Immunologie

Immunogénétique normale et pathologique

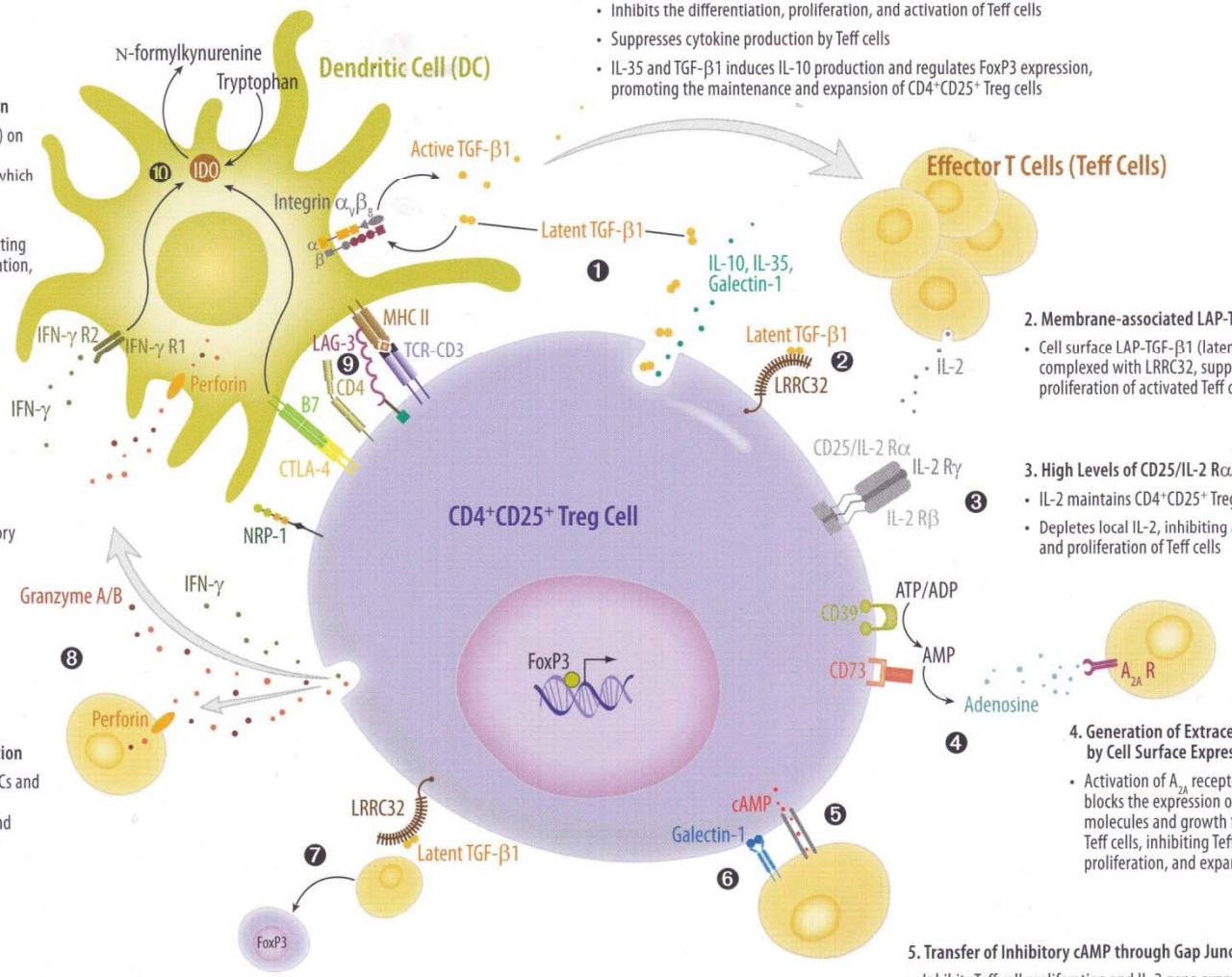
Gérard LEFRANC
Marie-Paule LEFRANC

Université Montpellier 2,
CNRS UPR1142, Institut de Génétique Humaine,
IMGT, Information System http://www.imgt.org

Cours Montpellier, février 2011

L3 et Master 1ère année

Suppressive Activities of Treg Cells



1. Secretion of TGF-β1, IL-10, IL-35, and Galectin-1

- Inhibits the differentiation, proliferation, and activation of Teff cells
- Suppresses cytokine production by Teff cells
- IL-35 and TGF-β1 induces IL-10 production and regulates FoxP3 expression, promoting the maintenance and expansion of $CD4^+CD25^+$ Treg cells

2. Membrane-associated LAP-TGF-β1 (Latent TGF-β1)

- Cell surface LAP-TGF-β1 (latent TGF-β1), complexed with LRRC32, suppresses the proliferation of activated Teff cells

3. High Levels of CD25/IL-2 Rα Expression

- IL-2 maintains $CD4^+CD25^+$ Treg cell populations
- Depletes local IL-2, inhibiting activation and proliferation of Teff cells

4. Generation of Extracellular Adenosine by Cell Surface Expression of CD39/CD73

- Activation of A_{2A} receptors by Adenosine blocks the expression of costimulatory molecules and growth factor receptors in Teff cells, inhibiting Teff cell activation, proliferation, and expansion

5. Transfer of Inhibitory cAMP through Gap Junctions

- Inhibits Teff cell proliferation and IL-2 gene expression

6. Membrane-associated Galectin-1

- Binds to GM1 ganglioside on Teff cells
- Induces cross-linking of associated integrins, triggering TRPC5 channel activation and calcium influx, inhibiting Teff proliferation

7. Induction of Infectious Tolerance

- In mice, membrane-associated LAP-TGF-β1 converts non-Treg cells into functional, FoxP3-expressing Treg cells

8. Granzyme A/B Secretion

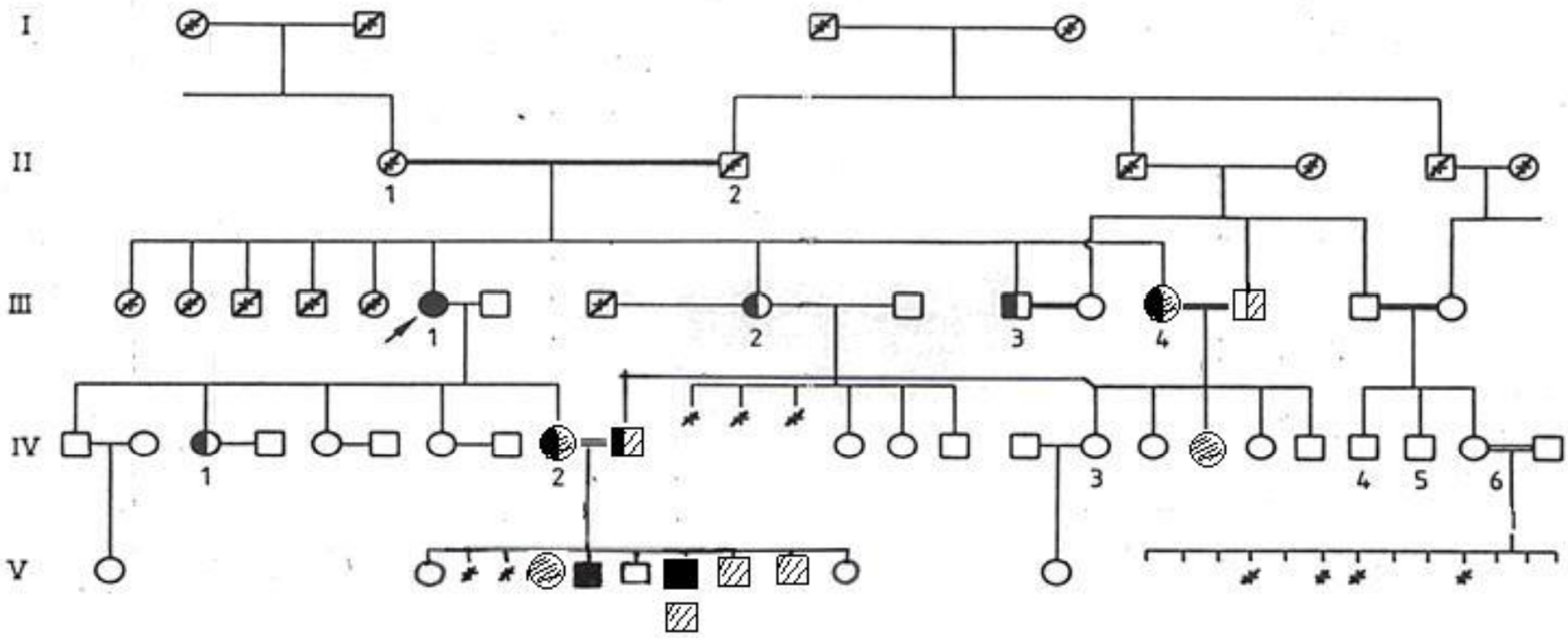
- Induces apoptosis in DCs and Teff cells in both a perforin-dependent and -independent manner

9. Binding of LAG-3 to MHC II

- Induces an immunoreceptor tyrosine-based activation motif (ITAM)-mediated inhibitory signaling pathway, blocking the maturation and immunostimulatory capacity of DCs

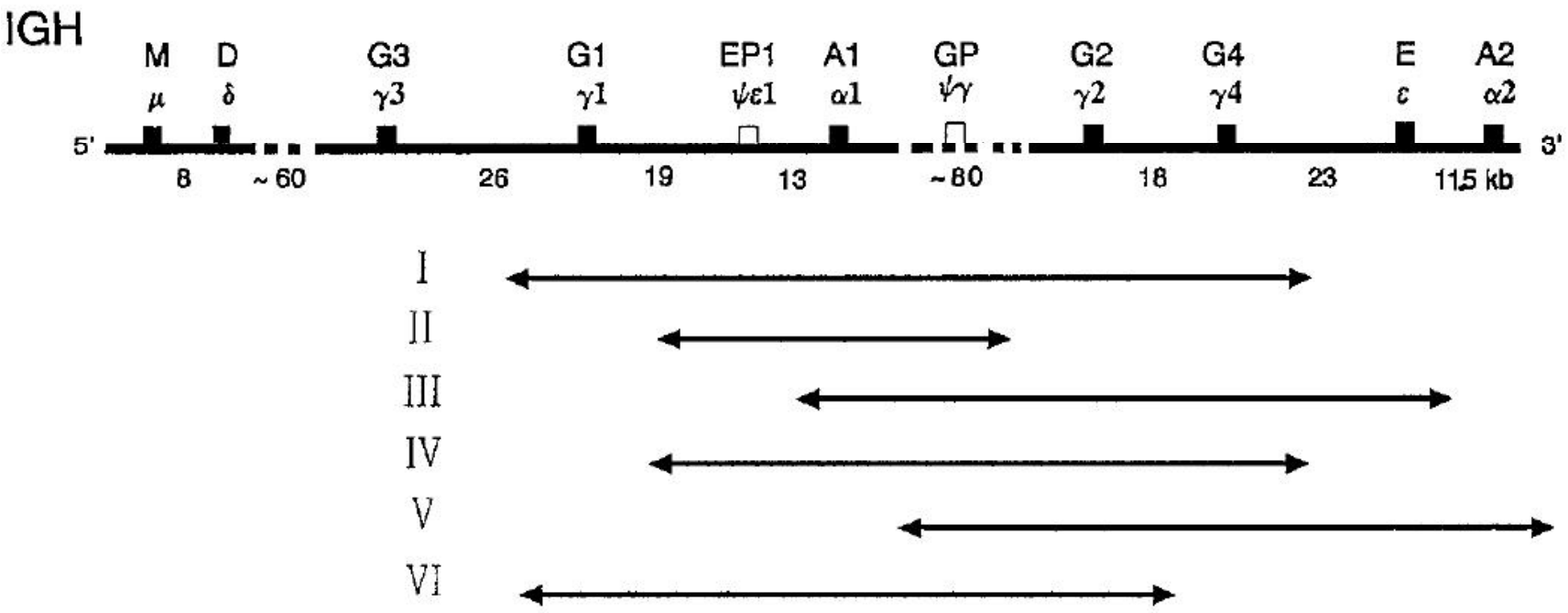
10. CTLA-4-dependent Suppression

- Interacts with B7 (CD80 and CD86) on DCs, triggering indoleamine 2, 3-dioxygenase (IDO) expression (which is also induced by IFN-γ receptor stimulation)
- IDO catabolizes tryptophan, depleting stores needed for Teff cell proliferation, and producing the pro-apoptotic metabolite N-formylkynurenine

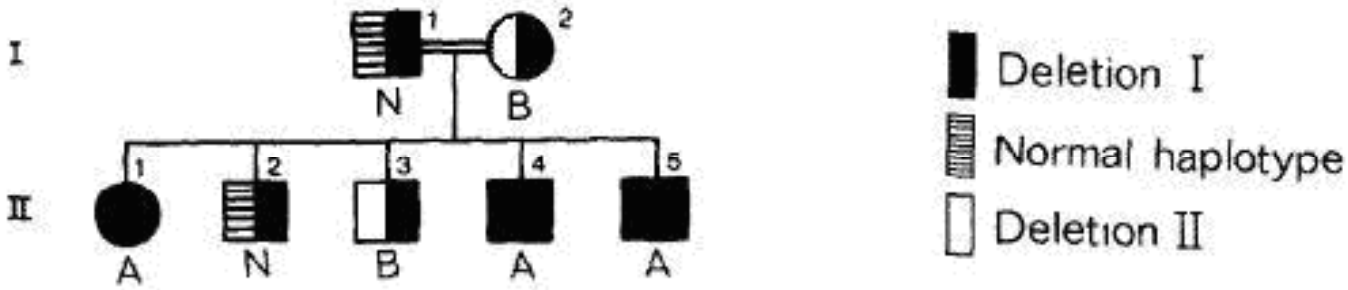


Genealogical tree of the Tunisian family HASS. In black: the family members III-1 and two of her grandsons (generation V) are autozygous for the deletion I. In hatched: family members of the generation IV and mainly generation V are also autozygous for a second very rare recessive allele (unidentified gene at the time of the study) and suffer of an Ataxia. The occurrence of two rare autosomal recessive diseases in consanguineous families is relatively frequent.

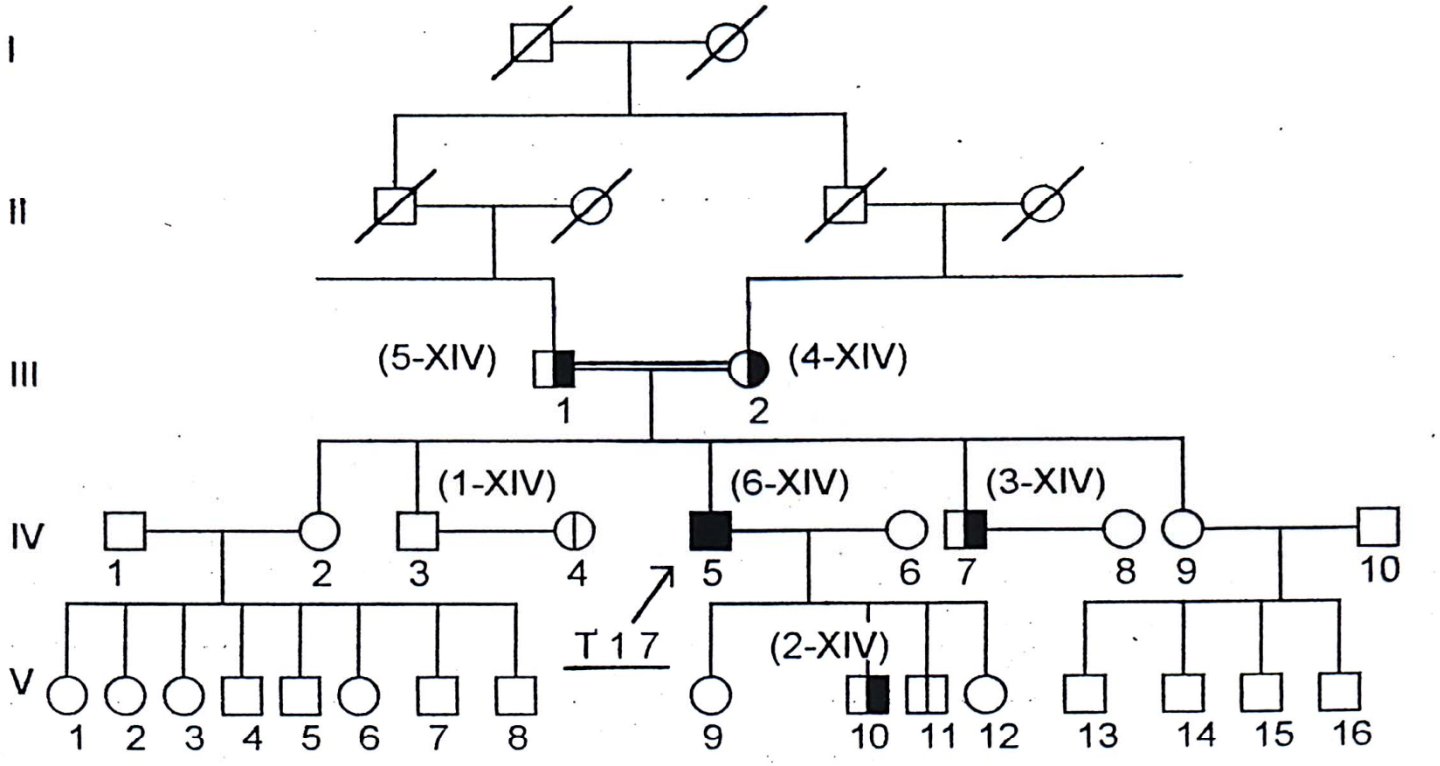
	Taux g/l sérums normaux	Absence de IgG1, IgG2, IgG4 et IgA1
IgG1	7,15	—
IgG2	3,80	—
IgG3	0,70	4,41
IgG4	0,55	—
IgM	1,32	1,41
IgA1	1,30	—
IgA2	0,14	0,56



