Glycosylphosphatidylinositol-specific, CD1d-restricted T cells in paroxysmal nocturnal hemoglobinuria

Lucia Gargiulo,1,2 Maria Papaioannou,1 Michelina Sica,2 Giulia Talini,2 Aristeidis Chaidos,3 Barbara Richichi,3 Andrei V. Nikolaev,4 Cristina Nativi,3 Mark Layton,1 Josu de la Fuente,6 Irene Roberts,1 Lucio Luzzatto,6 Rosario Notaro,2 and Anastasios Karadimitris1

1Centre for Haematology, Department of Medicine, Hammersmith Hospital, Imperial College London, London, United Kingdom; 2Cancer Genetics and Gene Transfer-Core Research Laboratory, Istituto Toscano Tumori, Firenze, Italy; 3Dipartimento di Chimica, Università di Firenze, Sesto Fiorentino, Italy; 4Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee, United Kingdom; 5Paediatric Haematology, Blood and Marrow Transplant Programme, St. Mary's Hospital, Imperial College Healthcare National Health Service (NHS) Trust, London, United Kingdom; and 6Istituto Toscano Tumori, Firenze, Italy

The mechanism of bone marrow failure (BMF) in paroxysmal nocturnal hemoglobinuria (PNH) is not yet known. Because in PNH the biosynthesis of the glycolipid molecule glycosylphosphatidylinositol (GPI) is disrupted in hematopoietic stem and progenitor cells by a somatic mutation in the PIG-A gene, BMF might result from an autoimmune attack, whereby T cells target GPI in normal cells, whereas PIG-A mutant GPI-negative cells are spared. In a deliberate test of this hypothesis, we have demonstrated in PNH patients the presence of CD8+ T cells reactive against antigen-presenting cells (APCs) loaded with GPI. These T cells were significantly more abundant in PNH patients than in healthy controls; their reactivity depended on CD1d expression and they increased upon coculture with CD1d-expressing, GPI-positive APCs. In GPI-specific T cells captured by CD1d dimer technology, we identified, through global T-cell receptor (TCR) analysis, an invariant TCRVα21 sequence, which was then found at frequencies higher than background in the TCR repertoire of 6 of 11 PNH patients. Thus, a novel, autoreactive, CD1d-restricted, GPI-specific T-cell population, enriched in an invariant TCRα chain, is expanded in PNH patients and may be responsible for BMF in PNH.

Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is caused by somatic mutations in the X-linked PIG-A gene in hematopoietic stem and progenitor cells (HSPCs).1,2 PIG-A mutations hinder the production of glycosylphosphatidylinositol (GPI), a glycolipid to which 2% of all proteins are posttranslationally attached before they are sorted and expressed on the cell membrane as GPI-linked molecules.

The clinical phenotype of PNH consists of a triad of intravascular hemolysis, prothrombotic diathesis, and cytopenias due to bone marrow failure (BMF).3,4 Whereas hemolysis and thrombosis are largely due to deficiency of the GPI-linked complement inhibitors CD55 and CD59,4 the pathogenesis of BMF has remained elusive. The close clinical relationship between PNH and idiopathic aplastic anemia (AA), a T-cell–mediated autoimmune BMF disorder characterized by depletion of HSPCs,5 points toward an immune mechanism mediating BMF in PNH as well. In fact, since the formulation of the “escape” hypothesis,6 it was suggested that a T-cell–mediated autoimmune process targeting GPI-positive but not GPI-negative HSPCs may underlie BMF in PNH.8,10 Accordingly, whereas GPI-negative HSPCs arise spontaneously,11 they do not contribute significantly to hematopoiesis unless a T-cell–mediated autoimmune process, by selectively targeting GPI-positive HSPCs, favors expansion and differentiation of GPI-negative HSPCs: this gives rise to a large proportion of GPI-negative hematopoiesis involving all blood cell lineages, which becomes manifest as classic hemolytic PNH.

