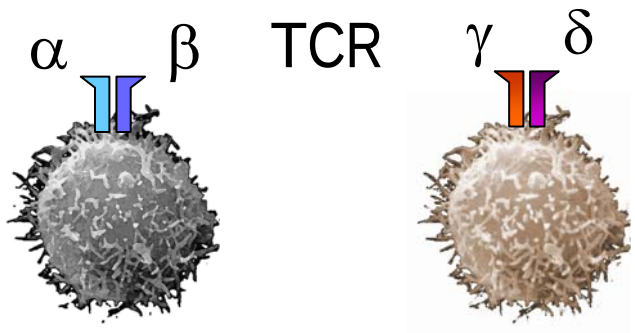
Activation et mode d'activation des lymphocytes T γ9δ2 humains

Les deux types de lymphocytes T

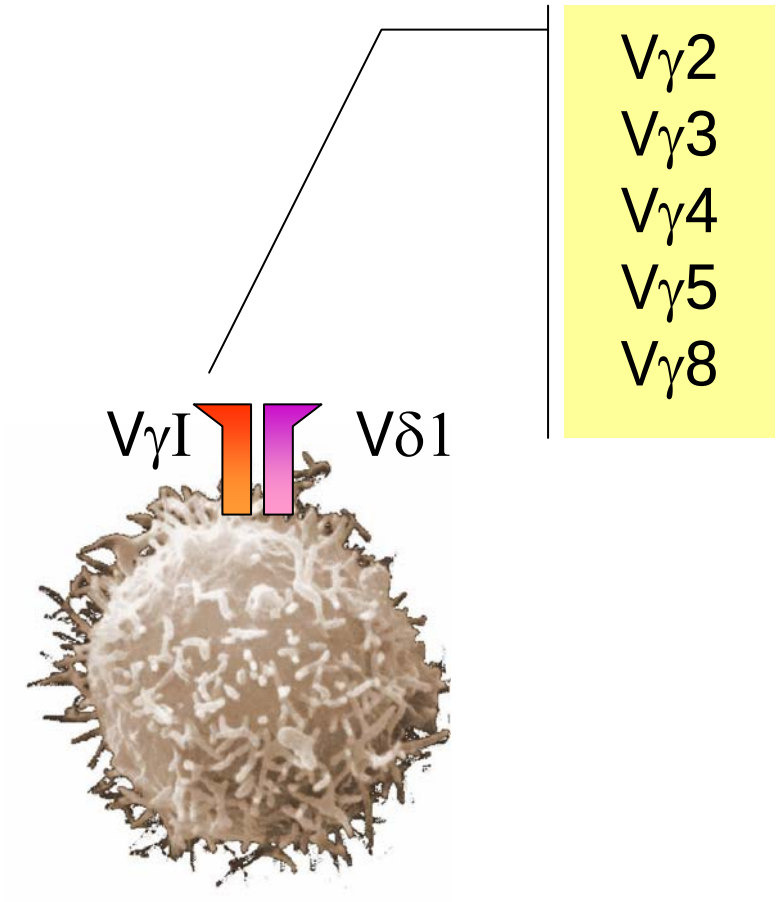


Lymphocyte Tαβ Lymphocyte Tγδ

Le répertoire Tγδ humain potentiel

Nombre de segments géniques fonctionnels	Chaînes			
	α	β	γ	δ
V	46	47	6	8
D	-	2	-	3
J	50	13	5	4
C	1	2	2	1

