ANNUAL REPORT

STUDENT: **ELISA RUFFO** CYCLE: **XXIX** YEAR: **2014-2015**

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1. Background

X-linked lymphoproliferative disease (XLP-1) is a heritable immune disorder caused by germline mutations in the *SH2D1A* gene, which encodes the Signaling Lymphocytic Activation Molecule (SLAM)-associated protein (SAP), an SH2 domain-containing adaptor primarily expressed in T, natural killer (NK), and invariant NKT (iNKT) cells (*1, 2*). XLP-1 is best recognized for the increased susceptibility of affected male individuals to develop overwhelming lymphoproliferation following primary Epstein Barr virus (EBV) infection (*3, 4*). Also known as fulminant infectious mononucleosis (FIM), this lymphoproliferative process is characterized by the massive accumulation of activated $CD8^+$ T cells, which infiltrate multiple organs and inflict severe tissue damage. FIM is the most common and clinically challenging manifestation of XLP-1, with up to 65% of patients dying despite the use of multi-agent chemo-immunotherapy and allogeneic hematopoietic stem cell transplantation (*5*). Accordingly, alternative and more effective treatment strategies are sorely needed for XLP-1 patients who develop FIM.

T lymphocytes derived from XLP-1 patients exhibit multiple functional defects, including reduced cytotoxic activity (*6*) and impaired restimulation-induced cell death (RICD) (*7*). RICD is a self-regulatory apoptosis program triggered by repeated TCR stimulation that maintains peripheral immune tolerance by constraining the accumulation of activated T cells (*8*). It is proposed that the defects in RICD, when combined with impaired clearance of EBV-infected B cells, likely sustain and amplifie the expansion of activated T cells that typifies FIM (*7, 8, 9*).

We recently observed that following TCR stimulation, SAP negatively regulates the activity of diacylglycerol kinase alpha (DGKα) that phosphorylates diacylglycerol (DAG) to generate phosphatidic acid, thereby modulating TCR signal strength by regulating DAG levels and downstream biochemical events (*10, 11*). In activated T cells, silencing SAP expression results in persistently active DGKα and impaired DAG-mediated PKCθ membrane recruitment, NFAT and ERK1/2 activation and interleukin 2 (IL-2) production (*12*). Based on those findings we demonstrate that the potentiation of TCR-induced DAG signaling by DGKα knockdown or DGK inhibition allows the TCR signaling to reach the threshold required for RICD onset *in vitro* (Ruffo el al., submitted to STM).

SAP binds to immunotyrosine-based switch motifs (ITSMs) present within the cytoplasmic domains of the SLAM-family receptors (*13, 14*) and thus competes with the binding of SH2 domain containing inhibitory lipid and tyrosine phosphatases such as SHIP and SHP1/SHP-2. A number of recent reports demonstrate that, besides SLAM family receptors, SAP interacts with other signaling proteins such as the CD3ζ chain (*15*), the adhesion receptor PECAM-1(*16*), the Lyn tyrosine kinase (*17*), the NCK1 adaptor (*18*), DOK1 and βPIX, a guanine nucleotide exchange factor for Rac and CDC42 (*19*) which influence certain signaling pathways downstream of the TCR, including extracellular signal-regulated kinase and nuclear factor of activated T-cell activation.
In particular NCK1, CDC42, and its guanine nucleotide exchange factor (GEF) Vav1, are integrated model for WASp (Wiskott–Aldrich syndrome protein) activation and recruitment (*20*). WASp belongs to a family of nucleation-promoting factors (NPFs) that facilitate actin polymerization by activating the actin-related protein 2/3 (ARP 2/3) complex (*21*). WASp is mutated in the Wiskott–Aldrich syndrome which is a severe X-linked primary immunodeficiency characterized by thrombocytopenia, bleeding, eczema, recurrent infections, and susceptibility to the development of autoimmune diseases and lymphomas (*22, 23*). Lymphocytes derived from WAS patients show an impaired TCR-induced proliferation and actin polymerization (*24, 25, 26*). The involvement of WASp in actin-filament formation depends on its functional activation and recruitment to the T cell/ antigen-presenting cell (APC) contact site and the immunological synapse (IS). Thus, WASp is essential for T-cell activation and effector functions, either by mediating actin remodeling or by transcriptional activity (*24*).
Some actin-associated proteins also regulate integrin activation, a mechanism that leads T cell to migrate. A complex constitute by SKAP-55, Rap1–GTP-interacting adapter molecule (RIAM), VASP and talin promotes β1 integrin activation through effects on the actin cytoskeleton and this increases the cell adhesion (*27*). Moreover, we recently showed that DGKα activity sustains the pro-invasive activity of metastatic p53 mutations, by promoting the recycling of α5β1 integrin to the tip of invasive protrusions in tridimensional matrix (*28*).
Another actin-interacting protein is Coronin 1a which is critical for the trafficking of näive T cell to secondary lymphoid organs, for TCR-signaling and T cell homeostasis. Mutations in CORO1A have been reported in a patient with combined immunodeficiency characterized by T cell lymphopenia and severe EBV-induced B cell lymphoproliferation (*29*). Coronin 1a has been shown to associate with filamentous F-actin and ARP 2/3 complex (*30, 31*).
Based on the evidence that actin and myosin contraction is important for T cell movement, we focused on Myosin light Chain Kinase (MLCK). MLCK is a kinase that by phosphorylatiing the myosin regulatory light chain (RLC) guides myosin contraction (*32*). In our laboratory MLCK was also identify as one of DGKα interactors during a proteomic study in other cell line. In particular we demonstrate that DGKα and MLCK co-precipitate and this complex is responsible for the rearrangement of cortical actin in growth factor stimulated endothelial cells (data from our lab unpublished).