What is not yet clear is how T cells might selectively target GPI-positive HSPCs. An attractive hypothesis is that GPI itself is a target of autoreactive T cells in PNH.12,13 In support of this hypothesis, analysis of the T-cell receptor (TCR) complementarity determining region (CDR) β sequence showed that the same identical or quasi-identical TCRβ chain was enriched in CD8+CD57+ T cells of the subset of human leukocyte antigen (HLA)–disparate PNH patients but not in normal controls.13 This is consistent with a T-cell response to the same antigen, restricted by a nonpolymorphic HLA-like molecule such as CD1d. CD1d is expressed on both human and murine HSPCs14,15 and has been shown to associate with GPI,16,17 in keeping with the possibility that CD1d-restricted, GPI-specific T cells might be responsible for targeting HSPCs in PNH. If such cells were involved in the pathogenesis of BMF in PNH, their frequency would be expected to be higher in patients than in normal controls and their TCR structure might resemble that of the...
CD1d-restricted, glycolipid-specific invariant natural killer T (iNKT) cells: that is, it might have an invariant TCR chain.18-22 To address these hypotheses, we searched for the presence and assessed the frequency of GPI-specific T cells in the blood of PNH patients and we analyzed the primary structure of their TCRα chain.

Patients, materials, and methods

Patients

Peripheral blood samples from patients with PNH and from age- and gender-matched healthy donors were obtained after written informed consent in accordance with the Declaration of Helsinki and appropriate institutional ethics committee approval. Patient characteristics are shown in supplemental Table 1 (available on the Blood website).

Cell lines and cell culture

The Epstein-Barr virus–transformed CIR B-cell line expresses no or low levels of HLA class I molecules, while CIR-CD1d cells were derived by retroviral transduction of CD1d.23 The KC cell line (a GPI-negative derivative of K562) was a gift from Dr R. W. Finnberg (University of Massachusetts Medical School, Worcester, MA). KC-CD1d cells were generated by retroviral transduction of human CD1d complementary DNA (cDNA) into KC cells as described.24 All cell lines were maintained in RPMI 1640 supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin (50 IU/mL), and streptomycin (50 μg/mL). Immunoophenotypic analysis of the above cell lines is shown in supplemental Figure 1A.

T-cell and APC coculture for ELISPOT assays

For trypanosomal GPI (t-GPI)25,26 loading of antigen-presenting cells (APCs; T-cell and APC coculture for ELISPOT assays above cell lines is shown in supplemental Figure 1A. For day 1 assays, T cells were placed directly into enzyme-linked immunospot (ELISPOT) wells for 24 hours. A similar approach was used for loading human GPI (h-GPI) onto patient-derived dendritic cells (DCs; see supplemental Methods).

For 7-day cocultures, T cells were cocultured with GPI-loaded CIR-CD1d until 24 hours before performing the ELISPOT assay. For 14-day cocultures, T cells were restimulated on day 7 with GPI-loaded CIR-CD1d cells and then kept in culture with the GPI-loaded CIR-CD1d until 24 hours before performing the ELISPOT assay. Specifically, after irradiation (6000 Rad), 2.5 × 10^5 CIR-CD1d were cocultured in 24-well plates with 0.5–1 × 10^5 CDB8+ T cells in 1 mL of RPMI medium (Invitrogen) supplemented with 5% human serum, t-glutamine (2 mM), penicillin (50 IU/mL), and streptomycin (50 μg/mL). Interleukin-2 (IL-2) (100 U/mL; Miltenyi Biotec) was added every 3 days. ELISPOT assay was carried out using an AID ELISPOT kit (Mabtech) according to the manufacturer’s instructions at days 1, 7, and 14 of the coculture. Briefly, CDB8+ T cells were collected and cocultured with freshly prepared GPI- or Veh-loaded irradiated CIR-CD1d at different effector-to-target (E:T) ratios, as indicated in individual experiments, in 96-well polyvinylidene difluoride membrane plates (Whatman) coated with anti-human interferon (IFN)γ monoclonal antibody (mAb). After 16 hours of incubation at 37°C and 5% CO2, secreted IFNγ was detected using biotinylated anti-human IFNγ mAb and revealed by development with streptavidin-alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate-toluidine salt/nitro blue tetrazolium chromogenic substrate. Spots were quantified using an AID ELISPOT reader.

