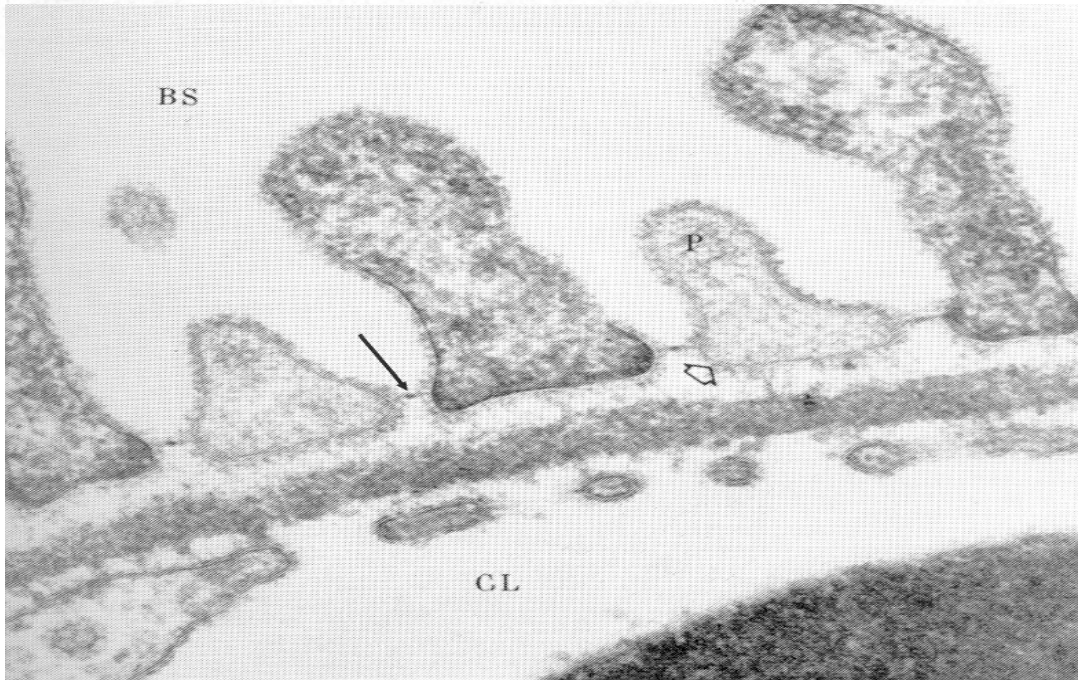
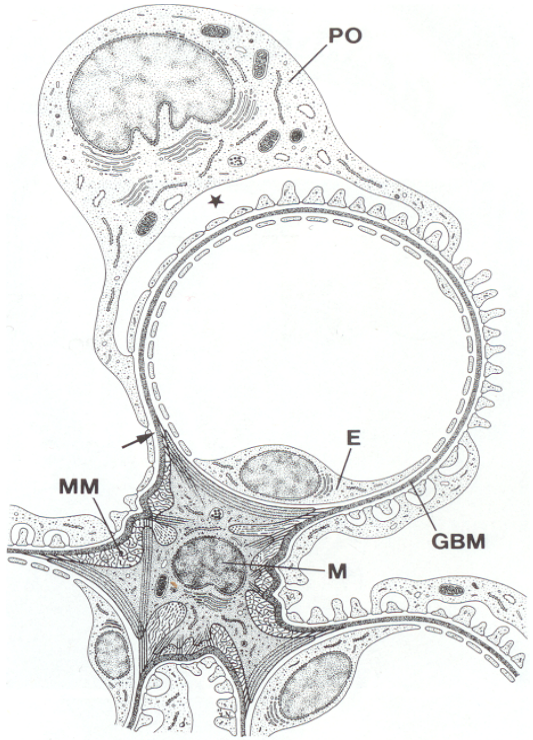
NUEVOS ENFOQUES DEL ROL DE LOS PODOCITOS EN LA GÉNESIS DE LA PROTEINURIA

HOSPITAL BRITANICO
DE BUENOS AIRES

La pared capilar del glomérulo, compuesta por:
la célula endotelial glomerular,
la membrana basal glomerular,
y los podocitos,
es la responsable de la ultrafiltración del plasma por el riñón.



