Chediak-Higashi syndrome: Lysosomal trafficking regulator domains regulate exocytosis of lytic granules but not cytokine secretion by natural killer cells

Aleksandra Gil-Krzewska, PhD,[a] Stephanie M. Wood, PhD,[b] Yousuke Murakami, PhD,[a] Victoria Nguyen, BSc,[a] Samuel Cern Cher Chiang, MSc,[b] Andrew R. Cullinane, PhD,[c] Giovanna Peruzzi, PhD,[a] William A. Gahl, MD, PhD,[c] John E. Coligan, PhD,[a] Wendy J. Introne, MD,[c] Yenan T. Bryceson, PhD,[b] and Konrad Krzewski, PhD[a] *Rockville and Bethesda, Md, and Stockholm, Sweden*

Background: Mutations in lysosomal trafficking regulator *(LYST)* cause Chediak-Higashi syndrome (CHS), a rare immunodeficiency with impaired cytotoxic lymphocyte function, mainly that of natural killer (NK) cells. Our understanding of NK cell function deficiency in patients with CHS and how LYST regulates lytic granule exocytosis is very limited.

Objective: We sought to delineate cellular defects associated with *LYST* mutations responsible for the impaired NK cell function seen in patients with CHS.

Methods: We analyzed NK cells from patients with CHS with missense mutations in the *LYST* ARM/HEAT (armadillo/huntingtin, elongation factor 3, protein phosphatase 2A, and the yeast kinase TOR1) or BEACH (beige and Chediak-Higashi) domains.

Results: NK cells from patients with CHS displayed severely reduced cytotoxicity. Mutations in the ARM/HEAT domain led to a reduced number of perforin-containing granules, which were significantly increased in size but able to polarize to the immunologic synapse; however, they were unable to properly fuse with the plasma membrane. Mutations in the BEACH domain resulted in formation of normal or slightly enlarged granules that had markedly impaired polarization to the IS but could be exocytosed on reaching the immunologic synapse. Perforin-containing granules in NK cells from patients with CHS did not acquire certain lysosomal markers (lysosome-associated membrane protein 1/2) but were positive for markers of transport vesicles (cation-independent mannose 6-phosphate receptor), late endosomes (Ras-associated binding protein 27a), and, to some extent, early endosomes (early endosome antigen 1), indicating a lack of integrity in the endolysosomal compartments. NK cells from patients with CHS had normal cytokine compartments and cytokine secretion.

Conclusion: LYST is involved in regulation of multiple aspects of NK cell lytic activity, ranging from governance of lytic granule size to control of their polarization and exocytosis, as well as regulation of endolysosomal compartment identity. LYST functions in the regulated exocytosis but not in the constitutive secretion pathway. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Chediak-Higashi syndrome, lysosomal trafficking regulator, natural killer cell, cytotoxicity, cytotoxic lymphocyte, lysosomes, lytic granules, exocytosis, immune deficiency*

Chediak-Higashi syndrome (CHS; OMIM 214500) is a rare lysosomal storage disorder caused by mutations in lysosomal trafficking regulator *(LYST)*, which encodes the LYST protein.[1,2] CHS is characterized by oculocutaneous albinism, bleeding diathesis, and immune dysregulation.[3-5] About 85% to 90% of patients have a severe form of the disease (classic CHS), with a fatal hyperinflammatory syndrome termed hemophagocytic lymphohistiocytosis (HLH).[3,6] Death usually occurs in the first decade of life from infection, bleeding, or development of HLH.[3,5] The remaining patients with CHS have a milder attenuated form of the disease without HLH (atypical CHS).[6] A cell biology characteristic of CHS is the presence of giant lysosomes or lysosome-related organelles in several cell types.[7,8] LYST is a 429-kDa protein with several distinct domains implicated in various aspects of vesicular trafficking: ARM/HEAT (armadillo/huntingtin, elongation factor 3, protein phosphatase 2A, and the yeast kinase TOR1), PH (pleckstrin homology), BEACH (beige and Chediak-Higashi) and WD-40 (tryptophan-aspartic acid dipeptide repeat),[2,5,9,10] but its exact function remains to be elucidated.