2. Summary of the first year

XLP-1 is a primary immunodeficiency associated with excessive $CD8^+$ T cell expansion following EBV infection. XLP-1 is caused by defects in SAP, an adaptor protein that facilitates signaling through the SLAM family receptors. Recent evidence showed that SAP-deficient cells exhibit impaired T cell receptor RICD a process that normally constrains T cell expansion during immune responses (*7*). SAP deficiency also impedes TCR-induced inhibition of DGKα, leading to increased DAG turnover and decreased RasGRP and PKCθ activation (*12*). DGKα is an enzyme that, by phosphorylating DAG to PA, acts as a negative regulator of TCR signaling and induces T cell anergy.

During the first year, we demonstrate that the downregulation of DGKα function by either RNAi-mediated silencing or pharmacological inhibition, fully restore RICD in SAP knocked-down primary T cells, as well as in primary T cells from XLP patients. In addition, we show that DGK-alpha silencing/inhibition is sufficient to correct the defective diacylglycerol signaling in SAP-deficient primary T cells, thereby enhancing IL-2 cytokine production and the IL2R expression. Furthermore we show that the rescue of RICD induced by DGKα silencing is fully dependent on the activity of DAG-dependent PKCθ and MAPK pathways. Finally, the inhibition of DGKα rescues the expression of the pro-apoptotic NR4A1 (Nur77) and NR4A3 (Nor1) proteins, whose antigen-stimulated induction is defective in SAP-deficient cells. Altogether these findings support the hypothesis that inhibition of DGKα, by enhancing DAG-mediated signaling, leads to the induction of pro-apoptotic genes, thus providing a constrain to the expansion of antigen-activated T cell clones. These data also indicate that DGKα inhibition could be a novel pharmacological strategy for treatment of XLP and others lymphoproliferative diseases characterized by defective RICD.

3. Project aim/objectives

During this second year of my PhD program, we proposed to investigate the molecular mechanisms by which the TCR regulates DGKα. We previously observed that when DGKα is constitutively active, due to the absence of SAP, there is a resistance in RICD and this defect should be compensated by DGKα silencing/inhibition. We studied the contribution of several SAP interactor proteins such as βPIX, DOK1, NCK1 and CDC42 in a RICD assay. NCK1 is one of a WASp interactor thus we decided to study actin polymerization and the cytoskeleton remodelling, by investigating the role of WASp and other actin interacting protein such as ARP 2/3 and Coronin 1a and on cell adhesion interactors like β1 intergrin and MLCK.

4. Experimental plan and methods

Cells culture and reagents

Peripheral blood lymphocytes (PBLs) were isolated from whole blood or buffy coat from normal subject by Ficoll-Paque PLUS (GE Health Care) density gradient centrifugation, washed, and resuspended at $2*10^6$ cell/ml in RPMI-GlutaMAX (GIBCO, Life technologies) containing 10% FCS heat inactivated, 2 mM glutamine, and 100 U/ml each of penicillin and streptomycin (Invitrogen). T cells were activated with 1 μg/ml of anti-CD3 (clone UCHT1) and anti-CD28 (clone CD28.2) for 3 days. After 3 days, activated T cells were thoroughly washed and then cultured in complete RPMI-GlutaMAX supplemented with 100 IU/ml rhIL-2 (Peprotech) at $1.2*10^6$ cells/ml for at least 7 days before apoptosis assays were conducted (the media was changed every 2-3 days).

Inhibitors

R59949 (DGK inhibitor), Cytochalasin D (actin depolymerizator), CK-666 (ARP 2/3 inhibitor), ML-7 inhibitor (MLCK inhibitor) (Sigma-Aldrich) were all dissolved in DMSO; equal amounts of DMSO were used in the control samples.

siRNA for transient silencing

Activated human PBLs were prepared as described above and transfected with 200 pmol of siRNA oligonucleotides specific for the target protein (Stealth Select siRNA; Life Technologies) or a NS control oligo (Life Technologies). Transient silencing was obtained by transfection using the Amaxa nucleofector kit "Human T cell nucleofector kit" (VPA-1002 Lonza) according to manufacturer

instructions. The cells were maintained in culture in presence of IL-2 (100 IU/ml) for 4 day to allow target gene knockdown.

siRNA	Sense strand	Antisense strand
DGKα	CGAGGAUGGCGAGAUGGCUAAAUAU	AUAUUUAGCCAUCUCGCCAUCCUCG
βPIX	CAGAUAGACAAGAUAUUCAtt	UGAAUAUCUUGUCUAUCUGta
DOK1	CCAUCUAUGAUGAACCUGAtt	UCAGGUUCAUCAUAGAUGGga
NCK1	CCUCAUUCGUGAUAGUGAAtt	UUCACUAUCACGAAUGAGGaa
CDC42	UGGUGCUGUUGGUAAAACAtt	UGUUUUACCAACAGCACCAtc
WAS	UGAACAACCUCGACCCAGAtt	UCUGGGUCGAGGUUGUUCAcg
CORONIN 1A	CCAUGUUCAGUUCCAAGGAtt	UCCUUGGAACUGAACAUGGag
β1 INTEGRIN	GGAGGAAUGUUACACGGCUtt	AGCCGUGUAACAUUCCUCCag

Stealth RNAi Negative Control Duplexes (# 12935-300, Life Technologies) was used as negative control.