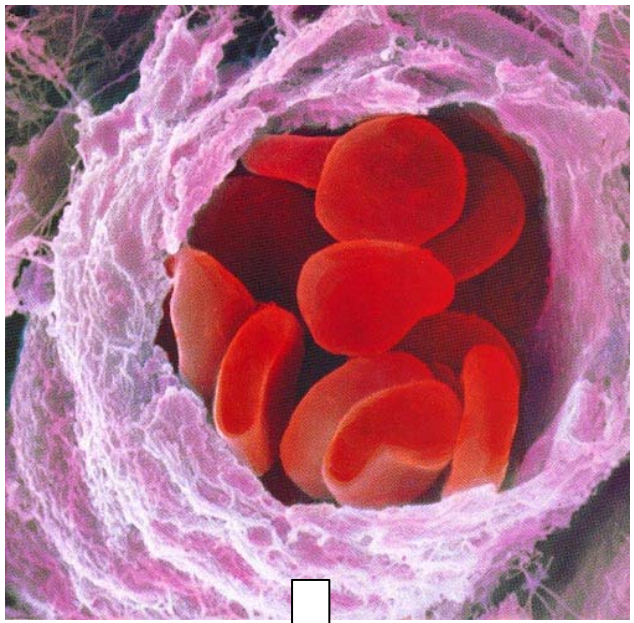
LE RÉPERTOIRE Tγδ MAJORITAIREMENT EXPRIMÉ EN PÉRIPHÉRIE

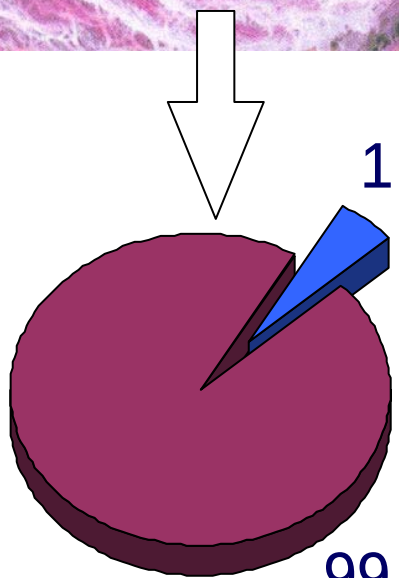


Lymphocyte TγIδ1

Lymphocyte Tγ9δ2

LES LYMPHOCYTES T DU SANG CIRCULANT CHEZ L'HOMME



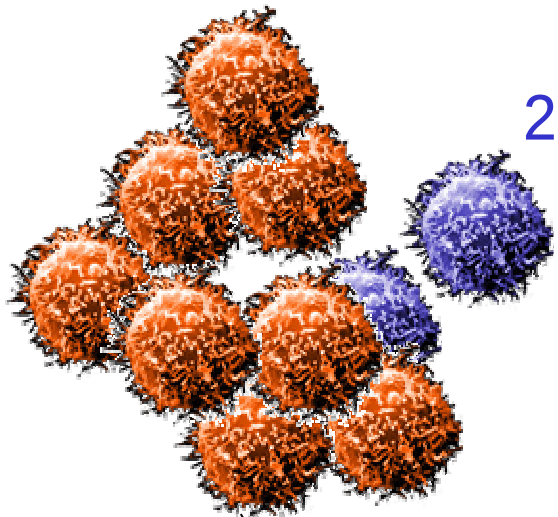


1 à 5% T γδ

99 à 95% T αβ

80 % T Vδ2+

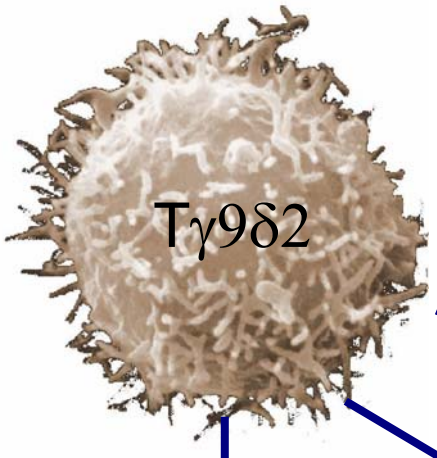
20 % T Vδ1+



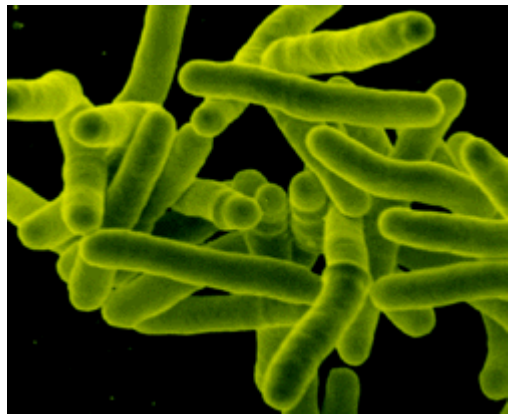
- CD4-
- CD8-
- CD28-

Les lymphocytes T γ9δ2

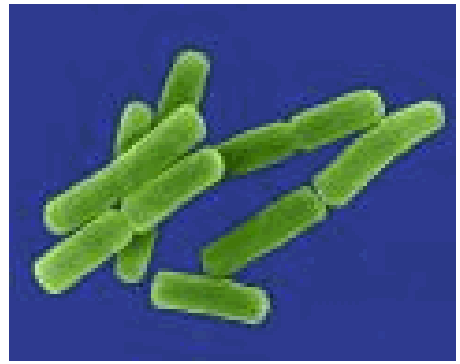
LA RÉACTIVITÉ γ9δ2



BACTÉRIES

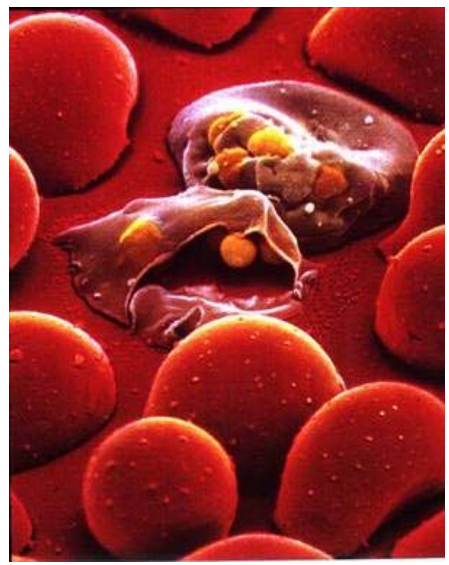


M.tuberculosis



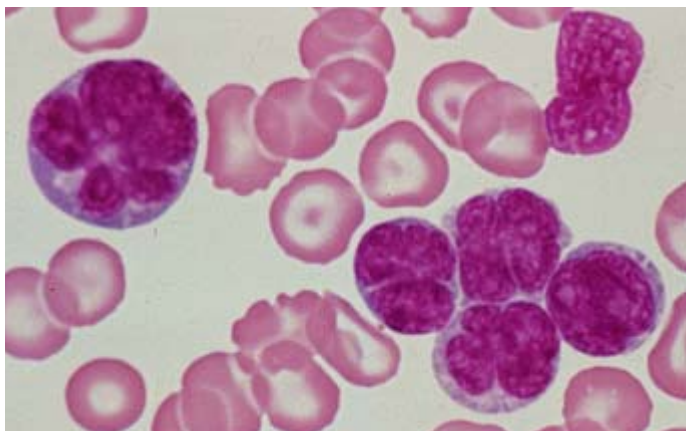
E. Coli

PROTOZOAIRES



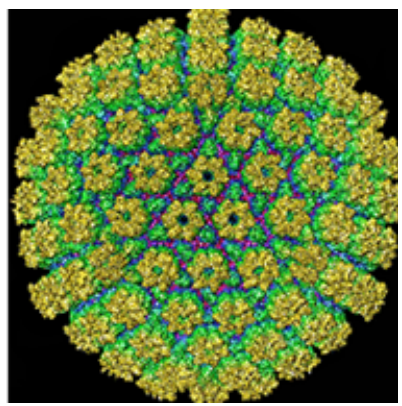
P.falciparum

CELLULES TUMORALES



Lymphomes B

VIRUS



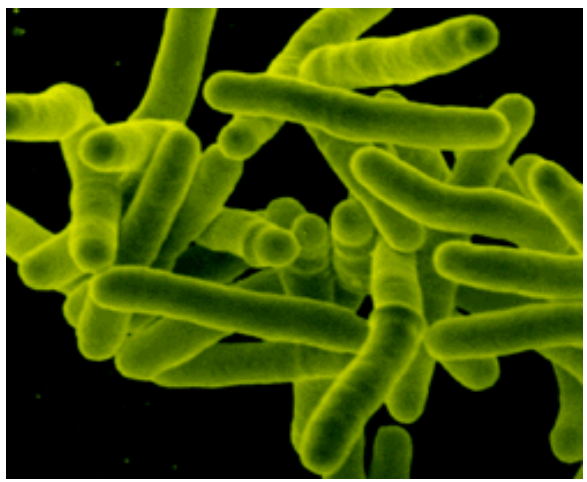
EBV

Les lymphocytes T γ9δ2

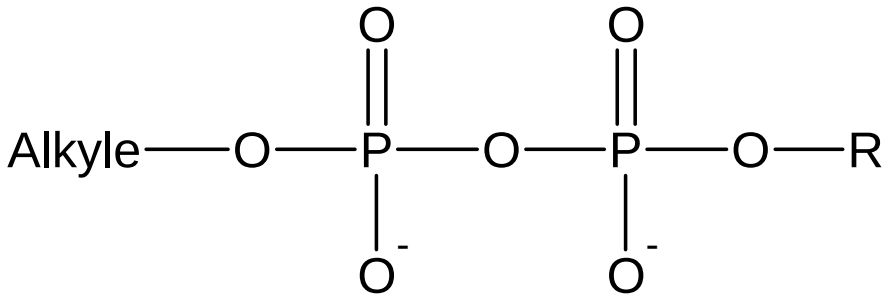
La réactivité γ9δ2

Les Phosphoantigènes

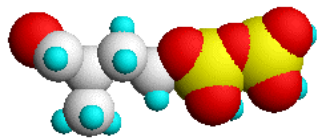
Lymphocyte Tγ9δ2



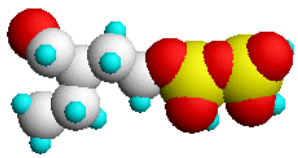
M.tuberculosis



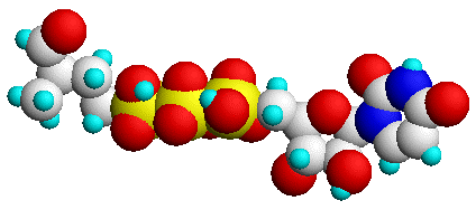
TUBag1



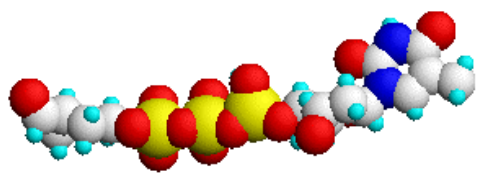
TUBag2



TUBag3

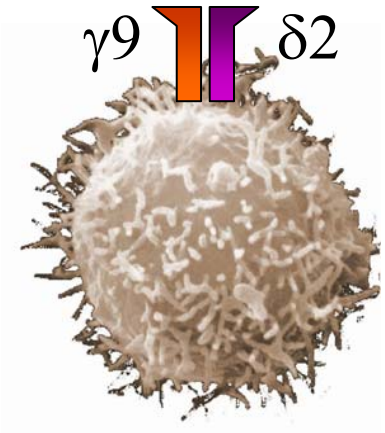


TUBag4

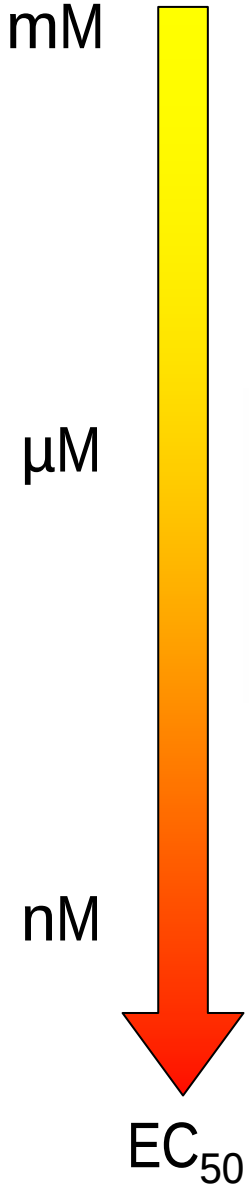


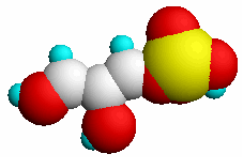
Les antigènes reconnus

LA RÉACTIVITÉ γ9δ2

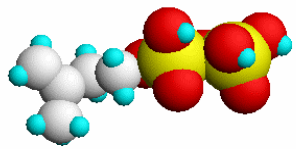


Lymphocyte Tγ9δ2

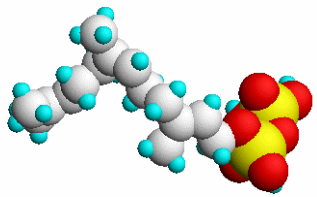




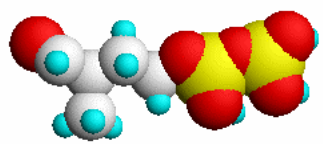
Glycérol-3-P



Isopentényl-PP



Farnésyl-PP

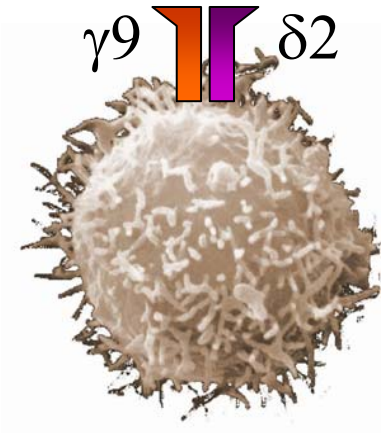


TUBag1

Les antigènes reconnus

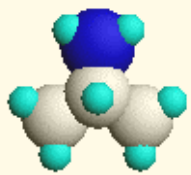
La réactivité γ9δ2

Les Alkylamines

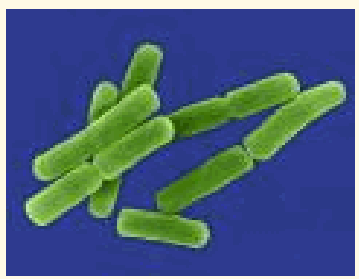


Lymphocyte Tγ9δ2

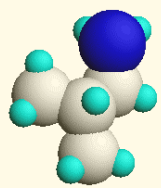
Alkyle——NH_2



isopropylamine



éthylamine



EC_{50}: 1 mM

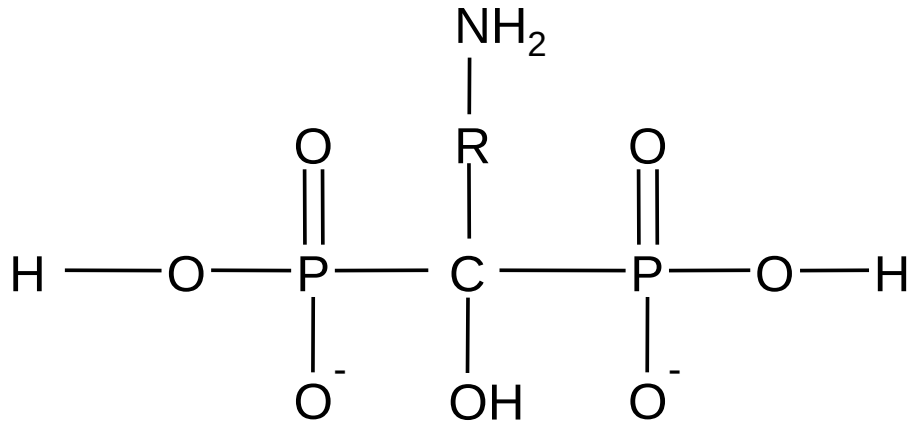
Sec-butylamine

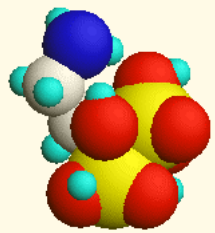
Les antigènes reconnus

La réactivité γ9δ2

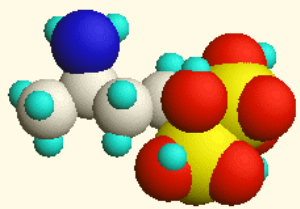
Les aminobiphosphonates

Lymphocyte Tγ9δ2





pamidronate

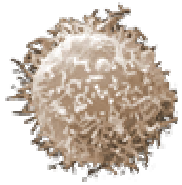


ibandronate

EC_{50}: 100 µM

Les antigènes reconnus

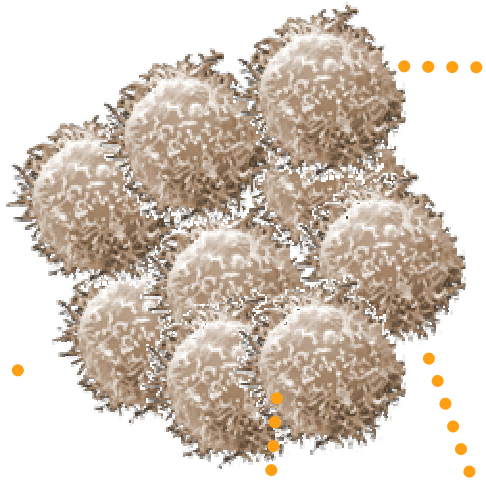
Fonctions cellulaires des Tγ9δ2 activés



Lymphocyte Tγ9δ2

+ Ag

+ IL2



Cytotoxicité
- Perforine/granzyme
- granulysine

Chimiokines
- MIP-1α
- MIP-1β
- RANTES

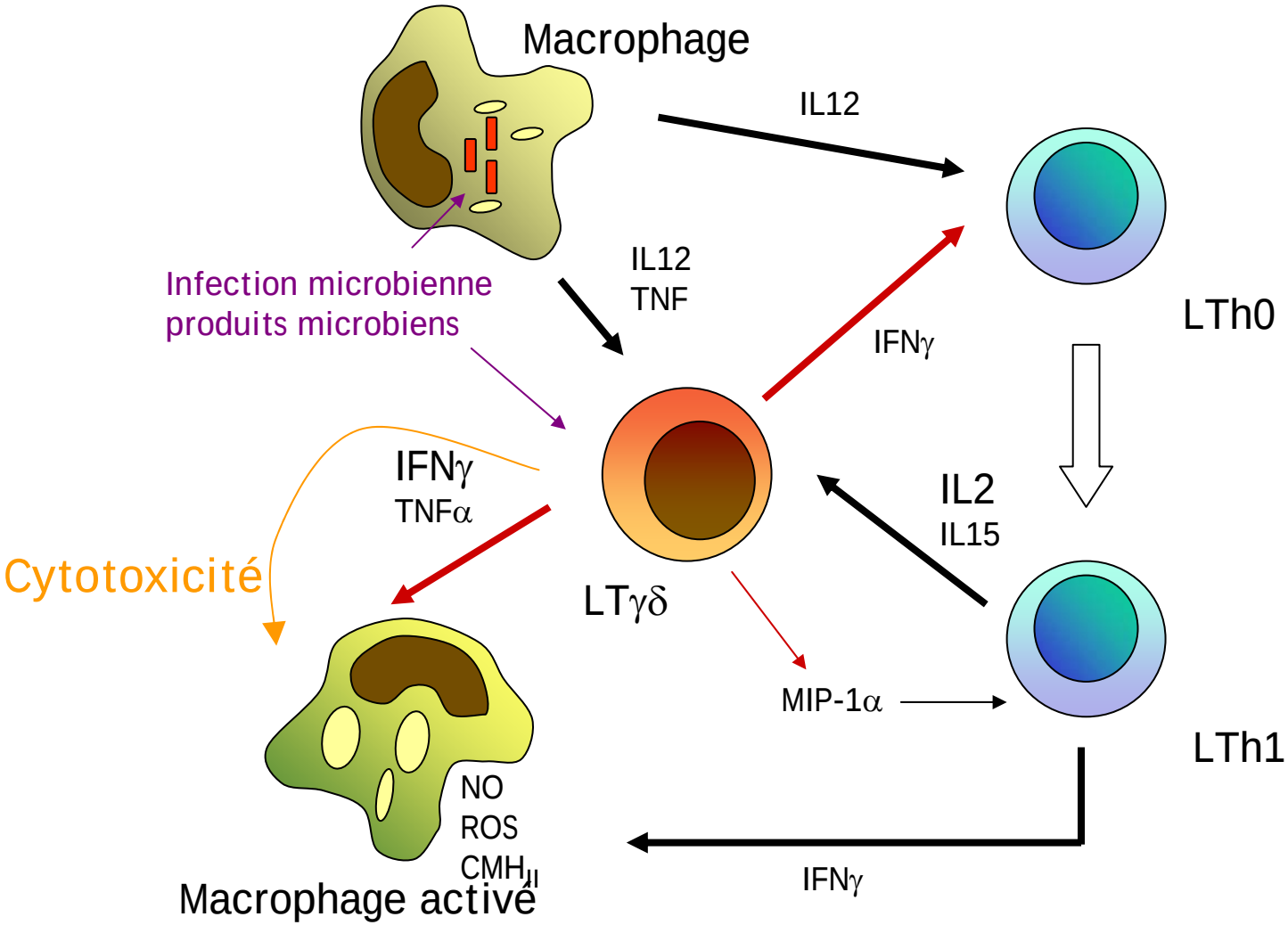
Cytokines Th1
- TNF-α
- IFN-γ

Différenciation

Mémoires indifférenciés

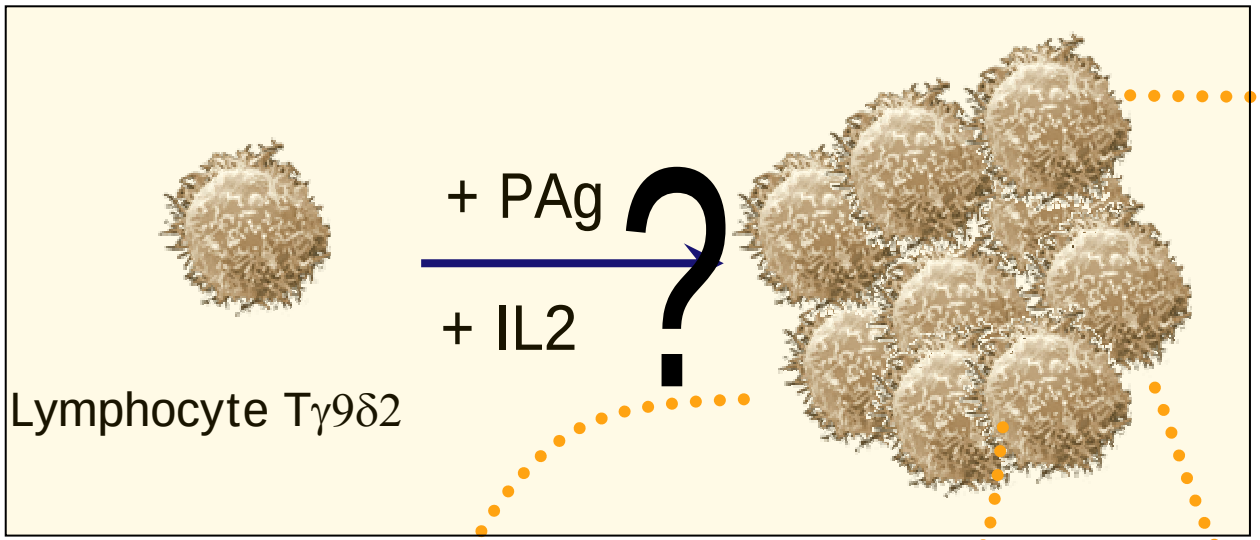
Mémoires effecteurs

INTÉGRATION DES Tγ9δ2 DANS LA RÉPONSE IMMUNITAIRE ANTIMICROBIENNE



Fonctions des T γ9δ2

Etude du mode d'activation des Tγ9δ2 par les PAg



Cytotoxicité
- Perforine/granzyme
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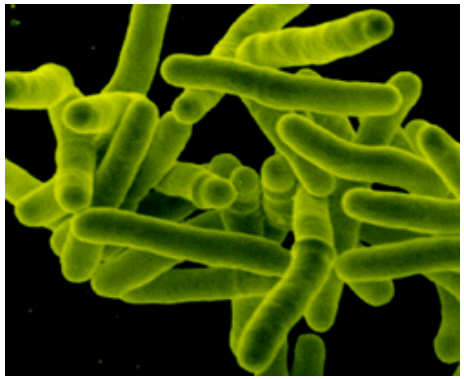
- MIP-1β
- RANTES

Cytokines Th1
- TNF-α
- IFN-γ

différenciation
- Mémoires indifférenciés
- Mémoires effecteurs

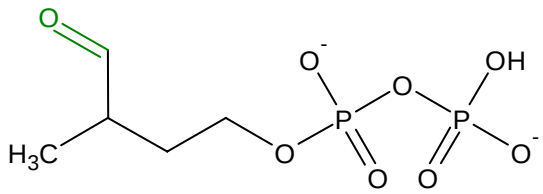
- **Caractérisation de phosphoantigènes de forte bioactivité**
- **Mode de reconnaissance des phosphoantigènes**
- **Interactions cellulaires lors de la réponse aux phosphoantigènes**

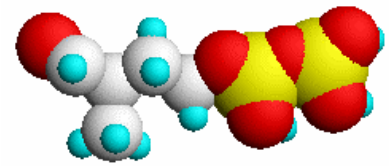
Les Phosphoantigènes naturels

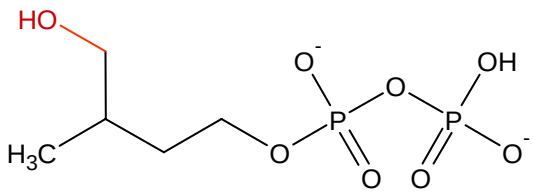


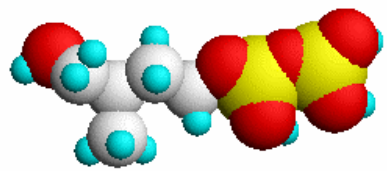
M.tuberculosis

TUBag1 : 3-formyl-1-butyl-PP

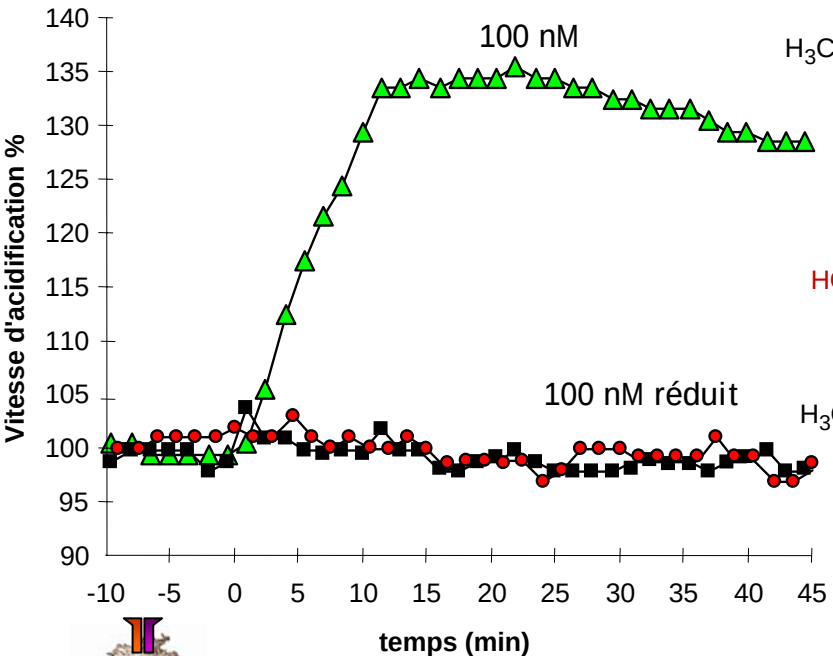








Forme réduite non bioactive



microphysiométrie

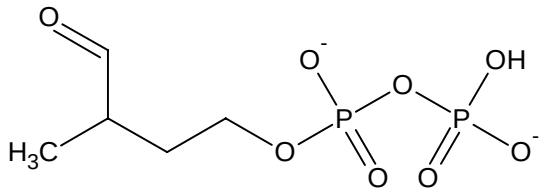
Lymphocyte Tγ9δ2

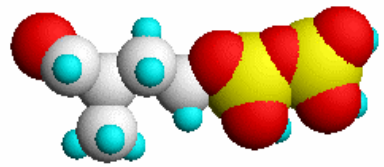
In Journal of Biological Chemistry 199

Caractérisation de phosphoantigènes de forte bioactivité

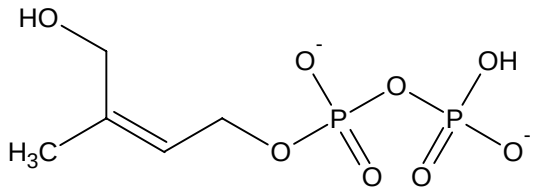
Lymphocyte Tγ9δ2

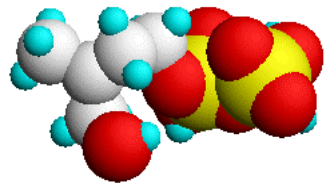
TUBag1 : 3 formyl-1 butyl-PP



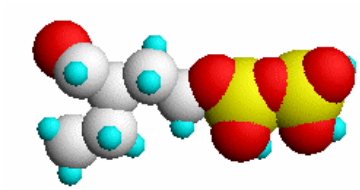


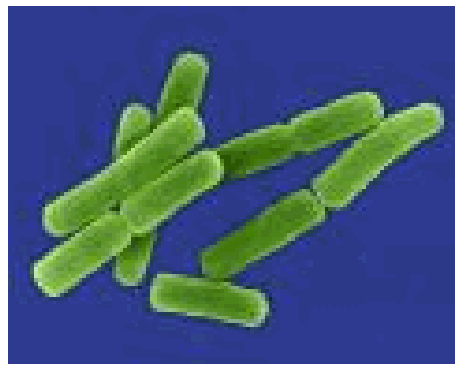
HMB-PP





TUBag2 : 3 formyl-1 pentyl-PP?





