The Wiskott-Aldrich syndrome: An Immunodeficiency due to a defective cytoskeleton

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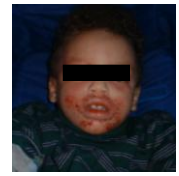
Harvard Medical School



Wiskott-Aldrich syndrome

An X-linked PID that affects 1 to 10 per million individuals.

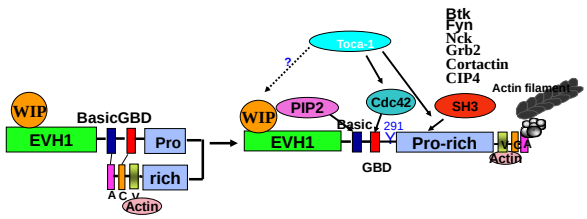
Clinical features

- **thrombocytopenia with small-sized platelets**
- **eczema**
- **immunodeficiency**
  - ***infections (encapsulated bacteria,***
  - ***autoimmunity: vasculitis colitis***
  - ***tumors (leukemia, lymphoma)***

Immunology

- **↓ IgM, ↑ IgA and IgE**
- **Impaired response to polysaccharide antigens**
- **Progressive decline of lymphocyte count**
- **Defective T cells**

WASP structure, interactions, and activation



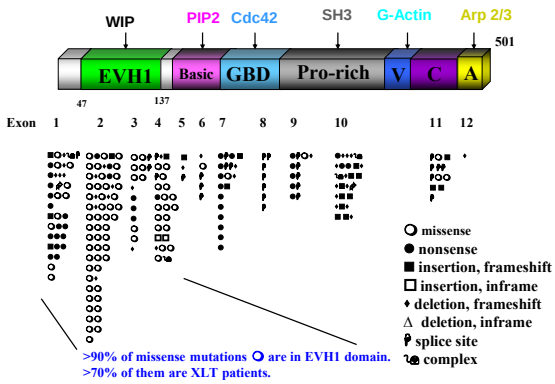
WASP is normally in an inactive state because of intra-molecular interactions.

Binding of Cdc42 to the GBD or of SH3 domains (Nck, Grb2, cortactin) to the proline-rich region of WASP results in breaking the intra-molecular bonds. This allows the acidic VCA domain of WASP to interact with the Arp2/3 complex and initiate actin polymerization.

Siseases associated with *WASP* mutations

- **Wiskott-Aldrich syndrome (WAS)**
- **X-linked thrombocytopenia (XLT)**
- **Intermittent X-linked thrombocytopenia (iXLT)**
- **X-linked neutropenia (XLN)**
- **X-linked myelodysplasia (XLM)**

WASP mutations

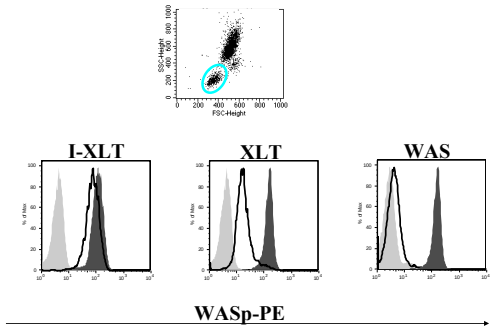


Diseases caused by mutations in WASP

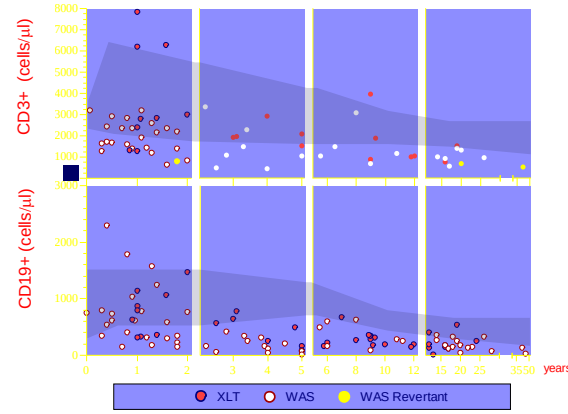
WAS is due to mutations in the gene that codes for the Wiskott-Aldrich Syndrome protein (WASP) resulting in a wide range of clinical phenotypes that depend on the position and nature of the mutation