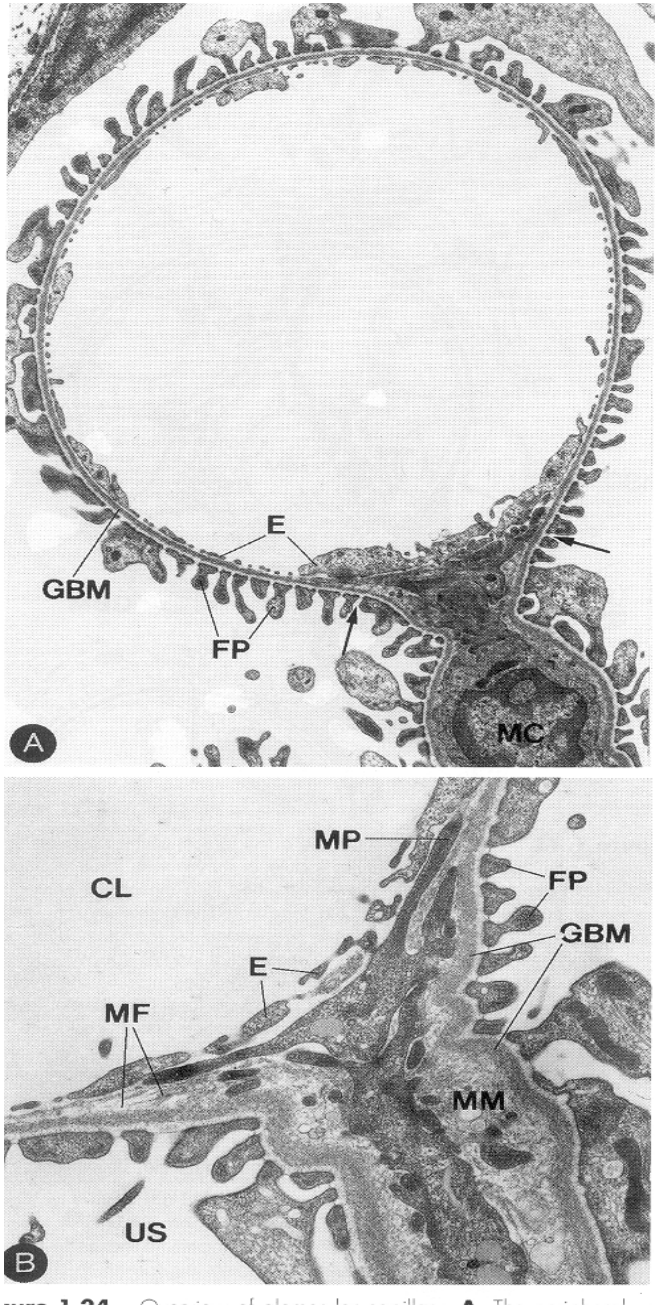


Muchos estudios han establecido que las moléculas transportadas en el plasma son sensadas y retenidas por la barrera de filtración en base a su tamaño, forma y carga.

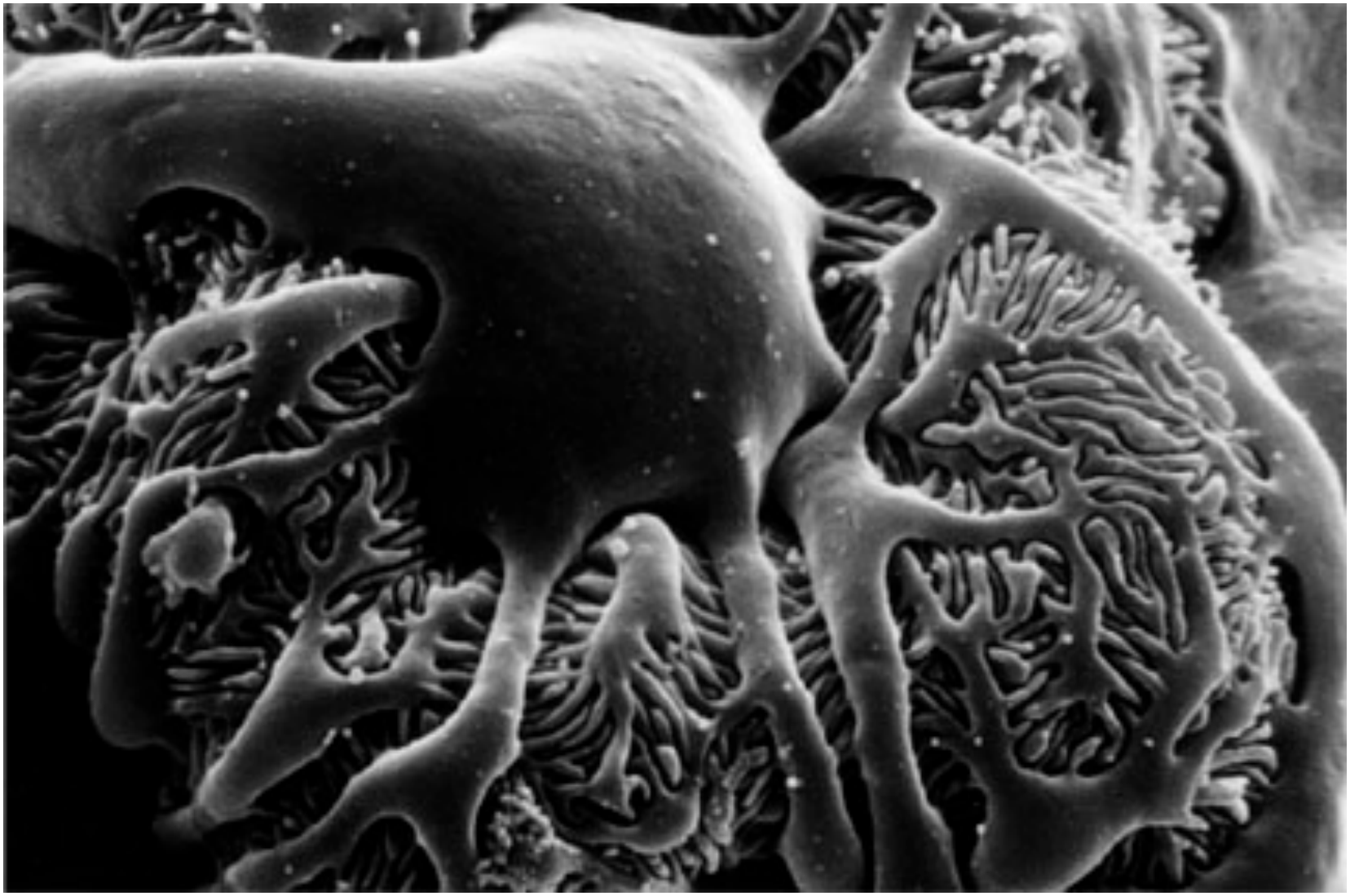
Sin embargo, la localización y la naturaleza de las capas filtrantes y los mecanismos exactos de filtración han sido materia de debate.