E.coli

In Journal of Biological Chemistry, 2002

Caractérisation de phosphoantigènes de forte bioactivité

Biosynthèse des isoprénoïdes

Voie du mévalonate

Cellules animales
levures

3 Acétyl-coA

Mévalonate

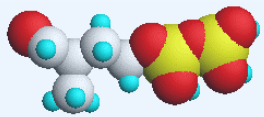
Voie de Rohmer

Bactéries
parasites

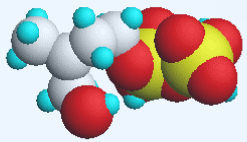
Pyruvate + glycéraldéhyde 3 P

1-deoxy-D-xylulose 5P

?

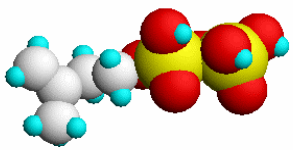


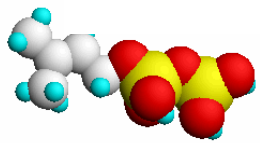
3fb-PP



HMB-PP

Isopentényl-PP

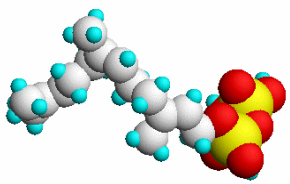




Diméthylallyl-PP

Phosphoantigènes
De forte bioactivité

Farnésyl-PP

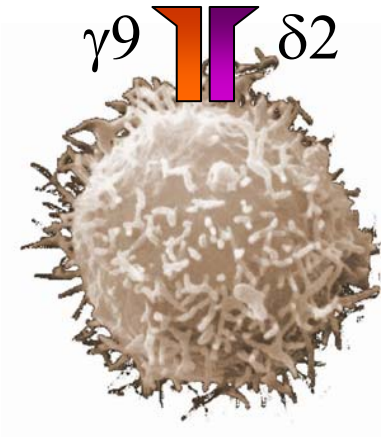


Cholestérol, isoprénylations…

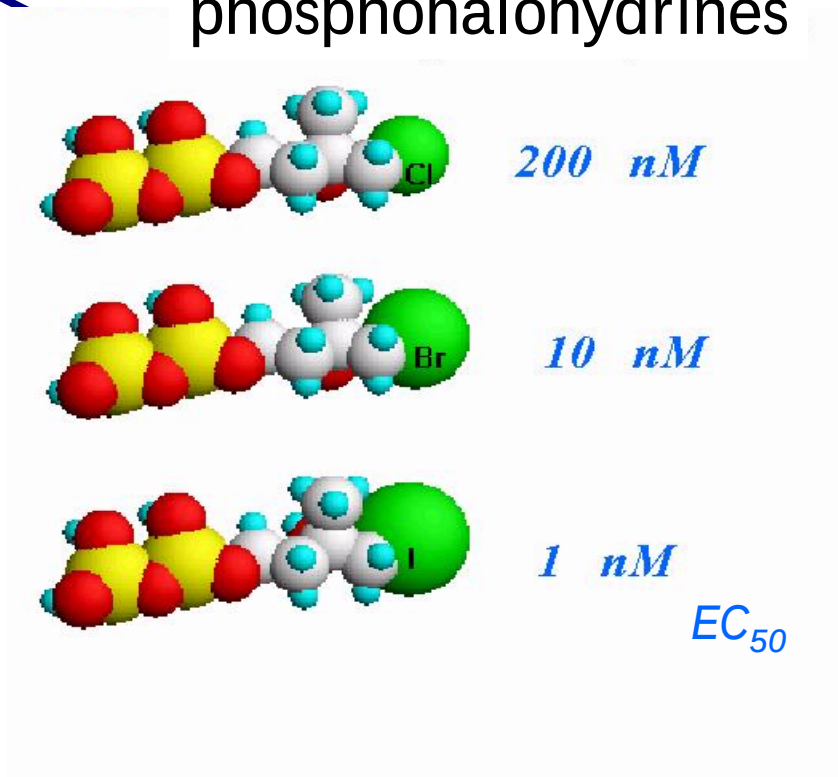
Caractérisation de phosphoantigènes de forte bioactivité

Les Phosphoantigènes de synthèse

Lymphocyte Tγ9δ2



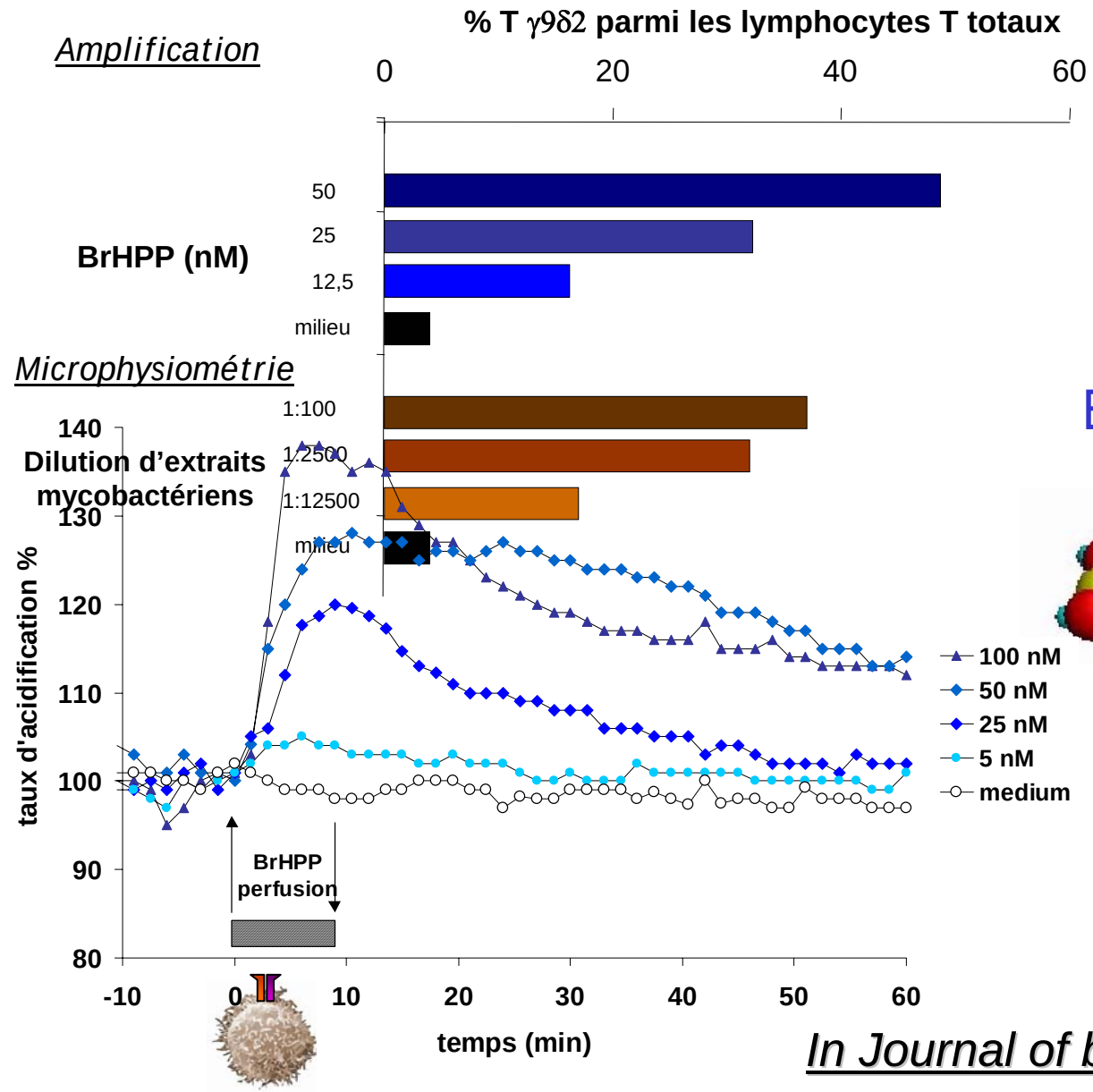
phosphohalohydrines



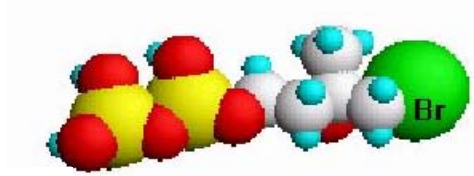
In Journal of biological chemistry 2001

Caractérisation de phosphoantigènes de forte bioactivité

Les Phosphoantigènes de synthèse



Bromohydrine : BrHPP



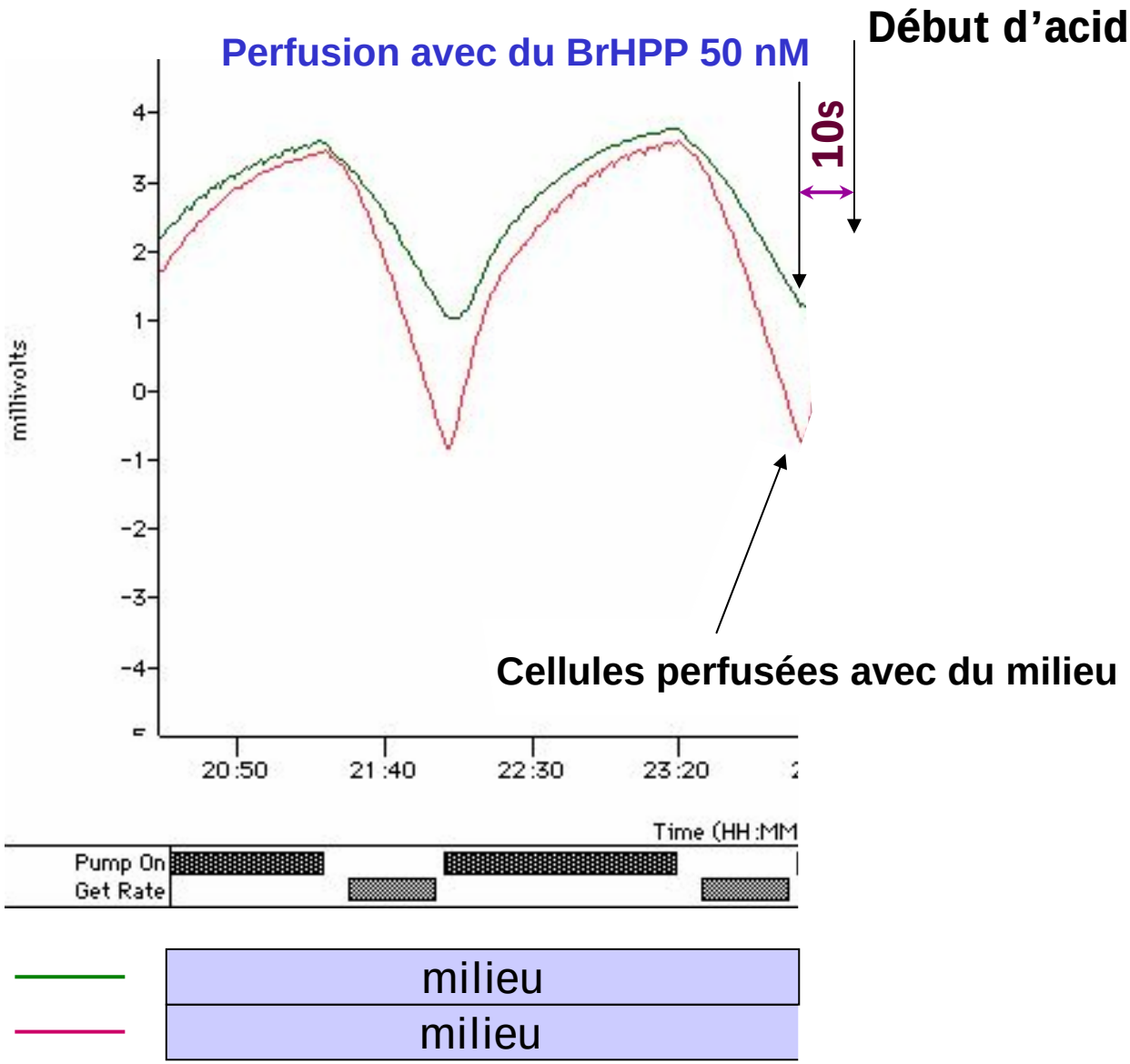
EC_{50}

10 nM

In Journal of biological chemistry 2001

Caractérisation de phosphoantigènes de forte bioactivité

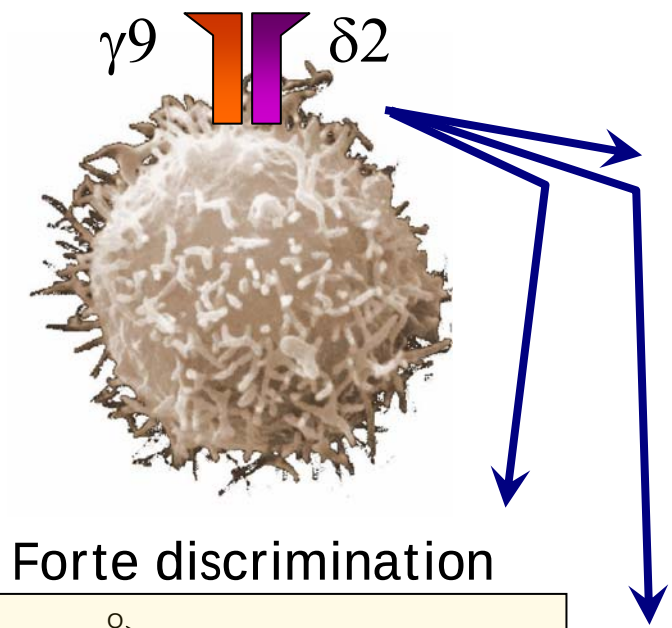
ETUDE CINÉTIQUE EN MICROPHYSIOMÉTRIE



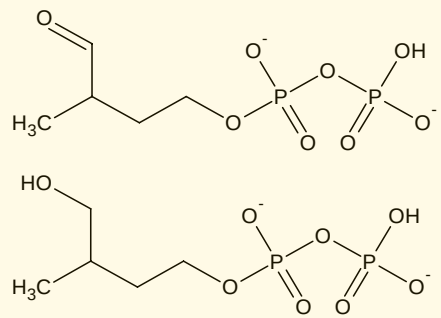
Caractérisation de phosphoantigènes de forte bioactivité