Disease	X-linked thrombocytopenia (XLT)	WAS	X-linked neutropenia (XLN)
Mutation	Missense mutations, in frame deletion or insertion in exons 1-3	Nonsense mutations, frame shift caused by deletions or insertions	Missense mutations, inframe deletion or insertion in the GBD domain
Phenotype	Thrombocytopenia and small platelets	Thrombocytopenia with small platelets, eczema, recurrent infections, increase incidence of autoimmunity and malignancies	Neutropenia

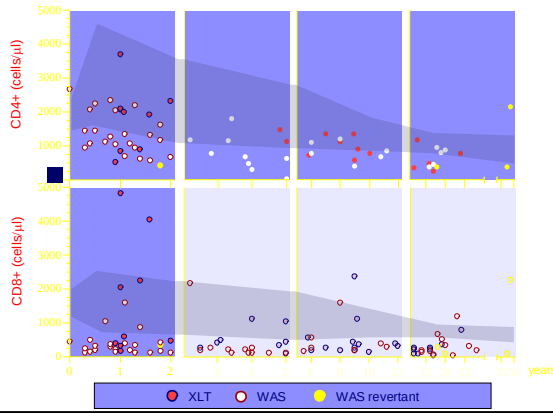
ANALYSIS OF WASP PROTEIN EXPRESSION BY FLOW CYTOMETRY



T and B cell lymphopenia in WAS/XLT



CD4 and CD8 Tcell lymphopenia in WAS/XLT



Factors that influence the phenotype

- **age.**
- **family history**

  (susceptibility genes for allergy/atoimmunity)
- **environment (microbes, hygiene)**
- **treatment/prevention**

Patients younger than 2 years often have a score ≤3, even if some of them develop severe symptoms later in life

T cell activation defects in WAS

- Impaired T cell proliferation, and IL-2 secretion after stimulation with immobilized anti-CD3, but

- Normal response to alloantigens, antigens and soluble anti-CD3 presented by APCs

- Defective regulatory T cell function

PEDIATRICS Vol. 111 No. 5 May 2003

Autoimmunity in Wiskott-Aldrich Syndrome: Risk Factors, Clinical Features, and Outcome in a Single-Center Cohort of 55 Patients

Sophie Dupuis-Girod, MD*‡; Jacques Medioni, MD§; Elie Haddad, MD, PhD*; Pierre Quartier, MD*; Marina Cavazzana-Calvo, MD, PhD‖; Françoise Le Deist, MD, PhD¶; Geneviève de Saint Basile, PhD#; Jean Delaunay, MD, PhD**; Klaus Schwarz, MD, PhD‡‡; Jean-Laurent Casanova, MD, PhD*§§; Stephane Blanche, MD*§§; and Alain Fischer, MD, PhD*‖#

TABLE 1. Autoimmune or Inflammatory Manifestations in a Cohort of 55 Patients With WAS

Condition	Patients		Age at Onset (Months)	
	n	%	Mean	Range
AIHA	20	36	13.7	0–58
Neutropenia	14	25	23.8	3–67
Arthritis	16	29	45.3	13–180
Skin vasculitis	12	22	53	11–186
Cerebral vasculitis	4	7	52	13–84
Inflammatory bowel disease	5	9	39.2	2–156
Renal disease	2	3.5	7	

The Journal of Experimental Medicine Vol. 204, No. 2, February 19, 2007 381–391

The Wiskott-Aldrich syndrome protein is required for the function of CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells

Michel H. Maillard,[1,5,7] Vinicius Cotta-de-Almeida,[1,5,8] Fuminao Takeshima,[1,5] Deanna D. Nguyen,[1,5] Pierre Michetti,[7] Cathryn Nagler,[2,3,5] Atul K. Bhan,[4,6] and Scott B. Snapper[1,5]