Cytofluorimetry

To test the restimulation induced cell death (RICD), activated T cells (10^5 cells/well) were plated in triplicate in 96-well round-bottom plate and treated with anti-CD3ε mAb OKT3 (1-10-100ng/ml) in RPMI-GlutaMAX supplemented with 100 IU/ml rhIL-2 for 24 hours. In some assays R59949 (5μM) and/or Cytochalasin D (5μM), ARP 2/3 inhibitor (60 μM), ML-7 inhibitor (30 μM) were added 30 minutes before the restimulation. 24 hours after treatment, cells were stained with 1 μg/ml propidium iodide and collected for a constant time of 30 seconds per sample on a FACScan flow cytometer (FACS calibur, BD). Cell death was analyzed with CellQuest software (BD) or Flowing software (Turku Bioimaging) as percentage of cell loss = (1 – [number of viable cells (treated) / number of viable cells (untreated)]) × 100.

Quantitative RT-PCR

Activated human PBLs were prepared and transfected as described above. The RNA concentration and purity were estimated by a spectrophotometric method using NanoDrop 2000c (Thermo Scientific). The RNA was retrotrascribed with High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems) and cDNA quantified by real time PCR (C1000 Thermal Cycler CFX96 realtime system, BIORAD) using GUSB as normalizer.

TaqMan gene expression assays were from Life Technologies: Hs00939627_m1 (glucuronidasi beta, GUSB), Hs00200039_m1 (CORO1A), Hs00187614_m1 (WAS), Hs00559595_m1 (ITGB1).

Statistics

Data are shown as the mean ± SEM. For statistical analysis, Student's t-test or *ANOVA* were used. Experiments shown are representative at least 3 independent experiments. A *p*-value less than or equal to 0.05 was deemed to be significant in all experiments.

5. Results

a) DGKα silencing restores RICD in NCK1 deficient T cells

A reduced TCR signaling strength promotes the resistance to RICD of XLP patients' SAP deficient lymphocytes (*7*) and we can rescue this defective phenotype by silencing or inhibiting DGKα. To better understand the signaling pathway by which the TCR induces the inhibition of DGKα through the activation or recruitment of different proteins, we investigate the role of SAP interactors such as βPIX,

DOK1, CDC42 and NCK1 (*18, 19, 20*). We first silenced DGKα in Peripheral blood lymphocytes (PBLs) from healthy donors along with βPIX, DOK1, CDC42, or NCK1. βPIX and DOK1 silencing results in a small reduction of the percentage of cell death comparing to control cells. The concomitant silencing of βPIX and DOK1 with DGKα does not affect cell death indicating that these two molecules proteins are not involved in the TCR-mediated signaling pathway, which induces inhibition of DGKα and cell death (Fig 1A and 1B). CDC42 deficient cells show a resistance to RICD, in particular to the highest dose of anti-TCR agonist antibody, OKT3 (100 ng/ml), and this defect is partially rescued by DGKα silencing (Fig. 1C). NCK1 silencing results in a severely reduced RICD over a wide concentration-range of OKT3. While DGKα silencing does not affect RICD in control cells, it fully restores agonist induced cell death in NCK1 silenced cells (Fig. 1D) demonstrating an involvement of NCK1 in TCR-induced DGKα inhibition and resistance to cell death.