T-cell and APC coculture for intracellular IFNγ staining

A total of 5 × 10^5 monocyte-depleted peripheral blood mononuclear cells (PBMCs; obtained by collection of nonadherent cells from Ficoll-fractionated PBMCs incubated in serum-free RPMI 1640 medium at 37°C for 2-4 hours) and irradiated 2.5 × 10^5 APCs were added to wells of a 24-well plate in 1 mL of RPMI medium supplemented with 5% human serum, t-glutamine (2 mM), penicillin (50 IU/mL), and streptomycin (50 μg/mL). IL-2 (100 U/mL; Miltenyi Biotec) was added every 3 days. On day 6, additional APCs (E:T ratio, 2:1) were added to the culture. On day 6, brefeldin A (E Bioscience) was added into the culture at a final concentration of 5 μg/mL, 5 hours after the addition of irradiated APCs. Finally, 16 hours later, intracellular staining for IFNγ was performed using the manufacturer’s reagents and instructions (Fix and Perm; Invitrogen). Briefly, cell suspensions were first stained with the following mouse anti-human mAbs: CD3-phycoerythrin (PE), CD8-PE-Cy7 (BD Pharmingen) for 30 minutes at 4°C, fixed in the dark at room temperature for 15 minutes, and then permeabilized in Medium B permeabilization buffer and stained with anti-human IFNγ Alexa Fluor 647 (BD Pharmingen) in the dark at room temperature for 15 minutes. Finally, cells were analyzed using a FACSCanto II flow cytometer (BD Biosciences) and FlowJo software (Tree Star Inc).

CD1d/GPI dimer generation, staining, and flow cytometric analysis and cell sorting

Recombinant CD1d dimeric complexes (dimer X: soluble dimeric human CD1dIg fusion) were from BD Biosciences. To stain 1 × 10^6 cells, we generated CD1d/GPI dimers according to the following protocol: 2 μg of CD1d dimer X were mixed in a glass vial with 2 μg of t-GPI (dissolved in 2 μL of isopropanol/water, 3:2 vehicle), h-GPI (dissolved in 4 μL of isopropanol:dimethylsulfoxide [DMSO], 10:1 vehicle), or vehicle alone for 16 hours at 37°C. Synthesis of t-GPI and h-GPI were previously described.27,28

For CD1d/GPI or CD1d/Veh dimer staining, cells were first incubated with Fc blocking reagent (Miltenyi Biotec) for 10 minutes at 4°C to reduce nonspecific staining. Loaded CD1d dimer was added for 1 hour at 4°C in phosphate-buffered saline plus 0.5% bovine serum albumin; samples were washed with phosphate-buffered saline and then incubated with 2.5 μL of a 1:100 dilution (5 ng) of PE-conjugated A85-1 anti-mouse immunoglobulin G1 (IgG1) Ab (BD Pharmingen) for 10 minutes a 4°C. After 2 washes, samples were stained with the following mouse anti-human mAb: fluorescein isothiocyanate isothiocyanate–CD19, fluorescein isothiocyanate–CD14, Alexa Fluor 405-CD3, allophycocyanin-CD8 (all from BD Pharmingen). Finally, 4',6-diamidino-2-phenylindole (DAPI) was used in order to exclude dead cells. We performed 6-color flow cytometry for analysis and cell sorting on a FACS Aria II cell sorter (BD Biosciences). Doublets were excluded based on FSC/SSC-A values. Data were analyzed using the FlowJo software (Tree Star Inc).

T-cell flow-sorting, TCR amplicon library preparation, and GS Junior NGS

Four T-cell subsets, CD3+CD48 TRBV19+ and CD3+CD48 TRBV19− were flow-sorted from patient PBMCs. CD3+ T cells from normal donor were flow-sorted in 2 fractions, that is, CD3+TRBV19+ and CD3+ TRBV19− T cells (supplemental Figure 4A). Total RNA was extracted from sorted cells using the RNeasy Plus Micro kit (QIAGEN) and transcribed to cDNA with the RevertAid First Strand cDNA Synthesis Kit (Fermentas).

Polymerase chain reaction (PCR) TCRVa21-24 amplification using cDNA from each sorted T-cell fraction (supplemental Figure 4A) was performed using fusion primers designed according to the Genome Sequencer (GS) Junior system, amplicon library preparation method (supplemental Figure 4B). The PCR products were run on an agarose 1.6% gel and the band of interest, ~450 bp, was cut and gel-purified using a Fertamers kit according to the manufacturer’s protocol (supplemental Figure 4C).