Por más de 2 décadas, a las cargas negativas de la MB se les adjudicó un rol protagónico como barrera a las macromoléculas;

Estudios en ratones genéticalmente modificados han desafiado esta teoría.



Actualmente, la pieza clave en estos mecanismos de filtración se cree que la juega el podocito.



La importancia de la barrera de filtración glomerular está basad por el mero hecho de que muchas enfermedades tanto renales como sistémicas resultan en proteinuria progresiva y enfermedad renal terminal.

La progresión de algunos tipos de proteinuria y sindromes nefróticos pueden ser enlentecidos o revertidos por esteroides, ciclosporina, ciclofosfamida, IECAS y ARA-II, pero estas drogas no están dirigidas a vías fisiopatológicas específicas.

Dado que la patogenia de las glomerulopatías es aún poco comprendida, la industria farmacológica no ha sido exitosa en desarrollar drogas que se dirijan específicamente a los procesos patológicos en juego.

Sin embargo, este campo de investigación se encuentra en un etapa muy activa y hay descubrimientos seminales que se han realizado.

Los procesos que llevan a la proteinuria son complejos, e involucran factores:

Hemodinámicos,

Tubulares

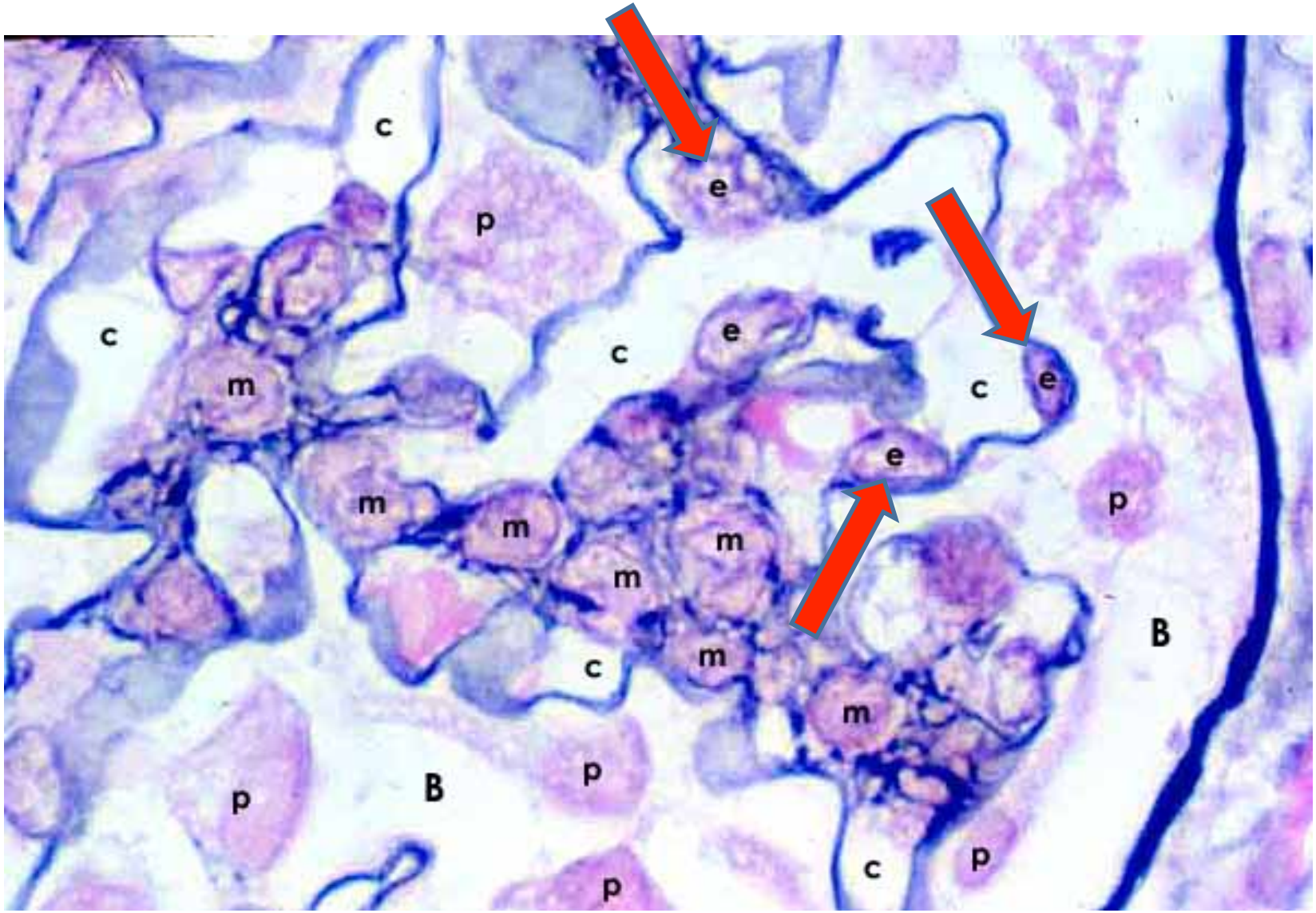
Gradientes de absorción

Gradientes de difusión

La idea es exponer una revisión y actualización de la estructura y función de la barrera de filtración glomerular y la patogénesis de la proteinuria, con un especial énfasis en los podocitos.

1

Célula endotelial glomerular (e)

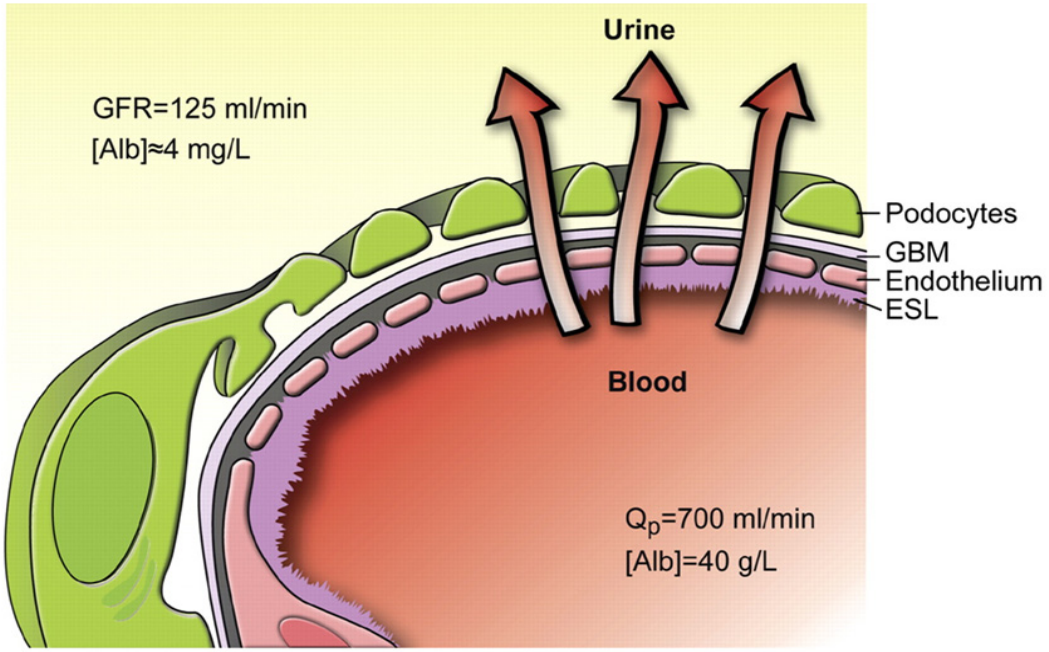


El endotelio del capilar glomerular contiene numerosas fenestras, que constituyen el 20–50% del área de la superficie capilar total.

Estas fenestras son enormes en tamaño en comparación con la albúmina.

Sin embargo, el endotelio presenta a nivel superficial de membrana el glicocáliz, que impediría el pasaje de albúmina y otras proteínas plasmáticas.

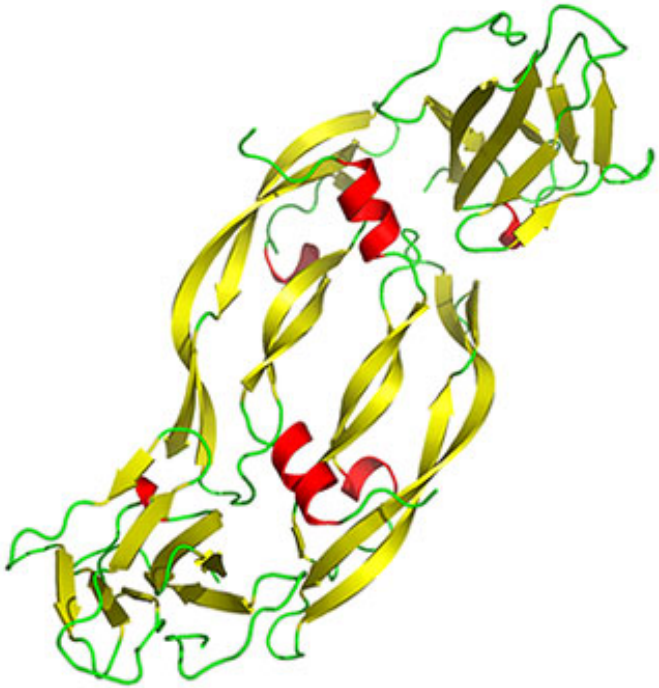
En este sentido, algunos estudios han sugerido que el glicocáliz endotelial podría ser la barrera a la filtración de albúmina, ya que se encontró en modelos animales de proteinuria un adelgazamiento del glicocáliz endotelial.