The Journal of Experimental Medicine Vol. 204, No. 2, February 19, 2007 369–380

WASP regulates suppressor activity of human and murine CD4$^+$CD25$^+$FOXP3$^+$ natural regulatory T cells

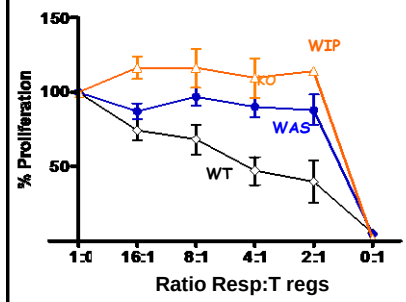
Francesco Marangoni,[1,2] Sara Trifari,[1] Samantha Scaramuzza,[1] Cristina Panaroni,[1] Silvana Martino,[3] Luigi D. Notarangelo,[4] Zeina Baz,[5] Ayse Metin,[6] Federica Cattaneo,[1] Anna Villa,[1,7] Alessandro Aiuti,[1] Manuela Battaglia,[1] Maria-Grazia Roncarolo,[1,2] and Loïc Dupré[1]

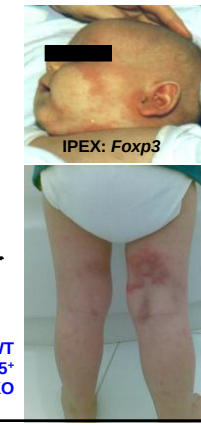
The Journal of Clinical Investigation http://www.jci.org Volume 117 Number 2 February 2007

Wiskott-Aldrich syndrome protein is required for regulatory T cell homeostasis

Stephanie Humblet-Baron,[1,2] Blythe Sather,[3,4] Stephanie Anover,[1] Shirly Becker-Herman,[1] Debora J. Kasprowicz,[4] Sochseth Khim,[1] Thuc Nguyen,[1] Kelly Huckins-Loya,[5] Charles E. Alpers,[5] Steve F. Ziegler,[3,4] Hans Ochs,[1] Troy Torgerson,[1] Daniel J. Campbell,[3,4] and David J. Rawlings[1,2]

Impaired Treg activity in WASP deficient mice





CD4$^+$CD25$^-$ responder T cells (Resp) from WT mice were co-cultured for 3 days with CD4$^+$CD25$^+$ T regs cells either from WT, WASP KO, or WIP KO mice at different ratios.

Significance of Treg cell defect

Lack of T regs results in autoimmune disease with colitis

Ulcerative colitis occurs in 3-40 % of patients. they also develop vasculitis and anti red cells and antiplts Abs

WASP KO mice develop chronic colitis with leukocytic infiltration in the intestinal mucosa. This is reversed by normal T regs

T cell cytoskeletal defects in WAS

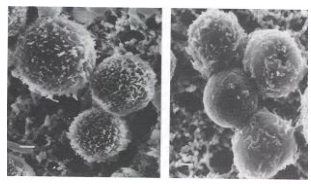
-Impaired F-actin polymerization after TCR ligation.

-Impaired capping of the TCR.

-Impaired spreading over anti-CD3 coated plates

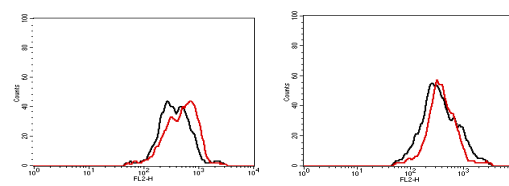
-Impaired homing to lymphoid tissues.

