Agammaglobulinaemia despite terminal B-cell differentiation in a patient with a novel LRBA mutation

Nashat Al Sukaiti1, Khwater AbdelRahman2, Jalila AlShekaili3, Sumaya Al Oraimi4, Aisha Al Sinani5, Nasser Al Rabhi6, Vicky Cho7, Matt Field7 and Matthew C Cook7,8

Mutations in lipopolysaccharide-responsive vesicle trafficking, beach and anchor-containing protein (LRBA) cause immune deficiency and inflammation. Here, we are reporting a novel homozygous mutation in LRBA allele in 7-year-old Omani boy, born to consanguineous parents. He presented with type 1 diabetes, autoimmune haematological cytopenia, recurrent chest infections and lymphocytic interstitial lung disease. The patient was treated with CTLA4-Ig (abatacept) with good outcome every 2 weeks for a period of 3 months. He developed complete IgG deficiency, but remarkably, histological examination revealed germinal centres and plasma cells in lymphoid and inflamed lung tissue. Further characterisation showed these cells to express IgM but not IgG. This ex vivo analysis suggests that LRBA mutation confers a defect in class switching despite plasma cell formation.

Clinical & Translational Immunology (2017) 6, e144; doi:10.1038/cti.2017.20; published online 26 May 2017

CASE REPORT

A 7-year-old Omani boy born to a consanguineous parent presented at the age of 9 months with type 1 diabetes, and was started on insulin replacement therapy. At the same time, he was found to have hepatosplenomegaly. He had two younger healthy male siblings. There was a family history of major histocompatibility complex class II deficiency due to a CIITA missense mutation (M1071T); neither proband nor his parents carried this mutation (Figure 1). At the age of 2 years, he developed repeated episodes of immune-mediated thrombocytopenia, which were managed successfully with high-dose intravenous immunoglobulin and prednisolone (Table 1). He remained in remission from immune-mediated thrombocytopenia for 2 months then developed Evan’s syndrome, (direct Coomb’s test-positive auto-immune haemolytic anaemia and immune-mediated thrombocytopenia), which failed to respond to either intravenous immunoglobulin or pulse methylprednisolone. At the age of 3 years, he was treated with four doses of rituximab, which induced a sustained remission. At the age of 4 years, a year after the last dose of rituximab treatment, however, he developed bacterial infections, which became recurrent. These included tonsillitis, otitis media, three bouts of pneumonia and one episode of acute salmonella gastroenteritis. At the age of 5 years, he was admitted with tachypnea and shortness of breath.

Chest X-ray at the age of 5 years showed interstitial lung infiltrates and hilar lymphadenopathy (Figure 2a). Bronchoalveolar lavage was positive for cytomegalovirus by PCR, however, there were no inclusion

1Department of Pediatrics, Allergy and Clinical Immunology Unit, Royal Hospital, Muscat, Oman; 2Department of Pediatrics, Allergy and Clinical Immunology, Royal Hospital, Muscat, Oman; 3Department of Pediatrics, Endocrine Unit, Royal Hospital, Muscat, Oman; 4Department of Pathology, Royal Hospital, Muscat, Oman; 5Department of Immunology, The John Curtin School of Medical Research, Australian National University, Acton, Australian Capital Territory, Australia and 6Department of Immunology, Canberra Hospital, Canberra, New South Wales, Australia.

Correspondence: Dr N Al Sukaiti, Department of Pediatrics, Allergy and Clinical Immunology Unit, Royal Hospital, Muscat 1331, Oman. E-mail: nashatalsukaiti@yahoo.com or Professor MC Cook, Department of Immunology, Canberra Hospital, Level 6 Building 10, PO Box 11, Woden ACT 2606, Canberra, New South Wales, Australia. E-mail: matthew.cook@anu.edu.au

Received 5 September 2016; revised 10 April 2017; accepted 10 April 2017
bodies seen by histopathology. At that time, cytomegalovirus DNA was detected at only 75 copies per ml in serum by PCR, however, this increased to 2434 copies per ml 1 month later. He was treated with intravenous ganciclovir 5 mg kg\(^{-1}\) twice a day followed by oral valganciclovir 15 mg kg\(^{-1}\) twice a day for a total of 6 weeks. Cytomegalovirus DNA was undetectable after treatment.

*Aspergillus niger* was isolated from bronchoalveolar lavage. Blood cultures were negative. He received 6 weeks treatment with voriconazole after which repeated bronchoalveolar lavage was negative for aspergillus. Despite administration of appropriate antimicrobial therapy, radiological changes persisted. High-resolution chest computed tomography scan was consistent with lymphocytic interstitial pneumonitis (Figure 2b).