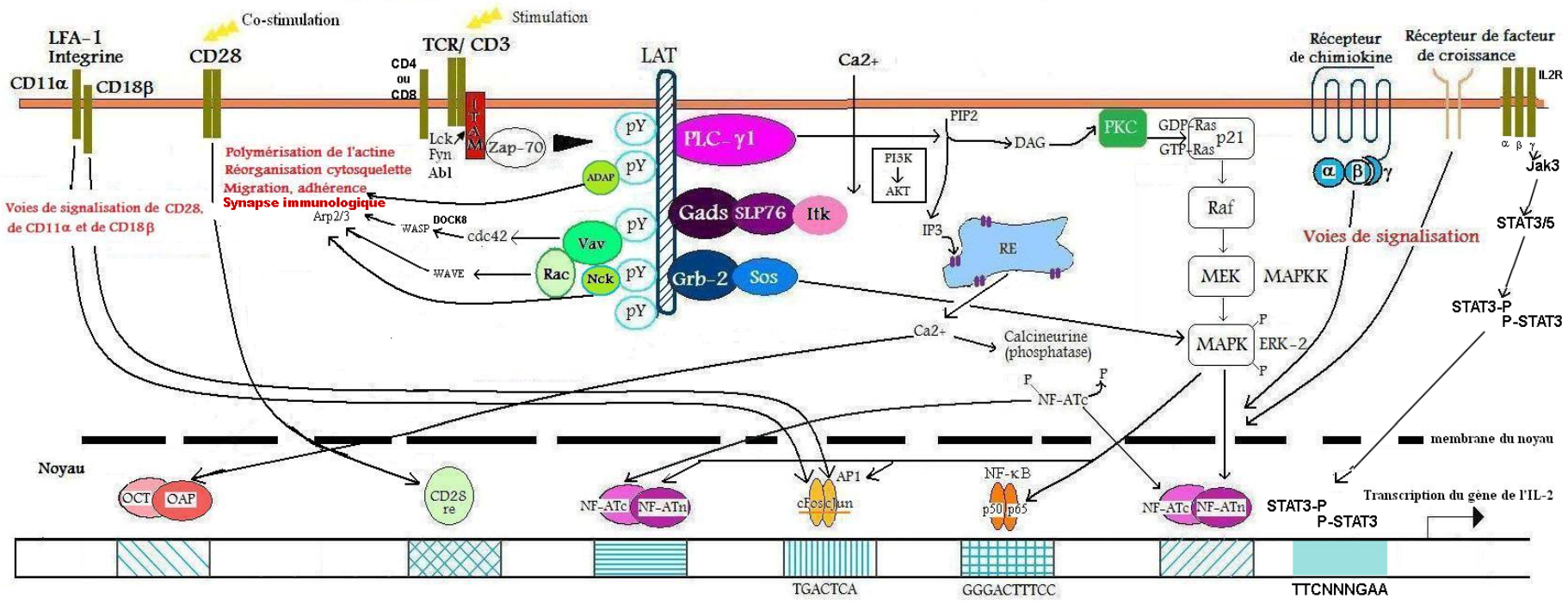
Organization of the human immunoglobulin heavy chain constant region locus (Flanagan and Rabbitts, 1982; Lefranc M-P et al., 1982, 1983). Nomenclature of the Ig CH genes is according to the recommendations of Human Gene Mapping HGM9. Multigene deletions in the Ig CH locus are designated I to VI according to the chronological order in which they were found.



Genealogical tree of the Tunisian family TOU. The family members II-1, -4 and -5 are autozygous for the deletion I. The mother and her son II-3 are heterozygous for the deletions I and II.



Genealogical tree of the Tunisian family H.: IV-5 is autozygous for the deletion III



MAPK	Mitogen-Activated Protein Kinase	ERK	Extracellular signal-Regulated Kinase
MAPKK	Mitogen-Activated Protein Kinase Kinase	LAT	Linker for activation of Tcells (9 Tyr)

Table 2. Summary of clinical differences between classic hyper-immunoglobulin E (IgE) syndromes (HIES) and AR-HIES

	HIES, classic form	HIES in consanguineous families
Inheritance pattern	Autosomal dominante négative	Autosomal recessive
Mutation de STAT3		TYK2 -/- DOCK8 -/-
Chronic eczema	Yes	Yes
Recurrent abscesses	Yes	Yes
Recurrent pneumonia	Yes	Yes
Pneumatoceles	Yes	No
IgE (IU/ml)	1875* to 58 200	1700–45 000
Absolute eosinophil count	726–2034/μl	2500–18 000/μl
Cerebral symptoms	Yes†	Yes
Vasculitis	No	Yes
Molluscum contagiosum	No	Yes
Herpes virus complications	No	Frequent
Recurrent bone fractures	Yes	No
Scoliosis	Yes	No
Hyperextensibility	Yes	No
Retained primary teeth	Yes	No
Lethality	Adulthood	Childhood

*IgE levels at diagnosis; authentic cases can have normal IgE levels in adulthood.

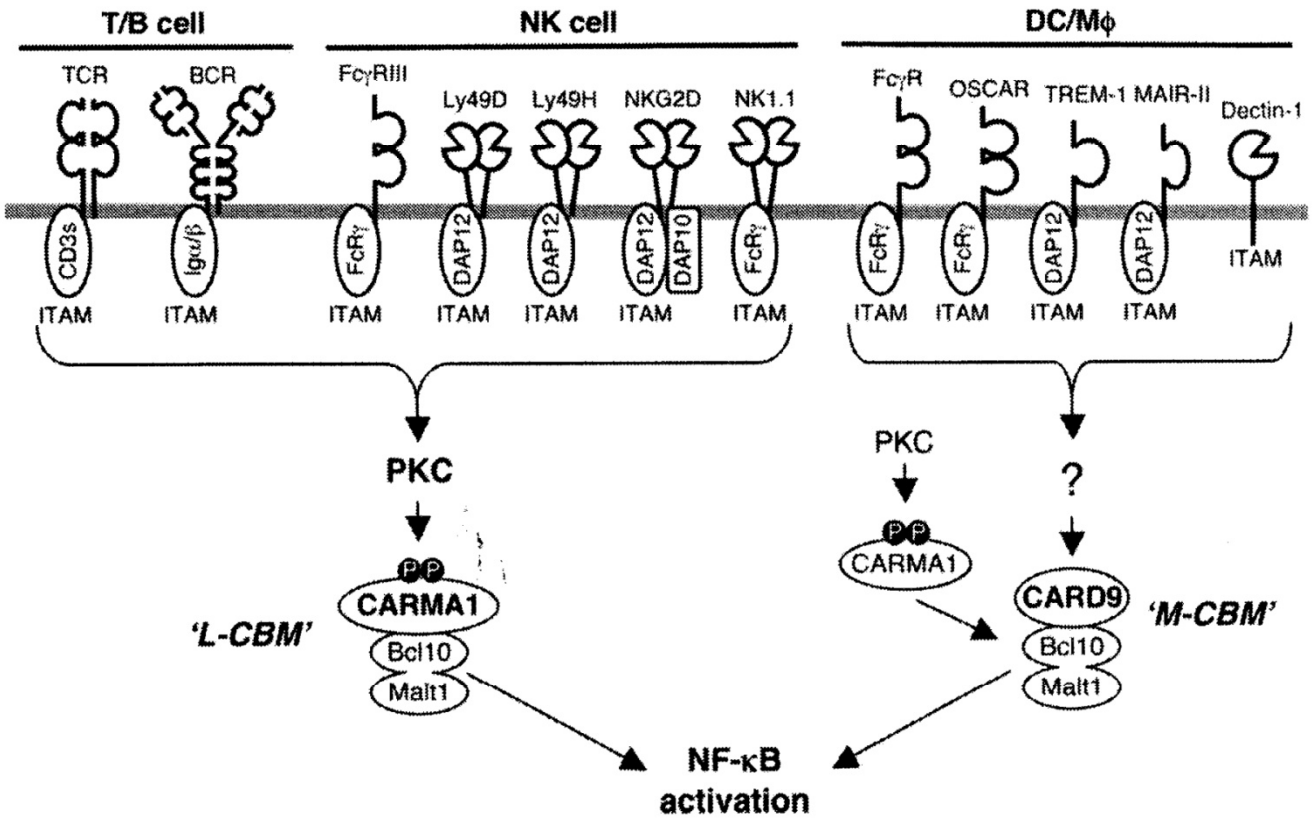


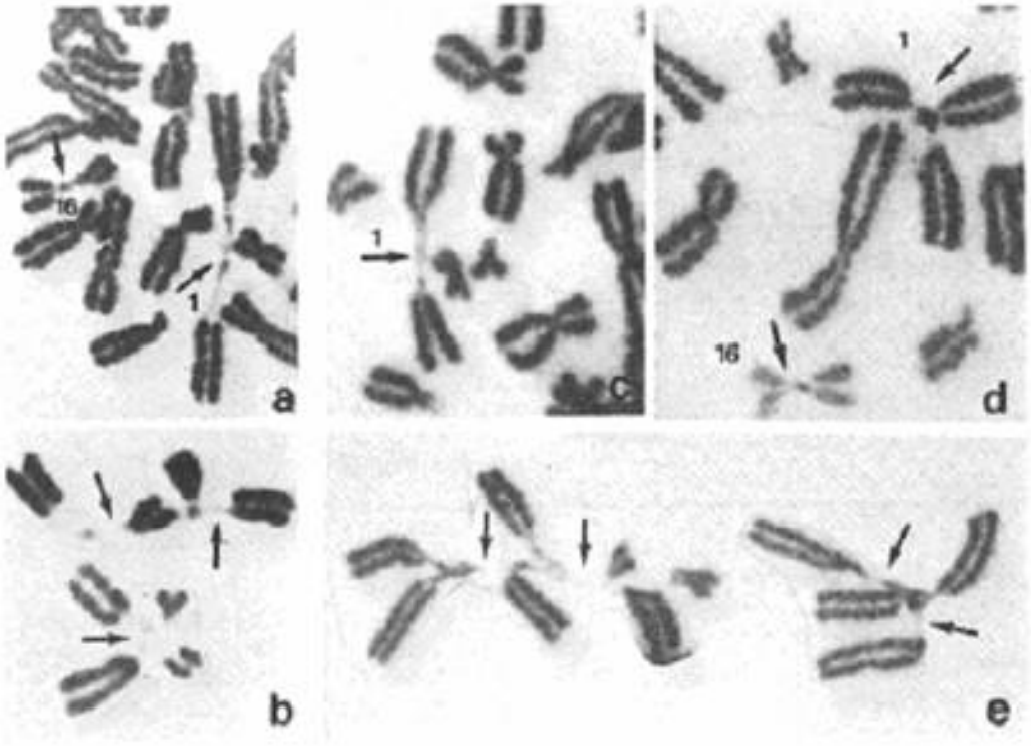
FIGURE 7. Cell type-specific regulation mechanism of ITAM receptor-mediated NF-κB activation through L-CBM and M-CBM. Activation of NF-κB in immune cells through ITAM receptors is mediated by two different complexes containing Bcl10-MALT1: the lymphoid type (L)-CARMA1-Bcl10-MALT1 (L-CBM) complex and the myeloid type (M)-CARD9-Bcl10-MALT1 (M-CBM) complex. L-CBM is regulated by PKC and acts in TCR, BCR, and activating NK cell receptor signaling, while M-CBM acts in ITAM receptor signaling independent of the PKC-CARMA1 system in macrophages and DCs.

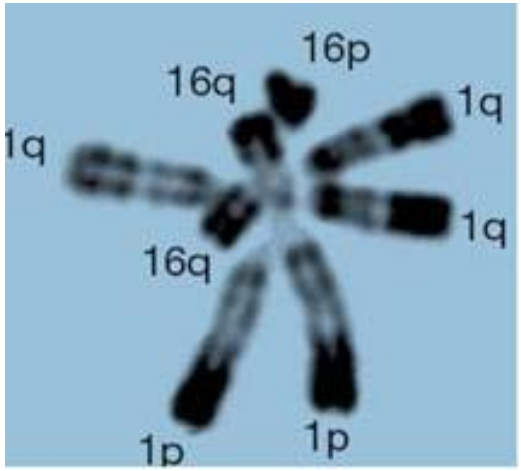
The adaptor molecule CARD9 is essential for tuberculosis control

The cross talk between host and pathogen starts with recognition of bacterial signatures through pattern recognition receptors (PRRs), which mobilize downstream signaling cascades. We investigated the role of the cytosolic adaptor caspase recruitment domain family, member 9 (CARD9) in tuberculosis. This adaptor was critical for full activation of innate immunity by converging signals downstream of multiple PRRs. *Card9*$^{-/-}$ mice succumbed early after aerosol infection, with higher mycobacterial burden, pyogranulomatous pneumonia, accelerated granulocyte recruitment, and higher abundance of proinflammatory cytokines and granulocyte colony-stimulating factor (G-CSF) in serum and lung. Neutralization of G-CSF and neutrophil depletion significantly prolonged survival, indicating that an exacerbated systemic inflammatory disease triggered lethality of *Card9*$^{-/-}$ mice. CARD9 deficiency had no apparent effect on T cell responses, but a marked impact on the hematopoietic compartment. *Card9*$^{-/-}$ granulocytes failed to produce IL-10 after *Mycobaterium tuberculosis* infection, suggesting that an absent antiinflammatory feedback loop accounted for granulocyte-dominated pathology, uncontrolled bacterial replication, and, ultimately, death of infected *Card9*$^{-/-}$ mice. Our data provide evidence that deregulated innate responses trigger excessive lung inflammation and demonstrate a pivotal role of CARD9 signaling in autonomous innate host defense against tuberculosis.

ICF syndrome (Immunodeficiency, Centromeric region instability and Facial anomalies)

ICF syndrome (OMIM 242860) is a very rare autosomal recessive disease characterized by a variable immunodeficiency, mild facial anomalies, centromeric decondensation and chromosomal instability involving chromosomes 1, 9, and 16. Chromosomal aberrations are recognized as breaks, deletions, isochromosomes and multiradial configurations in mitogen-stimulated lymphocytes. The most consistent finding is hypomethylation of the satellite 2 and satellite 3 regions of chromosomes 1, 9, and 16.
Other regions of heterochromatin, such as the centromeric, satellite repeats and the inactive X, may also be hypomethylated. Genomic methylation patterns play important roles in genome structure and expression. The predominant DNA methyltransferase in mammalian cells is DNMT1 (DNA MethylTransferase1).
Mutations of the *DNMT3B* (DNA MethylTransferase 3B) gene, in 20q11-13, is responsible for the ICF type 1. Mutations in another MethylTransferase gene, yet unidentified, cause the ICF type 2.
The B cells defects associated with a- or hypogammaglobulinemia in ICF type 1 are characterized by only naive and no memory B cells in peripheral blood (PB) due to B cell maturation blockage.





ICF: Centromeric decondensation and chromosomal instability involving chromosomes 1, 9, and 16: aberrations of chromosomes are recognized as breaks, deletions, isochromosomes and multiradial configurations in mitogen-stimulated lymphocytes.

Mutations in the gene encoding the PML nuclear body protein Sp110 are associated with immunodeficiency and hepatic veno-occlusive disease

We describe mutations in the PML* nuclear body protein Sp110 in the syndrome veno-occlusive disease with immunodeficiency, an autosomal recessive disorder of severe hypogammaglobulinemia, combined T and B cell immunodeficiency, absent lymph node germinal centers, absent tissue plasma cells and hepatic veno-occlusive disease. This is the first report of the involvement of a nuclear body protein in a human primary immunodeficiency and of high-penetrance genetic mutations in hepatic veno-occlusive disease.