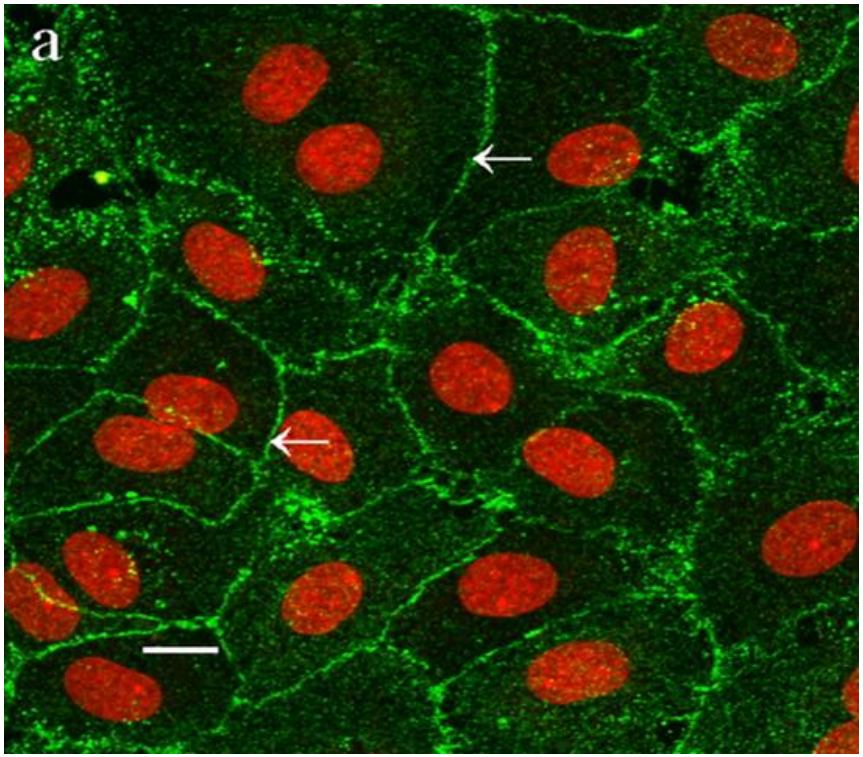


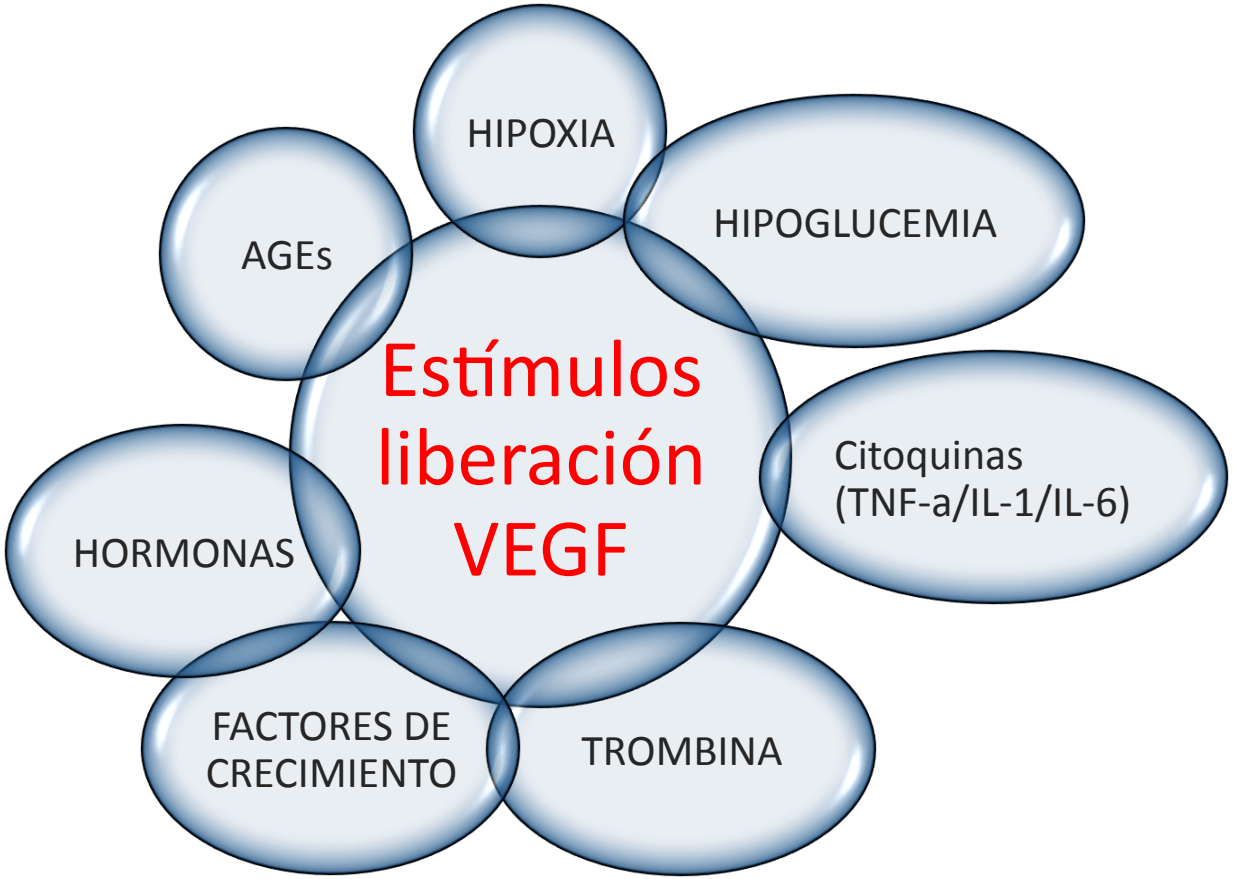
El Factor de crecimiento endotelial vascular (vascular endothelial growth factor (VEGF) es fundamental para el funcionamiento normal del endotelio.