TCR canonique Vγ9-JγP-Cγ1/Vδ2-Jδ1-Cδ
Variabilité jonctionnelle
Mais motifs récurrents

Le Paradoxe



Forte discrimination

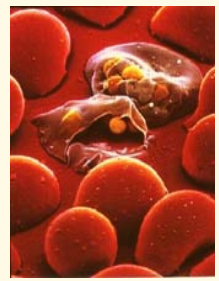


Forme réduite non bioactive

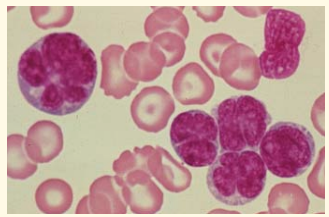
Très nombreux et divers Ag reconnus

Bactéries

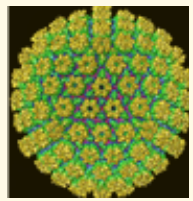
Protozoaires

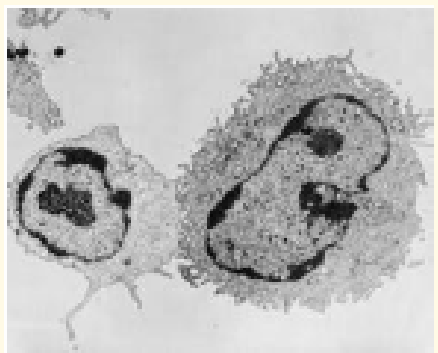


Cellules tumorales



Virus



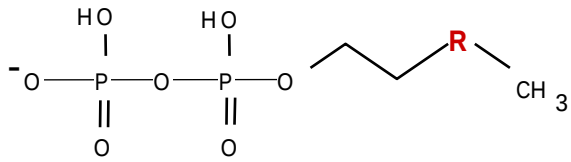


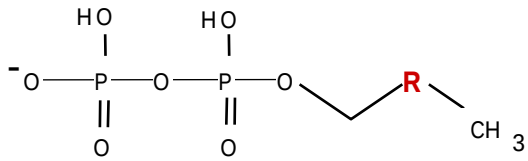
- Contact cellulaire nécessaire
- TCR γ9δ2 impliqué
- Pas de présentation par le CMH

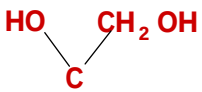
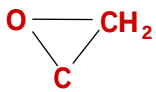
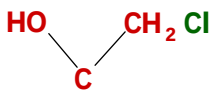
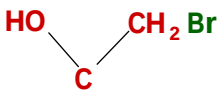
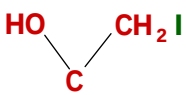
Le Mode de reconnaissance des PAg

Phosphoantigenes de synthèse :

Chaîne raccourcie d'un carbone





R:	Réactivité chimique	EC_{50} , (nM) :	EC_{50} , (nM) :
	*faible*	$>10^6$	nt
	A_n	50 000	nt
	E-1,E-2,S_n-2	5 000	nt
	S_n-2 $^{++}$	20	$>10^6$
	S_n-2 $^{+}$	100	$>10^6$
	S_n-2 $^{++}$	10	$>10^6$
	S_n-2 $^{+++}$	1	$>10^6$