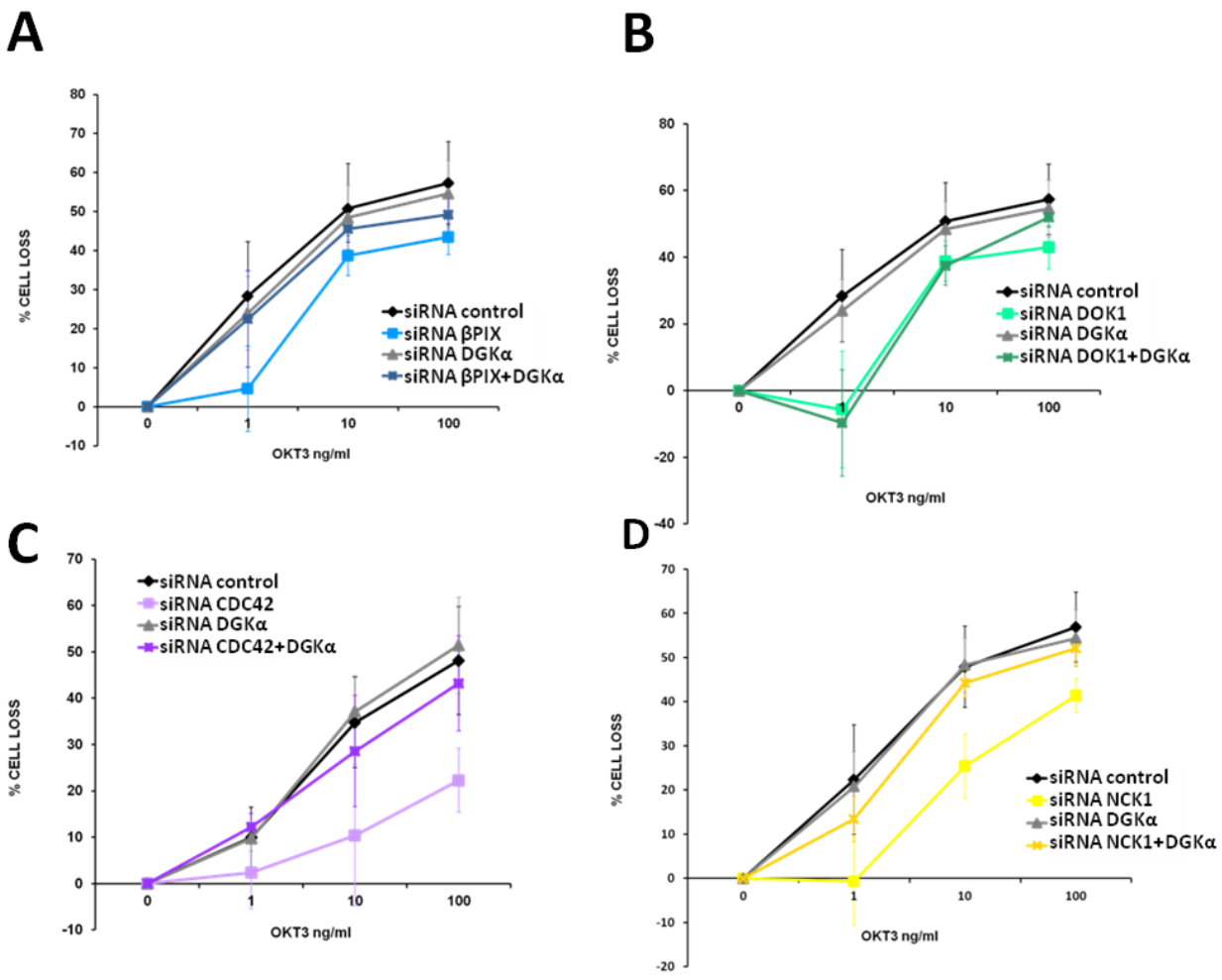

Fig.1 DGKα silencing restores RICD in NCK1 deficient T cells
Lymphocytes from normal subjects were transfected with different pairs of siRNA (**A**- βPIX and DGKα; **B**- DOK1 and DGKα; **C**- CDC42 and DGKα; **D**- NCK1 and DGKα) and after 4 days restimulated with increasing doses of CD3 agonist OKT3. After 24 hours the % of cell loss was evaluated by PI staining.
Data are the mean ± SEM of at least four independent experiments performed in triplicate.

b) DGKα silencing restores defective T cell RICD due to actin depolymerisation

NCK1 interacts with several proteins that induce cytoskeleton rearrangement and actin polymerization. First of all we wonder if actin polymerization is important to organize the cell and predispose the cell to the death. To verify such hypothesis we first used PBLs from healthy donors, in which SAP and/or DGKα was downregulated by RNAi-mediated silencing. Subsequently cells were treated with cytochalasin D (5μM), which is a cell-permeable and potent inhibitor of actin polymerization preventing polymerization of actin monomers (*33*).

The treatment of control cells with Cytochalasin D results in a severe reduction of RICD after TCR stimulation (Fig. 2A). DGKα silencing does not affect RICD in control cells while the treatment with Cytochalasin D decreases the percentage of cell death. SAP-deficient cells, which are resistant to cell death, show a reduction of cell death after Cytochalasin D treatment. RICD is fully restored in SAP and DGKα silenced cells but Cytochalasin D decrease this rescue indicating that actin polymerization is fundamental to determine the cell fate in a SAP/DGKα-dependent manner (Fig. 2A).

Based on our results, actin plays a central role in determining the cell fate, thus we investigated which actin interacting proteins could determine this process. Coronin 1a has been shown to associate with filamentous F-actin and the ARP 2/3 complex (*30, 31*). We performed RICD assay by silencing CORO1A and DGKα: silencing of CORO1A does not affect RICD indicating that this protein is not involved in the SAP-DGKα cascade to induce cell death (Fig. 2B).

Because NCK1 absence modify TCR signaling we decided to study NCK1 mediated-signaling pathway such as WASp and ARP 2/3 which induce actin reorganization. WASp deficiency presents some clinical and phenotipical features similar to the XLP-1 disease; WASp absence results in a defective target cell cytotoxicity by WASp CTLs (*34*). Thus, we verify if DGKα knockdown could at least partially restore WASp and ARP 2/3 deficient primary lymphocytes. We first used PBLs from healthy donors in which WASp function was downregulated by RNAi-mediated silencing and ARP 2/3 was inhibiting by using CK-666 inhibitor (60μM). We observed that WASp silencing results in a severely reduced RICD over a wide concentration-range of OKT3 while, again, DGKα silencing does not affect RICD in control cells, and it fully restores agonist-induced cell death in WASp silenced cells (Fig. 2C). These data demonstrate an essential and selective role of DGKα in the resistance to cell death of WASp deficient cells and possible cure of the disease. ARP 2/3 inhibited cells results in a reduced RICD after TCR stimulation and DGKα inhibition which does not affect RICD in control cells, it fully restores agonist induced cell death in ARP 2/3 inhibited cells (Fig. 2D). Those results suggest that the TCR-restimulation requires SAP and some SAP interacting protein such as NCK1, but not βPIX nor DOK1, and actin-interacting protein WASp and ARP 2/3 but not Coronin 1a.