Investigations at age 2 years (before rituximab treatment) revealed marked polyclonal hypergammaglobulinaemia (IgG > 32.2 g l\(^{-1}\)),

Table 1 Summary of immunological laboratory findings

| Age in years | 2 years | 5 years | 6 years | 7 years |
|--------------|---------|---------|---------|---------|
| Test         |         |         |         |         |
| IgG (3.5–12 g l\(^{-1}\)) | > 32.2 | < 1.0  | 4.5     | 6.9     |
| IgA (0.15–1.6 g l\(^{-1}\)) | 1.7    | < 0.05 | < 0.05  | < 0.05  |
| IgM (0.4–2 g l\(^{-1}\)) | 0.7    | < 0.05 | 1.65    | 4.99    |
| IgE (0–90 IU ml\(^{-1}\)) | < 15   |         |         |         |
| Hep B antibody |       | NR     |         |         |
| Measles, mumps and rubella antibodies | NR     | NR     |         |         |
| CD3 (1.0–2.0 x 10\(^{9}\)) | ND     | 5.210  | 3.260   | 0.730   |
| CD4 (0.5–1.3 x 10\(^{9}\)) | ND     | 1.230  | 0.860   | 0.820   |
| CD8 (0.3–1.0 x 10\(^{9}\)) | ND     | 3.510  | 2.030   | 1.730   |
| CD19 (0.2–0.5 x 10\(^{9}\)) | ND     | 0.410  | 0.300   | 0.190   |
| CD20 (6–23\(\%\)) | ND     | < 1    | 2.7     | 3.6     |
| CD16/56 (cells per \(\mu\)l) | ND     | 0.840  | 0.450   | 0.090   |
| %CD3\(^{-}\)CD4\(^{+}\) |       |         |         |         |
| %CD4\(^{-}\)CD45RA\(^{-}\)CCR7\(^{+}\) (naive CD4 cells) |       |         |         |         |
| %CD4\(^{-}\)CD45RA\(^{-}\)CCR7\(^{-}\) (central memory CD4T cells) |       |         |         |         |
| %CD4\(^{-}\)CD45RA\(^{-}\)CCR7\(^{-}\) (effector memory CD4T cells) |       |         |         |         |
| %CD4\(^{-}\)CD45RA\(^{-}\)CCR7\(^{-}\) (terminally differentiated CD4T cells) |       |         |         |         |
| %CD3\(^{-}\)CD8\(^{+}\) |       |         |         |         |
| %CD8\(^{-}\)CD45RA\(^{-}\)CCR7\(^{+}\) (naive CD8T cells) |       |         |         |         |
| %CD8\(^{-}\)CD45RA\(^{-}\)CCR7\(^{-}\) (central memory CD8T cells) |       |         |         |         |
| %CD8\(^{-}\)CD45RA\(^{-}\)CCR7\(^{-}\) (effector memory CD8T cells) |       |         |         |         |
| %CD8\(^{-}\)CD45RA\(^{-}\)CCR7\(^{-}\) (terminally differentiated CD8T cells) |       |         |         |         |
| %CD3\(^{-}\)CD4\(^{+}\)CD62L\(^{-}\)CD31\(^{-}\) (recent thymic emigrant) |       |         |         |         |
| %CD19\(^{+}\) |       |         |         |         |
| %CD19\(^{-}\)IgD\(^{-}\)CD27\(^{-}\) (naive B cell) |       |         |         |         |
| %CD19\(^{-}\)CD27\(^{-}\)IgD\(^{-}\) memory B cell |       |         |         |         |
| %CD19\(^{-}\)CD27\(^{-}\)IgD\(^{-}\) memory B cell |       |         |         |         |
| %CD19\(^{-}\)CD24\(^{-}\)CD38\(^{hi}\) transitional B cell |       |         |         |         |
| %CD19\(^{-}\)CD21\(^{-}\)CD38\(^{lo}\) plasmablast |       |         |         |         |
| Abbreviations: ND, not done; NR, not reactive. Reference ranges.\(^{17,18}\) |

Figure 1 Family pedigree of the proband (black and arrow) and the relatives with MHC class II deficiency (green).
with normal IgA, IgM and IgE (Table 1). At age 5 years (2 years after rituximab treatment), he was noted to be agammaglobulinemic. Lymphocyte subset analysis performed at age 5 years showed absence of CD20+ B cells, which started to recover by the age of 6 years old. Flow cytometric assessment of peripheral B-cell subsets at the age of 7 years revealed reduction in memory B cells and increased CD21+ B cells compared to normal controls of similar age group (Figure 3a). Transitional B cells were evident (CD24hi CD38hi). Flow cytometric analysis of different T-cell subsets revealed marked reduction of naïve cells in both CD4+ and CD8+ T-cell compartments, an inverted ratio of CD4+CD8+ T cells (Table 2). Moreover, there was an expansion of circulating follicular helper T cells (cTH) marked by high number of CD4+ PD-1+CD45RA− compared to normal controls (n=10) (Figures 3b and c).