Natural killer (NK) cells represent a subset of lymphocytes playing a key role in immunosurveillance and host defense against cancer and microbial pathogens.[11] Although contributing to the innate immune response, they also modulate the adaptive immune response.[12-14] NK cells recognize stressed cells through germline-encoded activating and inhibitory cell-surface receptors[15] and use their cytotoxic potential to eliminate abnormal cells and certain activated immune cells. Signals for activation, inhibition, or both are generated at a specialized contact site

From [a]the Receptor Cell Biology Section, Laboratory of Immunogenetics, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville; [b]the Center for Infectious Medicine, Department of Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Stockholm; and [c]the Office of the Clinical Director, National Human Genome Research Institute, National Institutes of Health, Bethesda.

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Corresponding author: Konrad Krzewski, PhD, 12441 Parklawn Dr, Twinbrook II, Rm 205, Rockville, MD 20852. E-mail: krzewskikj@niaid.nih.gov.

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Abbreviations used

ARM/HEAT domain:	Armadillo/huntingtin, elongation factor 3, protein phosphatase 2A, and the yeast kinase TOR1 domain
BEACH domain:	Beige and Chediak-Higashi domain
CHS:	Chediak-Higashi syndrome
CI-MPR:	Cation-independent mannose 6-phosphate receptor
EEA-1:	Early endosome antigen 1
F-actin:	Filamentous actin
HLH:	Hemophagocytic lymphohistiocytosis
IS:	Immunologic synapse
LAMP1/2:	Lysosome-associated membrane protein
LYST:	Lysosomal trafficking regulator
MTOC:	Microtubule organizing center
PH domain:	Pleckstrin homology domain
NK:	Natural killer
Rab:	Ras-associated binding protein
WD-40 domain:	Tryptophan-aspartic acid dipeptide repeat domain

formed between an NK cell and a target cell, which is known as the immunologic synapse (IS).[16] Target cell killing is a multistage process that concludes in exocytosis of perforin- and granzyme-containing lytic granules (secretory lysosomes) at the IS and induction of target cell apoptosis.[17,18] Defects in lytic granule secretion are associated with often fatal diseases, including familial HLH type 2 to 5, Griscelli syndrome type 2, and CHS.[18,19] NK cells in patients with CHS have abnormal morphology and function; a study of 2 patients with CHS showed profoundly impaired NK cell cytotoxic activity.[20,21] In line with the characteristic feature of CHS in other cell types, a single giant granule has been observed in NK cells from patients with CHS,[22] and enlarged lytic granules have been reported in cytotoxic T cells.[23] Nevertheless, our understanding of NK cell defects in patients with CHS is limited, and the role of LYST in cytotoxic lymphocyte biology is ill defined. Therefore we sought to dissect the effects of *LYST* mutations on different aspects of NK cell function.

METHODS

See the Methods section in this article's Online Repository at www.jacionline.org for detailed descriptions of methods and reagents.

Subjects and healthy donors

Patients with CHS were enrolled in protocol 00-HG-0153, which was approved by the National Human Genome Research Institute's Institutional Review Board, and provided written informed consent. CHS was considered based on clinical findings and confirmed by means of identification of giant inclusions within leukocytes on peripheral blood smears. Mutations in *LYST* were identified in all subjects and previously reported for some of the cases (see Table E1 in this article's Online Repository at www.jacionline.org).[6,24,25] Voluntary healthy donors were recruited at the National Institutes of Health, with informed consent provided in accordance with the Declaration of Helsinki.