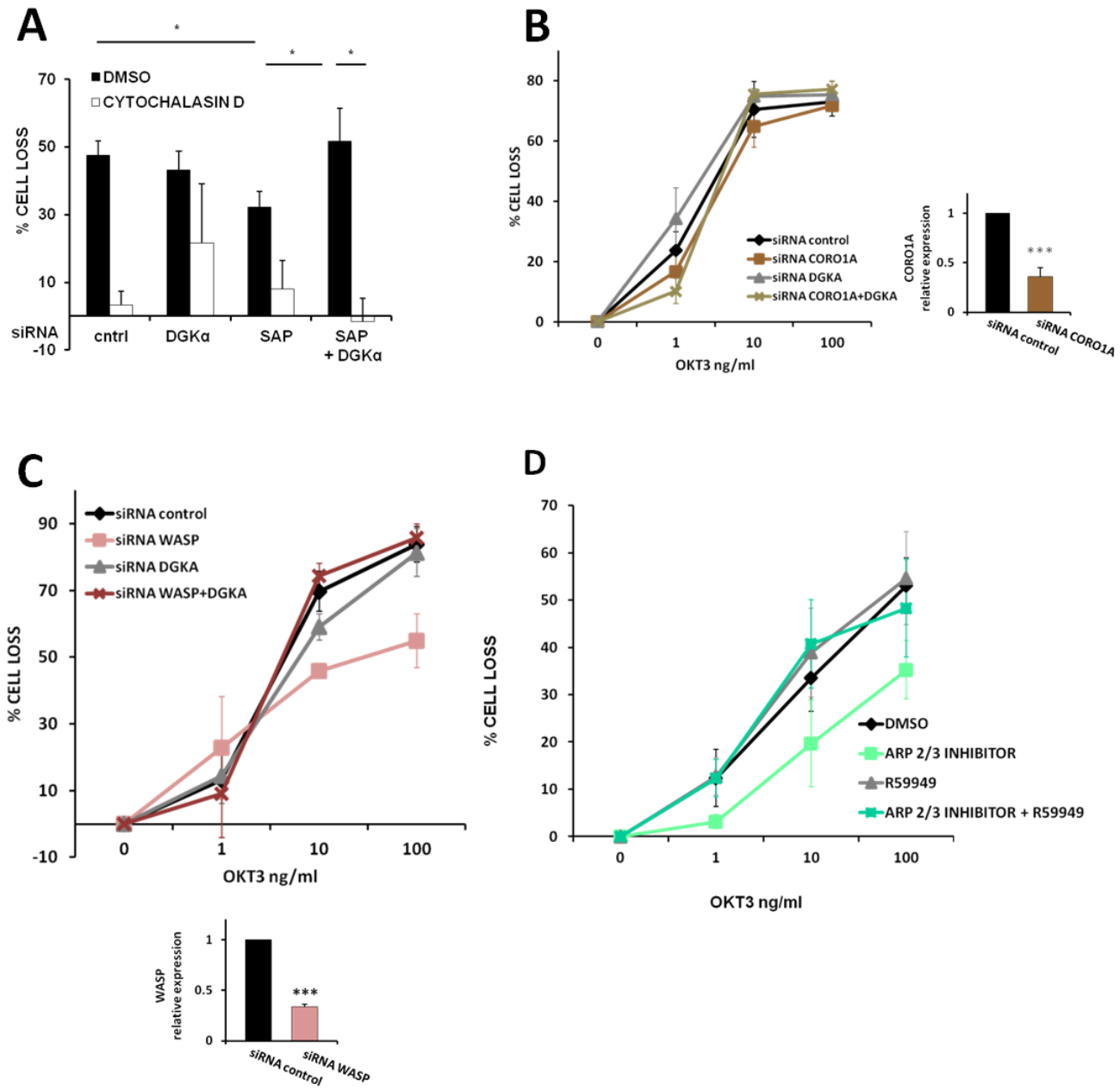

Fig.2 DGKα silencing restores defective T cell RICD due to actin depolymerisation

A) Lymphocytes from normal subjects were transfected with SAP and DGKα siRNA and after 4 days restimulated with increasing doses of CD3 agonist OKT3 in presence or absence of the Cytochalasin D (5 μM). After 24 hours the % of cell loss was evaluated by PI staining. Data are the mean ± SEM of three independent experiments performed in triplicate.

B) Lymphocytes from normal subjects were transfected with CORO1A and DGKα siRNA and after 4 days restimulated with increasing doses of CD3 agonist OKT3. After 24 hours the % of cell loss was evaluated by PI staining. Data are the mean ± SEM of three independent experiments performed in triplicate. Lymphocytes were also transfected with the indicated siRNA and after 4 days CORO1A expression was check by quantitative RT-PCR (right panel).