El VEGF es necesario tanto para la formación como para el mantenimiento de la célula endotelial glomerular como para la barrera de filtración.

La célula endotelial glomerular parecería estar involucrada en el desarrollo de la proteinuria en al menos ciertas glomerulopatías que se encuentran bajo revisión.





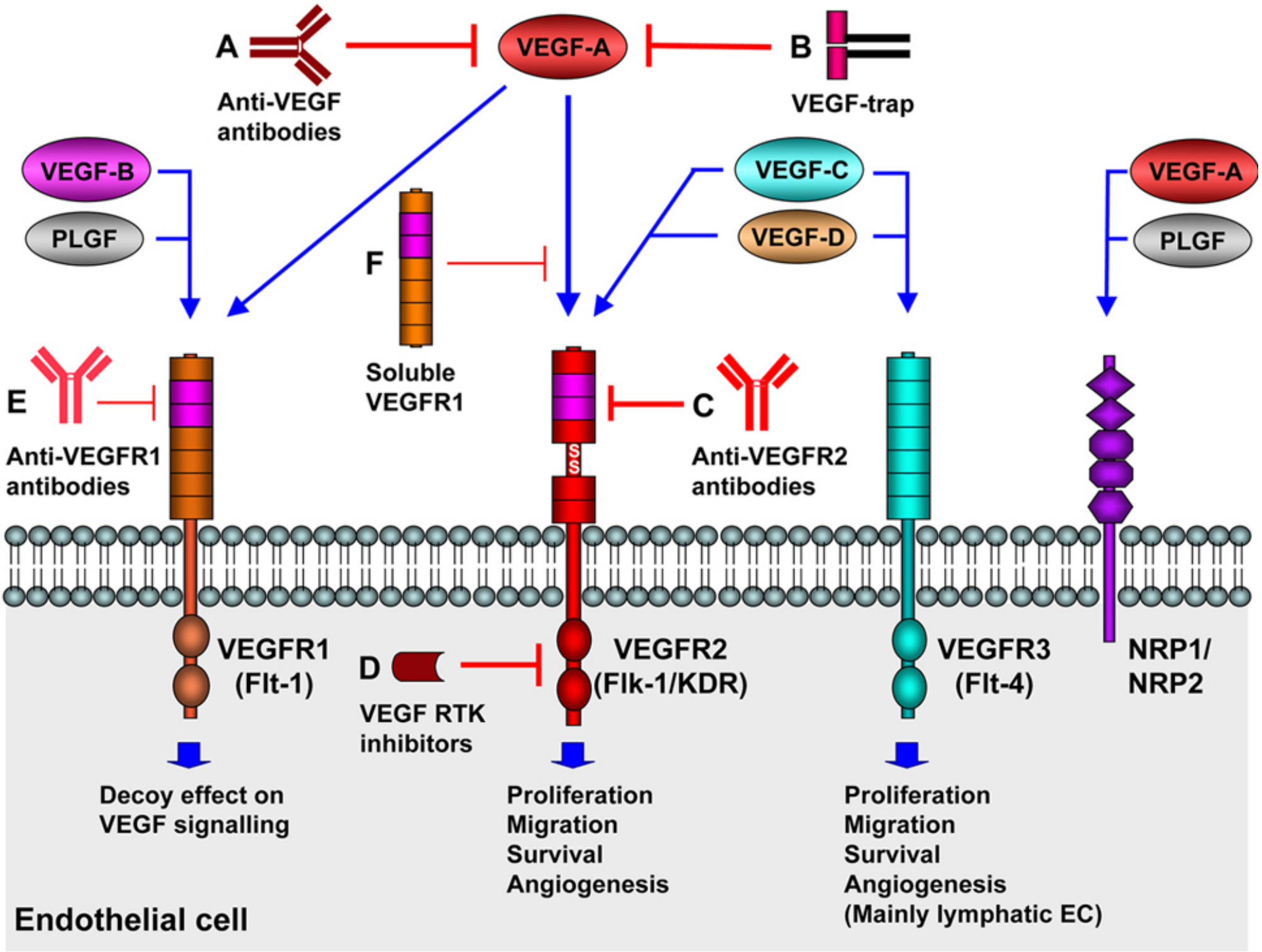


El VEGF juega un Rol fundamental en micro circulación glomerular

Deleción del gen del VEGF

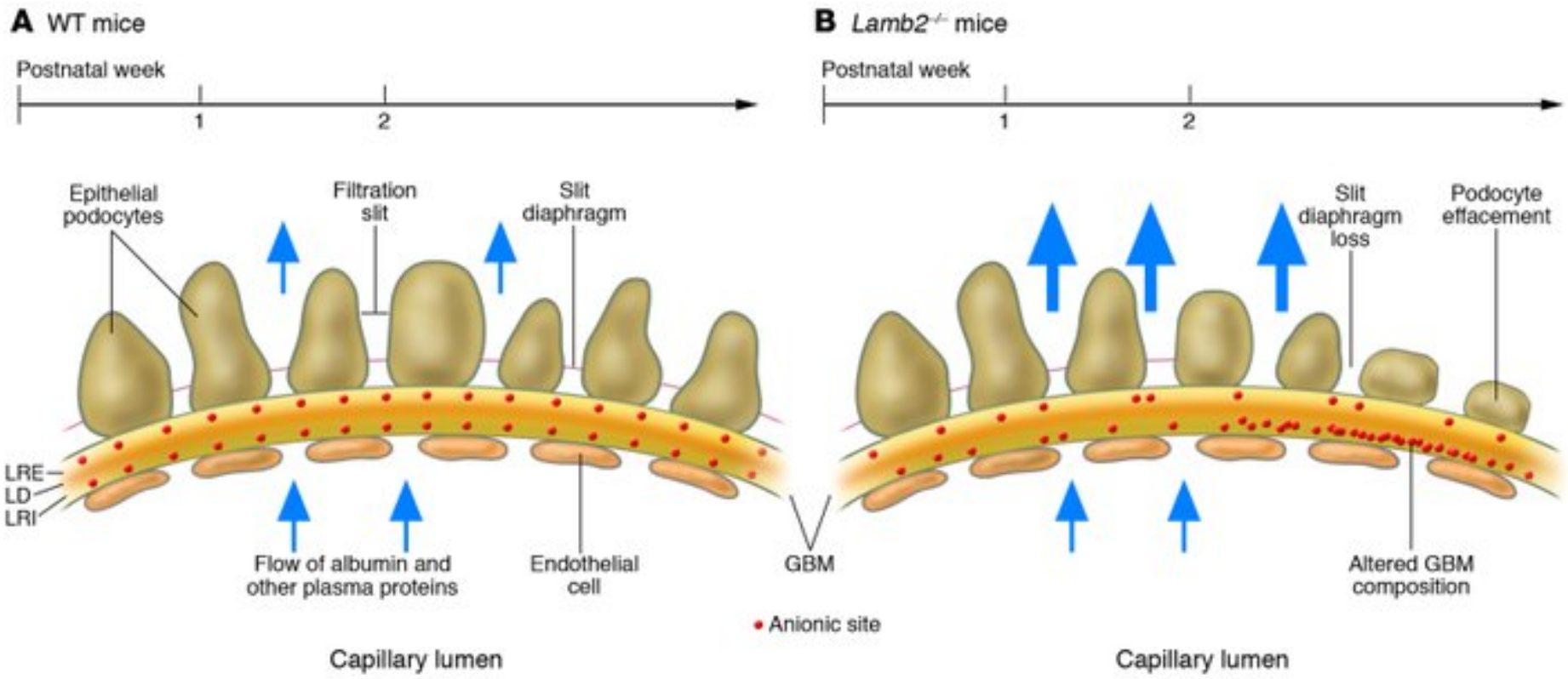
Tratamiento BEVACIZUMAB

Lesiones renales x microangiopatía trombótica


A
Anti-VEGF antibodies
VEGF-A
B
VEGF-trap
VEGF-B
PLGF
F
Soluble VEGFR1
VEGF-C
VEGF-D
VEGF-A
PLGF
E
Anti-VEGFR1 antibodies
C
Anti-VEGFR2 antibodies
S
S
VEGFR1 (Flt-1)
D
VEGF RTK inhibitors
VEGFR2 (Flk-1/KDR)
VEGFR3 (Flt-4)
NRP1/ NRP2
Decoy effect on VEGF signalling
Proliferation
Migration
Survival
Angiogenesis
Proliferation
Migration
Survival
Angiogenesis
(Mainly lymphatic EC)
Endothelial cell

2

Membrana basal glomerular (MBG)