PBMCs were isolated from whole blood by using the Ficoll-Paque method, and NK cells were isolated by using EasySep Human NK Cell Kits (STEMCELL Technologies, Vancouver, British Columbia, Canada) and cultured in X-VIVO medium (Lonza, Walkersville, Md) supplemented with IL-2 (100 U/mL). IL-2–cultured NK cells were used in experiments, unless otherwise noted.

Cytotoxicity assays

NK cell cytotoxicity was evaluated by using the DELFIA Assay (PerkinElmer, Waltham, Mass), as previously described.[26] Lytic units were calculated as described previously.[27] Delivery of granzyme B to target cells was assessed, as previously described.[26]

Cytokine production and release

For total cytokine levels, 2×10^5 NK cells were first mixed with K562 target cells at a 1:1 ratio for the indicated times at 37°C. The cells were next stained with anti-CD56–allophycoyanin, fixed, permeabilized, and stained with anti–TNF-α–phycoerythin or anti–IFN-γ–phycoerythin. Data acquisition and analysis were done with FACSort (BD Biosciences, San Jose, Calif) and FlowJo software (TreeStar, Ashland, Ore).

Cytokine secretion was evaluated by using the Human TNF-α or IFN-γ ELISA MAX Deluxe kit (BioLegend, San Diego, Calif) after stimulating 0.5×10^6 cells for 20 hours with IL-12 (20 ng/mL), IL-15 (100 ng/mL), and IL-18 (100 ng/mL).

Microscopy and image analysis

NK cells were left alone or mixed with target cells for 20 minutes at 37°C, followed by adherence to Excell Adhesion Slides (Thermo Scientific, Waltham, Mass) for 10 minutes at 37°C. Cells were fixed, permeabilized, and stained with anti–lysosome-associated membrane protein (LAMP) 1 or anti-LAMP2 antibody, followed by Alexa Fluor 647–conjugated anti-mouse antibody, and anti-pericentrin, followed by Alexa Fluor 568–conjugated anti-rabbit antibody or Alexa Fluor 568–conjugated phalloidin, and then stained with anti-perforin-AF488 antibody. In the experiments determining the location of vesicular compartments, fixed and permeabilized cells were stained with anti-LAMP1 antibody, followed by Alexa Fluor 488–conjugated anti-mouse antibody, and then anti-cation-independent mannose 6-phosphate receptor (CI-MPR), anti–early endosome antigen 1 (EEA-1), or anti–Ras-associated binding protein (Rab) 27a antibody, followed by Alexa Fluor 568–conjugated anti-rabbit antibody, and stained with anti-perforin–Alexa Fluor 647 antibody.

Cells mounted in ProLong Gold (Thermo Scientific) were visualized with a Zeiss LSM710 laser-scanning confocal microscope (Zeiss, Oberkochen, Germany). Images were obtained with a 63 × Zeiss Plan-Apochromat objective and Zeiss Zen software. Perforin polarization and colocalization were assessed, as described previously.[26,28]

NK cells were labeled for 30 minutes with 200 nmol/L LysoTracker and transferred to polylysine-coated Lab-Tek Chambered Coverglass (Thermo Scientific) in complete X-VIVO medium to assess the size and amount of lytic granules. Cells were imaged in all 3 planes at 37°C by using an Olympus IX81 spinning-disk confocal microscope with 100 × Olympus PlanApo objective (Olympus, Center Valley, Pa). Image acquisition was performed with MetaMorph software by using the streamlining function with the following parameters: 0.2-μm z-axial dimension, 100-ms exposure per frame, and 25 to 35 frames per image stack. The acquired images were deconvolved with Huygens software (Scientific Volume Imaging b.v., Hilversum, The Netherlands) with distilled experimental point spread function to correct image degradation as point spread function.[29] Deconvolved images were analyzed by using Imaris (Bitplane Inc, South Windsor, Conn) and its Spots function.

RESULTS

Subjects' characteristics

Eight subjects (6 male and 2 female subjects; age range, 21-43 years) from 5 families were studied (see Table E1). Most patients carried compound heterozygous *LYST* mutations with a

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