Brit, discussions with Dr Ray Watts, and secretarial assistance of Anita Mollica in preparing the manuscript.

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