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Allergen-specific CD8⁺ T cells in peanut-allergic individuals



To the Editor:

CD8⁺ T cells are uncommonly thought to be involved in IgE-mediated food allergy, but there are reasons to reconsider their role in this condition.¹ CD8⁺ T cells are present in skin, and in the respiratory and gut mucosae, where they may modulate the immune response in atopic conditions such as asthma and atopic dermatitis.²⁻⁴ In mice, CD8⁺ T cells attenuate food allergy in some experimental models, whereas in humans, CD8⁺ T cells have been shown to expand in response to wheat ingestion in

celiac disease.^{1,5,6} Despite the fact that these findings show that CD8⁺ T cells can recognize food antigen and participate in T_H2 immune responses, we are not aware of any previous evidence demonstrating whether CD8⁺ T cells are specifically activated by allergen in IgE-mediated food allergy in people. Here, we show that CD8⁺ T cells are activated by peanut peptide in a sequence-dependent manner in peanut-allergic individuals.

To determine whether CD8⁺ T cells respond to peanut, we examined blood samples from well-characterized and food challenge-proven peanut-allergic subjects using previously published methods,⁷ and phenotyped T cells using mass cytometry (Table I). PBMCs from 53 pediatric subjects and 20 adult subjects were incubated either with or without peanut protein solution for 24 hours, then analyzed by mass cytometry using published methods.⁸ To identify peanut allergen-specific responses, we measured surface expression of the early activation marker CD69. Incubation with peanut protein, but not media, was associated with a significantly greater percentage of activated CD69⁺ CD8⁺ T cells in both peanut-allergic pediatric and adult subjects (Fig 1, A; $P = 7.2 \times 10^{-10}$ pediatric, and .0003 adult). We conclude that CD8⁺ T cells become activated in the presence of peanut allergen in peanut-allergic individuals.

To determine whether an increase in peanut-specific CD8⁺ T cells is associated with peanut allergy, we tested blood samples from 16 peanut-allergic individuals and 16 peanut-tolerant controls (Table I) using the same methods. Peanut allergy was determined by standardized oral food challenge (Table I). In this set of experiments, PBMCs were incubated either with or without peanut protein for 3 days to reveal the expression of later activation markers. In the case of one such activation marker, the IL2RA chain (CD25), we found that incubating PBMCs with peanut protein was associated with a statistically significant increase in CD25⁺ CD8⁺ T cells in peanut-allergic subjects as compared with peanut-tolerant children (Fig 1, B; $P < .0001$). In addition, an increased proportion of these peanut-specific CD25⁺ CD8⁺ T cells expressed the T_H2-associated chemokine CCR4, consistent with their potential involvement in an allergic immune response (Fig 1, C; $P < .0001$). We conclude that peanut-specific CD8⁺ T cells exist at higher frequencies in peanut-allergic individuals, and express CCR4, suggesting involvement in a T_H2-type allergic immune response.