nt : non testé

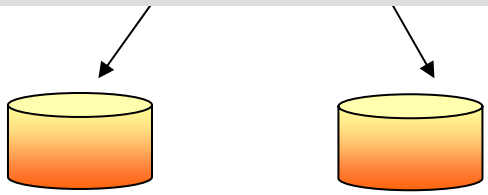
Les Mode de reconnaissance des Ag

In FASEB, 2000

Dégradation des Phosphoantigènes par les LT γ9δ2

Phosphoantigène : X-PP

13 nmoles

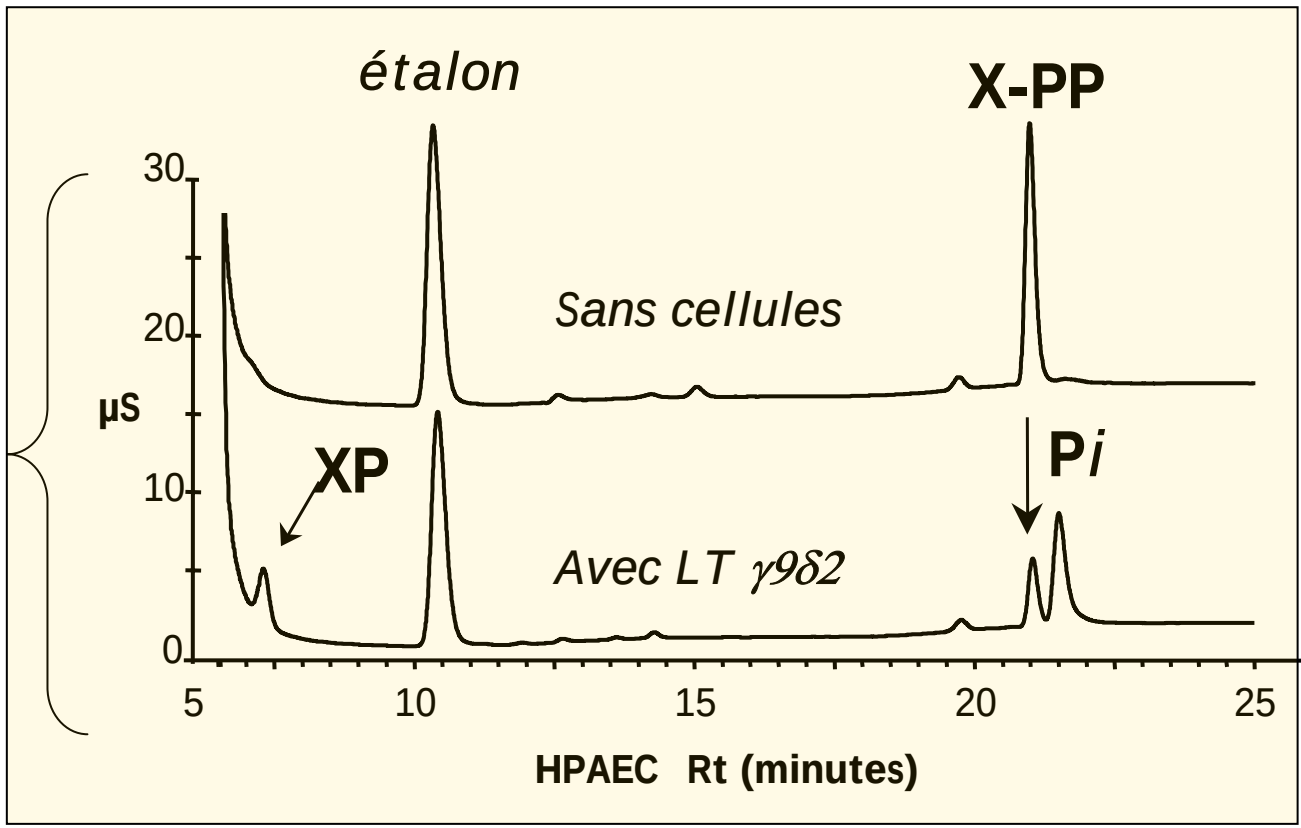


Sans cellules | 2 .10⁶ T γδ

1h 37°C

Analyse du surnageant
Par HPAEC

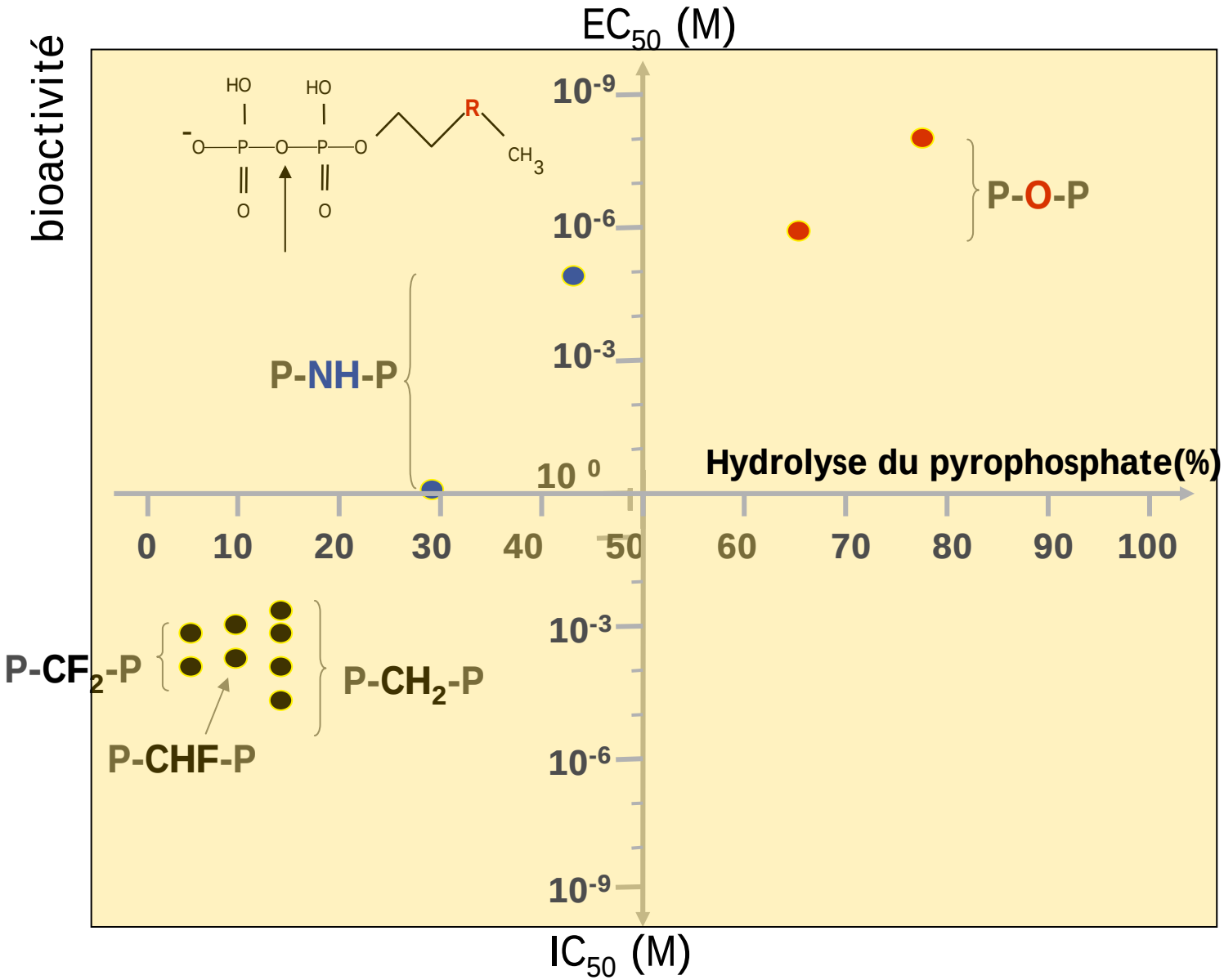
$$X\text{-}PP \longrightarrow XP + Pi$$



Mode de reconnaissance des Ag

In FASEB, 2000

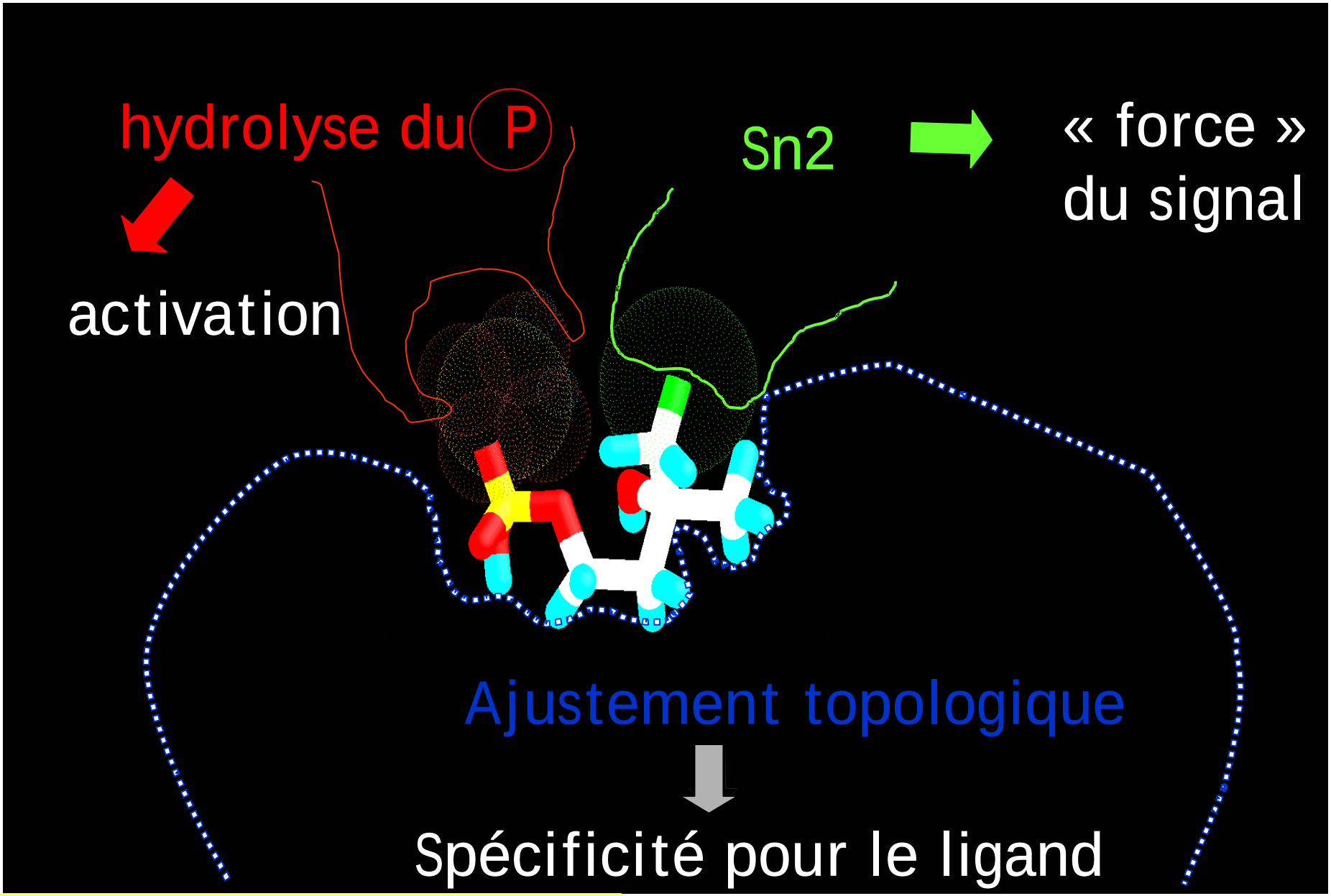
CORRÉLATION ENTRE BIOACTIVITÉ ET HYDROLYSABILITÉ



Mode de reconnaissance des Ag

In FASEB 2000

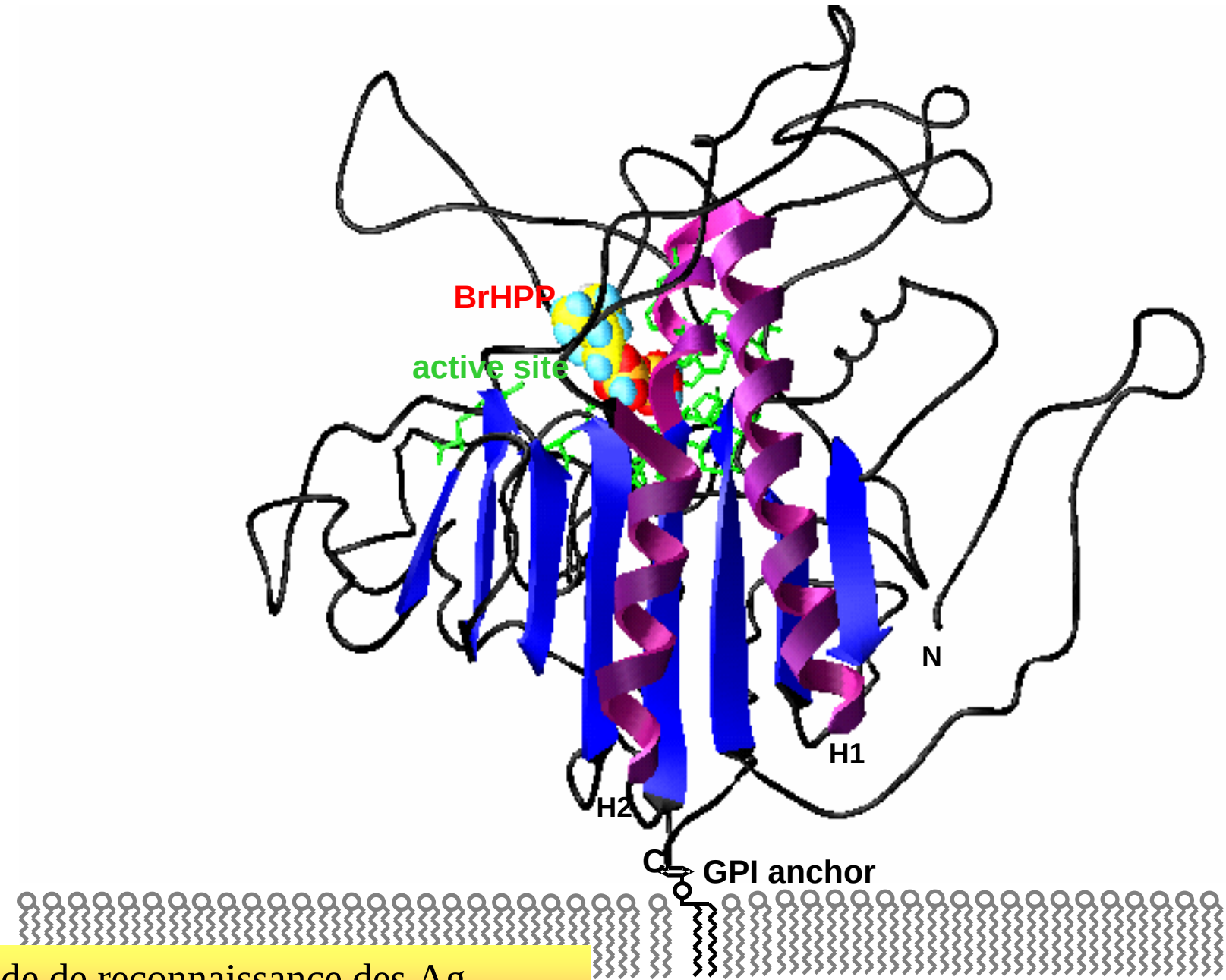
Les étapes de la reconnaissance d'un phosphoantigène



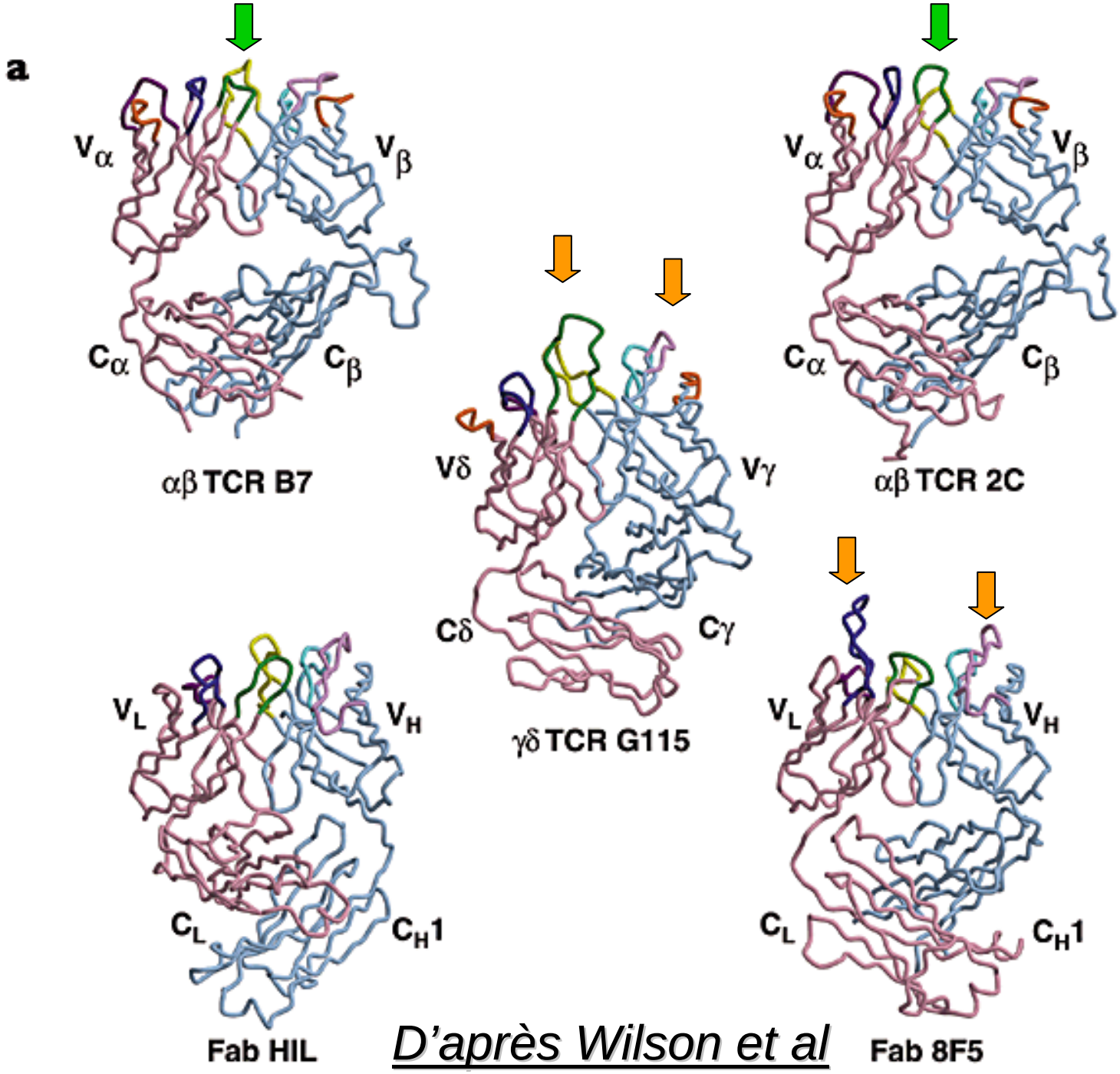
Mode de reconnaissance des Ag

In FASEB, 200

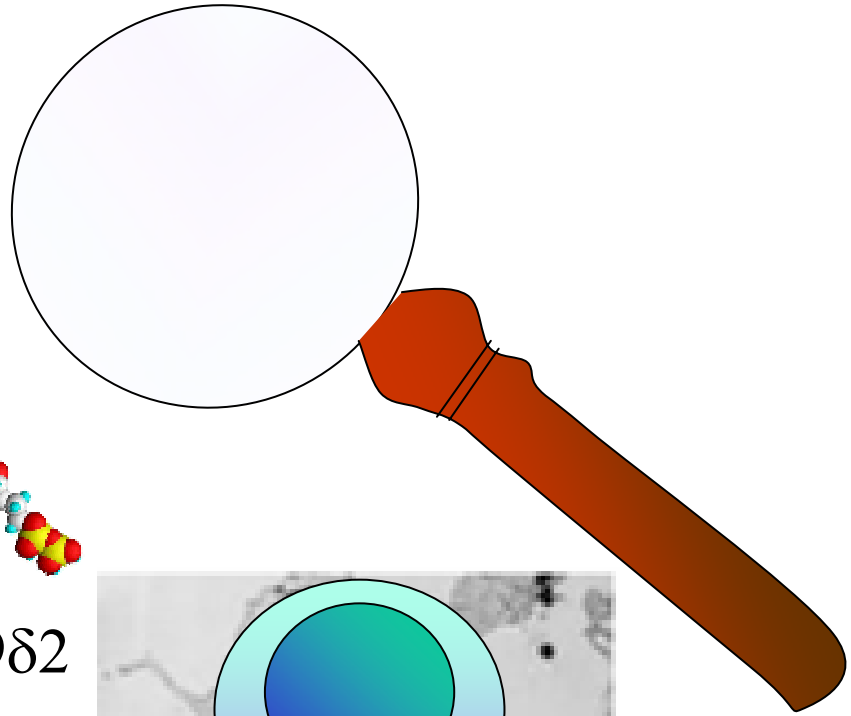
LA PHOSPHATASE ALCALINE HUMAINE TISSU-NON-SPÉCIFIQUE



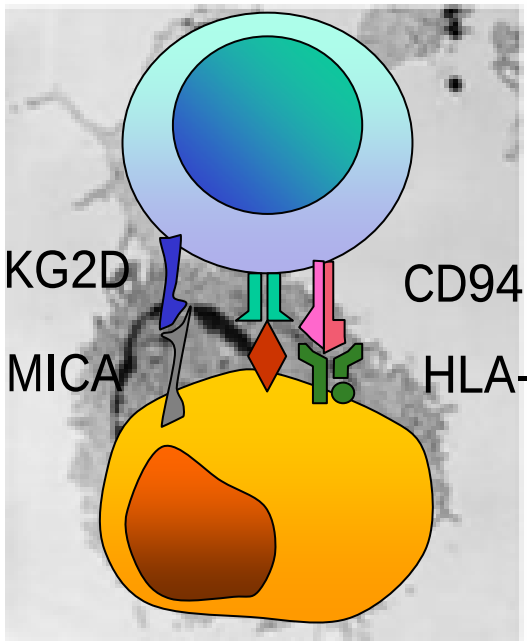
Mode de reconnaissance des Ag



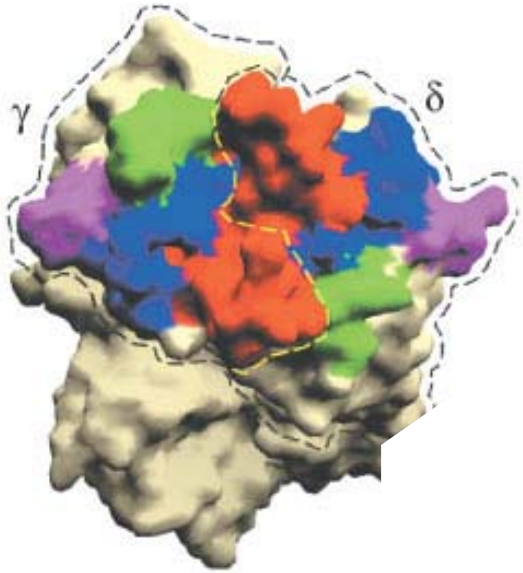
D'après Wilson et al

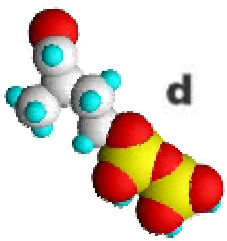


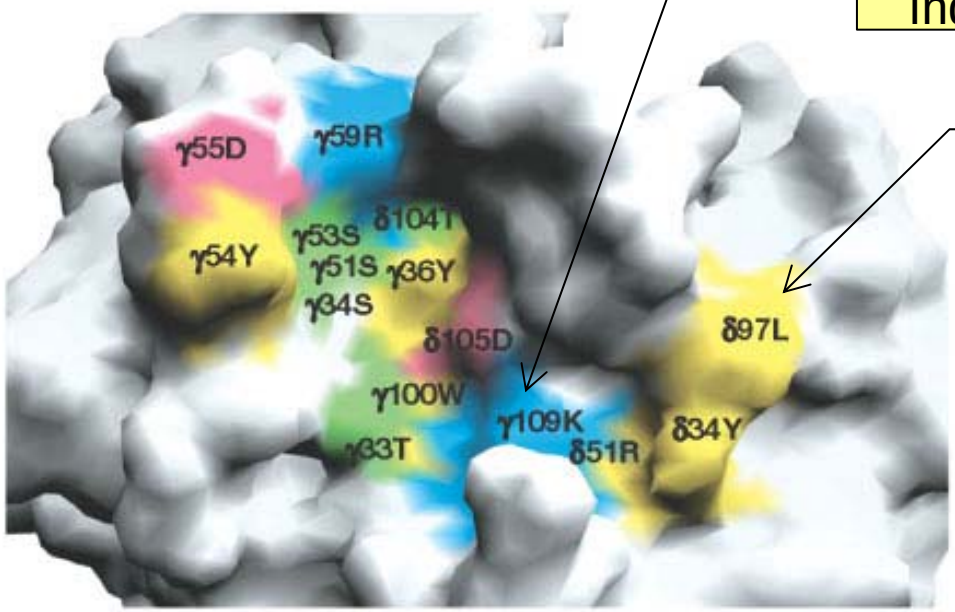
Lymphocyte Tγ9δ2



Cellule « cible »