Biopsies from lung obtained when the patient was 5 years old revealed type two pneumocyte hyperplasia. Alveolar septa were markedly expanded, with nodular lymphoid aggregates containing germinal centres identified by H&E. A cervical lymph node biopsy at the same time revealed follicular hyperplasia with histiocytic microgranulomas and aggregates of mononuclear cells. The lymphoid follicles showed prominent germinal centres confirmed by immunohistochemistry (CD10+). Abundant Ig kappa and Ig lambda positive plasma cells were identified, consistent with a polyclonal plasma cell response, although no IgG+ cells were present (Figure 3d). Bone marrow examination was hypercellular with plasma cells present and no other specific changes. Whole-exome sequencing was performed and analysed for known monogenic causes of primary immune deficiency or autoimmunity.
We identified a novel homozygous nonsense mutation in LRBA (Chr4: R1271X; g.163829C>T; c.4285C>T). The mutation was confirmed by Sanger sequencing, and both parents were confirmed as heterozygous for the same mutation (Figures 4b and c). LRBA R1271X has not been reported in 1000Genomes, dbSNP, ClinVar, ExAC or HGMD, and has not been detected in 4200 in-house exome sequences from unrelated individuals. The stop is predicted to result in loss of protein due to nonsense mediated decay of LRBA transcripts. After obtaining this molecular diagnosis, the patient was treated with abatacept (10 mg kg\(^{-1}\)) every 2 weeks for 3 months at the time of writing this article. He showed remarkable clinical improvement in terms of his shortness of breath, and his weight gain as well as clearance of X-ray (Figure 4a).

**DISCUSSION**

We have reported a novel allele of LRBA presenting with type 1 diabetes, autoimmune cytopenias and severe lymphocytic pneumonitis. At initial assessment, the patient had polyclonal hyperimmunoglobulinemia, but he subsequently became agammaglobulinemia. Although he had been treated with rituximab, agammaglobulinemia was noted >12 months after his treatment. He was commenced on intravenous immunoglobulin precluding further assessment of endogenous IgG production, but he remained IgA deficient for at least 4 years after rituximab. It is noteworthy that despite agammaglobulinemia, histological examination of lung and lymph node revealed abundant germinal centres with plasma cells being present. While plasma cells appeared polyclonal by Ig kappa and Ig lambda staining, they were negative for IgG.

BDCPs are thought to assemble macromolecular complexes that regulate vesicular trafficking and function.\(^1\) The ultra structural signatures of LRBA mutation include disordered autophagy, resulting in increased apoptosis\(^3\) and altered trafficking of CTLA4.\(^4\) The significance of the latter observation is borne out by the efficacy of abatacept therapy, as illustrated by our case. Nevertheless, manifestations of LRBA deficiency vary substantially from patient to patient. In Chediak-Higashi syndrome, which results from mutations in the BDCP family member LYST,\(^11\) phenotypic variation has been related to genotype, with milder phenotypes in patients with missense mutations.\(^11\) While there is genetic heterogeneity among the LRBA mutant cases reported to date (~50), all reported cases arise from nonsense mutations, indels or large deletions.

Clinical resemblance to autoimmune lymphoproliferative syndrome, deficiency of Tregs and response to abatacept indicate that T-cell dysregulation is an important cause of autoimmunity and inflammation in LRBA deficiency. Furthermore, clinical improvement has been noted in at least two cases treated with hematopoietic stem

| Table 2 Case histology chronology |
|----------------------------------|
| Age | Complication | Hb (g l\(^{-1}\)) | N (x10\(^9\) l\(^{-1}\)) | Plt (x10\(^9\) l\(^{-1}\)) | IgG (g l\(^{-1}\)) | IgA (g l\(^{-1}\)) | IgM (g l\(^{-1}\)) | CD19 (x10\(^9\) l\(^{-1}\)) | Treatment |
|-----|--------------|----------------|------------------|----------------|----------------|----------------|----------------|----------------|------------|
| 0.9 | T1D          | 13.3           | 1.8              | 250            |                |                |                |                | Insulin    |
| 2.3 | ITP          | 12.9           | 2.1              | 13             | >30            | 1.7            | 0.7            |                | IVIg       |
| 2.8 | AIHA, ITP    | 7.0            | 1.6              | 1              |                |                |                |                | IVIg, prednisolone |
| 3.0 | ITP          | 12.5           | 5.5              | 3              |                |                |                |                | IVIg, prednisolone |
| 3.2 | ITP          | 13.6           | 3.9              | 21             |                |                |                |                | RTX        |
| 4.2 | Pneumonia, OM, gastroenteritis | 12.9 | 3.2 | 255 | <1 | 0.05 | 0.05 | 0 | Antibiotics |
| 5.2 | Pneumonia (CMV), LIP, HS | 11.1 | 2.1 | 148 | 4.5 | 0.05 | 1.65 | 0.3 | GCV, VCZ, IVIg, MP |
| 6.5 | Pneumonia (Haemophilus influenzae), LIP, HS | 11.8 | 2.4 | 171 | 6.9 | 0.05 | 4.99 | 3.6 | IVIg, MP, MMF |
| 7.9 | LIP, HS      | 12.3           | 1.7              | 268            |                |                |                |                | ABCT, IVIg |