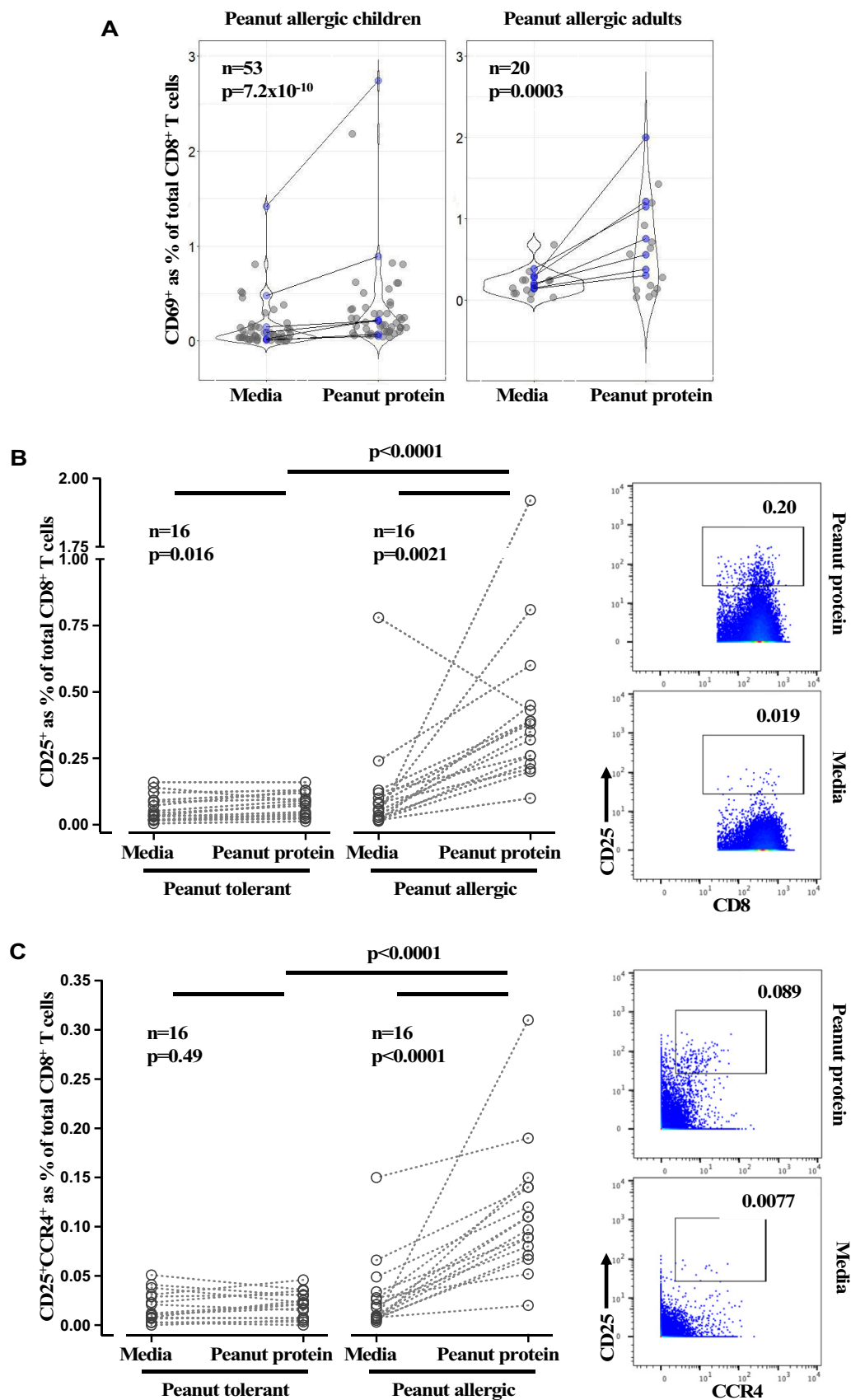
To determine whether the activation we observed in CD8⁺ T cells is peptide specific, we tested whether peanut peptide, as opposed to a mixture of peanut proteins, could also activate CD8⁺ T cells in peanut-allergic individuals. We chose

TABLE I. Patients' demographic characteristics

Characteristic	Subjects in Fig 1, A, and Fig 2		Subjects in Fig 1, B and C	
	Pediatric peanut allergic	Adult peanut allergic	Peanut allergic	Peanut tolerant
N	53	21	16	16
Age (y), GM (range)	10.5 (8-18)	29.7 (19-53)	7.45 (5-12)	6.20 (4-13)
Sex	20F:33M	5F:16M	7F:9M	8F:8M
Peanut IgE (kU _A /L), GM (range)	54.3 (0.41-869)	15.6 (0.62-221)	177.2 (13-676)	NA
Peanut allergy	All (+) by FC	All (+) by FC	All (+) by FC	None by hx $\pm$ IgE
History of atopic dermatitis, n (%)	40 (75)	11 (52)	8 (50)	0 (0)
History of asthma, n (%)	36 (68)	17 (81)	11 (69)	0 (0)
Race/ethnicity	32W:16A:0B:5O	13W:5A:1B:2O	5W:10A:1B:0O	9W:4A:1B:2O

A, Asian; B, black; F, female; FC, food challenge; GM, geometric mean; hx, history; M, male; NA, not applicable/available; O, other; W, white.

N = 21 for adult peanut-allergic patients because of the inclusion of subjects from Fig 1, A, and Fig 2, A.



to use peptide sequences derived from the peanut components Ara h 1, Ara h 2, and Ara h 3 because IgE specific for these proteins correlates with an increased risk of clinical IgE-mediated food allergy to peanuts.¹ Computer algorithms were used to identify peanut peptide sequences predicted to bind HLA-A*02:01 (iedb.org). We then performed HLA-typing at the Histocompatibility, Immunogenetics, & Disease Profiling Laboratory at Stanford University (Director, Dr Dolly Tyan) to identify peanut-allergic subjects who were also HLA-A*02:01⁺.