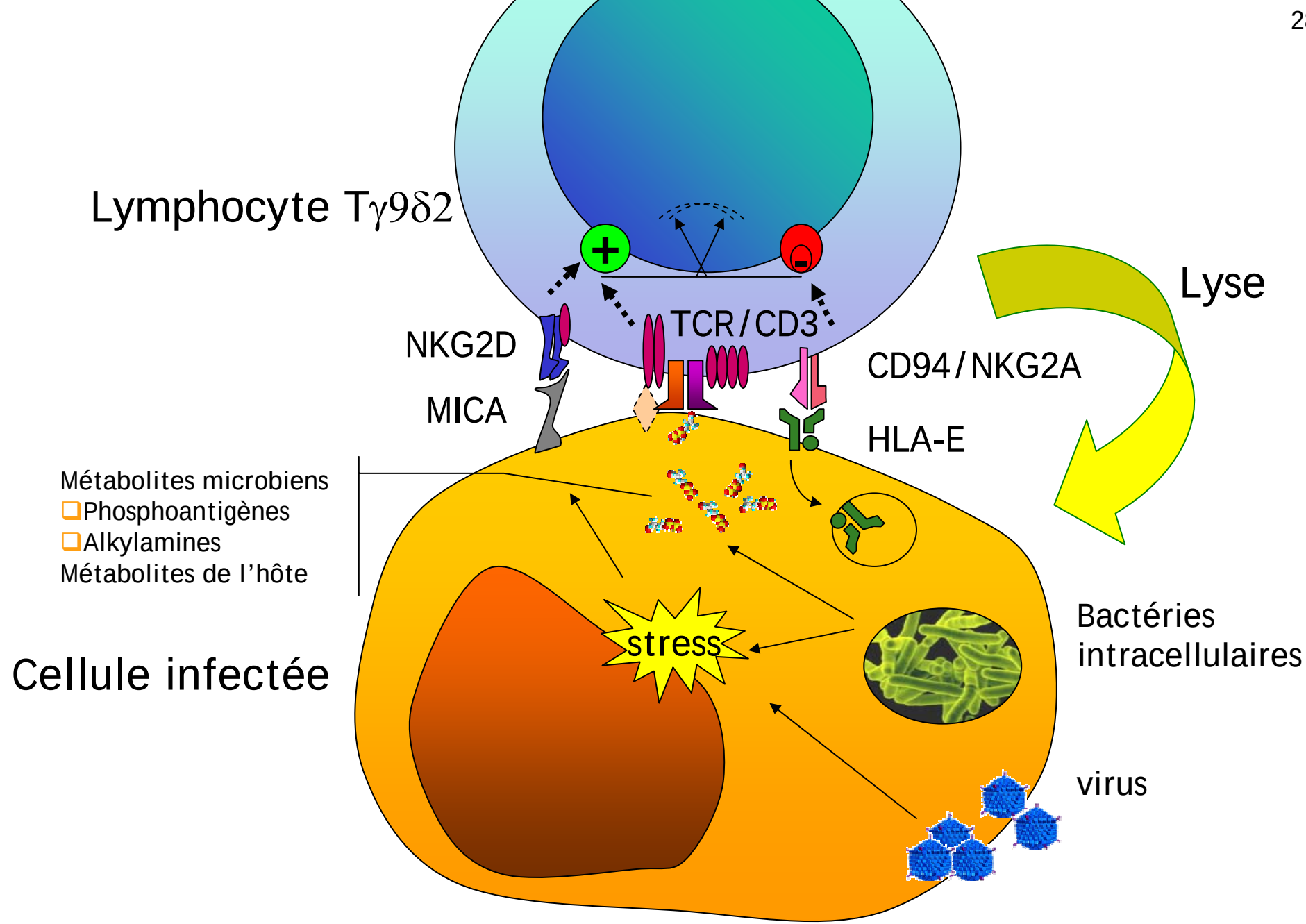




Conservé indispensable

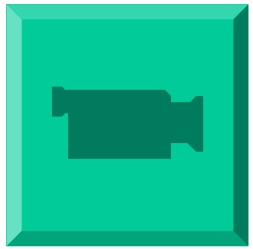
conservé

D'après Allison et a


Lymphocyte Tγ9δ2
+
-
TCR/CD3
NKG2D
MICA
CD94/NKG2A
HLA-E
Lyse
Métabolites microbiens
Phosphoantigènes
Alkylamines
Métabolites de l'hôte
stress
Cellule infectée
Bactéries intracellulaires
virus

La synapse immunologique entre CPA et Tαβ

Time lapse (3 min)
37°C



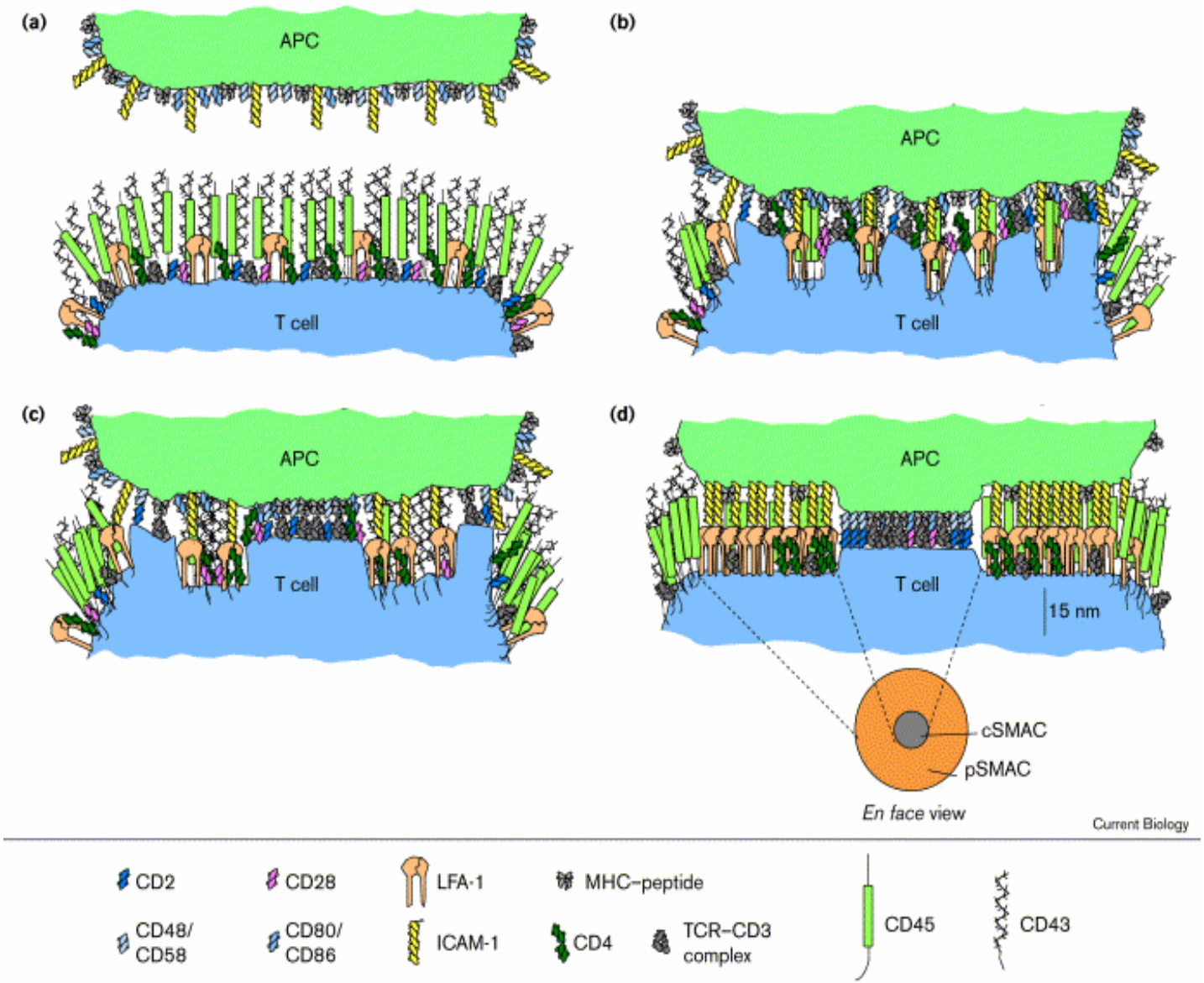
TCR-CD3ζ

CD4

CPA (LB)+agoniste

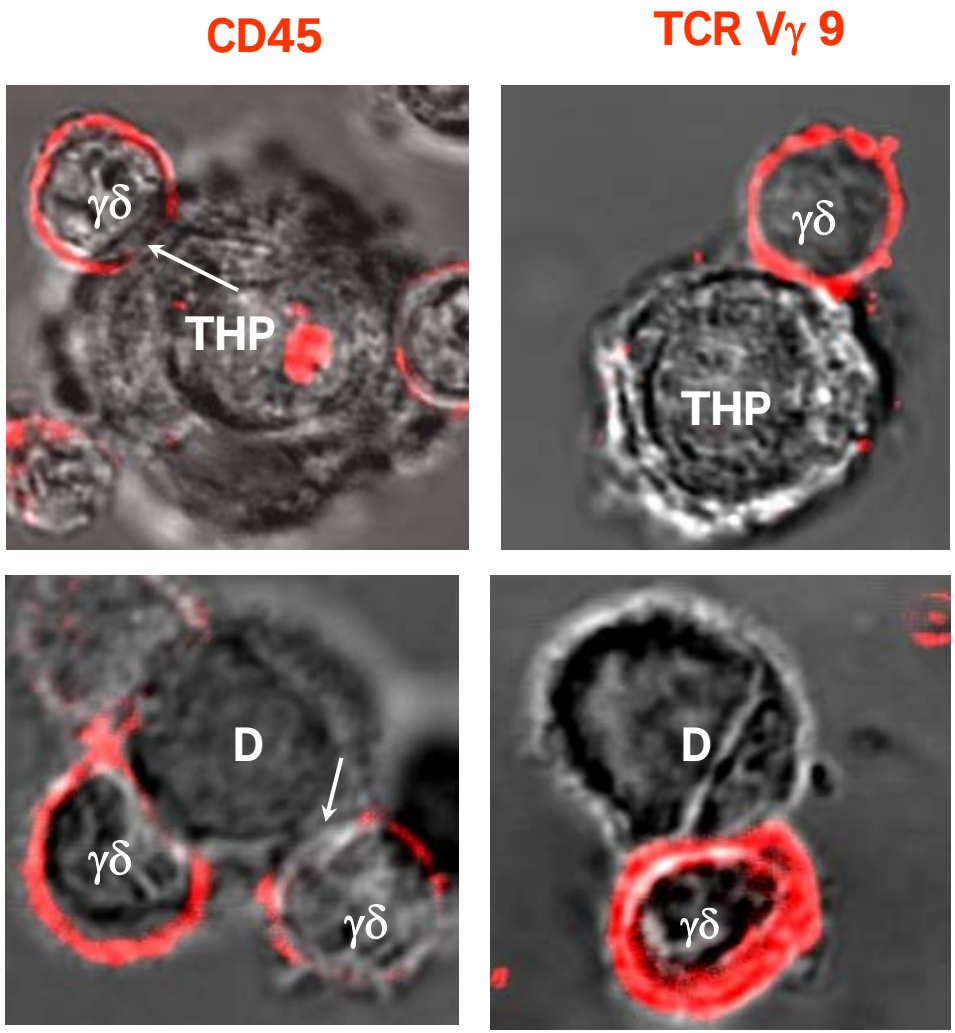
D'après Zal et al

La synapse immunologique entre CPA et Tαβ



D'après Dustin et al

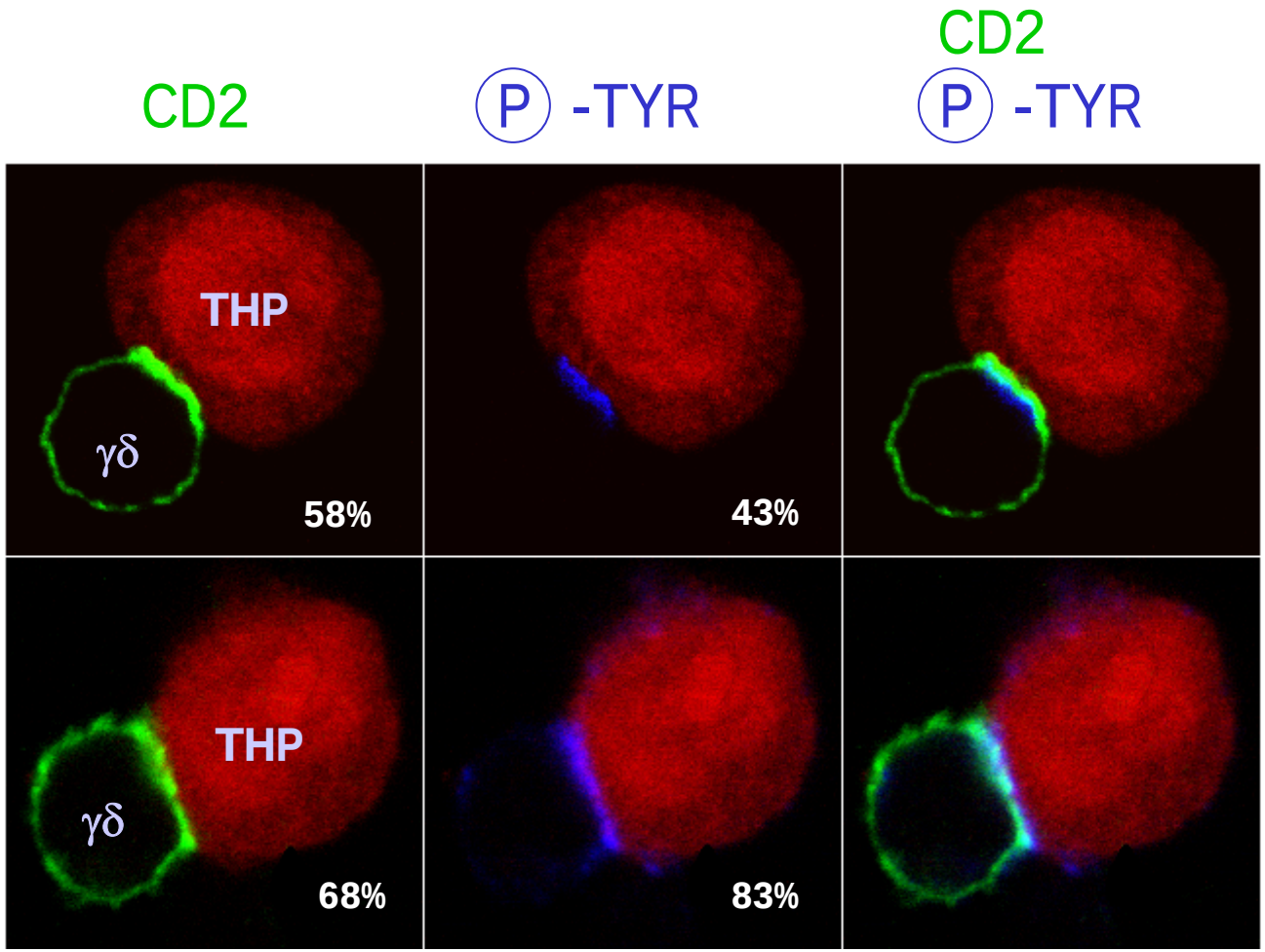
NATURE DE LA SYNAPSE DES LYMPHOCYTES Tγ9δ2



+BrHPP 100 nM

In Journal of Immunology, 200

NATURE DE LA SYNAPSE DES LYMPHOCYTES Tγ9δ2



Milieu

+BrHPP 100 nM

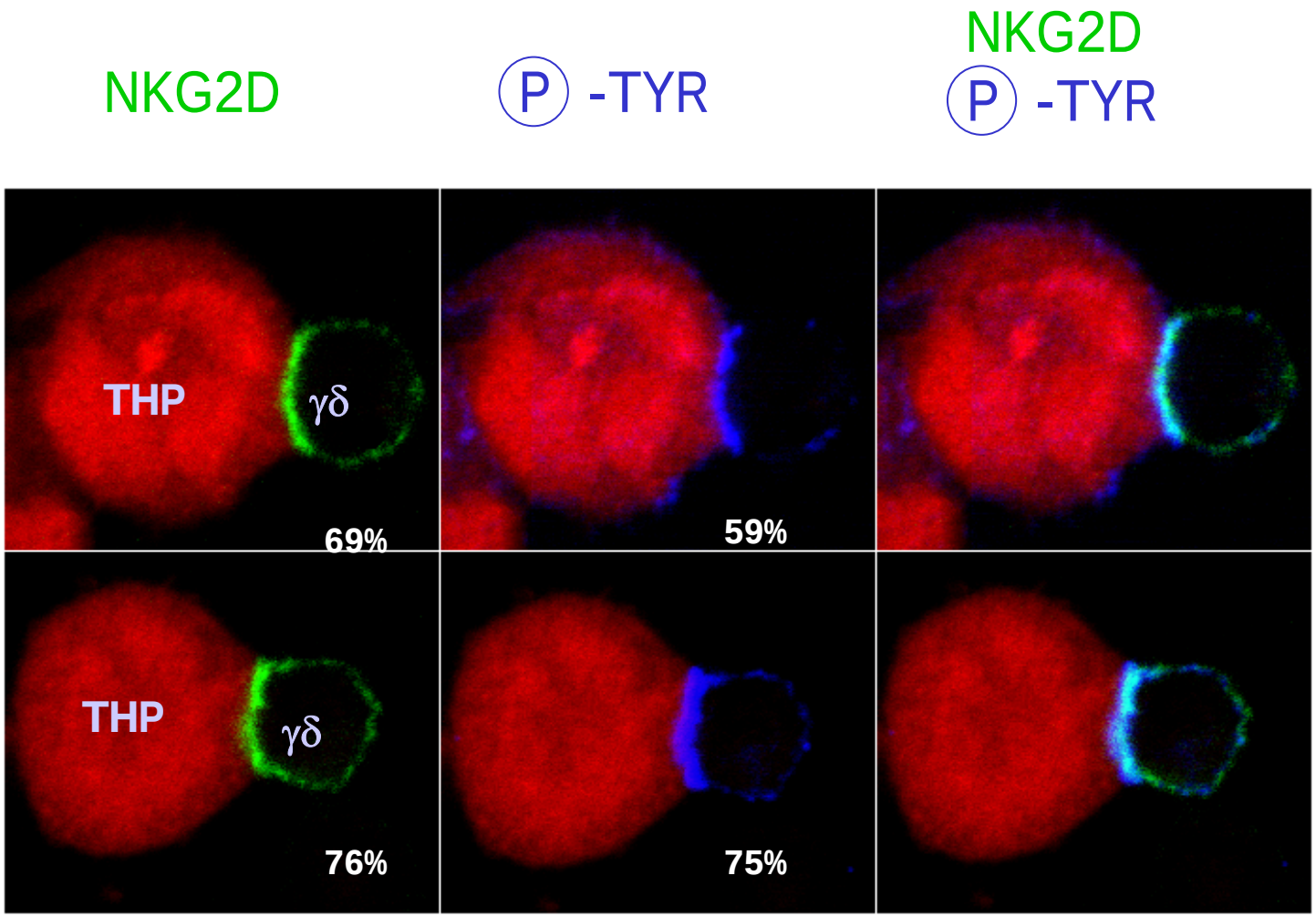
La synapse immunologique entre THP et Tγ9δ2

