X-linked primary immunodeficiency associated with hemizygous mutations in the moesin (*MSN*) gene

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Background: We investigated 7 male patients (from 5 different families) presenting with profound lymphopenia, hypogammaglobulinemia, fluctuating monocytopenia and neutropenia, a poor immune response to vaccine antigens, and increased susceptibility to bacterial and varicella zoster virus infections.

Objective: We sought to characterize the genetic defect involved in a new form of X-linked immunodeficiency.

Methods: We performed genetic analyses and an exhaustive phenotypic and functional characterization of the lymphocyte compartment.

Results: We observed hemizygous mutations in the moesin *(MSN)* gene (located on the X chromosome and coding for MSN) in all 7 patients. Six of the latter had the same missense mutation, which led to an amino acid substitution (R171W) in the MSN four-point-one, ezrin, radixin, moesin domain. The seventh patient had a nonsense mutation leading to a premature stop codon mutation (R533X). The naive T-cell counts were particularly low for age, and most $CD8^+$ T cells expressed the senescence marker CD57. This phenotype was associated with impaired T-cell proliferation, which was rescued by expression of wild-type MSN. MSN-deficient T cells also displayed poor chemokine receptor expression, increased adhesion molecule expression, and altered migration and adhesion capacities.

Conclusion: Our observations establish a causal link between an ezrin-radixin-moesin protein mutation and a primary immunodeficiency that could be referred to as X-linked moesin-associated immunodeficiency. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Leukopenia, primary immunodeficiency, moesin, ezrin-radixin-moesin protein, adhesion, migration*

Of the 300 or so genetic defects known to cause primary immunodeficiencies,[1,2] a subset concerns proteins involved in the function of the actin cytoskeleton; these include Wiskott-Aldrich syndrome protein, the Wiskott-Aldrich syndrome protein–interacting chaperon protein, dedicators of cytokinesis 8 and 2, β-actin, and the Rho GTPases RAC2 and RHOH.[3-5] These genetic defects highlight the cytoskeleton's particular role in the development and function of the hematopoietic system.

Ezrin, radixin, and moesin (MSN) are members of the ezrin-radixin-moesin (ERM) family of proteins that link the C-terminal

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Abbreviations used
7AAD: 7-Aminoactinomycin D
BCR: B-cell receptor
CFSE: Carboxyfluorescein succinimidyl ester
ERM: Ezrin-radixin-moesin
FERM: Four-point-one, ezrin, radixin, moesin
G-CSF: Granulocyte colony-stimulating factor
IS: Immunologic synapse
LFA-1: Lymphocyte function–associated antigen 1
PB: Sodium phosphate buffer
PBS: Phosphate saline buffer
SDF1a: Stromal cell–derived factor 1
TCR: T-cell receptor
VZV: Varicella zoster virus

domain of cortical actin filaments to the plasma membrane through an N-terminal four-point-one, ezrin, radixin, moesin (FERM) domain. The latter interacts variously with phospholipids, cytosolic adaptor proteins, membrane proteins (such as CD44), and intercellular adhesion molecules.[6] Although ezrin, MSN, and radixin are structurally and functionally similar, each protein can exert a number of unique functions related to differences in the respective expression patterns and physical-chemical properties (eg, sensitivity to calpain, an essential effector in Ca^{2+} signaling).[7] The ERM proteins are ubiquitously expressed, and there are tissue-specific and quantitative variations; for example, ezrin is the most prominent ERM protein in epithelial cells, whereas MSN predominates in endothelial cells.[8] In the hematopoietic system MSN and ezrin are expressed strongly, whereas radixin is mostly absent.[7]

ERM activity is regulated by a dynamic equilibrium between the active and inactive forms, in which the N- and C-terminal regions bind to each other and thus block access to actin and membrane proteins.[9] The active conformation of MSN predominates in circulating lymphocytes,[10,11] where it maintains cell rigidity. The results of *in vitro* studies have shown that chemotaxis and T-cell receptor (TCR)/B-cell receptor (BCR) signaling induce rapid dephosphorylation of ERM proteins (through a decrease in phosphatidylinositol 4,5-bisphosphate levels or through phosphatases) and thus cytoskeletal relaxation and cell polarization.[4,12] After TCR or BCR stimulation, ERM proteins are then rephosphorylated by kinases (eg, Rho-associated protein kinase, protein kinase Cθ, and lymphocyte-orientated kinase)[13-16] and relocate to the distal pole complex.[17] MSN and ezrin are involved in formation of the immunologic synapse (IS) through participation in microclustering of the antigen receptor complex and recruitment or exclusion of specific proteins.[18] In the case of chemokine stimulation, ERM proteins participate in the resorption of microvilli and relocate to the lamellipodial front and the uropods.[13,19] Although it was thought that ERM proteins are required for adequate TCR/BCR and chemokine responses (at least *in vitro*), the importance of each ERM protein in these processes is still subject to debate.