Sequencing of the TCRVα21-24 amplicons was carried out on a 454 GS Junior (Roche) platform targeting >500 reads per amplicon. Reads were processed with the Roche Amplicon Variant Analyzer Version 2.3 software according to the manufacturer’s instructions, and all variant sequences extracted in clusters per amplicon by the corresponding “MD” All per-amplicon clustered sequences were exported in fasta format to ImMunoGeneTics (IMGT, http://www.imgt.org) and processed by IMGT-HighV-QUEST software.28

Immunomagnetic bead selection, aerolysin selection to generate a CIR-CD1d/GPI-negative B-cell line, in vitro differentiation of GPI-negative monocytes to DCs, and TCR α- and β-gene mRNA repertoire amplification are described in supplemental Materials and Methods.

Data and statistical analysis were performed using GraphPad Prism software.
Results

CD1d-dependent T-cell reactivity in response to exogenous GPI

To test whether GPI-specific T-cell responses could be identified in patients with the classic, hemolytic form of PNH (supplemental Table 1), we cultured CD8+ T cells with CD1d-expressing C1R (C1R-CD1d) B cells (supplemental Figure 1A) as APCs loaded with chemically synthesized t-GPI25,26 (supplemental Figure 1B); 24 hours later, we carried out IFNγ ELISPOT assays. Because C1R cells express HLA class II but not class I molecules,29 to minimize CD4+ T-cell-mediated alloreactive responses, we used CD8+ but not CD4+ T cells. In pilot experiments (supplemental Figure 1C), we found that the number of CD8+ T cells able to form IFNγ spots after exposure to GPI-loaded APCs was higher in patients than in controls.

Similar results were obtained when CD8+ T cells were cocultured with GPI-loaded C1R-CD1d cells for 1, 7, and 14 days: at each time point, T cells from PNH patients formed 4.9-, 4.5-, and 5.2-fold, respectively, more IFNγ spots than similarly treated T cells from normal donors (Figure 1A; P < .005 at each time point).

To test whether IFNγ reactivity in response to GPI is dependent on CD1d expression on APCs, we used, along with its C1R-CD1d derivative, also the parental CD1d-negative C1R cell line (supplemental Figure 1A), with and without t-GPI loading. We found that CD8+ T cells from 4 of 6 patients tested formed the highest number of IFNγ spots in the presence of t-GPI–loaded C1R-CD1d cells. By contrast, no such effect was observed with T cells from 3 normal donors (Figure 1B). In another set of similar experiments, reactivity of T cells was tested against the CD1d-negative, GPI-negative K562 cell line KC and its derivative KC-CD1d (supplemental Figure 1A). In 7 of 9 PNH patients, the IFNγ spot-forming activity of T cells was highest against KC-CD1d cells loaded with GPI than in other conditions (Figure 1C). This effect was not observed in any of the 4 normal donor controls tested. Overall, in 7 of 8 PNH patients, the highest T-cell reactivity was observed against CD1d-expressing APCs loaded with t-GPI, while none of the 7 controls showed this pattern (P = .001, Fisher exact test).

Together, these results show that CD1d is required for induction of optimal T-cell responses elicited by exogenous GPI in PNH patients.

CD1d-dependent T-cell reactivity in response to endogenous GPI

Because a trend for higher T-cell reactivity was observed even in the absence of exogenous GPI (Figure 1A) and, similarly, T cells from patients P16 and P22 (Figure 1B) formed a high number of spots even when GPI had not been added to C1R-CD1d cells, we surmised that patient T cells might be responding to endogenous GPI. Indeed, after a 7-day coculture of monocyte-depleted PBMCs with APC, the number of reactive T cells, as determined by IFNγ-intracellular staining and flow cytometry, was 6-fold higher when APCs were C1R-CD1d/GPI-positive rather than when they were either C1R-CD1d/GPI-negative or C1R (ie, CD1d-negative; n = 9; P < .05; Figure 2A). Also consistent with reactivity elicited by endogenous GPI, in 3 of 4 patients tested, a higher number of IFNγ ELISPOTs formed after exposure of T cells to C1R-CD1d/GPI-positive than to C1R-CD1d/GPI-negative cells (Figure 2B). It should be noted that 6 of 9 patients shown in Figure 2A, and 3 of 4 patients in Figure 2B, had not received any blood transfusions at the time of testing or at any time prior to this (supplemental Table 1), suggesting that T-cell GPI reactivity is not influenced by blood transfusion. This is in line with our previous observation that blood transfusion does not skew the TCRβ repertoire.30