PBMCs from 15 HLA-A*02:01⁺ peanut-allergic subjects—of which 14 were also tested for CD69 expression (shown in blue in Fig 1, A)—were incubated with or without a pool of 6 to 21 peanut-derived peptides. The incubation time was increased up to 1 week to increase the activation signal by giving peptide-specific CD8⁺ T cells more time to expand.⁹ Antibody against CD28 was included regardless of whether pooled peptides were added or not. We observed a significant increase in CD8⁺ T-cell activation after incubation with peanut peptides versus media in 8 of 15 peanut-allergic subjects (Fig 2, A; $P = .0078$). This likely represents only a subset of the total peanut-specific CD8⁺ T-cell response to peanut, given that other peanut peptides and HLA alleles may be involved. We conclude that CD8⁺ T cells in peanut-allergic individuals can respond to peanut peptide.

To gather further evidence that the CD8⁺ T-cell allergen response is epitope specific, we attempted to determine the peptide specificity of individual T-cell clones. We took the approach of isolating single peanut-specific CD8⁺ T cells individually and then expanding these clones *in vitro* (Fig 2, B). Specifically, PBMCs from HLA-A*02:01⁺ peanut-allergic subjects were incubated with pooled peanut peptides and anti-CD28 antibody for 1 week. Activated (CD25⁺CD38⁺) CD8⁺ T cells were single cell sorted by fluorescence-activated cell sorting and then expanded *in vitro* as described previously.⁹

We grew T-cell clones, each derived from the expansion of a single CD8⁺ T cell, from the PBMCs of 3 subjects who were HLA-A*02:01⁺ (indicated in orange in Fig 2, A). These clones were retested for peptide specificity by the CD107 mobilization

assay. CD107a (LAMP1) is expressed on the cell surface of CD8⁺ T cells after T-cell receptor (TCR) ligation by a cognate peptide:MHC ligand, and so the detection of CD107a on the surface of CD8⁺ T-cell clones by flow cytometry indicates antigen recognition via the TCR.⁹ In the case of subjects I and III, we recovered CD8⁺ T-cell clones that mobilized CD107a after incubation with the same pool of peanut-derived peptides initially used to activate these cells and isolate them by fluorescence-activated cell sorting (Fig 2, B). Based on previous results, it is expected that not all clones derived in this manner will respond by the more stringent functional CD107 assay; for example, T-cell clones that robustly bind peptide MHC tetramer do not always mobilize CD107a when challenged with the same peptide.⁹

We were able to continue to propagate the CD8⁺ T-cell clones from one subject in sufficient numbers to narrow down which of the pooled peptides were recognized by TCR. This was done by dividing the original peptide pool and repeating the CD107 assay. An example of this iterative process is demonstrated in Fig 2 for one CD8⁺ T-cell clone, for which TCR recognition is first shown for the pool of peptides derived from Ara h 1 (Fig 2, C, top). A subsequent experiment (Fig 2, C, bottom) shows that this clone recognizes only 1 peptide (peptide 293:301) from the Ara h 1 peptide pool, demonstrating that the CD8⁺ T-cell activation is specific for peptide sequence. Interestingly, all 11 T-cell clones from subject III recognized the same peptide. To confirm that this peptide is recognized by CD8⁺ T cells, we incubated a new aliquot of PMBCs from subject III with or without this single Ara h 1–derived peptide for 1 week. In Fig 2, D, we observe robust CD8⁺ T-cell activation from a single peanut-derived peptide. It is difficult to conceive of any other mechanism of CD8⁺ T-cell activation with this degree of peptide specificity other than TCR recognition of peanut peptide presented by MHC. These results support the notion that CD8⁺ T cells respond to peanut peptide epitopes in peanut-allergic individuals.