Scanning EM of lymphocytes in Wiskott Aldrich syndrome



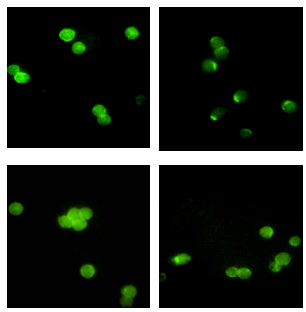
Normal WAS

F-actin polymerization in primary T-cells following anti-CD3 stimulation is deficient in WAS



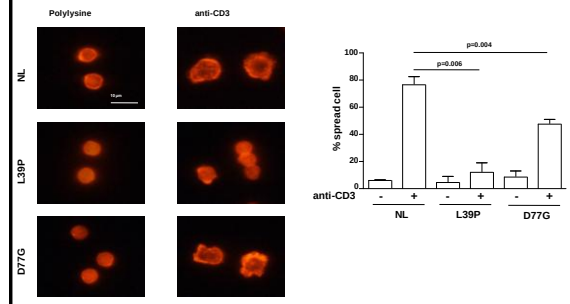
Control WAS Patient

Aberrant Capping in WASP$^{-/-}$ Tcells

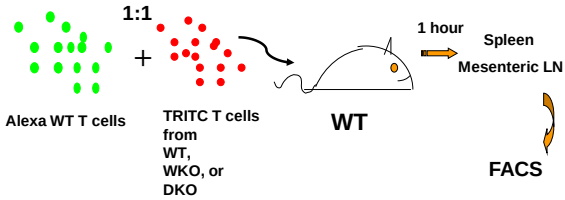


Immunity. 1998 Jul;9(1):81-91.

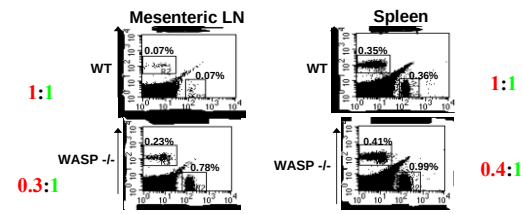
Defective spreading of WAS patient primary T cells stimulated with anti-CD3



In vivo homing of WASP$^{-/-}$ T cells



Severely impaired in vivo homing of WASP-/- T cells



Defects in other immune cells

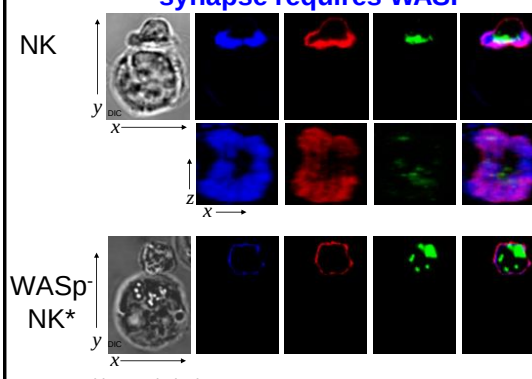
-NK cells :
- -defective cytolytic function
- -reduced F-actin at the immunological synapse

-B cells:
- - Defects in MZ B cells (TI Ab. responses to polyscaccharides) and memory B cells.

-Macrophages :
- -defective formation of the phagocytic actin cup

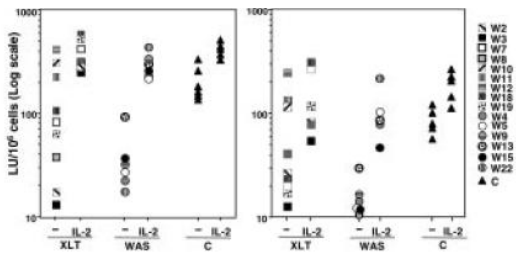
-Chemotactic and Migratory defects :
- - Neutrophils, Macrophages, Dendritic cells and B cells.

Formation of the cytolytic immunological synapse requires WASP



*same with cytochalasin-D

IL-2 corrects NK cell defect in vitro

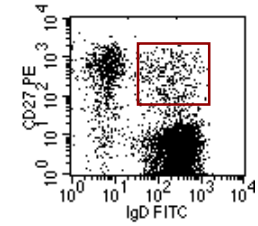


Impaired natural and CD16-mediated NK cell cytotoxicity in patients with WAS and XLT: ability of IL-2 to correct NK cell functional defect