C) Lymphocytes from normal subjects were transfected with WASp and DGKα siRNA and after 4 days restimulated with increasing doses of CD3 agonist OKT3. After 24 hours the % of cell loss was evaluated by PI staining. Data

are the mean ± SEM of three independent experiments performed in triplicate. Lymphocytes were also transfected with the indicated siRNA and after 4 days WASp expression was check by quantitative RT-PCR (lower panel).

D) Lymphocytes from normal subjects were restimulated with increasing doses of CD3 agonist OKT3 in presence or absence of ARP 2/3 inhibitor (CK-666 60 μM) and DGK inhibitor (R59949 5 μM). After 24 hours the % of cell loss was evaluated by PI staining. Data are the mean ± SEM of five independent experiments performed in triplicate.

c) DGKα silencing restores RICD in β1 integrin deficient T cells

The cytoskeleton remodeling is important to drive the cell to the death but also the interaction and the adhesion with microenvironment. To investigate the possibility that also the adhesion is important in the SAP-DGKα axis pathway, we performed RICD assay by silencing β1 integrin which activation has effects on both the actin cytoskeleton and cell adhesion (*27*). β1 integrin-silenced T cells results in a reduction in RICD while DGKα silencing does not affect RICD in control cells and it increases restoration of TCR-induced cell death in β1 integrin silenced cells (Fig. 3A) demonstrating an essential role of DGKα in the resistance to cell death of β1 integrin deficient cells and its involvement in cell adhesion.

To investigate T cell movement we focused on MLCK that phosphorylates myosin RLC and it guides myosin contraction (*32*). MLCK is also a DGKα interactors and this complex is responsible for the rearrangement of cortical actin in growth factor stimulated endothelial cells. To verify if MLCK has a role in the RICD we used PBLs from healthy donors and we treated them with MLCK inhibitor (ML-7 30 μM) and DGK inhibitor (R59949 5 μM). The treatment of control cells with MLCK inhibitor (ML-7 30 μM) does not affect RICD after TCR stimulation (Fig. 3B). DGK inhibitor (R59949 5 μM) does not affect RICD in control cells while the treatment with MLCK inhibitor (ML-7 30 μM) and DGK inhibitor (R59949 5 μM) does not influenced the percentage of cell death indicating that MLCK is not involved in the TCR signaling pathway that mediates resistance to cell death and DGKα inhibition.

