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The case of an APDS patient: Defects in maturation and function and decreased in vitro anti-mycobacterial activity in the myeloid compartment

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ABSTRACT

Activated PI3-kinase delta syndrome (APDS) was recently reported as a novel primary immunodeficiency caused by heterozygous gain-of-function mutations in *PIK3CD* gene.
Here we describe immunological studies in a 19 year old APDS patient for whom genetic diagnosis was discovered by Whole Exome Sequencing (WES) analysis. In addition to the progressive lymphopenia and defective antibody production we showed that the ability of the patient's B cells to differentiate *in vitro* is severely reduced. An in depth analysis of the myeloid compartment showed an increased expression of CD83 activation marker on monocytes and mono-derived DC cells. Moreover, monocytes-derived macrophages (MDMs) failed to solve the *Mycobacterium bovis* bacillus Calmette Guèrin (BCG) infection *in vitro*. Selective p110δ inhibitor IC87114 restored the MDM capacity to kill BCG *in vitro*. Our data show that the constitutive activation of Akt-mTOR pathway induces important alterations also in the myeloid compartment providing new insights in order to improve the therapeutic approach in these patients.

1. Introduction

Activated PI3-kinase delta syndrome (APDS) was recently reported as a novel autosomal dominant primary immunodeficiency caused by heterozygous gain-of-function mutations in *PIK3CD* gene [1,2] encoding p110δprotein, the catalytic component of the phospohoinositide 3-kinase delta complex (PI3Kδ). The p110δ function into the PI3Kδ heterodimer is stabilized by the presence of the regulatory subunit p85α encoded by *PI3KR1* gene and is mainly restricted to adaptive immunity [3,4]. The function of PI3Kδ is to convert phosphatidylinositol 4,5-bisphosphate (PIP2) into the second messenger phosphatidylinositol 3,4,5-triphosphate (PIP3) involved in many signal transduction pathways during the immune response [5]. p110δ gain-of function mutation increases PIP3 production in APDS patients, inducing constitutive activation of the Akt-mTOR pathway that leads to defects in T and B cell differentiation and function. Immunological alterations, as well as progressive lymphopenia and defective antibody response, predispose APDS patients to recurrent infections, lymph nodes enlargement and splenomegaly [1,2,6]. Moreover, two different studies [7,8] described a human immunodeficiency caused by mutations in the *PIK3R1* gene in which the loss of p85α-function induced a hyper-phosphorylation of the Akt protein with patients showing a clinical phenotype similar to that of APDS. By whole exome sequencing we identified a dominant gain-of-function mutation in *PI3KCD* gene (E1021K) in a 19 year old

Abbreviations: APDS, activated PI3-kinase delta syndrome; WES, whole exome sequencing; BCG, *Mycobacterium bovis* bacillus Calmette Guèrin; PBMC, peripheral blood mononuclear cells; CFSE, carboxyfluorescein succinimidyl ester; IVIg, intravenous immunoglobulin; PB, peripheral blood; moDC, monocye-derived dendritic cells; MDM, monocyte-derived macrophages; TREC, T-cell receptor excision circles; CFU, colony forming unit.

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Table 1
Hematological and immunological data.