In summary, we have provided evidence that individuals with IgE-mediated peanut allergy have an increase in allergen-specific CD8⁺ T cells, and that these CD8⁺ T cells recognize specific

FIG 1. A, CD8⁺ T cells are activated by peanut protein in peanut-allergic patients. PBMCs from peanut-allergic patients were incubated with or without peanut protein at 200 μ g/mL for 24 hours. Mass cytometry was then used to measure the percentage of CD69⁺CD8⁺ T cells, which is represented here with violin plots. Each pair of blue points connected by a line represents a peanut-allergic subject whose PBMCs were also used in Fig 2, A. Pairs of gray points (media vs peanut protein, not connected by lines) represent additional peanut-allergic subjects. P values calculated by Wilcoxon signed rank test. **B,** Peanut-specific CD8⁺ T cells are increased in peanut-allergic individuals in comparison to peanut-tolerant individuals. *Left:* PBMCs from 16 peanut-allergic and 16 peanut-tolerant individuals were incubated with or without peanut protein at 100 μ g/mL for 3 days. Mass cytometry was then used to measure the percentage of CD8⁺ T cells expressing the activation marker CD25. Each pair of points connected by a line represents 1 individual. The Wilcoxon signed rank test was used to calculate P values for paired media versus peanut protein incubation experiments using PBMCs from the same individuals. The Mann-Whitney test was used to calculate P values for unpaired PBMCs incubated with peanut protein from either peanut-allergic individuals or peanut-tolerant negative control subjects. *Right:* Example mass cytometry plots gated on CD8⁺ T cells showing activation by peanut protein for 1 peanut-allergic individual. Percent of activated CD8⁺ T cells, based on CD25 expression, is shown within each gate. **C,** CD8⁺ T cells activated by peanut protein in peanut-allergic individuals express the T_H2 chemokine receptor CCR4. *Left:* PBMCs from 16 peanut-allergic and 16 peanut-tolerant individuals were incubated as in Fig 1, B. Mass cytometry was then used to measure the percentage of CD25⁺CD8⁺ T cells expressing CCR4. Each pair of points connected by a line represents 1 individual. The Wilcoxon signed rank test was used to calculate P values for paired media versus peanut protein incubation experiments using PBMCs from the same individuals. The Mann-Whitney test was used to calculate P values for unpaired PBMCs incubated with peanut protein from either peanut-allergic individuals or peanut-tolerant negative control subjects. *Right:* Example mass cytometry plots gated on CD8⁺ T cells showing CCR4 expression in CD25⁺CD8⁺ T cells activated by peanut protein for 1 peanut-allergic individual. Percent of CCR4⁺CD25⁺CD8⁺ T cells, with or without peanut protein stimulation, is shown within each gate.