Caractéristiques de la synapse
d'un lymphocyte T γ9δ2 avec
THP-1 plus phosphoantigène

- Pas de polarisation caractéristique du TCR
- Polarisation de CD2 et LFA-1
- Exclusion de CD45

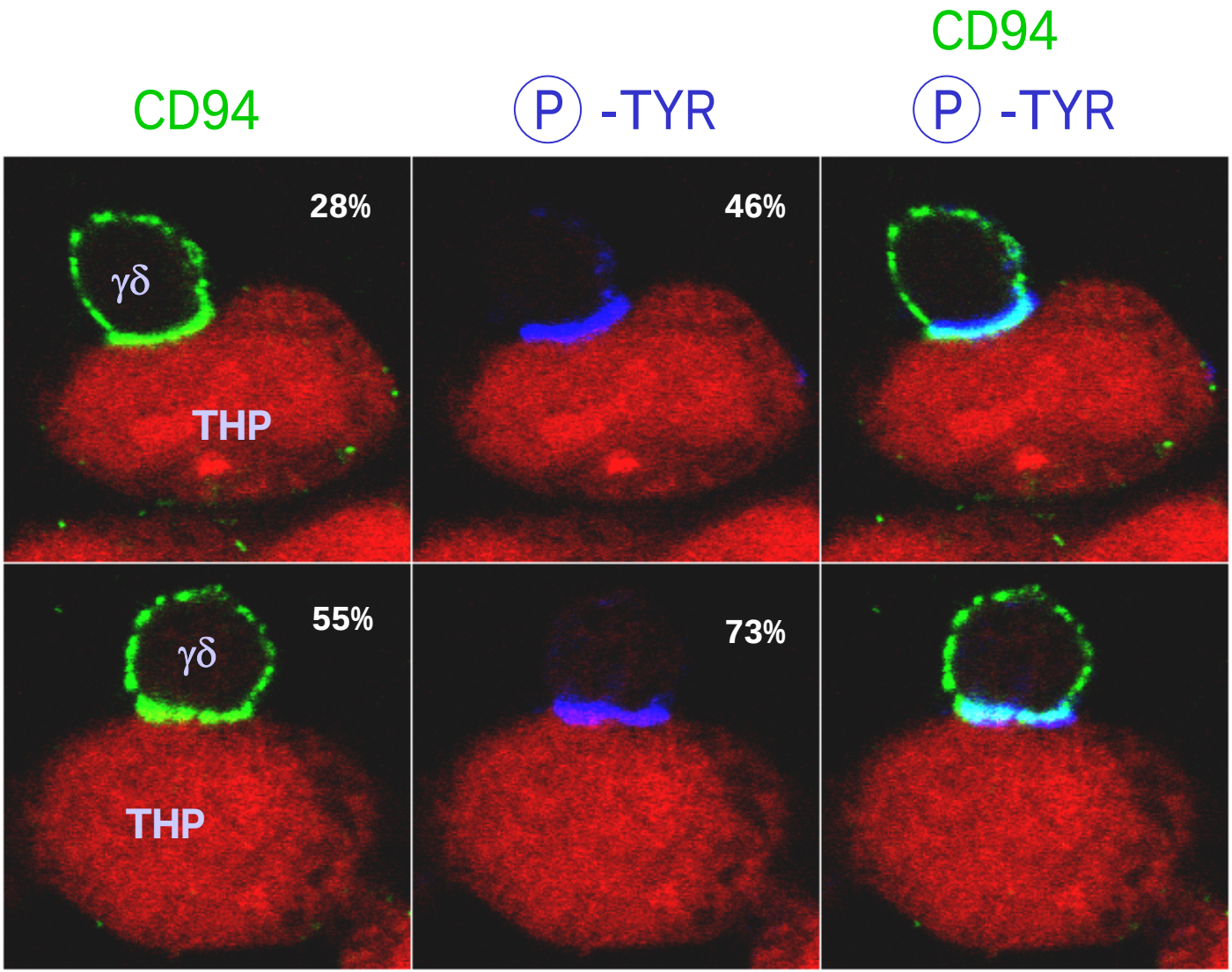
Cette synapse a lieu aussi sans phosphoantigène
Mais de manière moins fréquente

IMPLICATION DES RÉCEPTEURS DE L'IMMUNITÉ INNÉE



Soumis JI

IMPLICATION DES RÉCEPTEURS DE L'IMMUNITÉ INNÉE

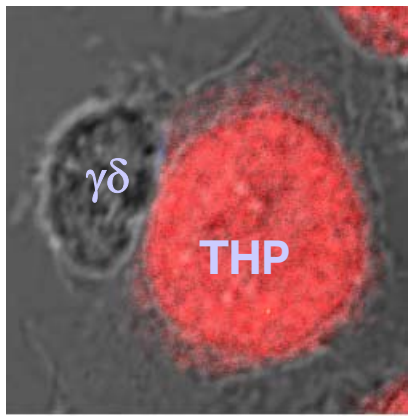


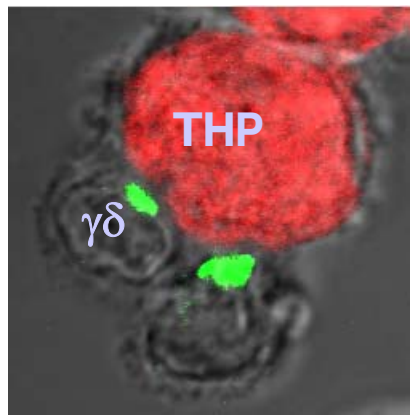
Soumis

La libération de Cytokines nécessite la présence De phosphoantigènes

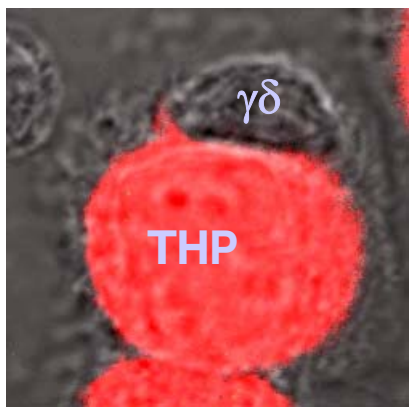
Milieu

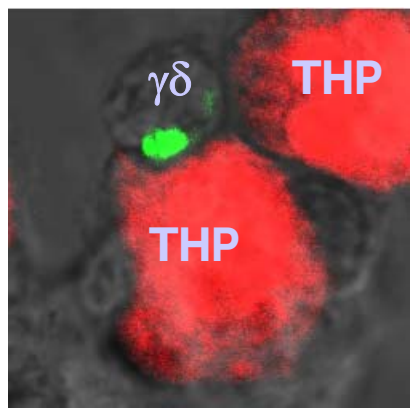
+BrHPP 100 nM





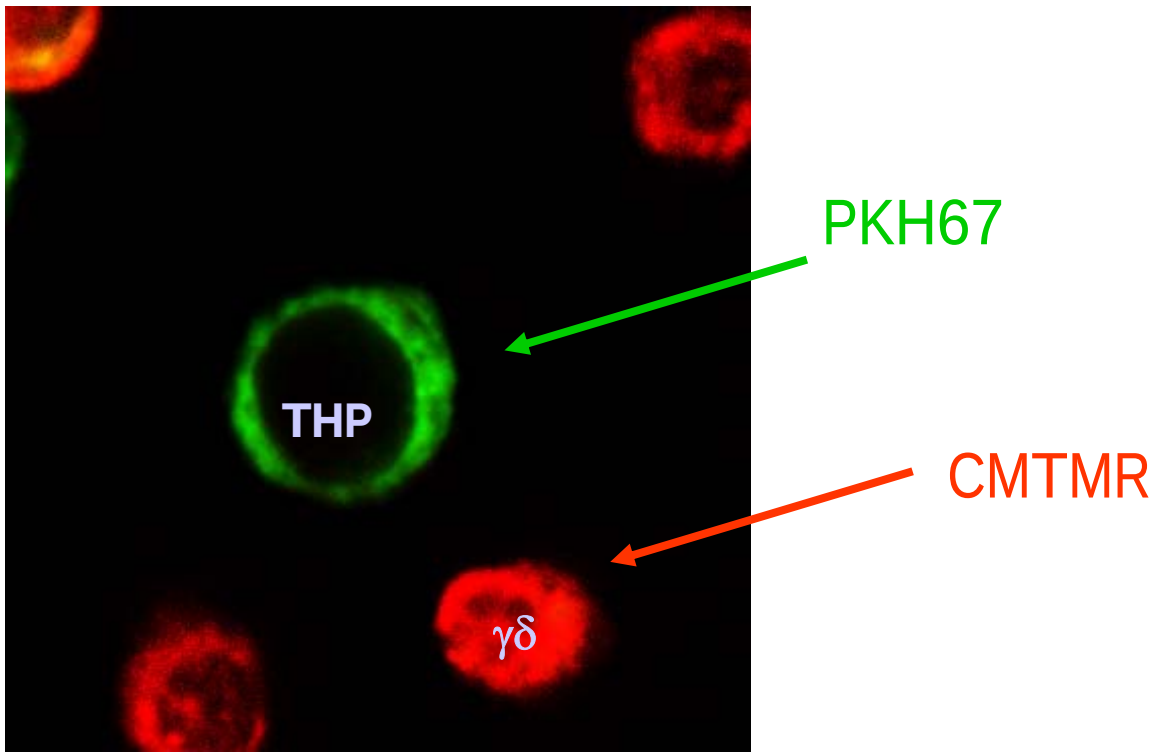
IFNγ





TNFα

LE TRANSFERT SYNAPTIQUE

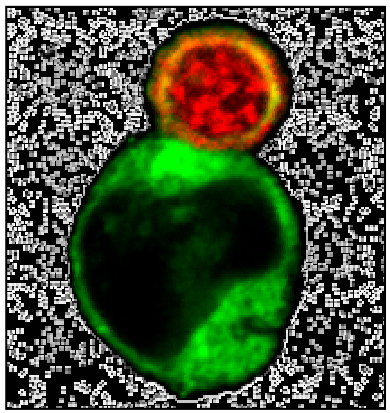


Le système de marquage

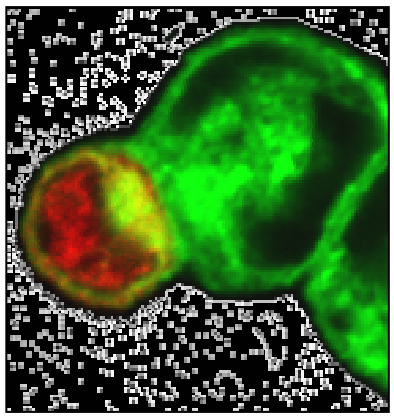
LE TRANSFERT SYNAPTIQUE

Transfert de fragments de membrane de THP-1
à la surface de lymphocytes T γ9δ2
dans différentes conditions de culture

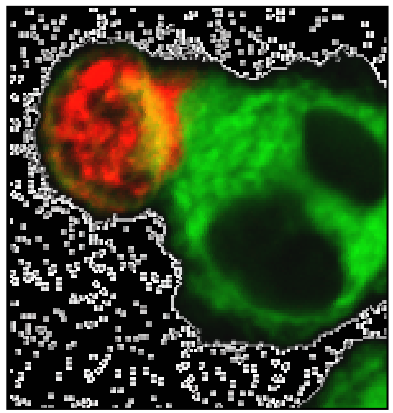
cellules seules



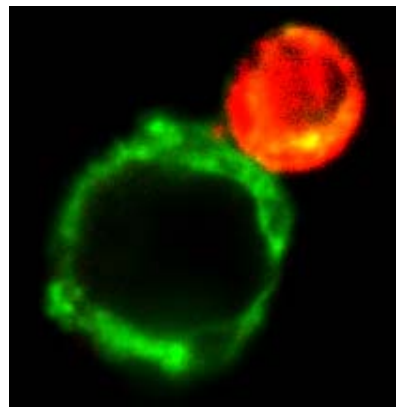
cellules+BrHPP



cellules+BrHPP+PP2



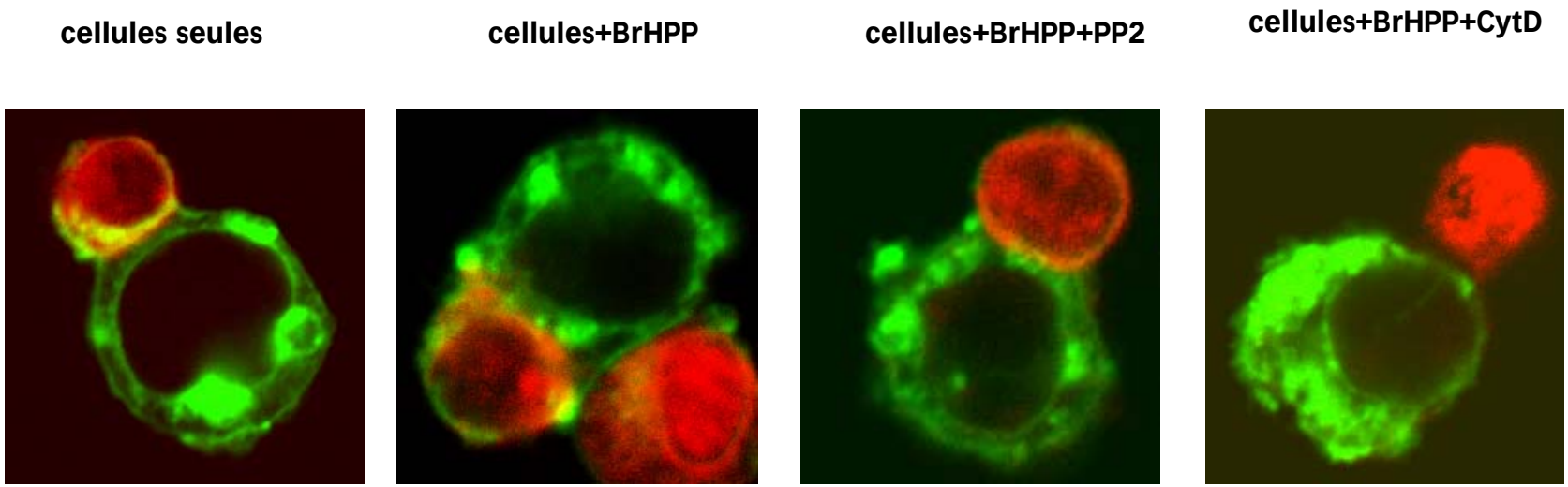
cellules+BrHPP+CytD



Journal of immunology 2002

LE TRANSFERT SYNAPTIQUE

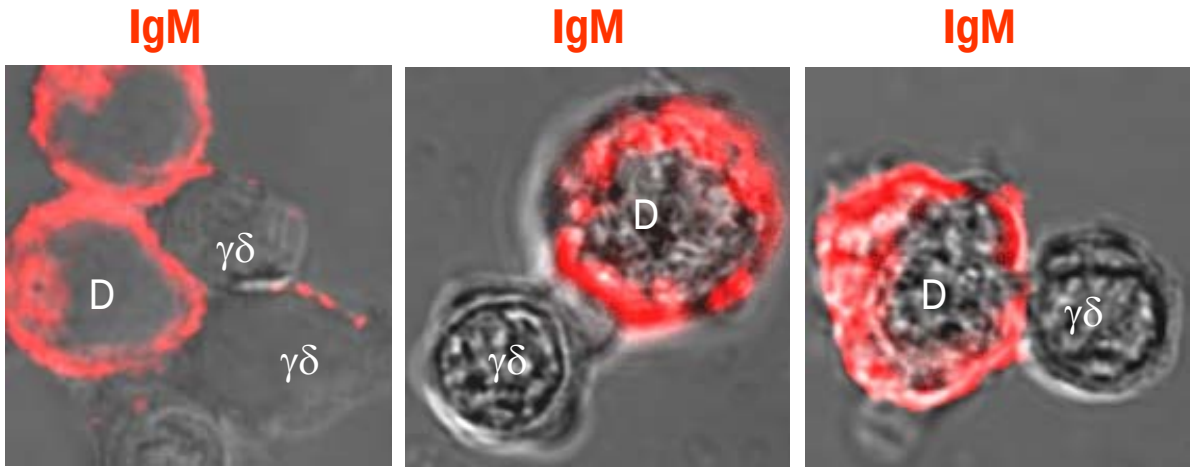
Transfert de fragments de membrane de Daudi
à la surface de lymphocytes T γ9δ2
dans différentes conditions de culture