* PML protein : ProMyelocytic Leukemia protein

3- Lymphocytes B

Protéine: Bruton tyrosine kinase

Gène: BTK (Xq21-22)

Déficit: XLA

Agammaglobulinémie liée à l'X

Pas d'immunoglobulines, pas de plasmocytes, pas de lymphocytes B circulants.

Le nombre de pré-B dans la moelle osseuse est normale.

Les lymphocytes T ne sont pas affectés.

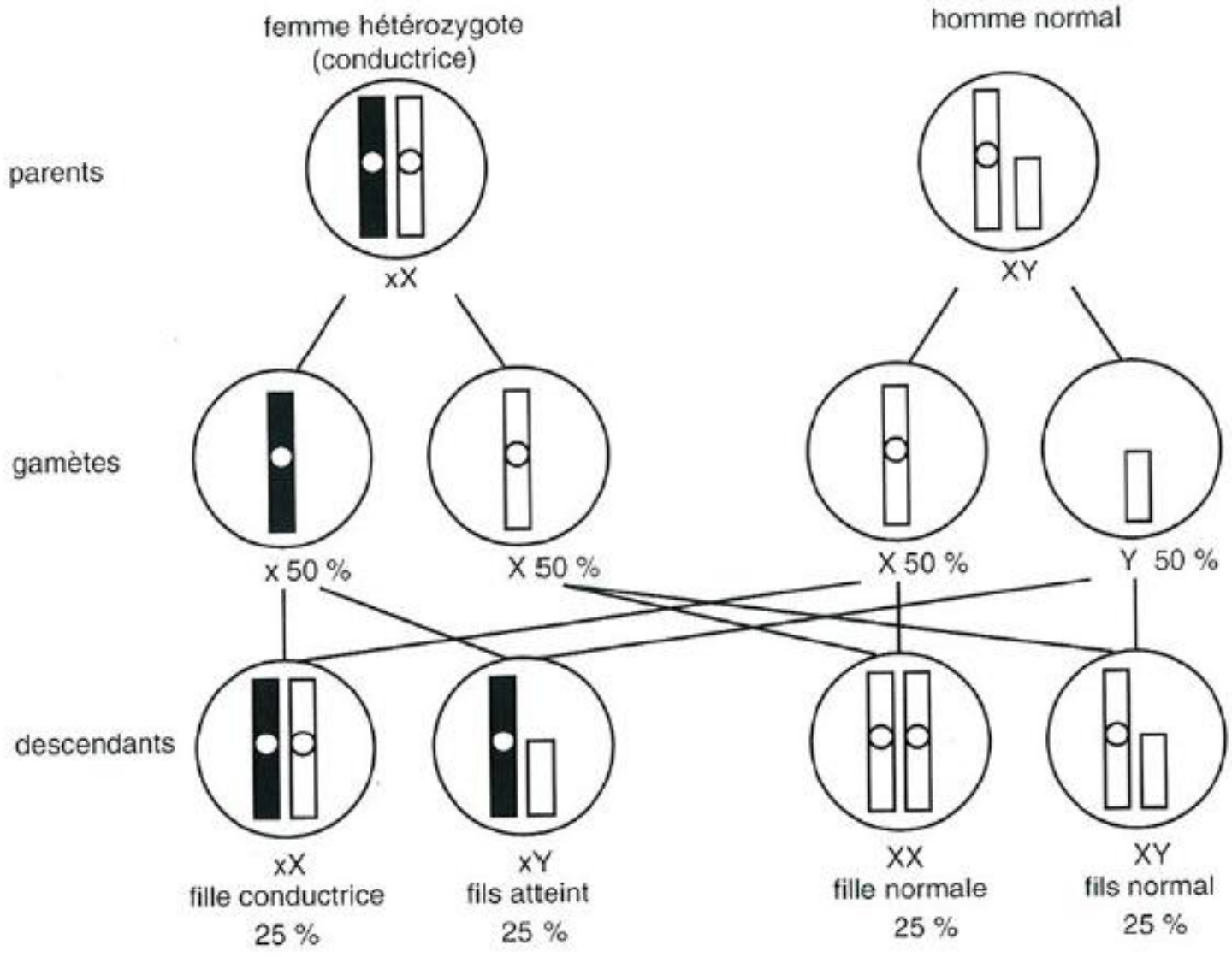


Figure 4.10 : Schéma de l'hérédité récessive liée au sexe ; mariage d'une femme conductrice et d'un homme normal.
Le chromosome X porteur de l'allèle morbide récessif, en noir, est dénoté x.

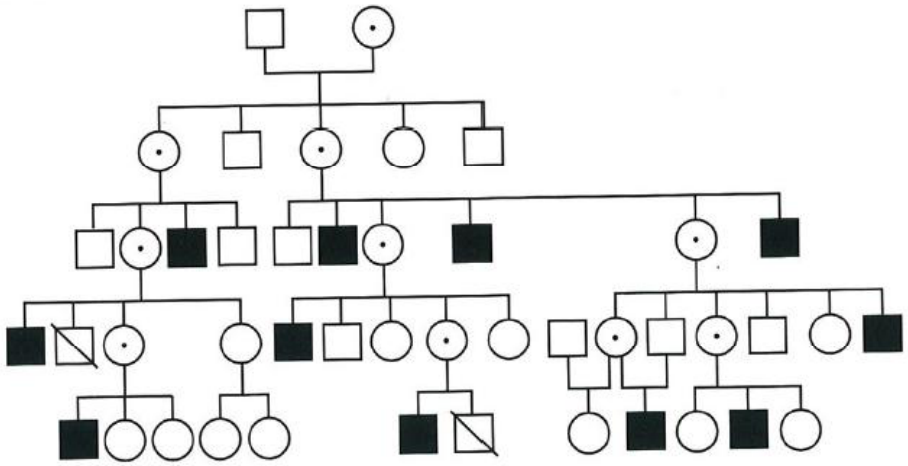


Figure 4.11 : Arbre généalogique d'une maladie récessive liée au sexe : maladie de Hunter, due à un déficit en αL-induronate sulfatase.

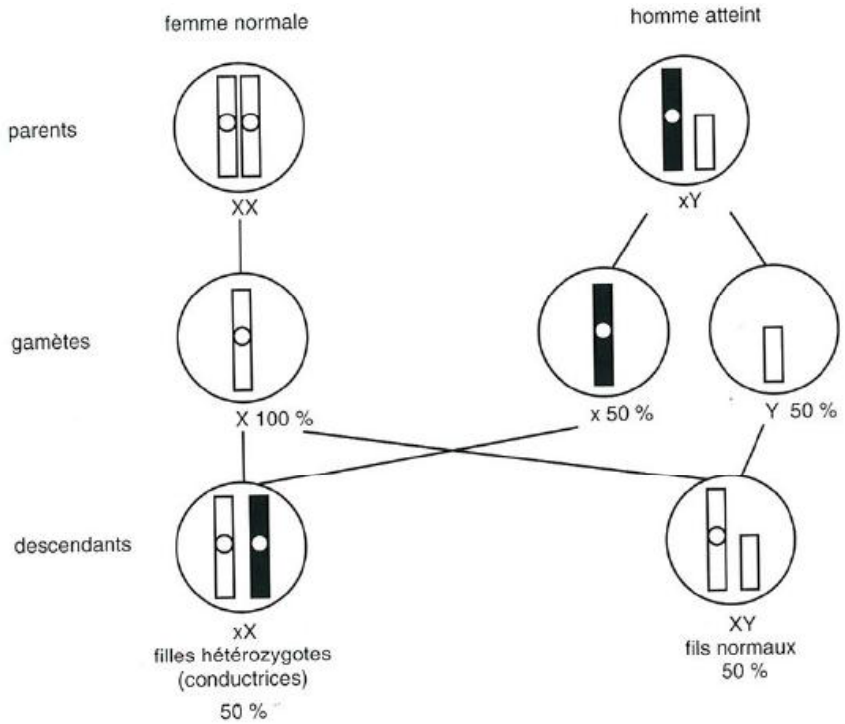
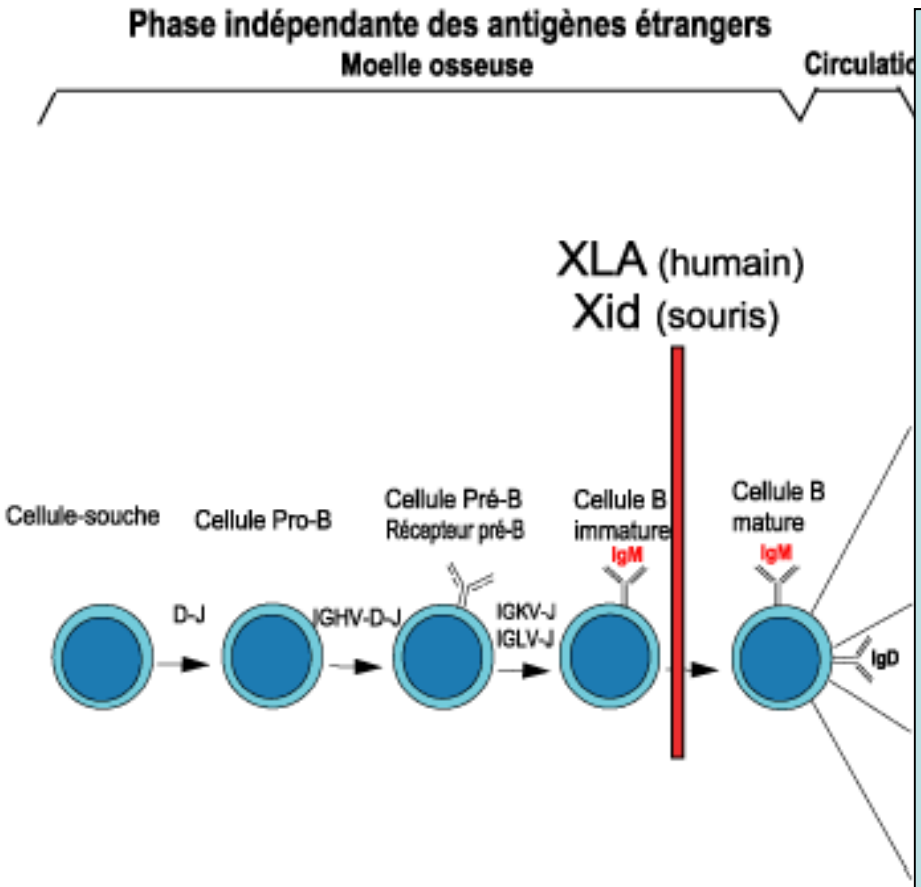


Figure 4.12 : Schéma de l'hérédité récessive liée au sexe : mariage d'un homme malade et d'une femme normale. Le chromosome X porteur de l'allèle morbide récessif, en noir, est dénoté x.

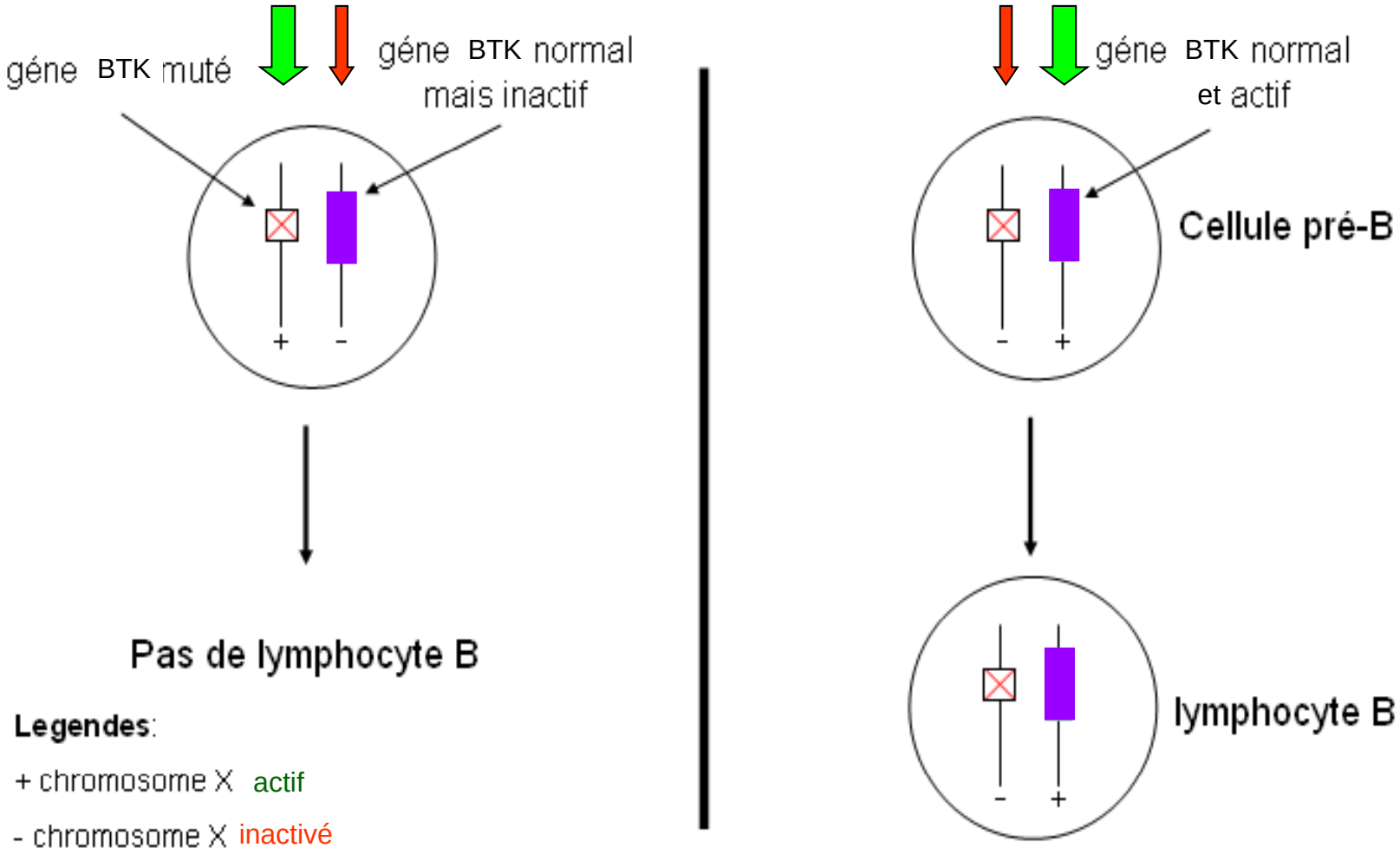

Phase indépendante des antigènes étrangers
Moelle osseuse
Circulatio
XLA (humain)
Xid (souris)
Cellule-souche
Cellule Pro-B
Cellule Pré-B
Récepteur pré-B
Cellule B
immature
Cellule B
mature
IgM
IgM
IgD
D-J
IGHV-D-J
IGKV-J
IGLV-J

3- Lymphocytes B

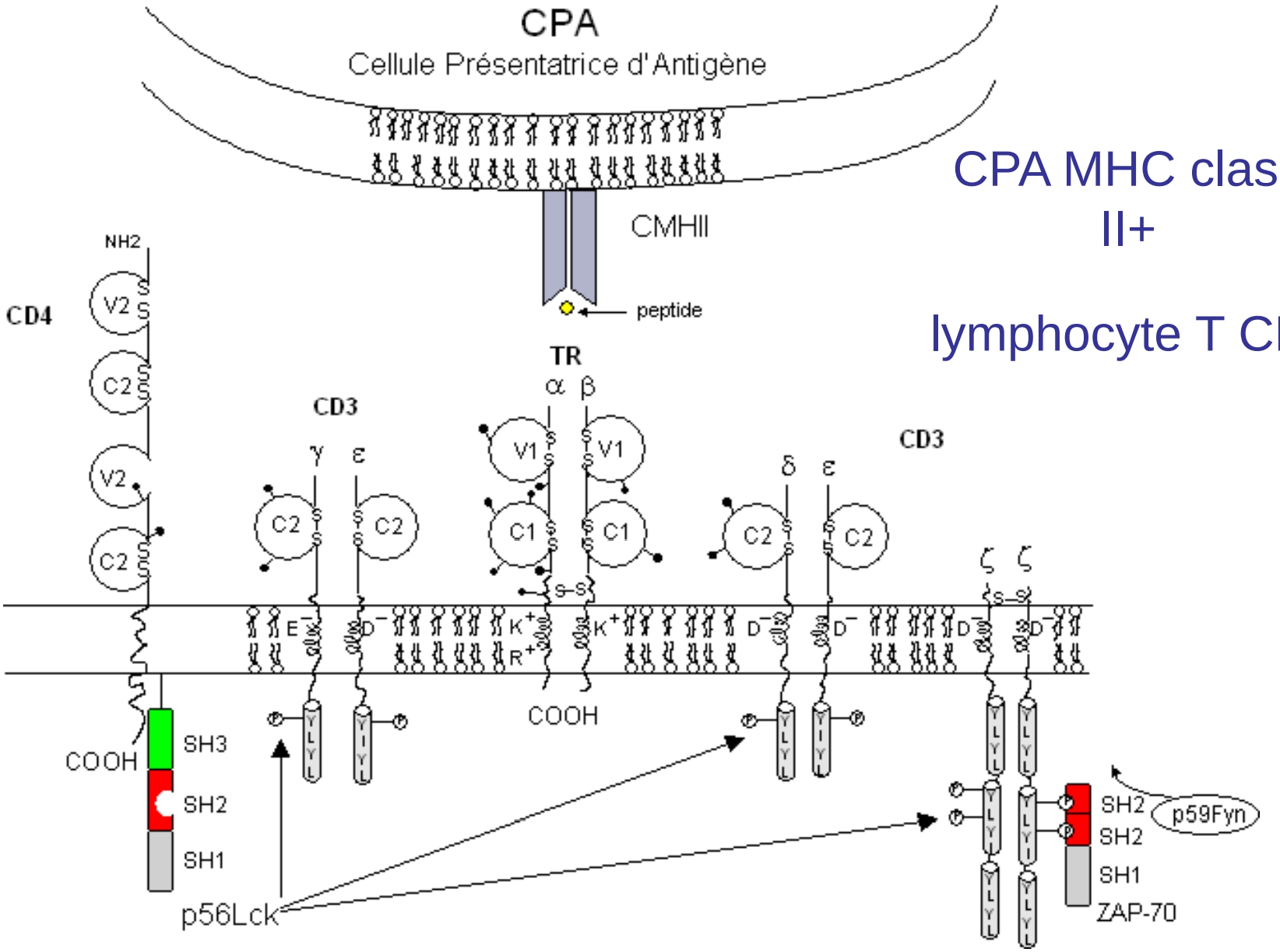
On observe un blocage de la maturation des cellules pré-B en lymphocytes B matures dans la moelle osseuse, d'où absence de lymphocytes B circulants

Traitement: Efficacité de la thérapie de substitution (perfusion intraveineuse d'immunoglobulines toutes les 3 semaines)

Chez les femmes hétérozygotes (porteuses de la mutation du gène BTK), un chromosome X est inactivé ( ) et l'autre chromosome X est **actif** ( ), au hasard, dans les cellules pendant l'embryogenèse.