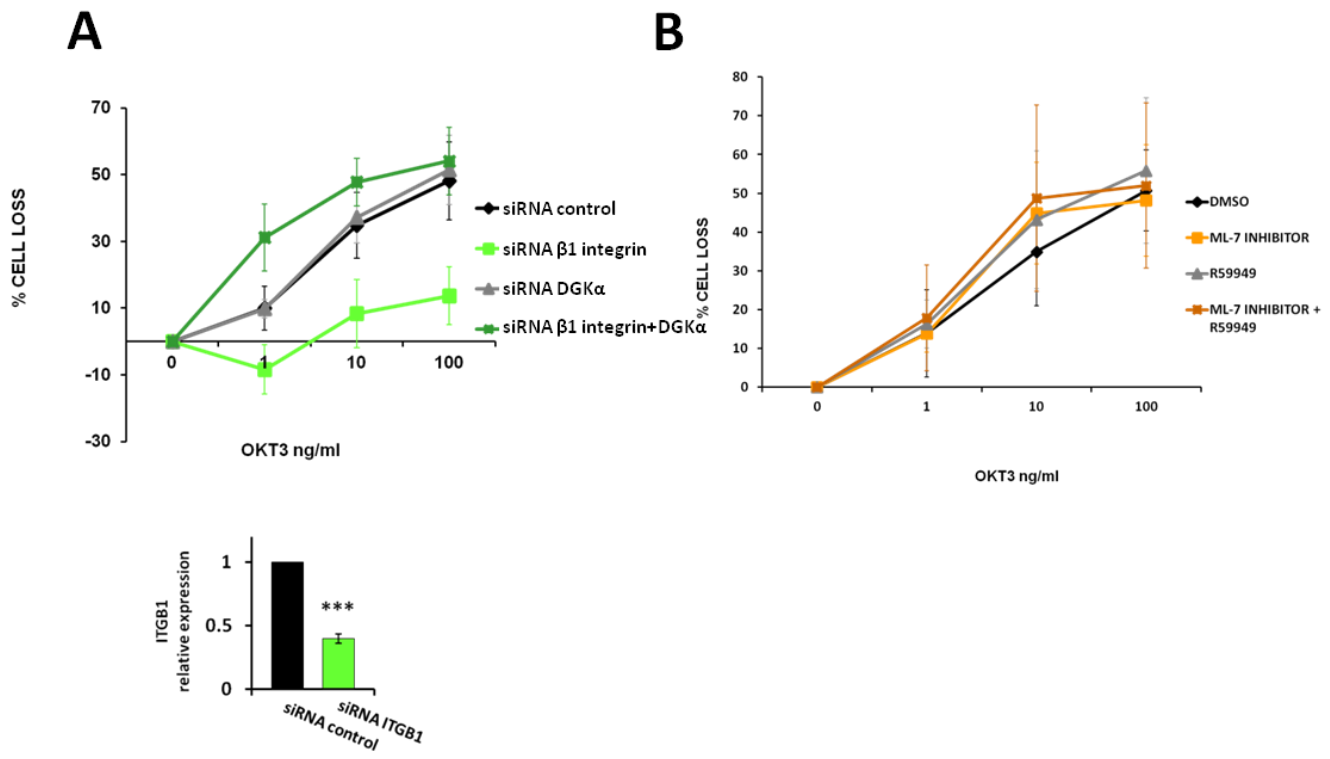

Fig.3 DGKα silencing restores RICD in β1 integrin deficient T cells

A) Lymphocytes from normal subjects were transfected with β1 integrin and DGKα siRNA and after 4 days restimulated with increasing doses of CD3 agonist OKT3. After 24 hours the % of cell loss was evaluated by PI staining. Data are the mean ± SEM of four independent experiments performed in triplicate. Lymphocytes were also transfected with the indicated siRNA and after 4 days β1 integrin expression was check by quantitative RT-PCR (lower panel).

B) Lymphocytes from normal subjects were restimulated with increasing doses of CD3 agonist OKT3 in presence or absence of MLCK inhibitor (ML-7 30 μM) and DGK inhibitor (R59949 5 μM). After 24 hours the % of cell loss was evaluated by PI staining. Data are the mean ± SEM of three independent experiments performed in triplicate.

6. Discussion

We previously observed that when DGKα is constitutively active, due to the absence of SAP, there is a resistance in RICD and this defect should be compensated by DGKα silencing/inhibition. We studied the contribution of several SAP interactor proteins such as βPIX, DOK1, NCK1 and CDC42. Based on the results that NCK1 induces resistance to RICD which is rescued by DGKα silencing and because NCK1 is a WASp interactor, we focused on actin polymerization and the cytoskeleton remodelling, by investigating the role of WASp and other actin interacting protein such as ARP 2/3 and Coronin 1a and on cell adhesion interactors like β1 intergrin.

WASp, ARP 2/3 and β1 integrin deficiency induce resistance to cell death which is rescue by DGKα silencing or inhibition while MLCK is not involved in this signaling pathway.

Our data suggest that upon TCR stimulation SAP mediates the inhibition of DGKα through a mechanism that requires NCK1, WASp and ARP2/3 which induces actin polymerization and induces cell death. The potentiation of TCR-induced DAG signaling by DGKα knockdown or DGK inhibition allows the TCR signaling to reach the threshold required for RICD onset *in vitro*. Those findings

elucidate the relevance of the DGKα inhibition as the possible treatment for XLP and WAS disease (Fig.4).

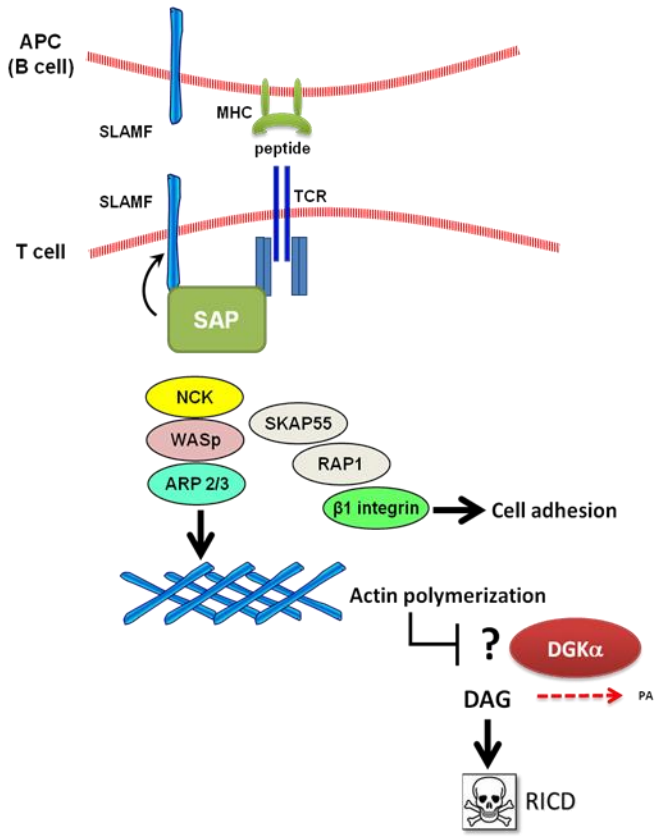

Fig.4 Model of signaling transduction
Upon TCR stimulation there is a recruitment of SAP that, through the inhibition of DGKα, activates DAG-dependent signaling pathway which induces cell death. The mechanisms by which SAP regulates DGKα is still unknown but we suggest that TCR-mediated inhibition of DGKα requires NCK1, WASp and ARP 2/3 to induce cell death.