Parameter	Age 6 years	Age 19 years
White blood cells/mm^3	7840	8870
Hemoglobin (g/dl)	13	14.5
Platelets (10^3/mm^3)	347,000	185,000
Serum immunoglobulin (mg/dl)		
IgG[a]	907	1.147
IgA	41	89
IgM	**363**	**243**
Neutrophils/mm^3	5041	6510
Lymphocytes/mm^3	1693	1060
T cells (%)		
CD3 +/CD45 +	72%	68%
CD19 +/CD45 +	13%	8%
CD3 −/CD56 +	11%	13%
CD3 +/CD4 +	20%	34%
CD3 +/CD4 +/CD45RO +	76%	80.6%
CD3 +/CD4 +/CD45RA +	24%	11.4%
CD3 +/CD4 +/CD27 + CD45RA + (naïve)	nd	10%
CD3 +/CD4 +/CD27 + CD45RA − (CM)	nd	82%
CD3 +/CD4 +/CD27 − CD45RA − (EM)	nd	7%
CD3 +/CD4 +/CD27 − CD45RA + (EMRA)	nd	0.10%
CD3 +/CD4 +/CD31 + CD45RA + (RTE)	nd	4%
CD3 +/CD8 +	46%	28%
CD3 +/CD8 +/CD45RO +	41%	77%
CD3 +/CD8 +/CD45RA +	59%	23%
CD3 +/CD8 +/CCR7 +/CD45RA + (naïve)	nd	11%
CD3 +/CD8 +/CCR7 +/CD45RA − (CM)	nd	6%
CD3 +/CD8 +/CCR7 −/CD45RA − (EM)	nd	71%
CD3 +/CD8 +/CCR7 −/CD45RA + (EMRA)	nd	11%
CD3 + TCR α/β	nd	97%
CD3 + TCR γ/δ	nd	2%
CD3 + CD4 − CD8 − (DN) TCR α/β +	nd	3%
Lymphoproliferation test (10^3 cpm)[b]		Value
Unstimulated	0.8	0.7
PHA (2.5 μg/mL)	69.1	36.5
PWM (0.35 μg/mL)	47.5	18.3
OKT3 (1.5 μg/mL)	24.7	33.5
OKT3 + anti-CD28 (0.15 μg/mL)		38.5
TCRVB spectratyping		
Percentage of polyclonal families (CD4 +)		Normal
Percentage of polyclonal families (CD8 +)		Normal
TRECs		Reduced (1.106–15.787)[c]
Treg (% of CD4 + T cells)		
Bona-fide (CD4 + CD25 + CD127lowFOXP3 +)		4.36% (5–7.1%;N = 37)
Among Treg FOXP3 +:		
Resting (CD45RAhighFOXP3low)		15.2% (32–58%; N = 14)
Activated (CD45RAlowFOXP3high)		56% (9.7–23.3%; N = 14)
Non-suppressive (CD45RAlowFOXP3low)		25% (25.4–50%; N = 14)
Helios +		73%
Helios −		26%
Th17		1.7%[d]
B cells (% of CD19 +)		
CD27 + IgD + IgM +	11%	5.10%
CD27 + IgD − IgM-	7.4%	10.70%
CD27 − IgD + IgM +	64%	72.60%
CD19 + CD27 + (memory)	17%	15.10%
CD19 + CD27 − (naïve)	83%	84.90%
CD21lowCD38 −	nd	5%
CD24 + +CD38 + + (transitional B cells)	59%	38%
CD38 + +CD24 − (plasmablast)	nd	4.40%

>18 cpm. OKT3 > 20 cpm. OKT3 + IL-2 > 30 × 10^3 cpm.
Numbers in brackets indicate 25th and 75th percentiles of healthy controls.
Normal reference values from [29,30].
[a] On IVIg.
[b] T-cell proliferation at day 3 by radiolabeled thymidine incorporation. Normal values: unstimulated <2 cpm. PHA > 30 cpm. PHA + IL-2 > 40 cpm. Pokeweed mitogen (PWM).
[c] N = 16 pediatric patients.
[d] 25th–75th percentiles 0.6%–1.85%. N = 60.

patient that explains clinical and immunological phenotype. No other genes were found to be associated with the disease. We identified a novel impact of this mutation on the myeloid compartment and on the response of the patient's monocyte-derived macrophages to BCG-infection *in vitro*.

2. Materials and methods

2.1. Patient and informed consent

All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the patient included in the study and was approved by the Institutional Ethical Committee of Ospedale Pediatrico Bambino Gesù and signed by his family.

2.2. Isolation of blood cells and cell purification

Peripheral blood mononuclear cells (PBMC) were isolated by density-gradient centrifugation with Ficoll-Paque PLUS (GE Healthcare). The protocol for the purification of different cell subsets was performed as described [9]. Purity of CD20 + B cells was >75%. Purity of CD14 + monocytes was 95%. DNA was extracted according to the manufacturer's instruction (QIAamp DNA Mini Kit). All the reported data were collected and analyzed when the patient was not under immunosuppressive therapy for at least 6 months.

2.3. Exome sequencing and bioinformatic analysis

Sequencing was performed on 100 ng DNA isolated from peripheral blood (PB). The protocol is detailed in the Online Repository.

2.4. Sanger sequencing

PI3KCD coding exon 24 amplification is detailed in the Online Repository.

2.5. Immunoblot analysis

PBMC were cultured in complete medium (RPMI-1640, 10% FBS, glutamine, penicillin and streptomycin) and activated with PHA (1 μg/ml, SIGMA) and IL2 (100 IU/ml, SIGMA) to generate T cell lines that were used for protein extraction and determination of p-AKT. Detailed protocol is reported in the Online Repository.

Table 2
Exome-sequencing summary.

PT	Total number of reads	107,301,248
	Coverage	103
	All rare variants	133
	Heterozygous	109
	Heterozygous non synonymous	81
	Heterozygous indels	11
	Heterozygous frameshift	7
	Heterozygous splice site	5
	Heterozygous stop gained	3
	Heterozygous exon	2
	Homozygous	24
	Homozygous non synonymous	5
	Homozygous indels	8
	Homozygous frameshift	4
	Homozygous splice site	1
	Homozygous stop gained	–
	Homozygous exon	1