5- Cellules présentatrices d'antigènes (peptides)



CPA MHC class II+

lymphocyte T CD4+

5- Cellules présentatrices d'antigènes

Protéines: CTAII (Class II Trans Activator), et facteurs de transcription (p36, RFX-5) des molécules HLA de classe II (HLA-DP, HLA-DQ, HLA-DR)

Gènes: 5 gènes

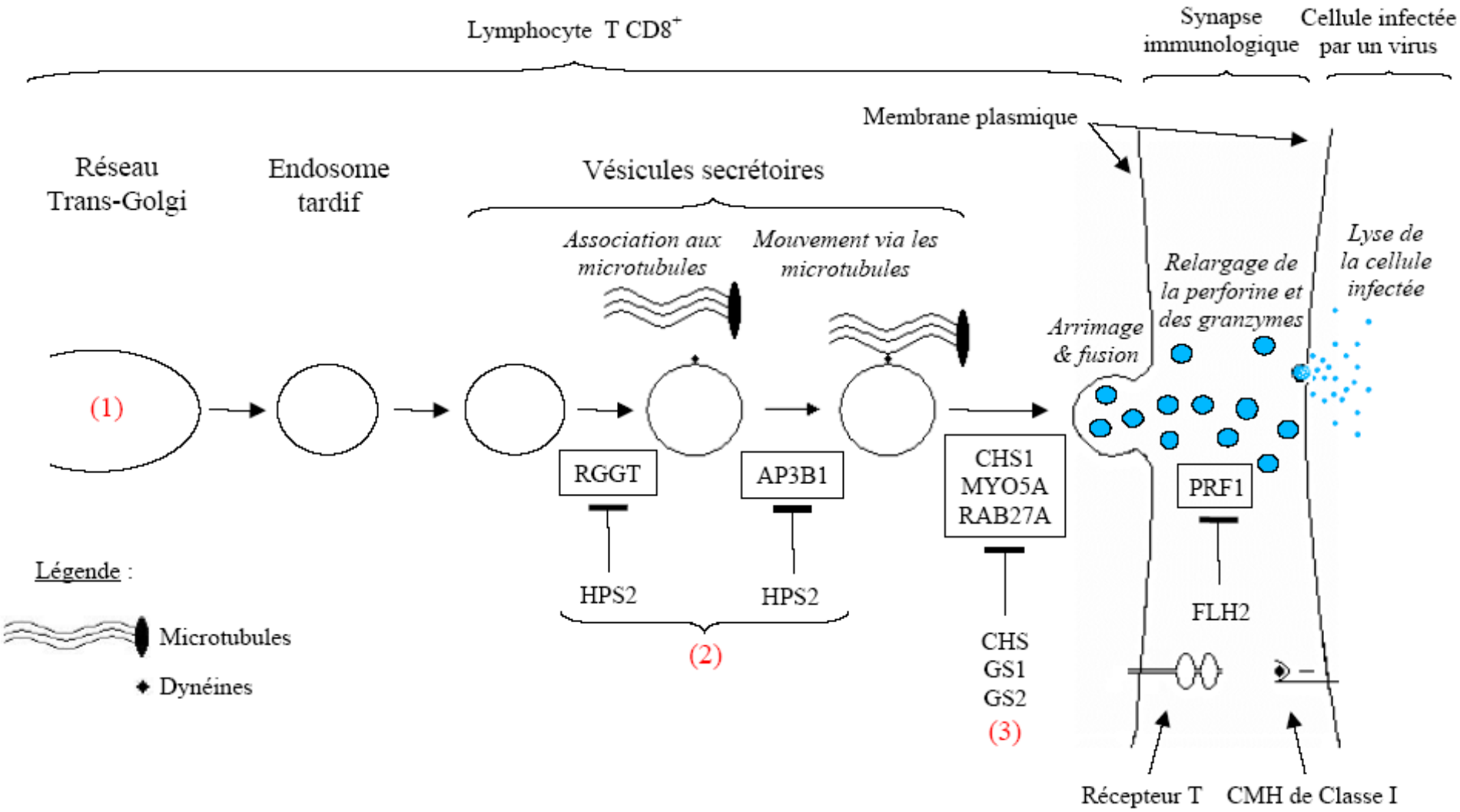
Déficits: maladies autosomales récessives

Signes cliniques: déficits immunitaires

Signes biologiques: pas d'expression de MHC classe II « syndrome des lymphocytes nus » (ou Bare Lymphocyte Syndrome BLS). Défaut de transcription (déficit en CTAII, ou de l'un des facteurs de trancription)

6- Propriété effectrices

Secrétion de la perforine et des granzymes par les lymphocytes T cytotoxiques CD8+



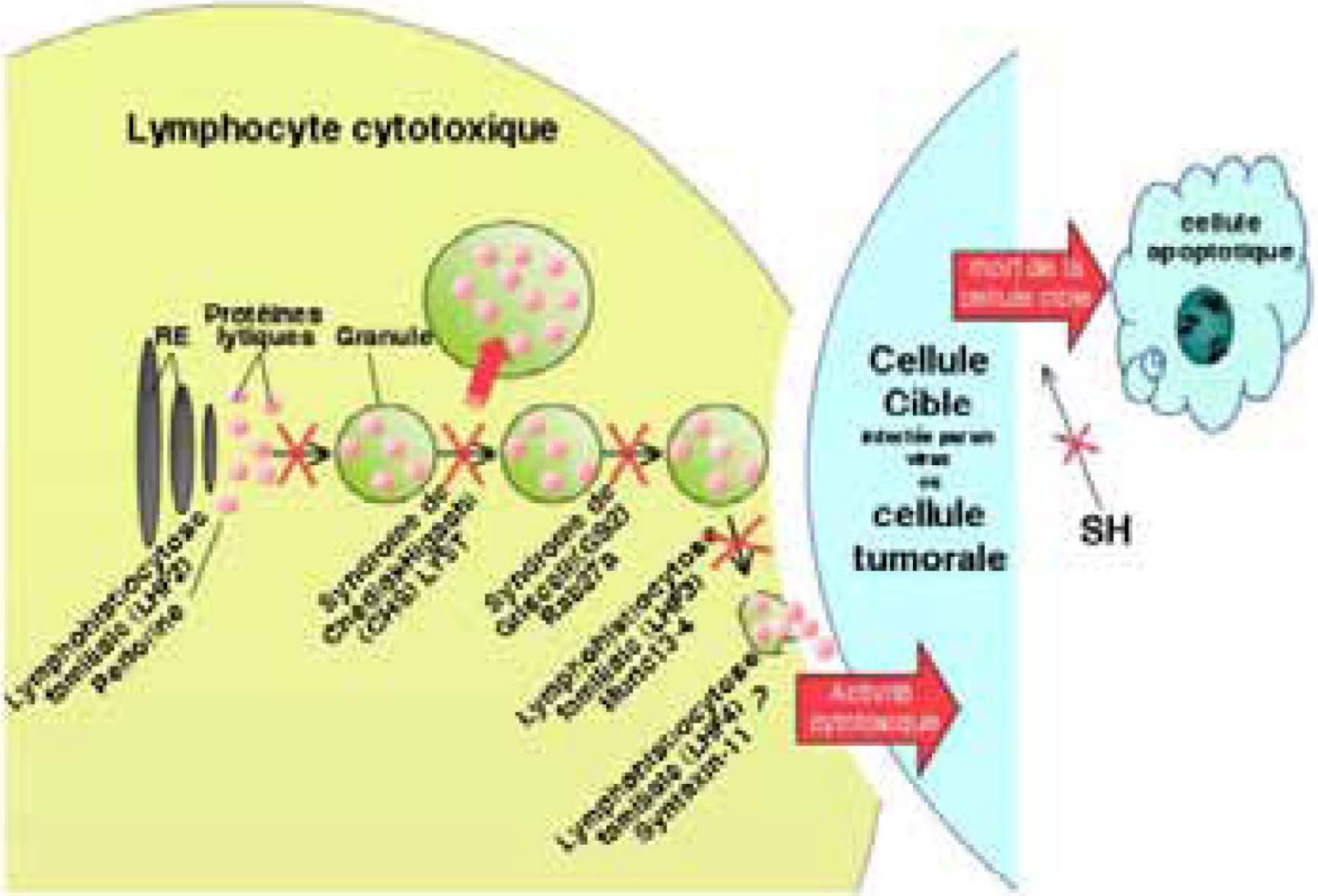
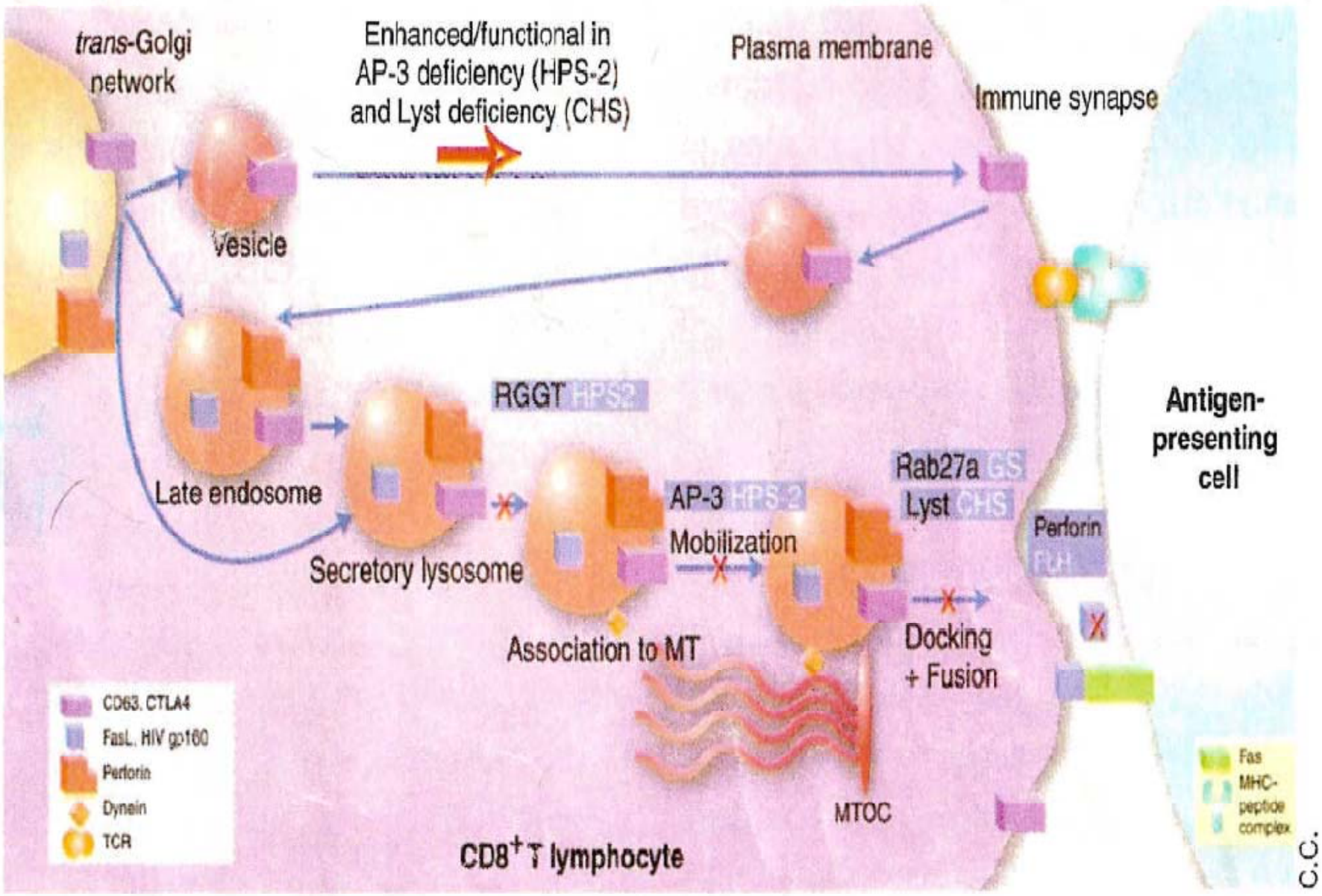


Figure 2: Causes moléculaires des syndromes hémophagocytaires associés à un défaut d'activité cytotoxique des lymphocytes. Défauts moléculaires responsables de syndromes hémophagocytaires héréditaires impliqués dans les différentes étapes de l'exocytose des granules cytotoxiques vers la cellule cible. RE : reticulum endoplasmic.


trans-Golgi network
Enhanced/functional in AP-3 deficiency (HPS-2) and Lyst deficiency (CHS)
Plasma membrane
Immune synapse
Vesicle
Late endosome
RGGT HPS2
Secretory lysosome
AP-3 HPS-2
Mobilization
Association to MT
Rab27a GS
Lyst CHS
Docking + Fusion
Perforin
RJH
MTOC
Antigen-presenting cell
CD8+ T lymphocyte
CD63, CTLA4
FasL, HIV gp160
Perforin
Dynein
TCR
Fas
MHC-peptide complex

C.C.

Manifestations Cliniques	Manifestations Biologiques
fièvre élevée	pancytopénie
hépatosplenomegalie	fibrinopénie
troubles neurologiques	hypertriglyceridémie
	hyponatrémie
	hémophagocytose

Tableau 1: Signes cliniques et biologiques du syndrome Hémophagocytaire

Etape d'intervention dans la cytotoxicité des lymphocytes	Syndrome	Gène	Protéine	Fonction	Transmission*	Activité cytotoxique
Activation cellulaire	XLP 1	SH2DIA	SAP	Protéine adaptatrice	X-L	+/-
Regulation de l'apoptose	XLP 2	XIAP	XIAP	Inhibiteur d'apoptose	X-L	+
Polarisation des granules cytotoxiques	HSP 2	AP3B1	AP3B1	Tri des protéines	AR	-
Arrimage des granules au niveau de la membrane plasmique	GS 2	Rab27a	Rab27a	Transport et arrimage des granules	AR	-
Amorçage de la fusion des granules cytotoxiques	FHL 3	UNC13D	Munc13-4	Amorçage des granules	AR	-
Exocytose	CHS	LYST	LYST	Tri des protéines /Fusion/ Fission membranaire	AR	-
Fusion avec la membrane plasmique	FHL 4	Syntaxin 11	Syntaxin 11	Fusion?	AR	+/ -
Induction de la mort cellulaire	FHL 2	PRF	Perforin	Formation de pores dans les cellules cibles	AR	-

Tableau 2 : Pathologies associées à un syndrome hémophagocytaire

*AR: transmission autosomique récessive, X-L: transmission liée au chromosome X.