7. References

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8. Attended seminars

SPEAKER	TITLE	DATE
Prof. Andrew L. Snow Department of Pharmacology Uniformed Services University of the Health Sciences Bethesda (Maryland, USA)	***"Dysregulated antigen receptor signaling: molecular lessons from two congenital lymphoproliferative disorders"***	06/11/2014
Dott. Francesca Boccafoschi Dipartimento di Scienze della Salute, Università del Piemonte Orientale	***"Tissue engineering: the state of the art"***	14/11/2014
Dott. Secco Gioel Gabrio Dipartimento di Scienze della Salute, Università del Piemonte Orientale	***"Optical coherence tomography from bench to bedside shening the light during percutaneous vascular intervention"***	17/11/2014
Prof. Maria Prat Dipartimento di Scienze della Salute, Università del Piemonte Orientale	***"Regenerative Medicine"***	21/11/2014
Dott. Roberta Arcidiacono Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale; Dott. Marta Ruspa Dipartimento di Scienze della Salute, Università del Piemonte Orientale	***"La scoperta del bosone di Higgs"***	25/11/2014
Prof. Laura Baglietto Inserm - Centre for Research in Epidemiology and Population Health, Unit: Nutrition, Hormones and Women's Health Parigi	***"Nuove sfide ed opportunità dell'epidemiologia molecolare per lo studio dei tumori"***	27/11/2014
Dott. Arvind Patel Programme Leader MRC Centre for Virus Research University of Glasgow (UK)	***"Humoral responses to HCV infection and clinical outcomes"***	28/11/2014
Dott. Roberto Furlan Università San Raffaele, Milano	***"Microglia microvescicles: messengers from the diseased brain"***	17/12/2014
Prof. Dr Yong-Sang Song, MD, PhD Director Cancer Research Institute, Gynecologic Oncology Chariman, Cancer Biology Interdisciplinary Program Professor, Obstetrics and Gynecology, College of Medicine Seoul National University	***"Anticancer strategy Targeting cancer cell metabolism in ovarian cancer"***	19/01/2015
Dott. Tonino Alonzi, PhD Lab. Espressione Genica ed	***"Different molecular mechanisms regulate hepatocyte***	20/01/2015

Epatologia Sperimentale Istituto Nazionale per le Malattie Infettive "L. Spallanzani" IRCCS, Roma	***differentiation during the transitions between epithelial and mesenchymal states"***	
Prof. Valeria Poli Department of Molecular Biotechnology and Health Sciences Molecular Biotechnology Center University of Turin	***"Targeting the liver to cure myocarditis: a lesson from a model of STAT3-dependent auto-immune myocarditis"***	21/01/2015
Prof. Antonio Sica DiSCAFF, UPO, Novara	***"Myeloid cells as therapeutic target in cancer"***	27/01/2015
Prof. Darko Bosnakovski Associate Professor University "Goce Delcev" Stip Faculty of Medical Sciences Krste Misirkov bb, 2000 Stip R. Macedonia	***"Proof of principle for cell therapy: from autologous transplantation of tissue specific progenitors to gene corrected patient specific injured pluripotent stem cells"***	11/03/2015
Prof. Xiaoping Zhong, MD, PhD Associate Professor Department of Pediatrics-Allergy and Immunology Duke University Medical Center Durham (North Carolina, USA)	***"Signal control in iNKT cell development and function"***	09/04/2015
Prof. Piergiorgio Percipalle Associate Professor Department of Cell and Molecular Biology Karolinska Insitute (Stockholm, Sweden)	***"Actin-based mechanisms in the control of gene expression and cell fate"***	21/04/2015
Prof. John McDonald PhD Integrated Cancer Research Center, School of Biology and Parker H. Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology Georgia Tech University, Georgia (Atlanta, US)	***"An Integrated Approach to the Diagnosis and Treatment of Ovarian Cancer"***	07/05/2015
Kathleen Ruff RightOn Canada Founder&Senior Advisor to the Rideau Insitute	***"Conflicting interests and scientific communication"***	14/05/2015
Prof. Steven R. Ellis Department of Biochemistry and Molecular Biology, University of Louisville (Kentucky, USA)	***"Ribosomopathies"***	25/05/2015
Dr. Nicoletta Filigheddu Dipartimento di Medicina Traslazionale, Università del Piemonte Orientale "A.Avogadro"	***"Basis of scientific research"***	10/06/2015

MINIWORKSHOP on "BIOTECHNOLOGY FOR DERMATOLOGY" Dipartimento di Scienze della Salute, Università del Piemonte Orientale "A.Avogadro"	***"Biotechnology for Dermatology"***	09/07/15

9. Partecipations to conferences

- 20th-24th August 2015, "Genetic control of immune cell activation. Implication for autoimmune disease/IV Nordic meeting on Genetics and Pathogenesis of Immunopathological diseases", Henningsvær, Lofoten, Norway.

Selected oral presentation: Inhibition of Diacylglycerol kinase alpha restores TCR-induced diacylglycerol signaling and restimulation-induced cell death in XLP-1 T lymphocytes

10. Publications

- The diacylglycerol kinase α/atypical PKC/β1 integrin pathway in SDF-1α mammary carcinoma invasiveness.

Rainero E, Cianflone C, Porporato PE, Chianale F, Malacarne V, Bettio V, **Ruffo E**, Ferrara M, Benecchia F, Capello D, Paster W, Locatelli I, Bertoni A, Filigheddu N, Sinigaglia F, Norman JC, Baldanzi G, Graziani A.
PLoS One. 2014 Jun 2;9(6):e97144. doi: 10.1371/journal.pone.0097144. eCollection 2014.

- Inhibition of diacylglycerol kinase alpha restores restimulation-induced cell death and reduces immunopathology in XLP-1

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