Finally, we tested the ability of a form of h-GPI obtained by organic synthesis27 (supplemental Figure 1B) to elicit patient T-cell reactivity. We used as APCs mature myeloid DCs obtained from autologous, GPI-negative monocytes (supplemental Figure 2). We took advantage of the fact that in PNH patients the large majority of monocytes are CD14-negative. In the absence of added GPI, we

![Figure 1. CD1d-dependent T-cell reactivity in response to exogenous GPI.](Image)
could not demonstrate autologous reactive T cells; however, when h-GPI was added there was a sixfold increment in reactive autologous T cells, (n = 11; P = .005; Figure 2C), thus providing direct evidence that primary, professional APCs, when GPI-loaded, are also able to elicit IFNγ T-cell responses.

Thus, endogenous as well as exogenous GPI activates CD1d-dependent, GPI-reactive T cells, and such reactive cells are increased in patients with PNH.

CD1d/GPI dimer staining of T cells

To prospectively identify, enumerate, and physically isolate GPI-reactive, CD1d-restricted T cells ex vivo, we stained patient or control PBMCs with fluorescently labeled CD1d-dimer complexes loaded with t-GPI. To minimize nonspecific staining, we used a stringent protocol that excludes monocytes, B cells, and dead cells. The frequency of CD8+ CD1d/t-GPI dimer+ T cells was 10-fold higher in patients than in controls (P = .02; Figure 3A-B). In vitro, after 14 days of coculture of CD8+ T cells with C1R-CD1d cells loaded with t-GPI, the frequency of CD1d/t-GPI dimer+ T cells was fourfold higher in patients than in controls (P < .01), and significantly higher than the background staining frequencies of patient and control CD1d/Veh dimer+ T cells (P < .01; Figure 3C-D). Similar results were obtained using CD1d dimer loaded with h-GPI (Figure 3E-F). Therefore, CD1d/GPI dimer staining provides independent evidence that compared with controls, the frequency of CD1d-restricted, GPI-specific T cells is increased in patients with PNH.

Structural characterization of the TCR in CD1d/GPI dimer+ T cells

Although T-cell clones from flow-sorted CD1d/GPI dimer+ cells were obtained at high frequency in response to mitogens, IL-2, IL-7, or IL-15 in parallel experiments, several attempts to generate clones or cell lines from CD1d/GPI dimer+ T cells were unsuccessful. We therefore sought insights into their function by analyzing the primary structure of their TCR. For this purpose, after flow-sorting CD8+CD1d/GPI dimer+ T cells from 3 patients, the whole TCR...
α- and β-chain mRNA repertoire was analyzed using an approach successfully tested previously in amplifying TCR mRNA from as few as 3 T cells (supplemental Figure 3).

A novel invariant TCRα21Jα31-1 sequence was identified in 9 of 9 clones in 1 of the 3 patients (P5; supplemental Table 2). Unlike in the majority of TCRα chains, the CDR3 of an invariant TCRα chain is defined by not having any J segment N insertions or deletions: indeed, our Jα31-1 segment is germline and therefore invariant (Figure 4A). Interestingly, analysis of the CDR3β sequence in the same population of flow-sorted CD8+CD1d/GPI dimer+ T cells also revealed the same variant TCRβ19-D2-Jβ2-7 sequence in all 8 clones sequenced (Figure 4B).