Syndrome de Griscelli (GS)	Hypopigmentation	Défaut neurologique primitif	Syndrome hémophagocytaire	Défaut moléculaire
GS 1	+	+	-	Myo5a
GS 2	+	-	+	Rab27a
GS 3	+	-	-	Melanophiline (Mlph)

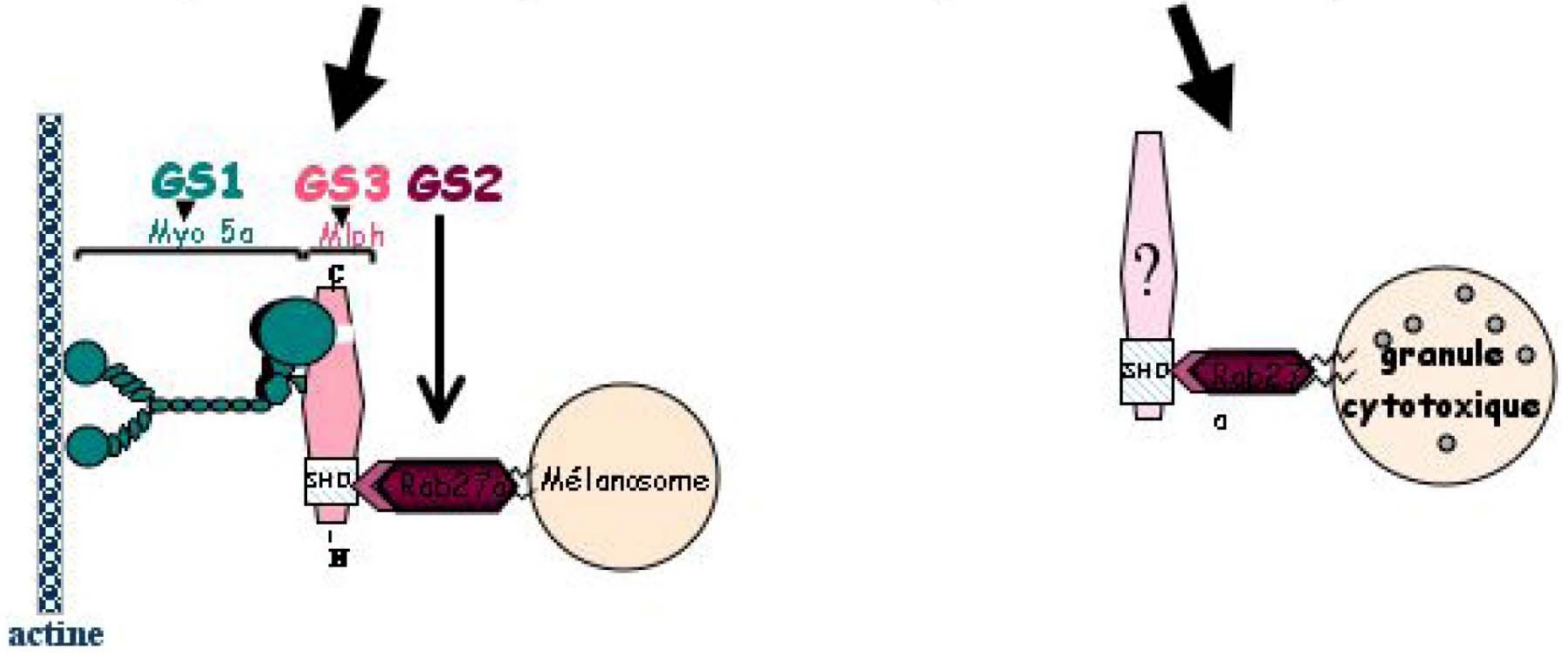


Figure 3 : Expression phénotypique et défauts moléculaires associés aux trois formes du syndrome de Griscelli (GS). Les mutations des gènes codants pour les protéines Myo5a, Rab27a ou la Mélanophiline sont responsables des défauts pigmentaires dont souffrent les patients atteints du syndrome de Griscelli. La formation d'un complexe hétérotrimerique permet le transport des melanosomes le long des fibres d'actine. L'expression phénotypique des différentes formes génétiques suppose que le transport des organelles au niveau des melanocytes, des neurones, et des cellules T cytotoxiques utilisent divers effecteurs associés à Rab27a et Myo5a. Certains effecteurs restent à identifier.

6- Propriété effectrices

Protéines: protéines de migration des vésicules et de relargage de la perforine et des granzymes

Gènes: MYO5A, AP3B1, …

Déficit: maladies autosomiques récessives

Signes cliniques: immunodéficience et albinisme partiel

Signes biologiques: défaut de la **voie d'exocytose** des **vésicules sécrétoires** dans les **lymphocytes cytotoxiques** et les **mélanocytes**

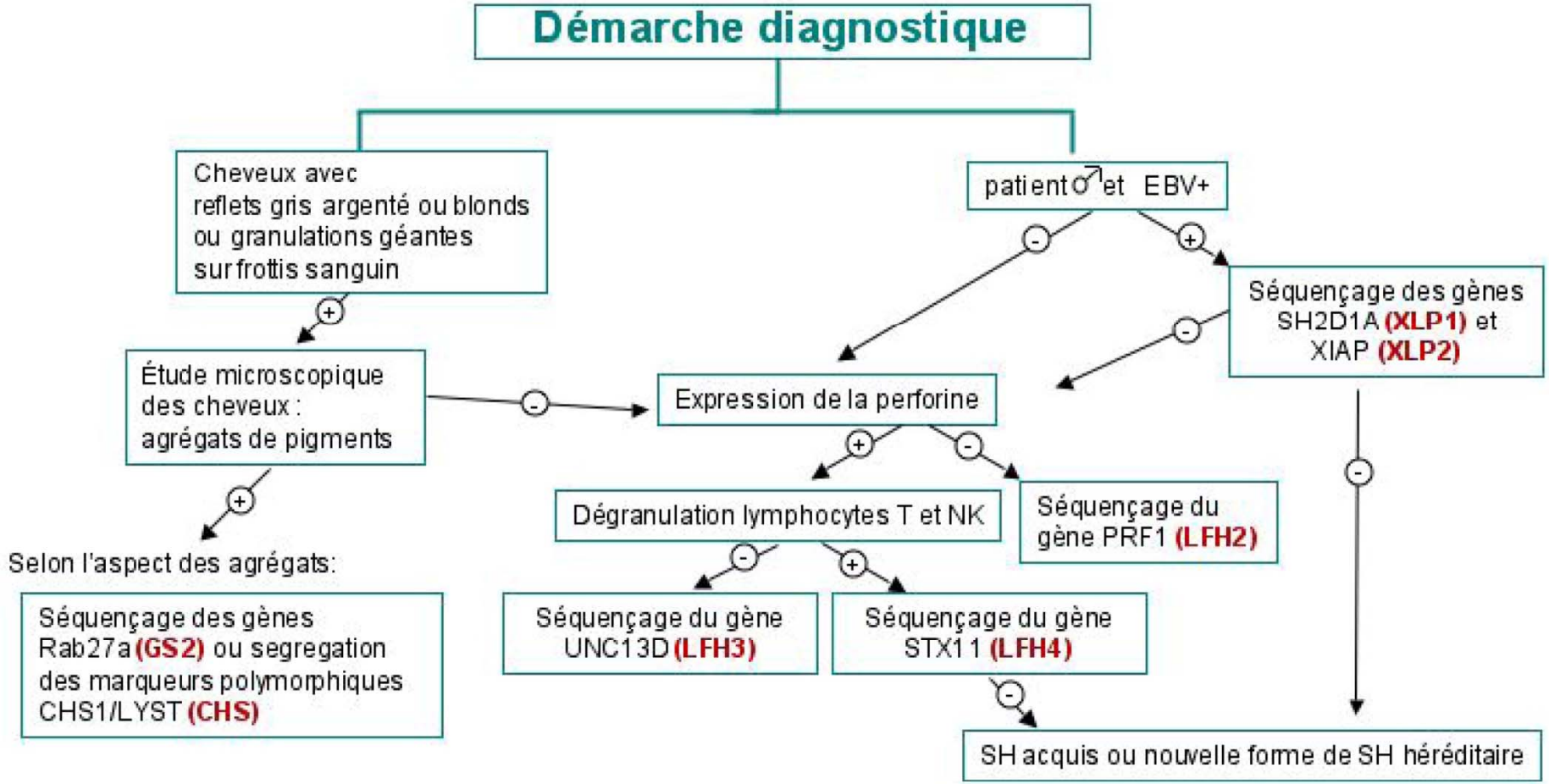


Figure 4: Démarche diagnostique devant un cas pédiatrique suspect de syndrome hémohagocytaire primitif. Une étude microscopique des cheveux du patient permet d'orienter vers un syndrome de Chediak-Higashi (CHS) ou de Griscelli (GS). La présence de granulations intracytoplasmiques géantes permet de confirmer le diagnostic de CHS alors qu'une mutation du gène Rab27a confirme le diagnostic de GS associé à un SH. En l'absence d'anomalie pigmentaire des cheveux, le diagnostic de lymphohistiocytose familiale (LHF) sera orienté par l'étude de l'expression de la perforine dans les lymphocytes du patient, la ségrégation des marqueurs polymorphiques liés aux différents loci dans les familles consanguines, le séquençage ciblé des différents gènes connus comme responsables de cette affection (perforine, Munc13-4 et syntaxin 11), et l'étude de la dégranulation du contenu des granules cytotoxiques. Un défaut de dégranulation *in vitro* des cellules T et NK caractérise une anomalie de Munc 13-4, alors que seul un défaut de dégranulation des cellules NK est mis en évidence avec une anomalies de STX11 en utilisant les tests standards. Cette dégranulation est déficiente dans les cellules T *et* NK dans le cas d'une mutation du gène Munc13-4, et déficiente uniquement dans les cellules NK dans le cas d'une mutation de la STX11 avec les tests *in vitro* actuellement utilisés,. L'expression d'un SH faisant suite à une infection par l'EBV chez un jeune garçon orientera en priorité vers un syndrome de Purtilo mais n'élimine pas le diagnostic de LHF.

Figure 1 Inherited diseases resulting from defects in CD8+ T cell secretory lysosomes. Sorting begins in the *trans*-Golgi network, where proteins destined for secretory lysosomes are targeted to late endosomes. Secreted proteins and some membrane proteins (such as FasL and human immunodeficiency virus (HIV) gp160) are directly targeted in this way, whereas other membrane proteins (such as CD 63 and CTLA-4) also use an alternative pathway from the cell surface back to late endosomes (blue arrows). After T cell activation at the immune synapse (IS) with an antigen-presenting cell, lytic granules attach to microtubules (MT) emanating from the microtubule-organizing center (MTOC), which migrates close to the immune synapse. Dynein motor proteins deliver the vesicles to the immune synapse, where they dock and release their contents. Hereditary autosomal recessive diseases involving immunodeficiency and albinism result from defects in the sorting pathway of secretory lysosomes in immune cells and melanocytes. These include HPS2, Griscelli syndrome (GS) and Chediak-Hagashi syndrome (CHS). The gene products defective in these diseases are shown as black text in blue box; the sites of the resulting blockade are red crosses. Patients with HPS2 have been described with defects in the β3A or δ subunits of AP-3, or in Rab-geranyl-geranyltransferase (RGGT). The defect in RGGT does not completely abrogate perforin secretion. Griscelli syndrome is associated with defects in Rab27A or the myosin motor protein MyoVa. Patients with Chediak-Hagashi syndrome have a defect in the lysosomal trafficking molecule Lyst.

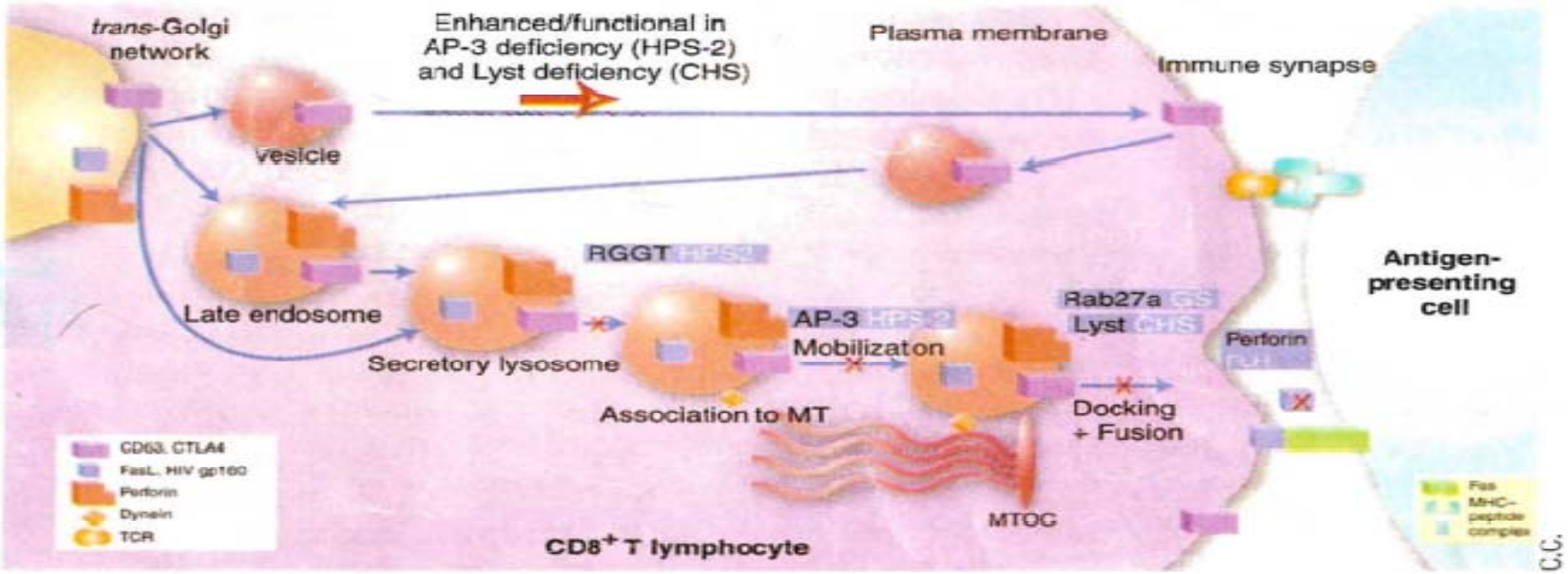


Figure 1 Inherited diseases resulting from defects in CD8+ T cell secretory lysosomes. Sorting begins in the *trans*-Golgi network, where proteins destined for secretory lysosomes are targeted to late endosomes. Secreted proteins and some membrane proteins (such as FasL and human immunodeficiency virus (HIV) gp160) are directly targeted in this way, whereas other membrane proteins (such as CD 63 and CTLA-4) also use an alternative pathway from the cell surface back to late endosomes (blue arrows). After T cell activation at the immune synapse (IS) with an antigen-presenting cell, lytic granules attach to microtubules (MT) emanating from the microtubule-organizing center (MTOC), which migrates close to the immune synapse. Dynein motor proteins deliver the vesicles to the immune synapse, where they dock and release their contents. Hereditary autosomal recessive diseases involving immunodeficiency and albinism result from defects in the sorting pathway of secretory lysosomes in immune cells and melanocytes. These include HPS2, Griscelli syndrome (GS) and Chediak-Hagashi syndrome (CHS). The gene products defective in these diseases are shown as black text in blue box; the sites of the resulting blockade are red crosses. Patients with HPS2 have been described with defects in the β3A or δ subunits of AP-3, or in Rab-geranyl-geranyltransferase (RGGT). The defect in RGGT does not completely abrogate perforin secretion. Griscelli syncrome is associated with defects in Rab27A or the myosin motor protein MyoVa. Patients with Chediak-Hagashi syndrome have a defect in the lysosomal trafficking molecule Lyst.