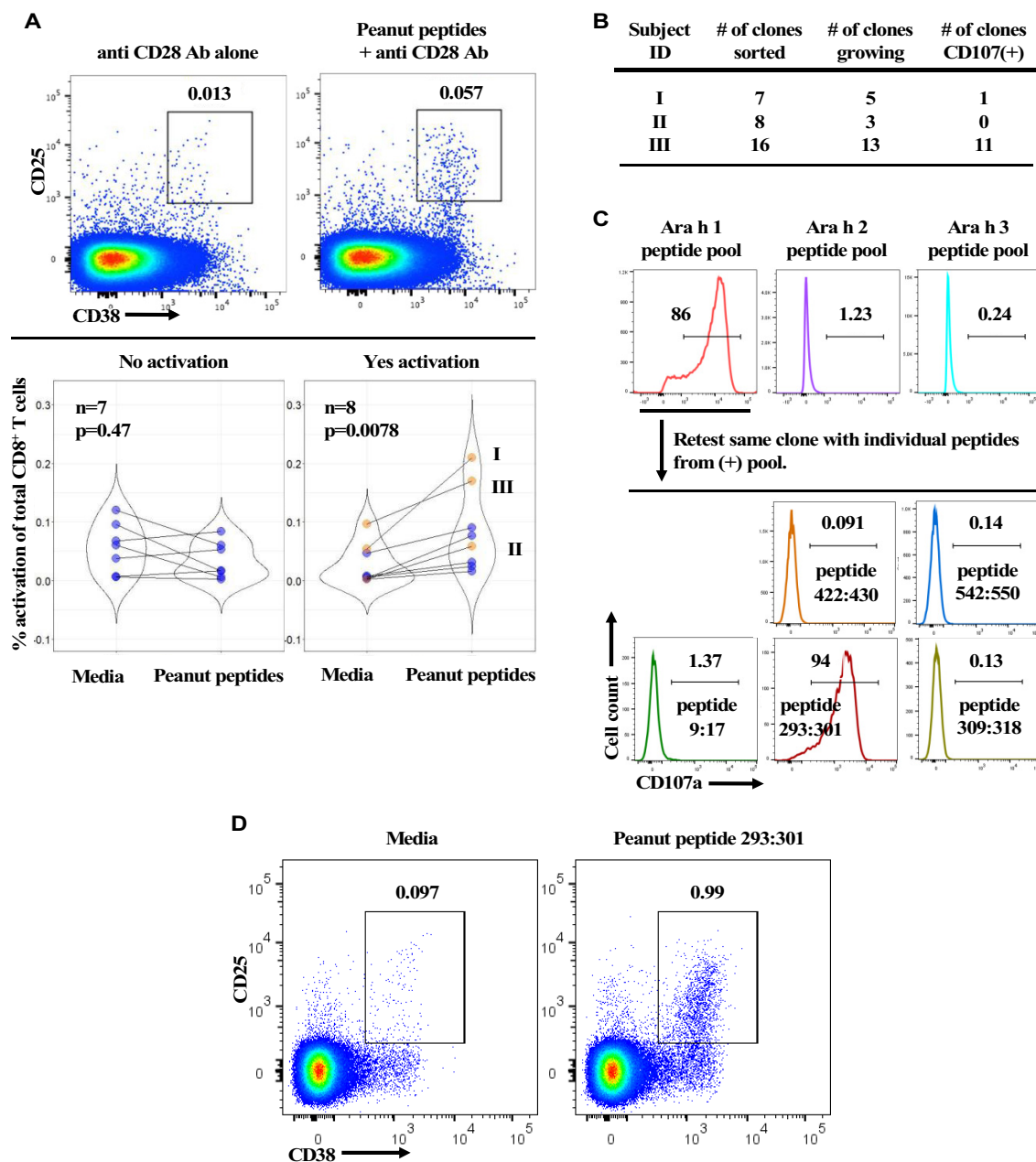


FIG 2. **A**, Pooled, peanut-derived peptides activate CD8⁺ T cells from peanut-allergic individuals. *Top*: Example flow cytometry plots gated on CD8⁺ T cells from 1 of 15 HLA-A*02:01⁺ peanut-allergic subjects whose PBMCs were incubated up to 1 week with media and anti-CD28 antibody (1-5 μ g/mL), $\pm$ pools of 6 to 21 peanut peptides (each at 1 μ g/mL) predicted to bind HLA-A*02:01. Percent of activated CD8⁺ T cells within each gate is shown. *Bottom*: Violin plots showing that pooled peanut peptide increases the percentage of activated CD25⁺CD38⁺CD8⁺ T cells in PBMCs from over half of the 15 peanut-allergic, HLA-A*02:01⁺ subjects tested. Each pair of points connected by a line represents 1 individual. Orange points represent the 3 subjects whose PBMCs were used in Fig 2, *B*. *P* values calculated by Wilcoxon signed rank test. **B**, Summary of *in vitro* cloning and expansion of CD8⁺ T cells activated by pooled peanut peptides. PBMCs from peanut-allergic individuals were activated as in Fig 2, *A*. Activated CD8⁺ T cells were then single cell sorted into individual wells by fluorescence-activated cell sorting, and expanded *in vitro* in the presence of mixed, irradiated PBMC feeder cells, PHA, and IL-2 (50 U/mL). Expanded T-cell clones were retested for recognition of the same pool of peanut peptides by the CD107 mobilization assay. **C**, CD8⁺ T-cell clones from peanut-allergic subjects differentiate between specific peanut peptides. Example flow cytometry plots of the CD107 mobilization assay for 1 T-cell clone from subject III showing TCR recognition of peptide from 1 of 3 peptide pools (*top*), followed by a subsequent round of screening showing TCR recognition of a single peptide (*bottom*). Clones are CD8⁺CD3⁺CD4⁺ $\gamma\delta$ TCR⁺. Peptide numbers separated by a colon indicate the starting and ending amino acid position of the peptide within the parent Ara h 1 sequence. **D**, A single peanut peptide activates CD8⁺ T cells from a peanut-allergic subject. A fresh aliquot of PBMCs from peanut-allergic subject III was incubated with anti-CD28 antibody $\pm$ peanut peptide 293:301 (see Fig 2, *B* and *C*). Flow cytometry plots gated on CD8⁺ T cells show an increase in CD8⁺ T-cell activation based on CD25 and CD38 expression in the presence of peanut peptide 293:301. Percent of activated CD8⁺ T cells within each gate is shown.

peanut-derived peptides. Although in this report we do not address questions about clinical sequelae, our results justify further investigation of the phenotype and role of CD8⁺ T cells, often overlooked, in IgE-mediated food allergy.