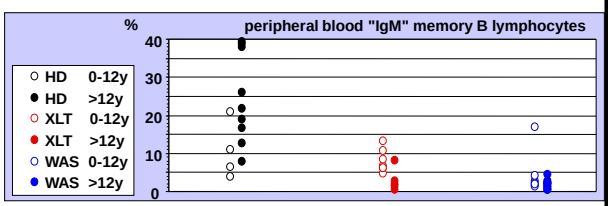
Angela Gismondi, Loredana Cifaldi, Cinzia Mazza, Silvia Giliani, Silvia Parolini, Stefania Morrone, Jordan Jacobelli, Elisabetta Bandiera, Luigi Notarangelo, and Angela Santoni

Blood. 2004;104:436-443

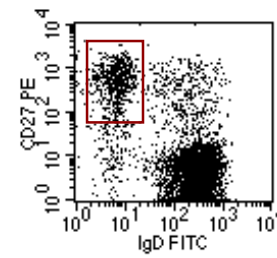
IMMUNOPHENOTYPE OF IgM MEMORY B cells :CD19+ CD20+ IgD+ IgM+ CD27+



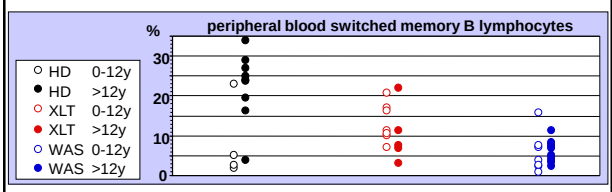
"IgM" memory B cells are markedly decreased in XLT and WAS patients



IMMUNOPHENOTYPE OF SWITCHED MEMORY B cells :CD19+ CD20+ IgD- IgM- CD27+



Switched memory B cells are decreased in XLT and WAS patients



TREATMENT

1. **Splenectomy, IVIG**

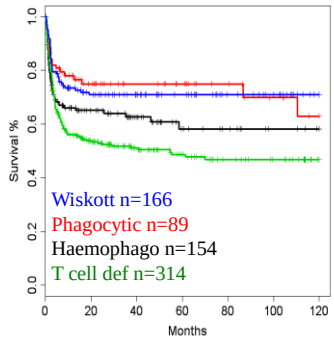
2. **BMT**

Complete correction of the Wiskott-Aldrich syndrome by allogeneic bone-marrow transplantation.
Parkman et al. N Engl J Med. 1978 Apr 27;298(17):921-7.