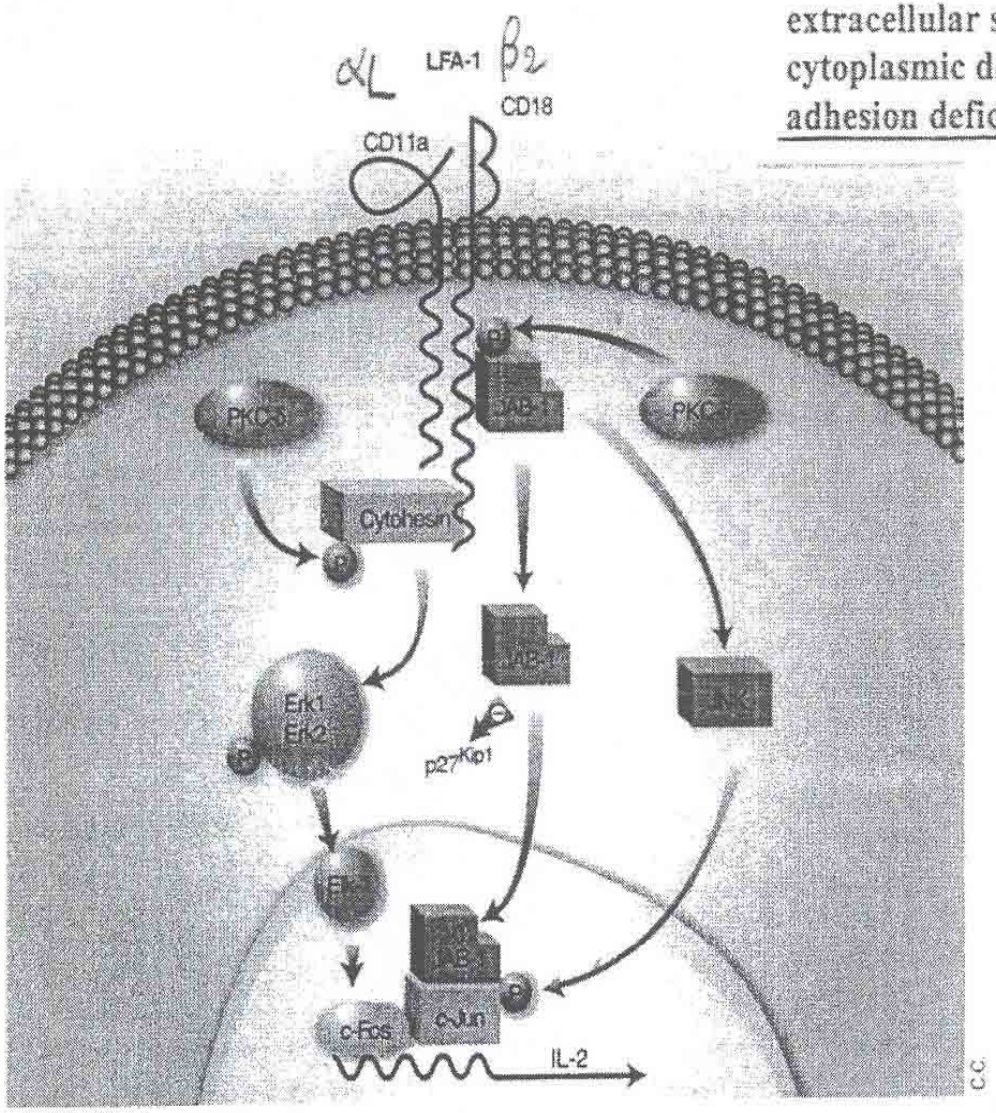
(Rab

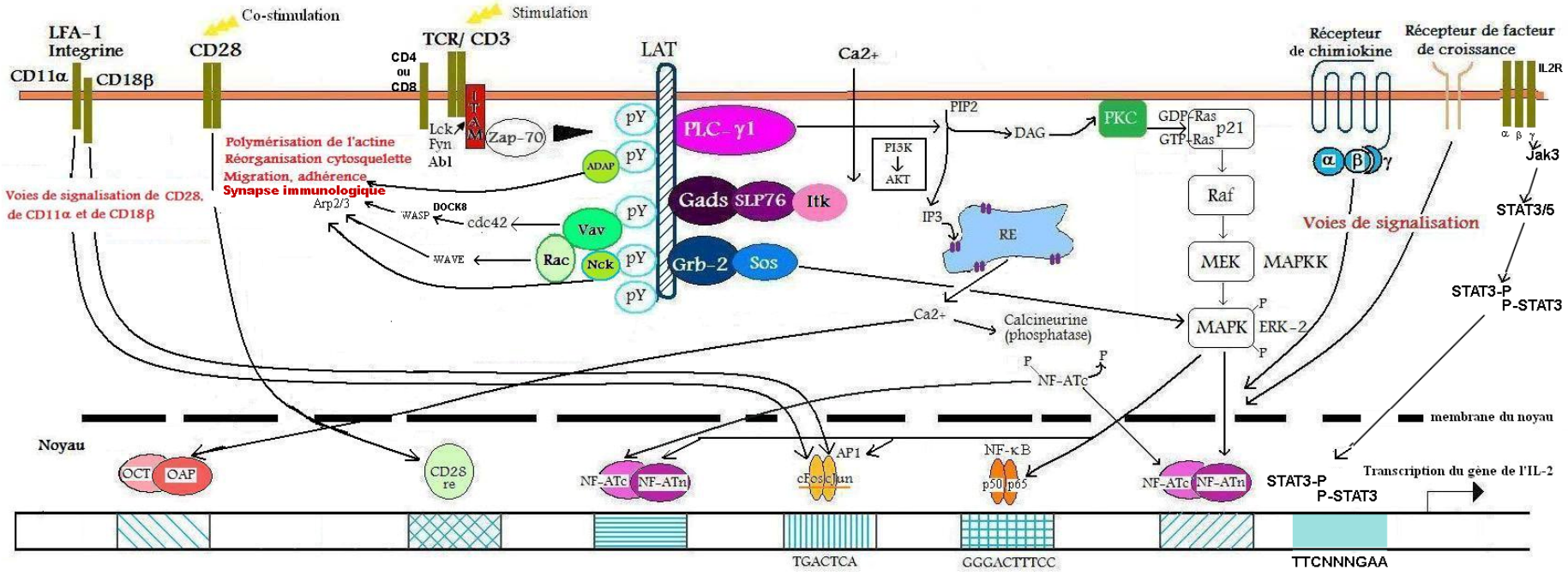
t.1

LFA-1: more than just T cell Velcro

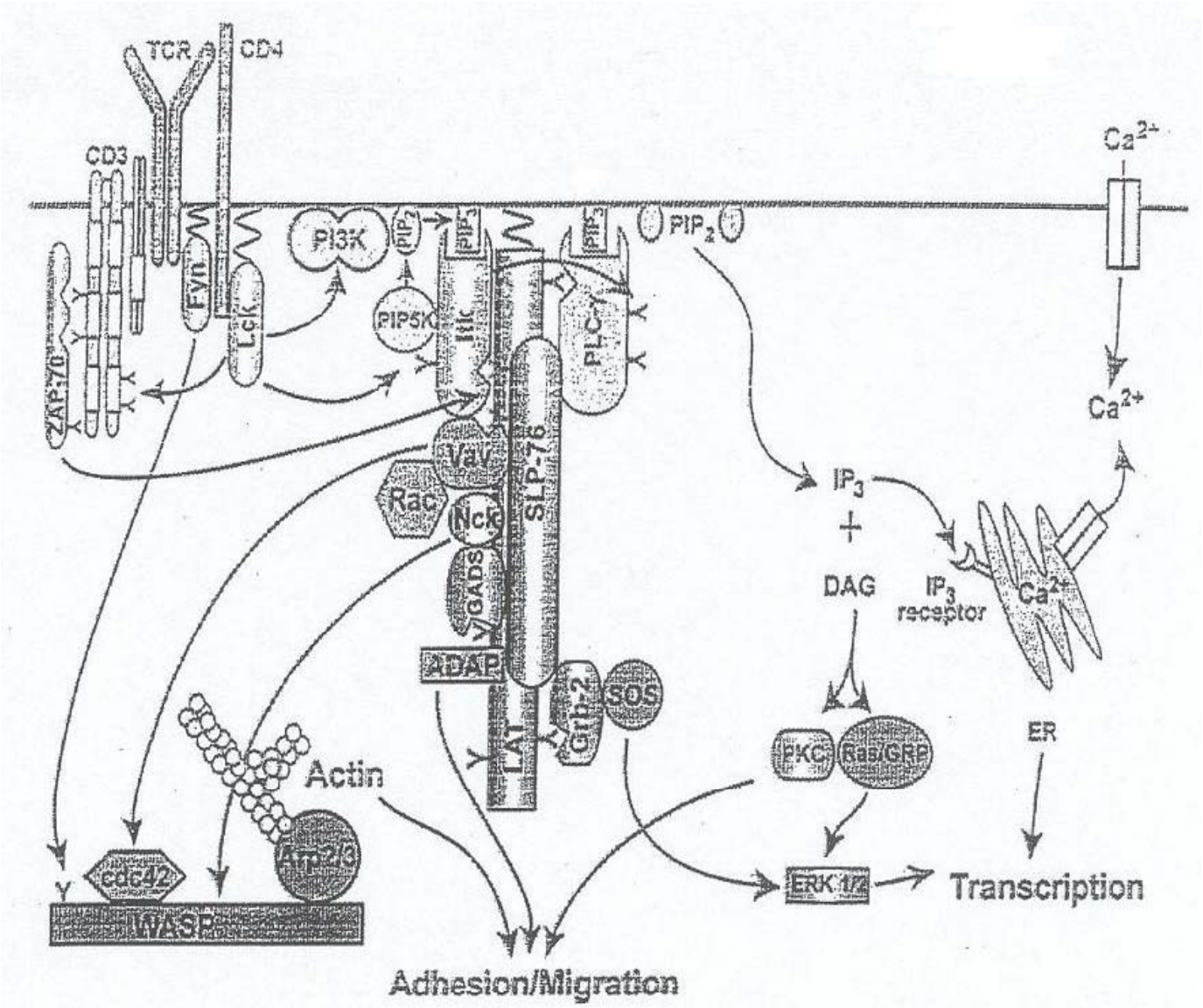
The LFA-1 integrin is known to mediate adhesion between T cells and antigen-presenting cells. New evidence, however, is provided for its role in transmitting signals that promote T cell activation and differentiation.

Unique CD18 mutations involving a deletion in the extracellular stalk region and a major truncation of the cytoplasmic domain in a patient with leukocyte adhesion deficiency type 1 (LAD type 1).

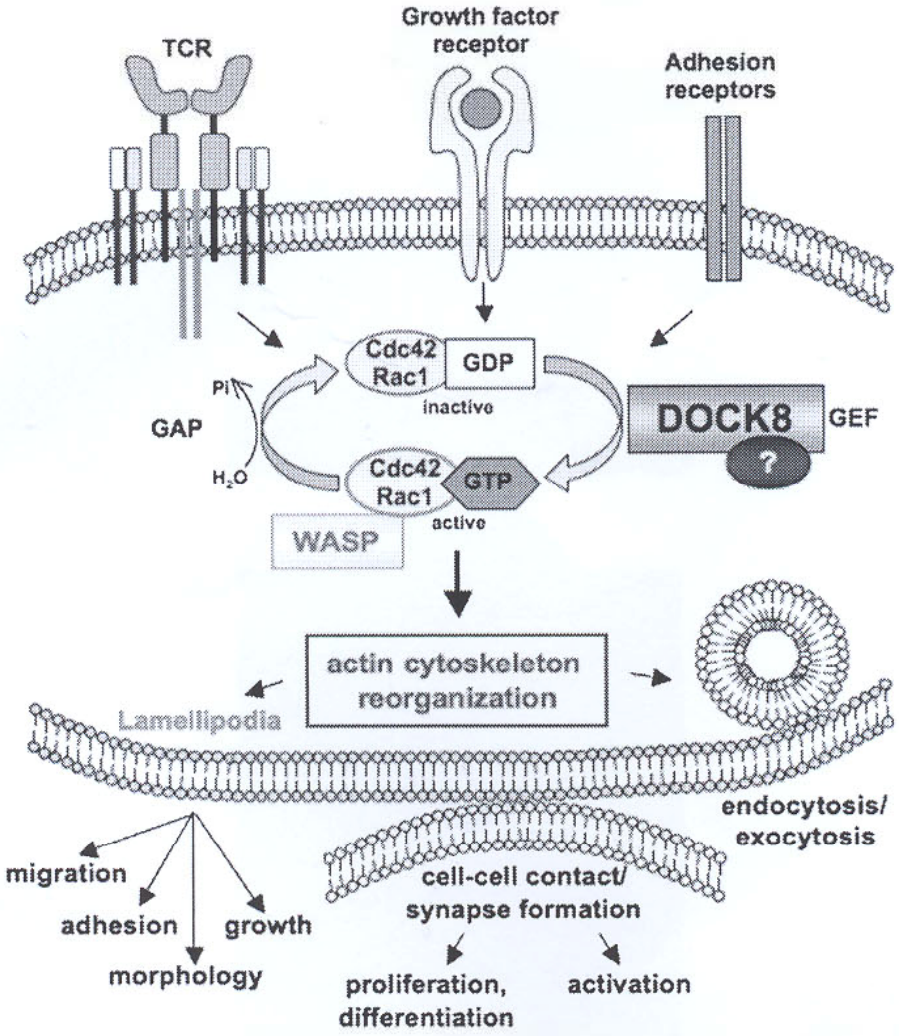




MAPK	Mitogen-Activated Protein Kinase	ERK	Extracellular signal-Regulated Kinase
MAPKK	Mitogen-Activated Protein Kinase Kinase	LAT	Linker for activation of Tcells (9 Tyr)


TCR
CD4
CD3
ZAP-70
Fyn
Lck
PI3K
PIP2
PIP3
PIP5K
Itk
PLC-γ
SLP-76
Vav
Rac
Nck
GADS
ADAP
LAT
Grb-2
SOS
PIP2
Ca2+
Ca2+
IP3
+
DAG
IP3 receptor
Ca2+
ER
PKC
Ras/GRP
ERK 1/2
Transcription
Actin
Arp2/3
cdc42
WASP
Adhesion/Migration

Wiskott-Aldrich syndrome (WAS) is a primary human X-linked immunodeficiency, caused by mutations in the WAS protein (WASP) (33), and characterized by recurrent infections and thrombocytopenia. A high proportion of WAS patients develop eczema and have high IgE levels (34), suggesting a Th2 unbalance. This is in line with the recent finding that WAS T cell lines have a selective defect in Th1 cytokine production (35). Moreover, the majority of WAS patients lacking WASP expression develop autoimmune diseases including hemolytic anemia, cutaneous vasculitis, IgA nephropathy, inflammatory bowel disease and arthritis (34, 36). WASP is a tightly regulated hematopoietic-specific protein, which controls actin nucleation (37), immunological synapse assembly (38) and migration to secondary lymphoid organs (39). In addition, WASP regulates apoptosis (40), proliferation and IL-2 production in effector T cells (41-44). Although these defects contribute to the immunodeficiency, it cannot be excluded that defects in Treg populations and function are associated with the development of autoimmunity in WAS patients.


TCR
Growth factor receptor
Adhesion receptors
Cdc42
Rac1
GDP
inactive
Pi
GAP
H_2O
Cdc42
Rac1
GTP
active
DOCK8
GEF
?
WASP
actin cytoskeleton reorganization
Lamellipodia
endocytosis/ exocytosis
migration
adhesion
growth
morphology
cell-cell contact/ synapse formation
proliferation, differentiation
activation

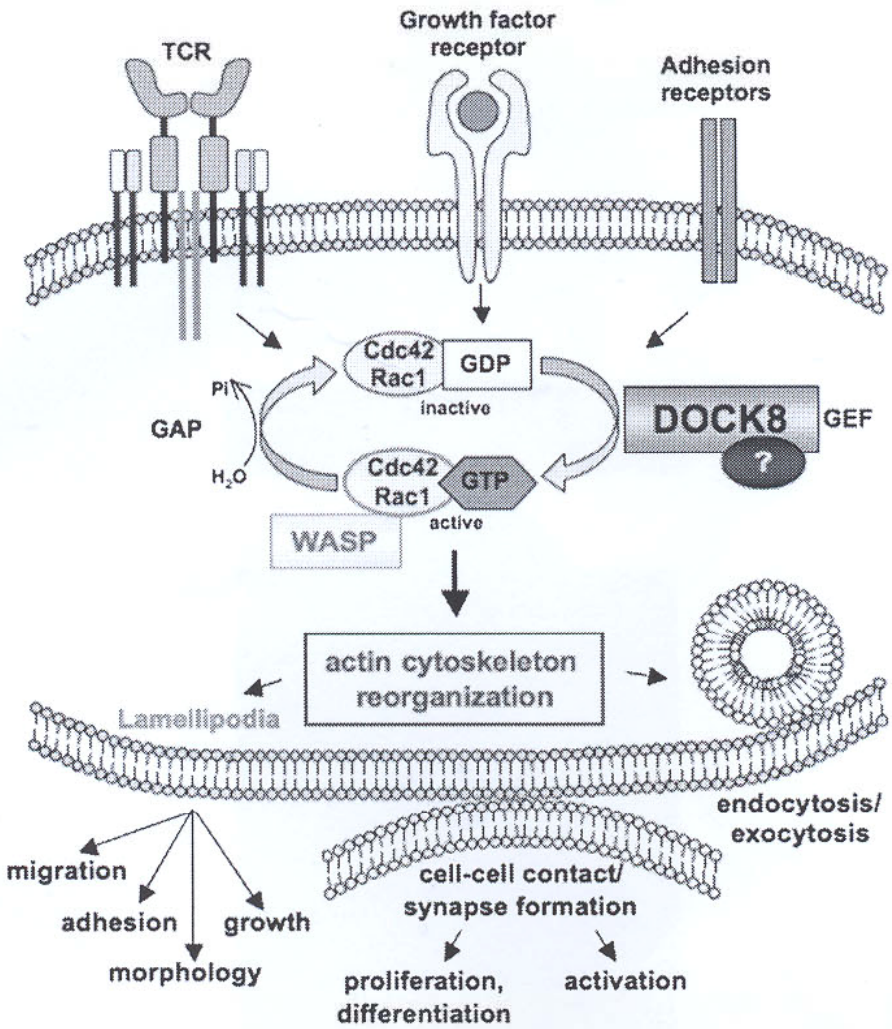


FIG 7. Hypothetical function of DOCK8. DOCK8 is likely to function as a GEF for the Rho-GTPases Cdc42 and Rac1, turning them into the active, GTP-bound form on receptor engagement (eg, receptor tyrosine kinases, antigen receptors, and adhesion receptors). An unknown protein possibly stabilizes the interaction of DOCK8 with Cdc42 and Rac1. GTPase activation induces dynamic filamentous actin rearrangements and lamellipodia formation, possibly *via* WASP, leading to cell growth, migration, and adhesion. Given the clinical phenotype of the AR-HIES patients with DOCK8 deficiency, we propose an important role of DOCK8 in T-cell actin dynamics, which might be important for the formation of the immunologic synapse, leading to T-cell activation, proliferation, and differentiation. *TCR,* T-cell receptor.