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Combined liver and hematopoietic stem cell transplantation in patients with X-linked hyper-IgM syndrome



To the Editor:

X-linked hyper-IgM syndrome (XHIGM [or HIGM1]) is a combined immunodeficiency caused by mutations in the gene encoding CD40 ligand (*CD40LG*), leading to an impairment of both cellular and humoral immunity.¹ Lack of CD40 ligand (CD40L) and hence of interaction between CD40L and CD40 results in defective T-cell function and costimulation, with

ensuing impaired class-switch recombination and somatic hypermutation in B cells, hindering an effective secondary antibody response. Moreover, activation of natural killer cells, dendritic cells, and monocytes is impaired, which results in an impaired inflammatory response.¹ Around 40% of patients present with *Pneumocystis jirovecii* interstitial pneumonia, and recurrent respiratory tract infections are present in 80% of cases. Other pathogens include mainly bacteria; mycobacteria; fungi, such as *Histoplasma*, *Cryptococcus*, and *Candida* species; and viruses, especially cytomegalovirus (CMV).¹⁻³

Gastrointestinal manifestations are described in 40% of patients and include diarrhea with failure to thrive, inflammatory bowel disease, and oral ulcers; neuroendocrine tumors of the gastrointestinal tract have been reported.^{1,2,4,5} Liver involvement can manifest as an increase in levels of liver enzymes, infectious hepatitis, and sclerosing cholangitis. *Cryptosporidium* species infection has been reported in as many as half of the cases of sclerosing cholangitis, often leading to cirrhosis and liver failure.¹⁻⁵ Central nervous system disease can be present in up to 11% of patients at presentation in the form of central nervous system infections or neurodegenerative disease.¹⁻⁵ Hematologic abnormalities, such as neutropenia and anemia, are described in more than 60% and 15% to 20% of patients, respectively.^{1,3-5}

Despite immunoglobulin substitution and antimicrobial prophylaxis, the overall prognosis is poor, with a median survival time from diagnosis of 25 years.⁶ Both European and US registries report mortality of 10% to 20% before the age of 30 years in their patient cohorts.^{1,3-6}

A recent retrospective observational study analyzed the survival rate of 176 patients with a diagnosis of XHIGM between 1964 and 2013 and compared outcomes for those patients treated with or without hematopoietic stem cell transplantation (HSCT).⁶ Liver or biliary involvement at diagnosis represented the only significant predictor of mortality, and survival was not influenced by HSCT.⁶ However, a survival benefit for patients undergoing transplantation was noted starting from 1987, suggesting improvements in transplantation practice over the last 4 decades. Also, patients treated with HSCT demonstrated improvement on scales of daily living (Lansky or Karnofsky) when compared with the group not undergoing transplantation.⁶

A recent retrospective study from the United Kingdom of 24 patients with XHIGM reported a crude mortality of 38% in patients with liver disease compared with 6% in patients without liver disease.⁷ Mortality as high as 80% was reported for patients with XHIGM with liver disease treated with HSCT compared with 10% for those treated with HSCT without liver pathology.⁶ Therefore it is generally recommended that patients with XHIGM patients receive HSCT upfront and before the onset of liver disease.^{6,7}

Liver transplantation (LT) has been performed in small numbers of patients with XHIGM who have end-stage liver disease with or without concomitant HSCT. Mortality in patients with XHIGM only receiving LT is high. This is likely because the underlying immune defect is not corrected and could be aggravated further by the immunosuppression needed for LT, as previously reported.^{6,8} Improved outcome is noted when both LT and HSCT are performed.^{6,7}

The aim of this study was to systematically report and compile the data on published and unpublished patients with XHIGM who received LT with or without HSCT. Patients were included from the previous study by de la Morena et al,⁶ as well as through