3. **Gene therapy: ongoing**

4. **Peptide therapy to stabilize WASP**

The European experience with HSCT in PID: disorders other than SCID



10 years Survival rate

Wiskott : 71%

Phagocytic : 63%

Haemophagocytic : 58%

T cell def : 47%

p<0.0001

The European experience with HSCT in WAS: impact of donor type

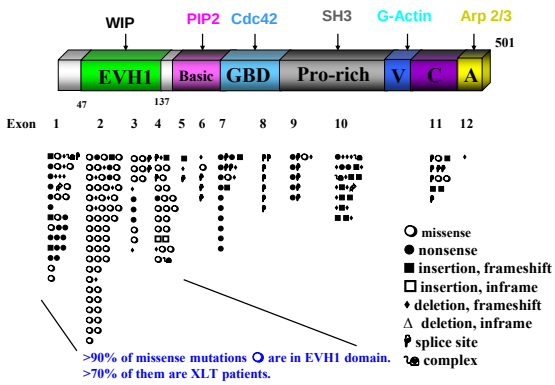
10-year survival

MRD: 88%

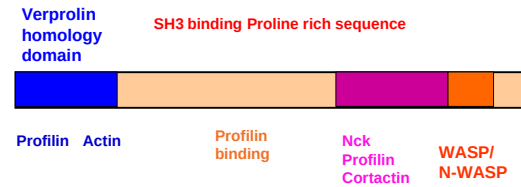
MUD: 77%

MMRD: 45%

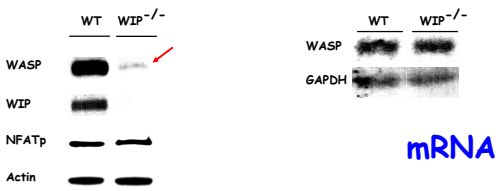
WASP mutations



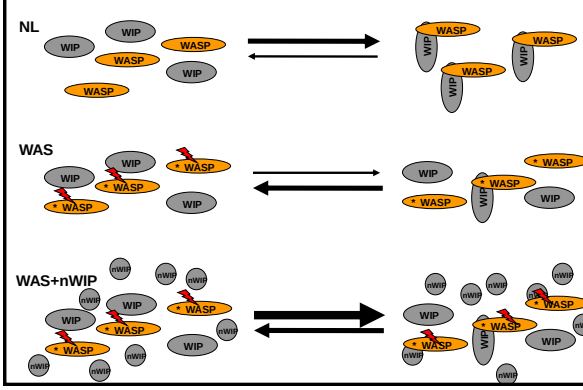
WIP (WASP Interacting Protein)



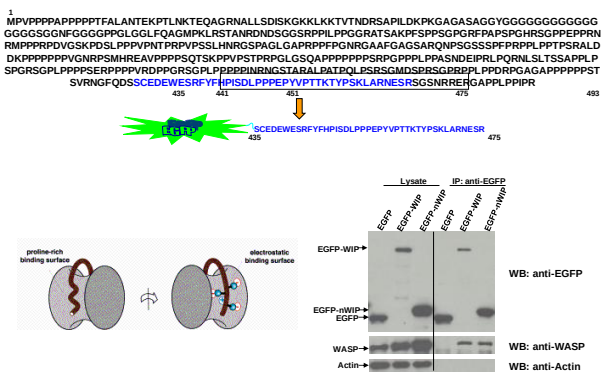
WIP stabilizes WASP:



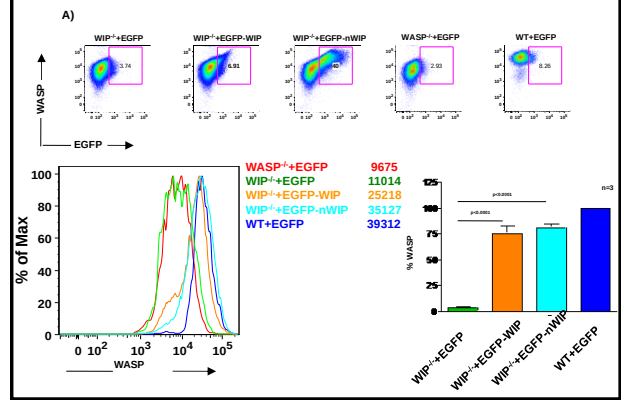
Rationale for stabilization of WASP by overexpression of WIP



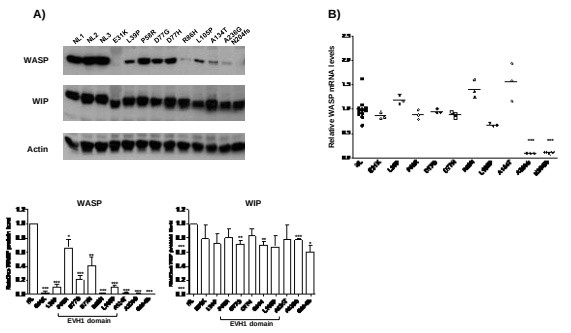
Interaction of EGFP-nWIP with WASP



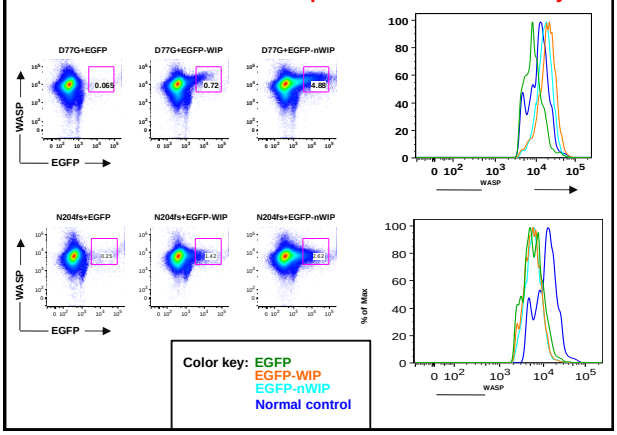
WASP levels are increased by expression of the WASP-binding domain of WIP in WIP$^{-/-}$ T cells