L pour Ligand

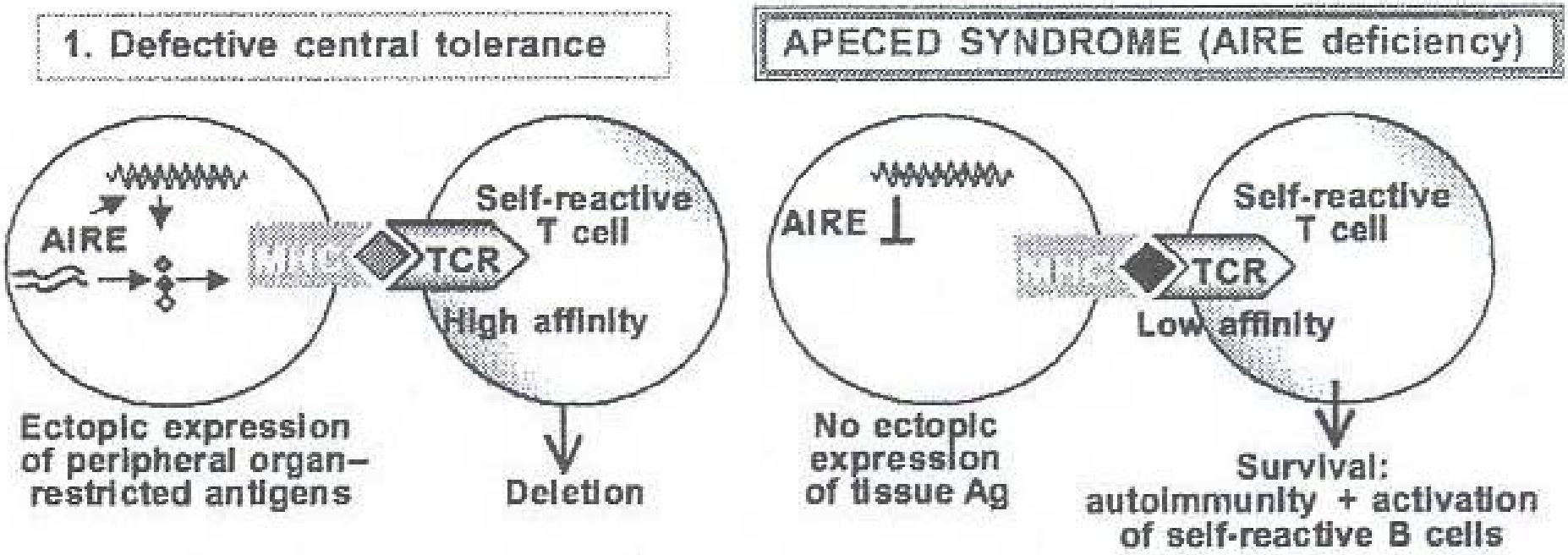
LES CHIMIOKINES ET LEURS RÉCEPTEURS

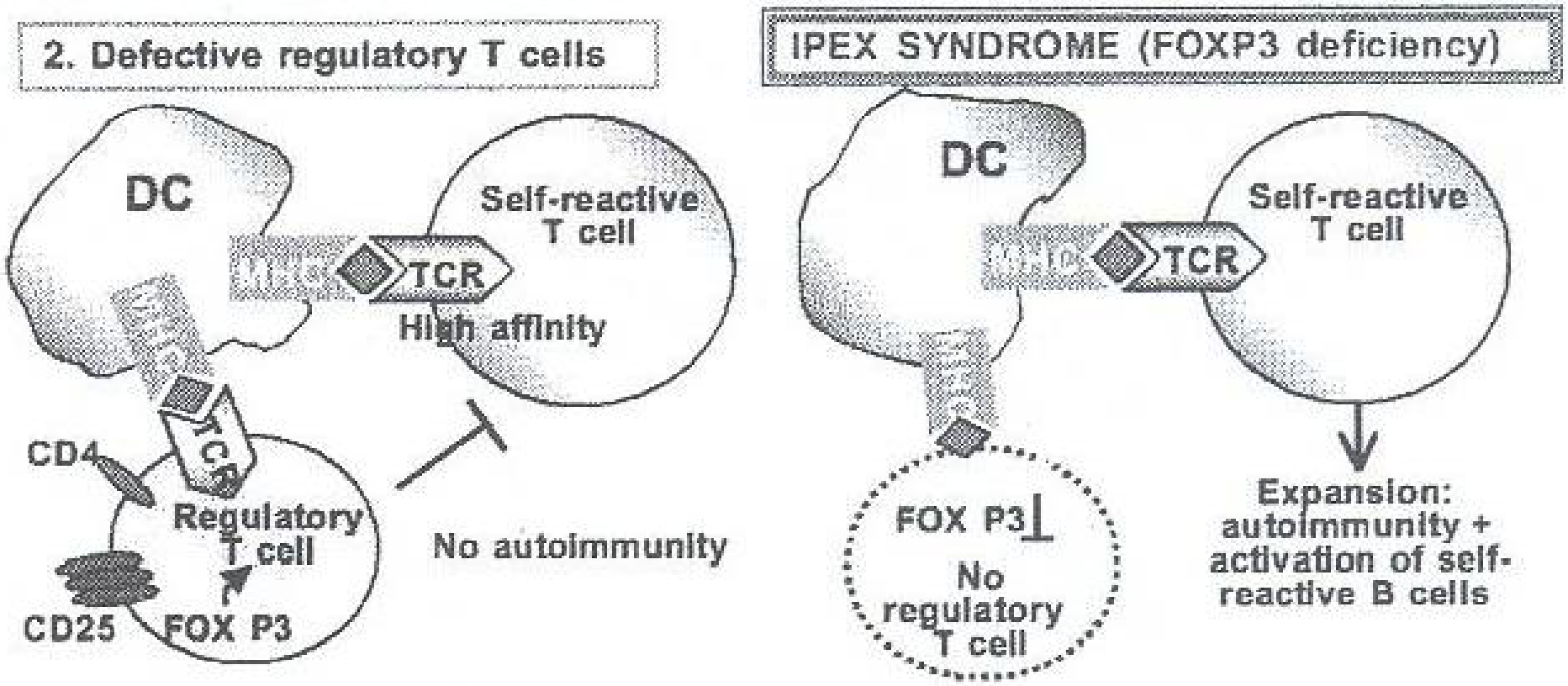
CC chimiokine	Récepteurs
CCL1, I-309	CCR8
CCL2, MCP-1	CCR2
CCL3, MIP-1α	CCR1, CCR5
CCL4, MIP-1β	CCR5
CCL5, RANTES	CCR1, CCR3, CCR5
CCL6, C10	?
CCL7, MCP-3	CCR1, CCR2, CCR3
CCL8, MCP-2	CCR1, CCR2, CCR3, CCR5
CCL9, MIP-1γ	?
CCL11, éotaxine	CCR3
CCL12, MCP-5	CCR2
CCL13, MCP-4	CCR2, CCR3, CCR5
CCL14, HCC-1	CCR1
CCL15, MIP-1δ	CCR1, CCR3
CCL16, HCC-4	?
CCL17, TARC	CCR4
CCL18, DC-CK1	?
CCL19, ELC	CCR7
CCL20, MIP-3α	CCR6
CCL21, SLC	CCR7
CCL22, MDC	CCR4
CCL23, MPIF-1	CCR1
CCL24, éotaxine-2	CCR3
CCL25, TECK	CCR9
CCL26, éotaxine-3	CCR3
CCL27, skinkine	?

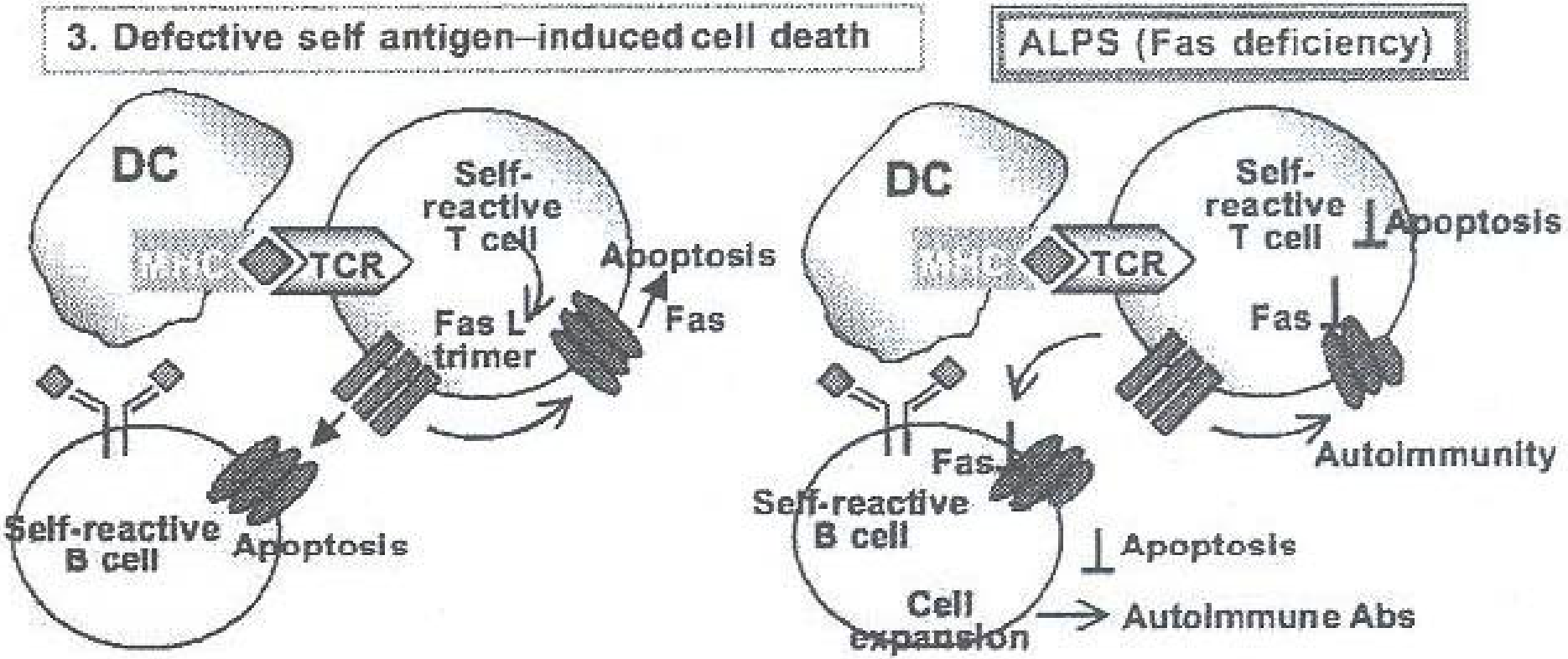
CXC chimiokine	Récepteurs
SDF1	CXCR4
CXCL1	CXCR2
CXCL2, GROb	CXCR2
CXCL3, GROg	CXCR2
CXCL4, PF4	?
CXCL5, ENA-78	CXCR2
CXCL6, GCP-2	CXCR1, CXCR2
CXCL7, NAP-2	CXCR2
CXCL8, IL-8	CXCR1, CXCR2
CXCL9, Mig	CXCR3
CXCL10, IP-10	CXCR3
CXCL11, I-TAC	CXCR3
CXCL13, BLC	CXCR5

Mutations in the chemokine receptor gene *CXCR4* are associated with WHIM syndrome, a combined immunodeficiency disease

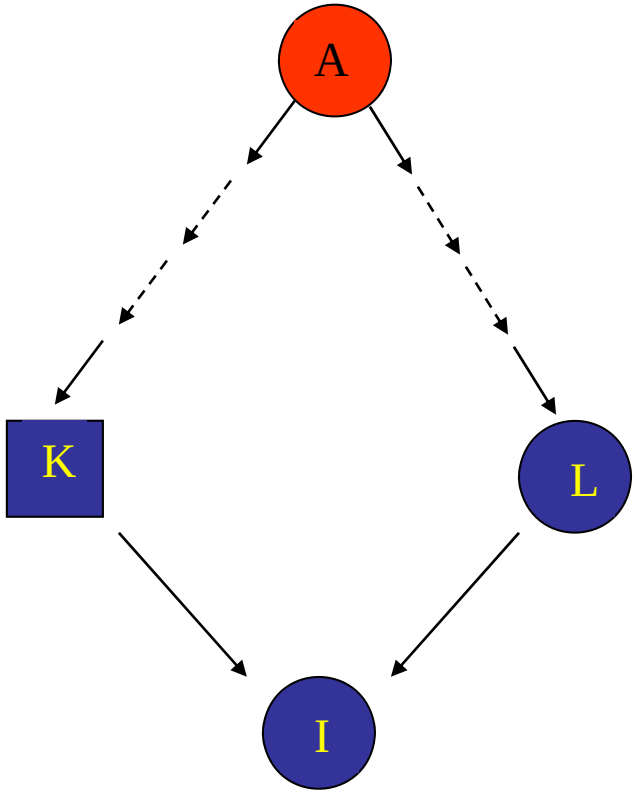
WHIM syndrome is an immunodeficiency disease characterized by neutropenia, hypogammaglobulinemia and extensive human papillomavirus (HPV) infection[1]. Despite the peripheral neutropenia, bone marrow aspirates from affected individuals contain abundant mature myeloid cells, a condition termed myelokathexis[2]. The susceptibility to HPV is disproportionate compared with other immunodeficiency conditions, suggesting that the product of the affected gene may be important in the natural control of this infection. We describe here the localization of the gene associated with WHIM syndrome to a region of roughly 12 cM on chromosome 2q21 and the identification of truncating mutations in the cytoplasmic tail domain of the gene encoding chemokine receptor 4 (*CXCR4*). Haplotype and mutation analyses in a pedigree transmitting myelokathexis as an apparently autosomal recessive trait support genetic heterogeneity for this aspect of the WHIM syndrome phenotype. Lymphoblastoid cell lines carrying a 19-residue truncation mutation show significantly greater calcium flux relative to control cell lines in response to the CXCR4 ligand, SDF-1, consistent with dysregulated signaling by the mutant receptor. The identification of mutations in *CXCR4* in individuals with WHIM syndrome represents the first example of aberrant chemokine receptor function causing human disease and suggests that the receptor may be important in cell-mediated immunity to HPV infection.


1. Defective central tolerance
APECED SYNDROME (AIRE deficiency)
AIRE
MHC
TCR
Self-reactive
T cell
High affinity
Ectopic expression
of peripheral organ–
restricted antigens
Deletion
AIRE
MHC
TCR
Self-reactive
T cell
Low affinity
No ectopic
expression
of tissue Ag
Survival:
autoimmunity + activation
of self-reactive B cells


2. Defective regulatory T cells
DC
Self-reactive
T cell
TCR
High affinity
TCR
CD4
Regulatory
T cell
CD25
FOX P3
No autoimmunity
IPEX SYNDROME (FOXP3 deficiency)
DC
Self-reactive
T cell
TCR
FOX P3
No
regulatory
T cell
Expansion:
autoimmunity +
activation of self-
reactive B cells


3. Defective self antigen–induced cell death
ALPS (Fas deficiency)
DC
TCR
Self-reactive T cell
Apoptosis
Fas L trimer
Fas
Self-reactive B cell
Apoptosis
DC
TCR
Self-reactive T cell
Apoptosis
Fas
Autoimmunity
Fas
Self-reactive B cell
Apoptosis
Cell expansion
Autoimmune Abs

Situation de consanguinité



… sont apparentés, si ils ont au moins un ancêtre commun, A.