Journal of immunology 2002

LE TRANSFERT SYNAPTIQUE

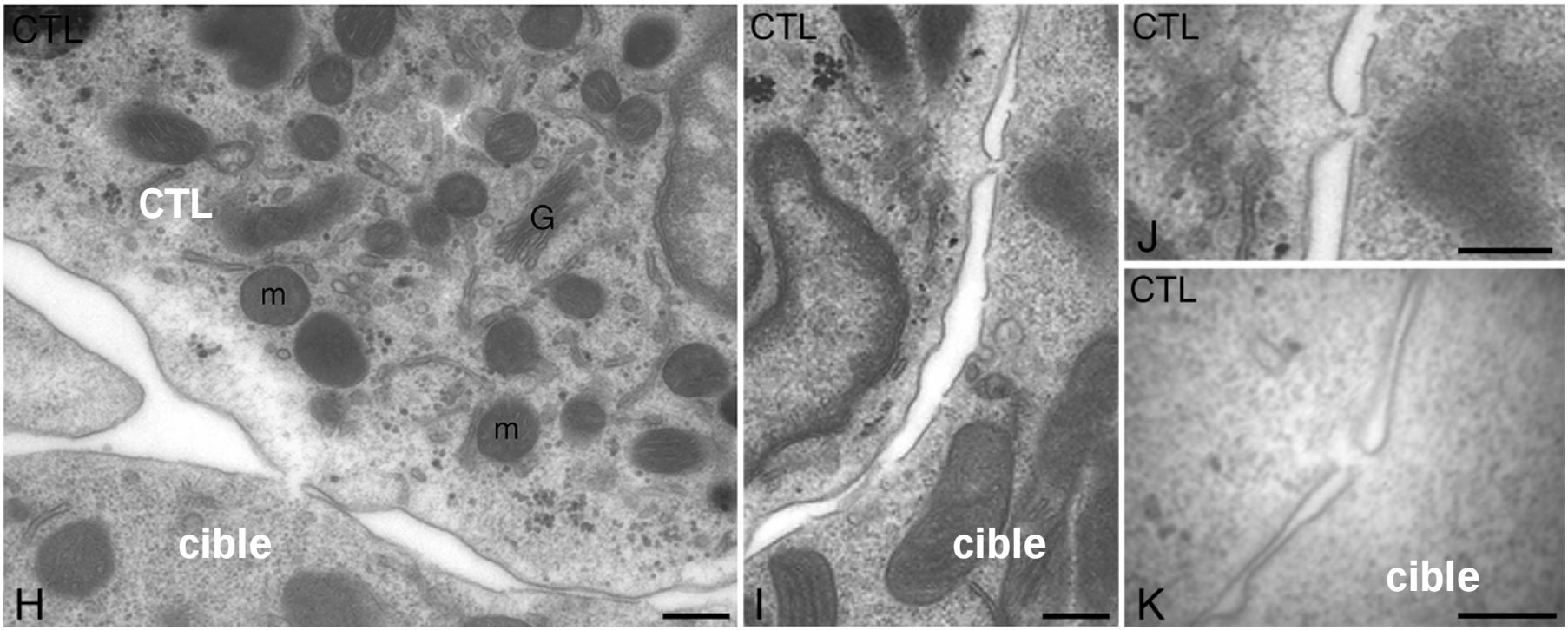
Transfert de molécules membranaires (IgM) de Daudi
à la surface de lymphocytes T γ9δ2
dans différentes conditions de culture



milieu PP2 CytD

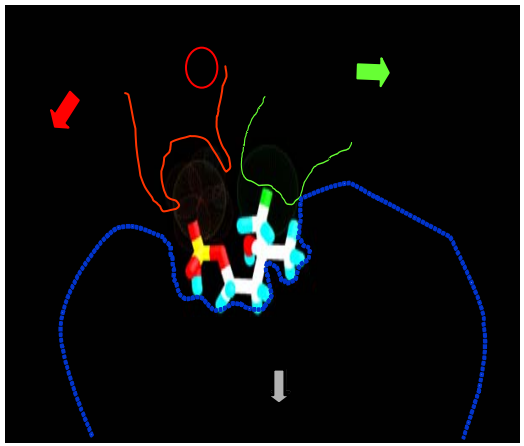
Journal of immunology 2002

Le transfert synaptique

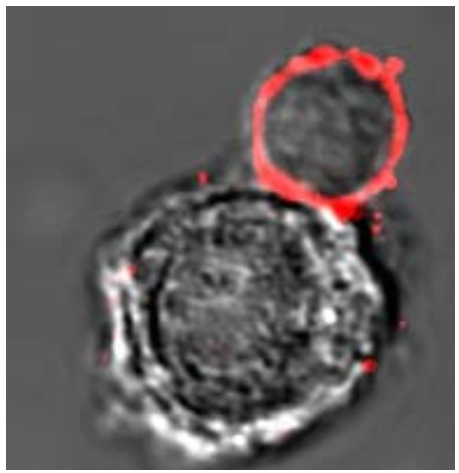


D'après Stinchcombe et al

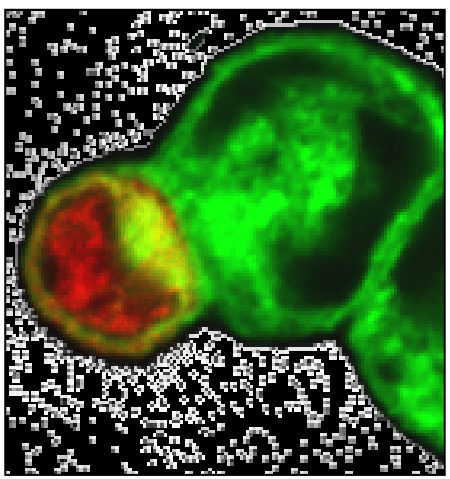
CONCLUSION



Les lymphocytes T γ9δ2 reconnaissent des Ag solubles,
Les phosphoantigènes, de manière particulière.
Les phosphoantigènes naturels sont extrêmement bioactifs et leurs analogues de synthèse sont de bons candidats pour l'immuno-intervention



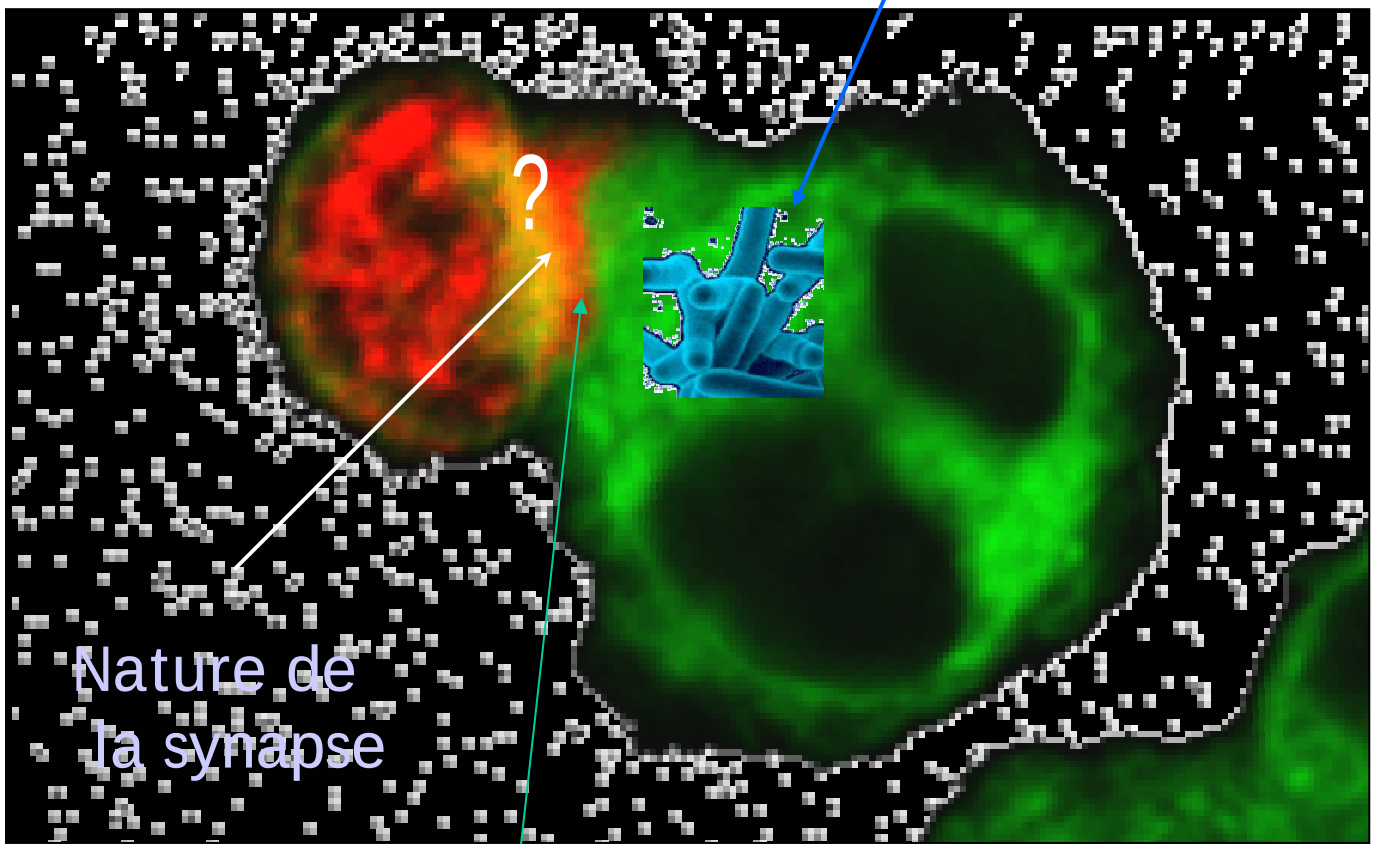
Une synapse immunologique proche des autres lymphocytes s'établit face à une cible potentielle, elle implique le TCR mais aussi les NKR.



Un transfert synaptique de fragments de membrane et de protéines membranaires a lieu activement lors de l'interaction avec une cible en présence de phosphoantigènes

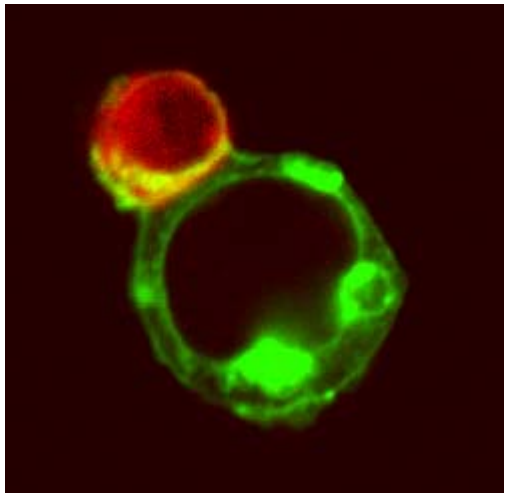
PERSPECTIVES

lors d'infections

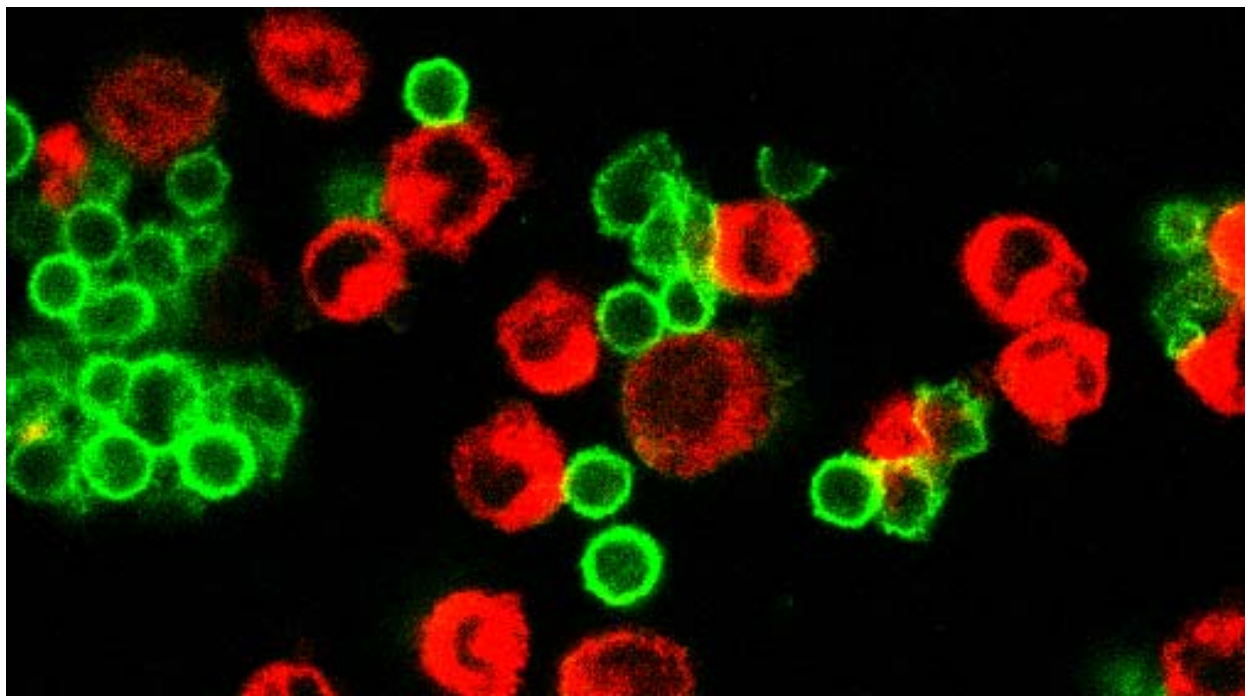


Caractéristiques du transfert synaptique

PERSPECTIVES



Rôle physiologique du transfert synaptique
dans la réponse immunitaire



Caractéristiques de la synapse lors de l'interaction
avec des cibles résistantes à la lyse

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