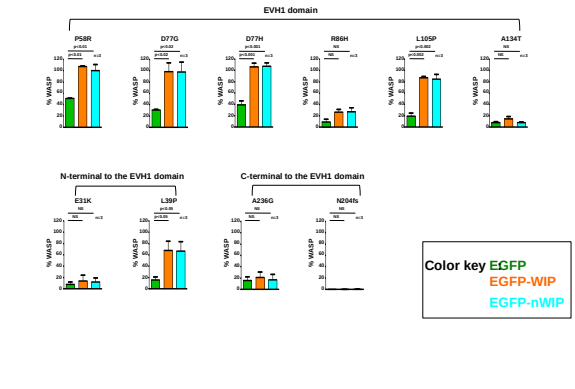
WASP protein and mRNA levels in WAS patient EBV-transformed B cell lines.



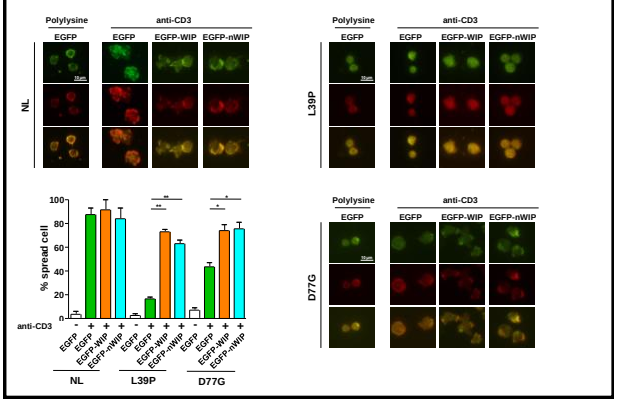
Stabilization of WASP in WAS patient EBV-B cell lines by WIP



Stabilization of WASP in WAS patient EBV-B cell lines by WIP



Expression of WIP or nWIP increases spreading of WAS patient primary T cells stimulated with anti-CD3



Conclusion

- WIP stabilizes WASP
- A 34 a.a long WASP binding peptide fragment of WIP restores WASP levels in EBV B cells and corrects the T cell cytoskeletal defect in T cells from WAS patients with mutations in WASP that destroy WIP binding.
- This may provide a novel approach to therapy of WAS patients.

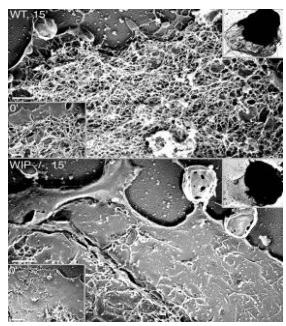
Phenotype of WIP$^{-/-}$ mice

- T and B cell phenotype is normal
- Severe colitis develops in in 100 % of mice
- WIP$^{-/-}$ T cells
  - Fail to extend membrane protrusions in response to anti-CD3 stimulation.
  - Impaired in their ability to form an IS.
  - Fail to increase their F-actin after TCR ligation.
  - Have severely defective T-reg cell number and function
  - Have a disrupted actin network
  - Have drastically low levels of WASP
  - Fail to proliferate to both immobilized and soluble anti-CD3 and to antigens, and fail to develop DTH
  - Fail to respond to IL-2 by STAT5 phosphorylation, c-myc expression and proliferation,…