Furthermore, purified TCRβ19+ T cells from P5 were 6.5 times more reactive after coculture with GPI-loaded than with unloaded GPI-SPECIFIC T CELLS IN PNH

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**Figure 3. CD1d/GPI dimer staining of T cells.** (A) Representative flow cytometry plots showing ex vivo staining of patient and control PBMCs with anti-CD8 and CD1d/t-GPI dimer followed by analysis by multiparameter flow cytometry. Plots shown are gated on DAPI-CD14-CD19-CD3+ T cells. (B) Cumulative data showing frequency of CD8+CD1d/t-GPI dimer+ T cells from 4 PNH patients and 4 healthy controls after ex vivo staining of PBMCs as described in panel A (staining with CD1d/Veh dimer was not performed in this set of experiments). (C) Representative flow cytometry plots showing staining of patient CD8+ T cells with (left) CD1d/t-GPI or (right) unloaded dimer after 14 days of coculture with C1R-CD1d cells loaded with t-GPI. Gating strategy as described in panel A. (D) Cumulative data showing frequency of CD8+CD1d/t-GPI dimer+ vs CD1d/Veh dimer+ T cells from patients (n = 7) and controls (n = 11), after 14 days of coculture of CD8+ T cells with C1R-CD1d cells loaded with t-GPI (1-way analysis of variance with Tukey multiple comparison test; NS, not significant). (E) Staining of CD8+ T cells with CD1d/h-GPI dimer or CD1d/Veh dimer after 14 days of coculture with C1R-CD1d cells loaded with h-GPI. Gating strategy as described in panel A. Staining of T cells from a PNH patient is shown. (F) Cumulative data from 10 PNH patients and 7 normal controls.
we used a TCRV frequency of invariant TCRV then quantify, by next-generation sequencing (NGS), the frequency of GPI. (C) Flow-sorted TCRV T cells. We postulated that if the invariant TCRV T cells.

Figure 4. Characterization of the TCR α and β chains of CD1d/GPI dimer+ T cells. Flow-sorted CD1d/GPI dimer+ T-cell fractions were subjected to a 2-step reverse transcription PCR to amplify the whole TCR α- and β-chain mRNA repertoire followed by plasmid cloning and sequencing of individual clones (see also supplemental Figure 4). (A) Nucleotide (top) and amino acid (bottom) sequence of the TCRVα21-Jα31-1 mRNA within the normal repertoire (Figure 4D; Table 1).

Discussion

The notion that PNH develops thanks to the escape of GPI-negative hematopoietic from T-cell–mediated autoimmune attack against normal (GPI-positive) hematopoietic cells was first outlined over 20 years ago, and has been supported by several studies.30,33 It has been further suggested that the target of the autoimmune attack may be, rather than a GPI-linked protein, the GPI molecule itself.12 Here, we provide direct evidence for this hypothesis. We have used a number of complementary methodologies (ie, ELISPOT and intracellular staining of IFNγ-producing cells and CD1d/GPI dimer staining), different types of APCs (B cells, DCs, and K562 cells) and 2 different GPI molecules (i-GPI and h-GPI). All of these approaches converged in enabling us to identify and quantify a novel population of GPI-specific, CD1d-restricted T cells that is expanded in a substantial proportion of PNH patients. Given that GPI has been previously demonstrated in the presentation groove of CD1d16,17,34 and given that CD1d is expressed on HSPCs,14,15 these findings provide strong support for the pathogenetic model which posits that CD1d-restricted, GPI-specific T cells target and deplete GPI-positive but not GPI-negative HSPCs in PNH.12

We found small numbers of GPI-specific T cells in normal controls, suggesting that they are part of the normal T-cell repertoire. However, their frequency does not increase in response to in vitro–added exogenous GPI, consistent with a state of functional anergy. By contrast, in PNH patients, the frequency of GPI-reactive T cells was not only higher at baseline, but also increased in the course of cocultures carried out for up to 14 days with GPI-loaded APCs. Our results with 2 different GPI-deficient cell lines and their isogenic GPI-positive counterparts, as well as with patient-derived autologous DCs, are consistent with the notion that GPI-specific T cells are autoreactive.