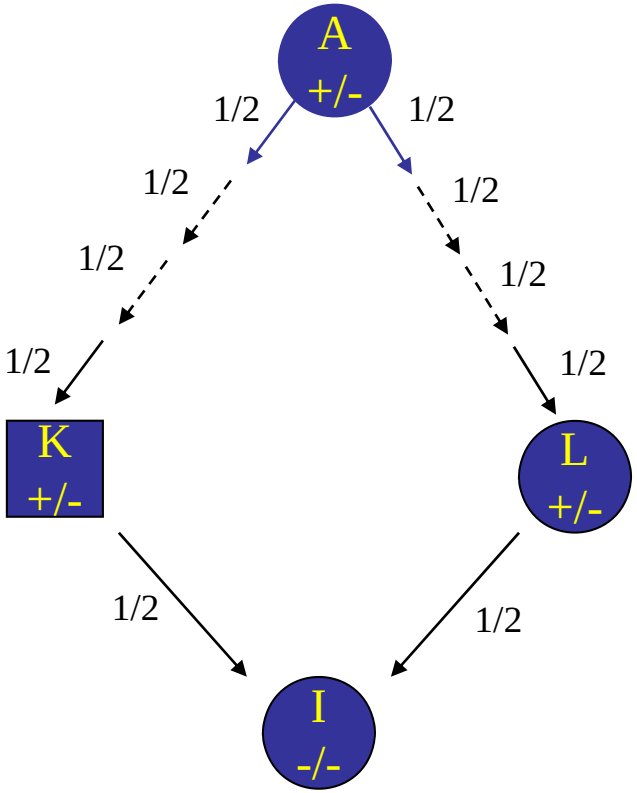
Deux individus, K et L …

qui sont apparentés ont des enfants consanguins, I.

Ce n'est pas le mariage qui est consanguin mais le produit (l'enfant) du mariage.

Le mariage ou l'union est dite entre apparentés.

Conséquence possible de la consanguinité



Si l'ancêtre A est porteur sain d'une mutation pathologique récessive très rare, telle que par panmixie, la maladie est pratiquement absente ($q^2 = \varepsilon$)

Les deux individus K et L, ont pu recevoir chacun une copie de l'allèle muté et, du fait de cette parenté et de leur union, ils peuvent …

… transmettre ces deux copies, identiques par descendance (IBD) à l'allèle muté ancestral, à l'enfant, qui sera alors atteint de la maladie

Les unions entre apparentés :

- augmente le niveau d'homozygotie chez les enfants, par la réunion d'allèles identiques par descendance (autozygotie) favorisent, pour des mutations pathologiques récessives rarissimes, l'émergence d'une maladie qui serait inexistante chez les couples panmictiques.

STRUCTURE GENOTYPIQUE DE CETTE POPULATION

	A (p)	a (q)
A (p)	AA (p^2)	Aa (pq)
a (q)	Aa (pq)	aa (q^2)

AA : p^2

Aa : 2pq

aa : q^2

$p+q=1$

Exemples : coefficients de parenté entre cousins germains et doubles cousins germains

entre cousins germains

$f_{K,L} = 1/16$

entre doubles cousins germains

$f_{K,L} = 1/16 + 1/16 = 1/8$

Conséquences génétiques au sein des populations

La consanguinité dans quelques populations

Population (date de l'étude)	Nombre de mariages	% mariages apparentés	% cousins germains	% oncle-nièce
Egypte (1991)	9 073	37%	31%	0
Soudan (1990)	5 860	66%	57%	0
Koweit (1987)	4 175	53%	30%	0
Inde (1982)	3 350	34%	10%	16%

Liban : environ 25% de mariages entre cousins germains avec d'importantes variations selon les communautés.

Écarts à la panmixie
&
Consanguinité

Conséquences génétiques au sein des populations

Risque collectif et santé publique

Conséquences génétiques au sein des populations
3- Risque collectif et santé publique

L'augmentation de la fréquence des maladies récessives est considérable même si le risque individuel est minime pour chaque couple de cousins germains parce que <u>ce risque est cumulé à l'échelle collective</u>

$$f(m/m) = q^2 + Fq$$

Maladies Récessives recensées	Rarissimes	Très rares	Rares	Assez Fréquentes (phénylcétonurie)	Fréquente (mucoviscidose)	Total
Nombre	5000	1 000	100	2	1	6 103
Fréquence (q) de l'allèle pathologique	10^{-5}	10^{-4}	10^{-3}	10^{-2}	2.10^{-2}	
Risque panmictique (q^2)	**10^{-10}**	**10^{-8}**	**10^{-6}**	**10^{-4}**	**4.10^{-4}**	
Nombre annuel de cas pour 10^6 naissances par maladie	0,0001	0,01	1	100	400	
Nombre annuel de cas pour 10^6 naissances pour toutes	0,5	10	100	200	400	710,5
Supplément de risque Fq, pour F = 1% (16% d'unions CG)	**10^{-7}**	**10^{-6}**	**10^{-5}**	**10^{-4}**	**2.10^{-4}**	
Supplément annuel de cas pour 10^6 naissances par maladie	0,1	1	10	100	200	
Supplément annuel de cas pour 10^6 naissances pour toutes les maladies	**500**	**1 000**	**1 000**	**200**	**200**	**2 900**
Nombre total de cas pour 10^6 naissances pour toutes les maladies	**500,5**	**1 010**	**1 100**	**400**	**600**	**3610,5**

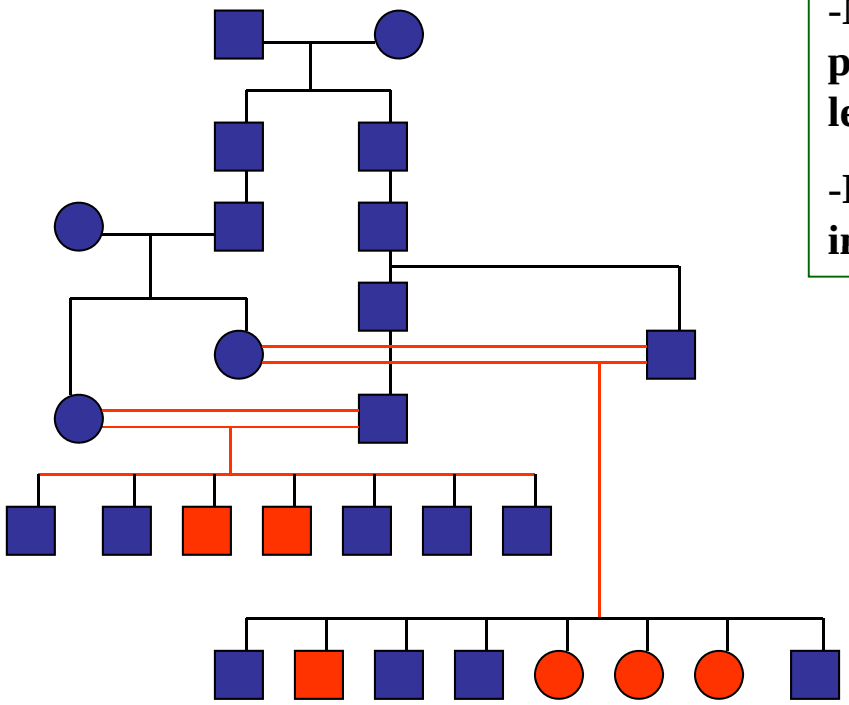
Écarts à la panmixie
&
Consanguinité

Conséquences génétiques au

sein des populations

Consanguinité et recherche médicale et scientifique

Conséquences génétiques au sein des populations
4- Consanguinité et recherche médicale et scientifique



-Mise en évidence de pathologies, donc de phénomènes biologiques, qui n'émergent pas dans les populations panmictiques

-Localisation génomique plus facile des gènes impliqués par « homozygosity mapping ».

Les enfants atteints dans la zone du « locus maladie » :

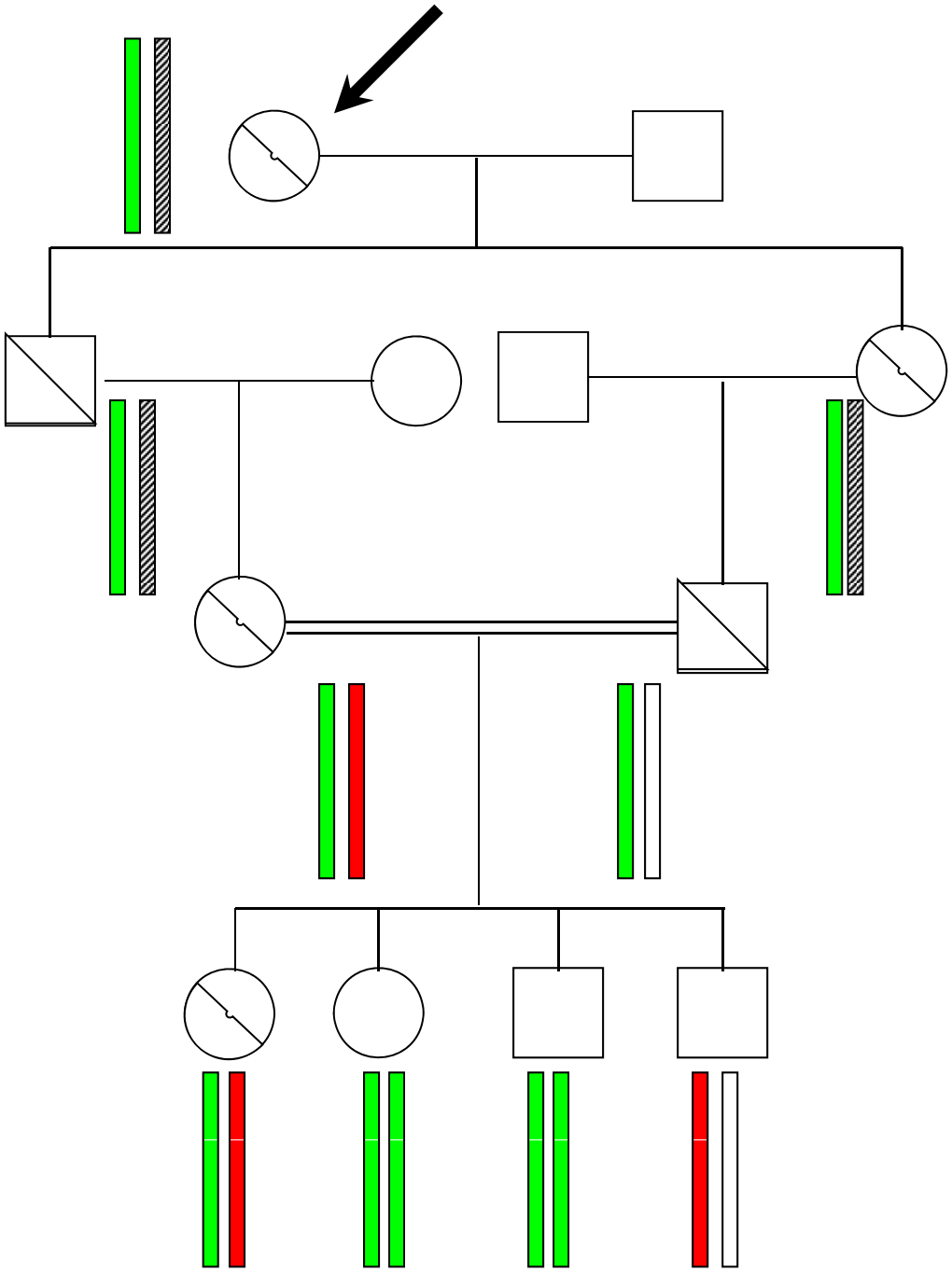
• Sont homozygotes pour une même mutation ancestrale en raison de la consanguinité

• Sont aussi homozygotes pour des marqueurs polymorphes adjacents transmis avec la mutation

Principes de la localisation par homozygosity mapping

•Analyse des marqueurs polymorphes de l'ADN, répartis sur tout le génome, chez les enfants atteints et les enfants sains.

•Recherche de la zone de génome systématiquement homozygote chez les atteints et hétérozygotes chez les sains

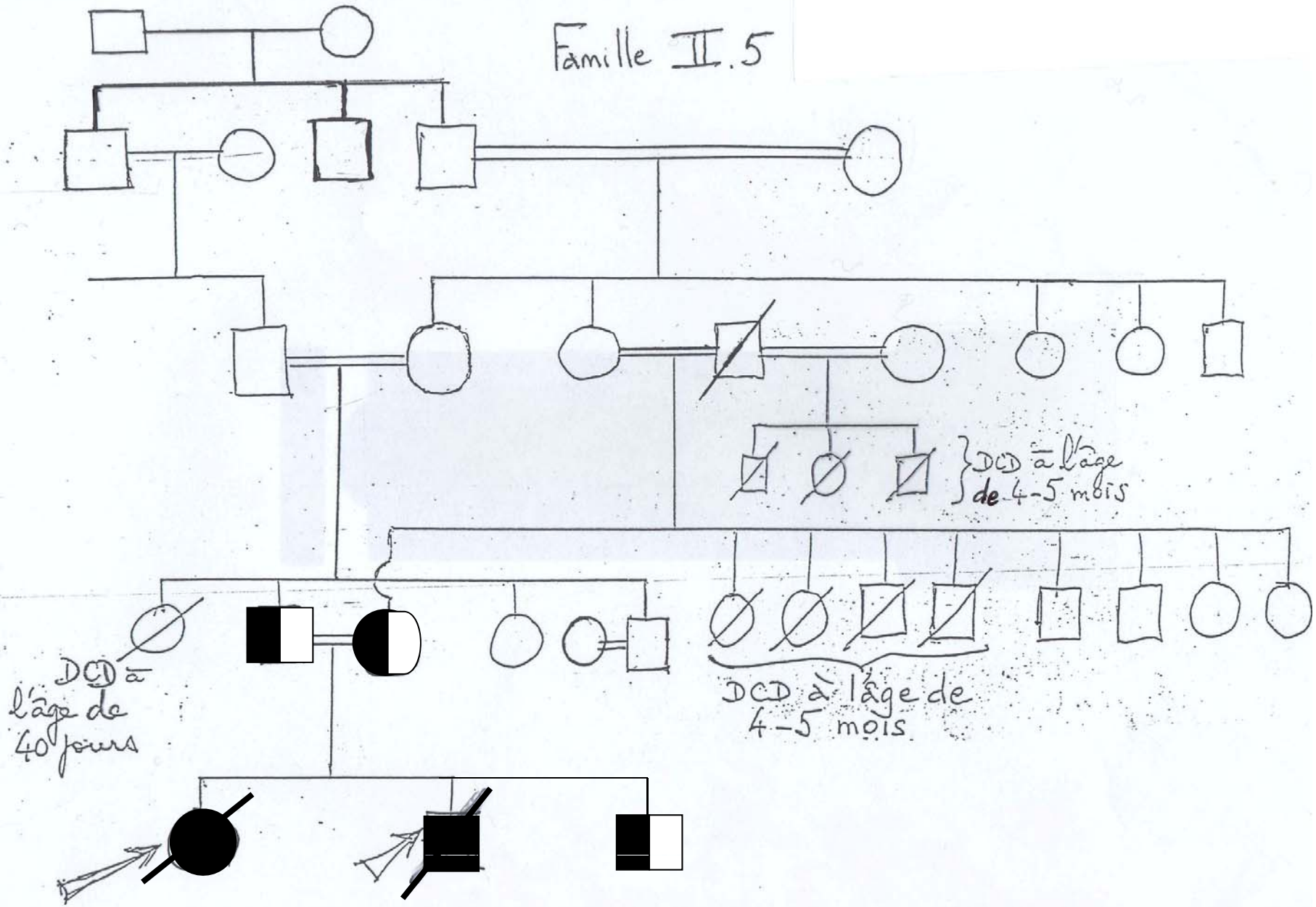


Calcul de la fraction k (en %) des malades issus de mariages entre cousins germains dans l'ensemble des malades, pour différentes fréquences q de l'allèle récessif concerné et pour différentes fréquences c de mariages entre cousins germains :

Formule de DALHBERG

$$K = \frac{C\,(1 + 15q)}{16q + C(1-q)} \quad \text{ou} \quad q = \frac{c(1-K)}{K(16-c)-15c}$$

c \ q	0,01	0,001	0,0001	0,00001
1 %	6,77	39,05	86,34	98,44
0,22 %	1,56	12,27	57,98	93,2
0,1 %	0,7	6	38,52	86,2


Famille II.5
DCD à l'âge de 4-5 mois
DCD à l'âge de 40 jours
DCD à l'âge de 4-5 mois