Electron micrographs of T cells

WT

WIP-/-



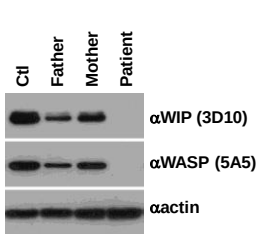
- Actin network is disrupted

Presentation

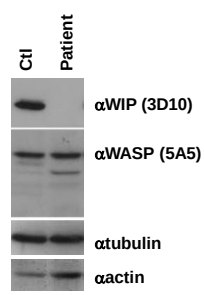
- Family History:
  - Moroccan consanguineous healthy parents (II grade cousins)
  - 4 m/o sister dead of sepsis in Morocco
- Failure to thrive and feeding difficulties
  - Bullous lesions on the abdomen and impetiginized vesicular lesions
  - RSV penumoioa
  - Colitis
  - Platelet Count: 59 x 10^3/µL
  - CRP 100.3 mg/L
  - Low CD3 CD4 and CD8

Absent WIP protein

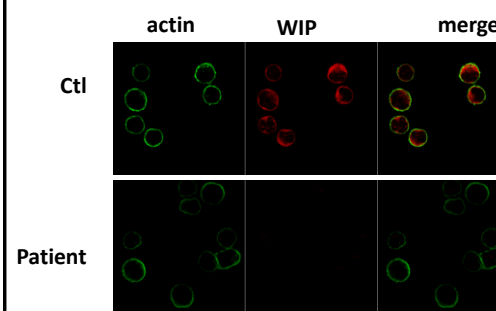
T cells



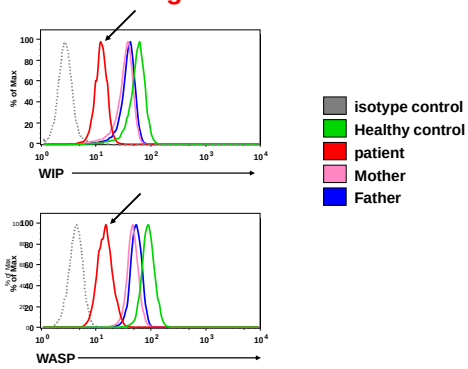
Fibrobalsts

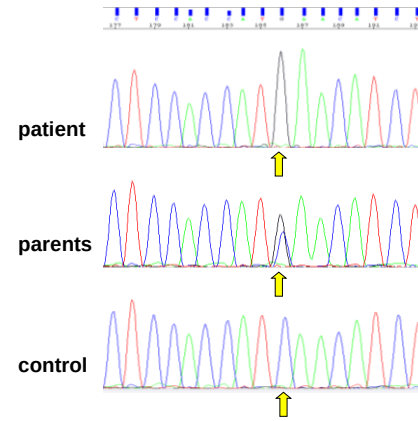


Intracellular staining

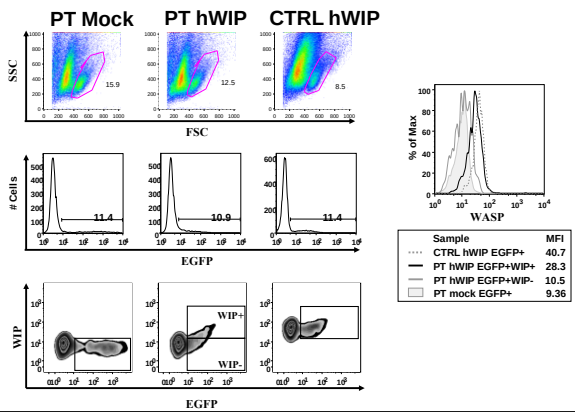


Intracellular staining for WIP and WASP





WIP transduction rescues WASP levels in patient T cells



Sample	MFI
CTRL hWIP EGFP+	40.7
PT hWIP EGFP+WIP+	28.3
PT hWIP EGFP+WIP-	10.5
PT mock EGFP+	9.36

Conclusion

- WIP deficient mice have a similar but more severe phenotype than WASP deficient mice and 100% develop colitis
- They have severely diminished WASP levels in T cells, which can be corrected by overexpressing WIP
- WIP deficient patient presents with T cell lymphopenia, poor T cell proliferation to mitogens and severe colitis
- She had severely diminished WASP levels in T cells, which can be corrected by overexpressing WIP