Hereditary disorders caused by *ERM* gene mutation have not been previously described. In the mouse, ezrin deficiency leads to intestinal/gut defects, early death,[20] and defective lymphoid development, which was attributed to malnutrition.[21] In contrast, *Msn* knockout mice do not display any major developmental defects[22,23] but do show poor egress of T and B lymphocytes from primary organs and lymph nodes (because of impaired chemotaxis).[24]

Here we report on a new form of human primary immunodeficiency observed in 7 male patients. The condition is characterized by profound leukopenia and impaired T-cell migration and proliferation. All 7 patients had hemizygous mutations in the *MSN* gene coding for the ubiquitously expressed MSN protein.

METHODS

Patient samples and cell preparations

PBMCs were harvested from patients and their families or healthy adult donors after the provision of written informed consent and in accordance with the Declaration of Helsinki. The study was approved by the regional investigational review board (reference: DC 2011-1338, CPP Ile-de-France II "Hôpital Necker-Enfants malades").

Leukocytes were counted in an automated hematologic analyzer (ABXMicrosES60; Horiba Medical, Kyoto, Japan). Mononuclear cells were isolated by means of Ficoll-Hypaque density gradient centrifugation (d = 1.077 g/mL; Lymphoprep; Axis-Shield, Oslo, Norway). The antibodies used for flow cytometric analysis are listed in the Methods section in this article's Online Repository at www.jacionline.org.

Mutation detection and X-chromosome inactivation assays

Genomic DNA was isolated by means of phenol/chloroform extraction. For patients P1, P2, and P7, whole-exome sequencing was performed with an Illumina TruSeq Exome Enrichment Kit (Illumina, San Diego, Calif) by using 100-bp paired-end reads. Variants were annotated by using both ANNOVAR33 (http://www.openbioinformatics.org/annovar/annovar_input.html) and custom scripts. Sanger sequencing confirmed mutations in P1, P2, and P7. For P3, P4, P5, and P6, DNA samples were amplified by using PCR with *MSN*-specific primers. The PCR products were purified with QIAquick PCR (Qiagen, Valencia, Calif), sequenced, and analyzed with a BigDye Terminator sequencing kit and an ABI Prism 3700 sequencer (Life Technologies, Carlsbad, Calif). PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org/), and MutationTaster (http://www.mutationtaster.org) software packages were used to predict the mutations' functional effects.

An X-chromosome inactivation assay was performed on DNA isolated from blood lymphocytes, polymorphonuclear neutrophils, and buccal epithelial cells (exposed or not to *Hpa*II restriction enzyme) by using a PCR-based method to probe the human androgen receptor gene locus (Xq12) with specific primers, as previously described.[25]

Lymphocyte cultures and proliferation assays are detailed in the Methods section in this article's Online Repository.

Protein blotting

Cells were lysed in RIPA buffer (Cell Signaling, Danvers, Mass) supplemented with protease inhibitors (Roche Applied Science, Penzberg, Germany) and phosphatase inhibitors (Sigma-Aldrich, St Louis, Mo). Cell extracts were separated on a NuPAGE 4-12% Bis-Tris gel (Life Technologies), blotted, and then stained with the following primary antibodies: anti-MSN (clone EB1863Y) from Cell Signaling and β-actin antibody (clone N21) from Santa Cruz Biotechnology (Dallas, Tex). After staining with anti-rabbit IgG and horseradish peroxidase–Fab fragments (GE Healthcare, Pittsburgh, Pa), the immunoblots were developed with Fusion Fx-7 Spectra (Vilber Lourmat, Collegien, France).

Transduction of human PBMCs

A full-length *MSN* cDNA clone (clone ID IOH2457; Thermo Fisher Scientific, Waltham, Mass) was subcloned into the pLVX-EF1a-ires-mcherry vector (Clontech, Mountain View, Calif). Empty pLVX-EF1a-ires-mcherry vector was used as a control. PBMCs were activated with anti-CD3/anti-CD28 beads and transduced overnight with lentiviral vector supernatants (produced at the Lentiviral Vector Production Facility, SFR Biosciences Gerland, Lyon,