The numbers of GPI-reactive T cells, although higher than in controls, comprised a small percentage of all T cells in PNH patients. There could be several reasons for these low numbers. First, GPI anchors undergo several structural modifications3 and we do not...
Because both chains were identified at clonal frequencies in the same CD1d/GPI dimer+ T cells, it is very likely that they form a TCR heterodimer in a configuration that is similar to that of the TCR of iNKT cells. In support of this, CD8+ TCRVβ19+ T cells from the same patient displayed their highest reactivity in the presence of CD1d-expressing APCs pulsed with GPI; this establishes a link between TCRVα21-Jα31-1-expressing T cells with GPI-specific reactivity. The frequency distribution of the invariant TCRVα21-Jα31-1 chain in normal controls is consistent with the low frequency of GPI T-cell reactivity identified in healthy individuals. A higher frequency pattern was seen exclusively in 6 of 11 patients with PNH: in patient P22, the invariant TCRVα21-Jα31-1 chain was as high as 75% of the total TCRVα21 repertoire in TCRVβ19+ T cells, consistent with a process of GPI-dependent expansion of the TCRVα21-Jα31-1 T cells. A TCRVα21-specific mAb and TCR transfer experiments will be needed to further define the functional, phenotypic, and structural features of these T cells in health and in disease, including AA. These will include characterization of the complete repertoire of TCRβ chains that can pair with the invariant TCRVα21-Jα31-1 chain, functional validation of the CD1d dimers in complex with different structural variants of GPI, and testing the effect of the GPI-specific T cells on GPI-positive and GPI-negative HSPCs. In the meantime, the higher frequency of CD1d-restricted, GPI-specific T cells in patients than in controls strongly supports the potential of these cells to target CD1d-expressing GPI-positive HSPCs and thus contribute to the pathogenesis of BMF in PNH. This notion offers a rather straightforward explanation as to why PNH patients have often been previously diagnosed with AA. If AA was mediated by T cells that recognize a “conventional” (non-GPI) antigen, then this expansion would not take place. Future work will focus on a group of AA patients who do not have evidence of a PNH clone: we expect that in this group, the GPI-reactive T cells will not be above background.

Table 1. NGS analysis of the TCRVα21-Cα mRNA repertoire

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NGS analysis of the TCRVα21-Cα mRNA repertoire in flow-sorted TCRVα19+ and TCRVα19− T cells from PNH patients and normal controls (see also supplemental Figure 4).

mRNA, messenger RNA; NGS, next-generation sequencing.

†Due to very low frequencies of GPI-negative T-cell fractions and small sample sizes, deep sequencing of TCRVα21-Cα amplicons was not possible for all 4 populations in all patients with PNH.
‡Represent productive TCR reads.
HSCs, may exist at very low frequencies both before and after the expansion of the PNH hematopoietic clone. Once an autoimmune process has been triggered, both GPI-positive and GPI-negative TCRVβ21Jα3-1 T cells may become autoreactive against GPI-positive HSCs and expand in an antigen-dependent manner, either concurrently or sequentially.

In conclusion, we have demonstrated, in patients with PNH, expansion of a novel, autoreactive, CD1d-restricted, GPI-specific T-cell population enriched in an invariant TCRα chain. These T cells may be responsible for BMF in PNH.

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Authorship

Contribution: L.G. and M.P. performed research, analyzed data, and wrote the manuscript; M.S. performed research and analyzed data; G.T. and A.C. performed research; B.R., C.N., and A.V.N. contributed vital new reagents; M.L. and J.d.I.F. contributed to the writing of the manuscript; I.R. designed and supervised research and wrote the manuscript; and L.L., R.N., and A.K. were in charge of the clinical management of some of the patients, designed and supervised research, analyzed data, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Anastasios Karadimitris, Centre for Haematology, Department of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Rd, London W12 0NN, United Kingdom; e-mail: a.karadimitris@imperial.ac.uk.

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21. Kronenberg M. Toward an understanding of the clinical management of some of the patients, designed and supervised research, analyzed data, and wrote the manuscript.


Glycosylphosphatidylinositol-specific, CD1d-restricted T cells in paroxysmal nocturnal hemoglobinuria

Lucia Gargiulo, Maria Papaioannou, Michela Sica, Giulia Talini, Aristeidis Chaidos, Barbara Richichi, Andrei V. Nikolaev, Cristina Nativi, Mark Layton, Josu de la Fuente, Irene Roberts, Lucio Luzzatto, Rosario Notaro and Anastasios Karadimitris