Abbreviations: ABCT, abatacept; AIHA, autoimmune haemolytic anaemia; CMV, cytomegalovirus; GCV, ganciclovir; HS, hepatosplenomegaly; ITP, immune-mediated thrombocytopenia; IVIg, intravenous immunoglobulin; LIP, lymphocytic interstitial pneumonitis; MMF, mycophenolate mofetil; MP, methylprednisolone; OM, otitis media; RTX, rituximab; T1D, type 1 diabetes; VCZ, voriconazole.

Figure 4 Diagnosis and therapy. (a) Comparison of chest X-rays taken before and 3 months after abatacept therapy. (b) DNA chromatograms of LRBA showing heterozygous mutations in the parents (upper panels) and homozygous mutation in the proband. (c) Location of nonsense mutation in exon diagram of LRBA.
cell transplantation, although it is interesting to note that recurrent or persistent thrombocytopenia and progressive vitiligo was reported even in the face of documented microchimerism.12,13

Hypogammaglobulinaemia is common but not universal with LRBA deficiency. Indeed, as illustrated in our case, an increase in immunoglobulin can be observed early in the natural history. The pathophysiology of immune deficiency related to LRBA mutation remains incompletely understood. Remarkably, we have shown agammaglobulinaemia in the face of germinal centre formation, and present plasma cells. This suggests that within the B-cell compartment, immunoglobulin deficiency arises as a result of plasma cell dysfunction not deficiency. Future studies will be necessary to determine the nature of this functional defect.

METHODS
Approved hospital consent was taken for the genetic analysis for the patient and his parents.

Cellular T and B-cell phenotype (flow cytometry): the following antibodies were used for flow cytometry staining from Beckman Coulter (Brea, CA, USA): anti-CD45-Krome Orange (clone J33), anti-CD19-Red-X (ECD) (clone J3-82), anti-CD62L-APC750 (clone DREG56), anti-CD3-FITC (clone UCHT-1), CD4-PE (clone 13B8.2) and CD45-PCS.5.5 (clone J33). Anti-CD45RA-Paci119, and anti-CD21-PE (clone BL13), anti-CD27-PC7 (clone 1A4CD27), and anti-CD45-Krome Orange (clone J33), anti-CD3-APC-A750 (clone UCHT-1), antiCD4-APC (clone 13B8.2), antiCD8-A700 (clone B9.11), antiCD45RA-PE (clone 2H4), CCR7-PE (clone G043H7), anti-PD-1-PC5.5 (clone 1D3), and anti-CD27-PE (clone G043H7), anti-PD-1-PC5.5 (clone PD13.5), and anti-CD57-PB (NC1) as Dura clone IM T-cell subsets from Beckman Coulter. Hundred microlitres of blood was added to the desired cocktail of antibodies and incubated for 20 min at room temperature. Hundred microlitres of lysing solution optilyse-B or VersaLyse was added according to the manufacture recommendation. This was followed up with a wash step and then acquisition of the sample using Navios flow cytometer (Brea, CA, USA).

Whole-exome capture sequencing
The Illumina paired-end genomic DNA sample preparation kit (PE-102-1001, Illumina, San Diego, CA, USA) was used for preparing the libraries including end repair, A-tailing and ligation of the Illumina adapters. Each sample was prepared with an index using the Illumina multiplexing sample preparation oligonucleotide kit (PE-400-1001, Illumina) and then pooled in batches of six in equimolar amounts prior to exome enrichment. The Illumina TruSeq exome kit (FC-121-1008, Illumina) was used to capture the human exome for each sample pool. Each 6-plex exome enriched library was sequenced in two lanes of an Illumina HiSeq 2000 with version 2 chemistry as 100 bp paired-end reads.

Sequence reads were mapped to the GRCh37 assembly of the reference human genome using the default parameters of the Burrows–Wheeler Aligner (bio-bwa.sourceforge.net).14 Untrimmed reads were aligned allowing a maximum of two sequence mismatches and reads with multiple mappings to the reference genome were discarded along with PCR duplicates. Sequence variants were identified with SAMtools (samtools.sourceforge.net)15 and annotated using Annovar (http://annovar.openbioinformatics.org).16

CONFLICT OF INTEREST
The authors declare no conflict of interest.