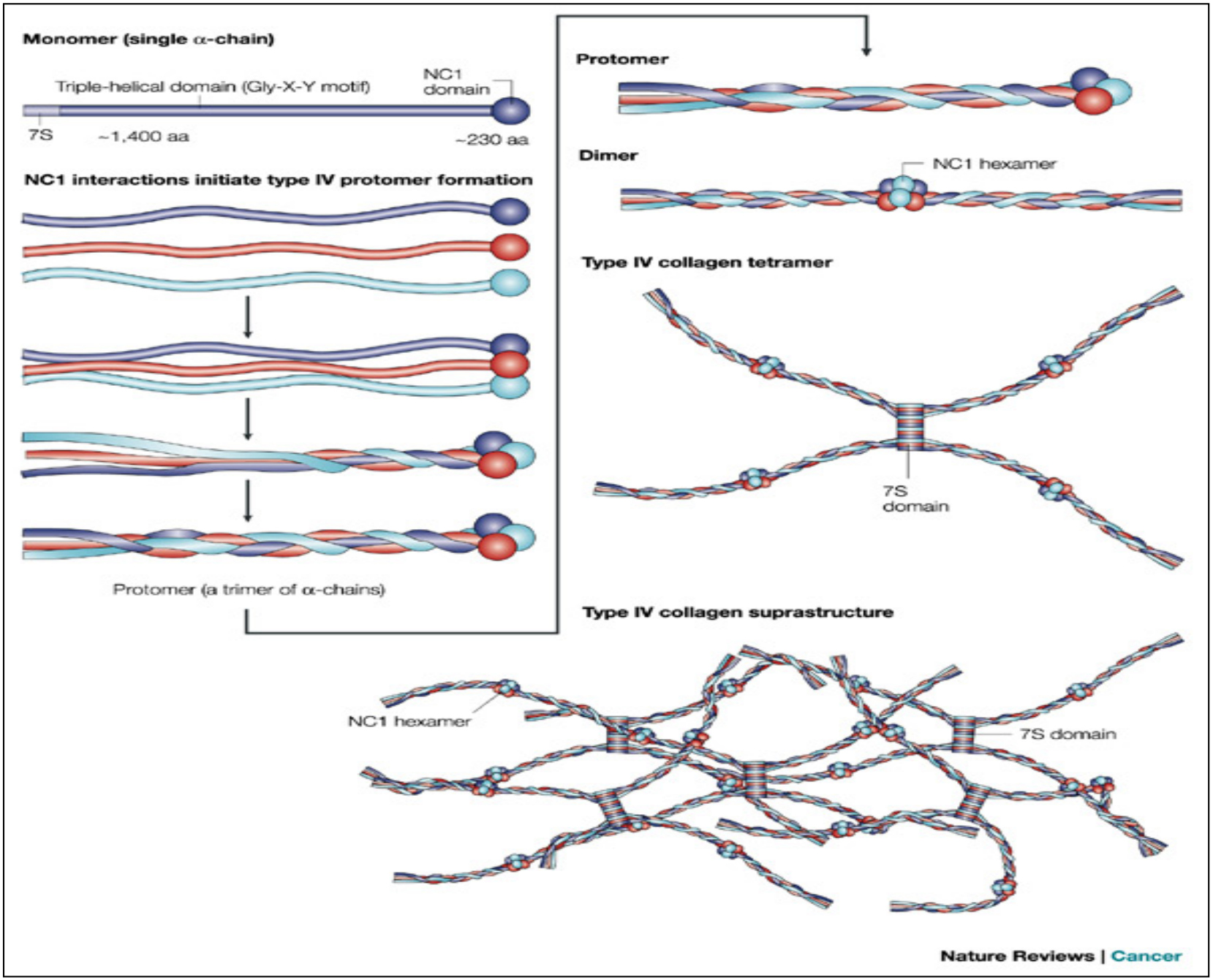
La MBG es una matriz acelular a la cual se adhieren los podocitos y las células endoteliales.

Los componentes de la MBG son el colágeno tipo IV, los proteoglicanos y las lamininas.

El colágeno tipo IV está organizado como una malla entrecruzada de moléculas triple-hélice que provee primariamente soporte estructural a la pared capilar glomerular y contribuye escasamente a la selectividad del tamaño, forma o carga de la MBG.

Esta observación está resaltada por el hecho de que las mutaciones de los genes que codifican para los colágenos de tipo IV alterados en la enfermedad de Alport resultan en una alteración de la MBG que se traduce clínicamente en forma inicial con proteinuria leve en conjunción con hematuria marcada

COLÁGENO


Monomer (single α-chain)
Triple-helical domain (Gly-X-Y motif)
NC1 domain
7S
~1,400 aa
~230 aa
NC1 interactions initiate type IV protomer formation
Protomer (a trimer of α-chains)
Protomer
Dimer
NC1 hexamer
Type IV collagen tetramer
7S domain
Type IV collagen suprastructure
NC1 hexamer
7S domain
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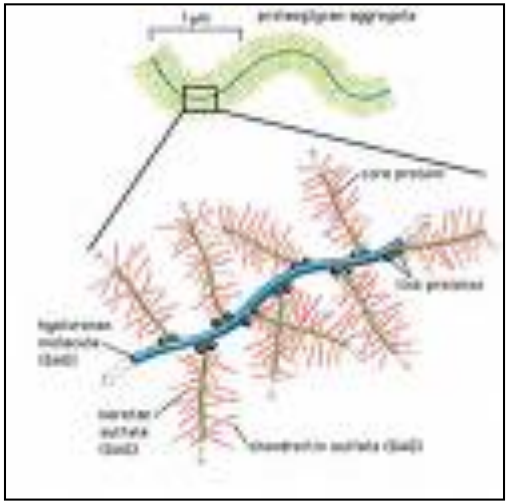
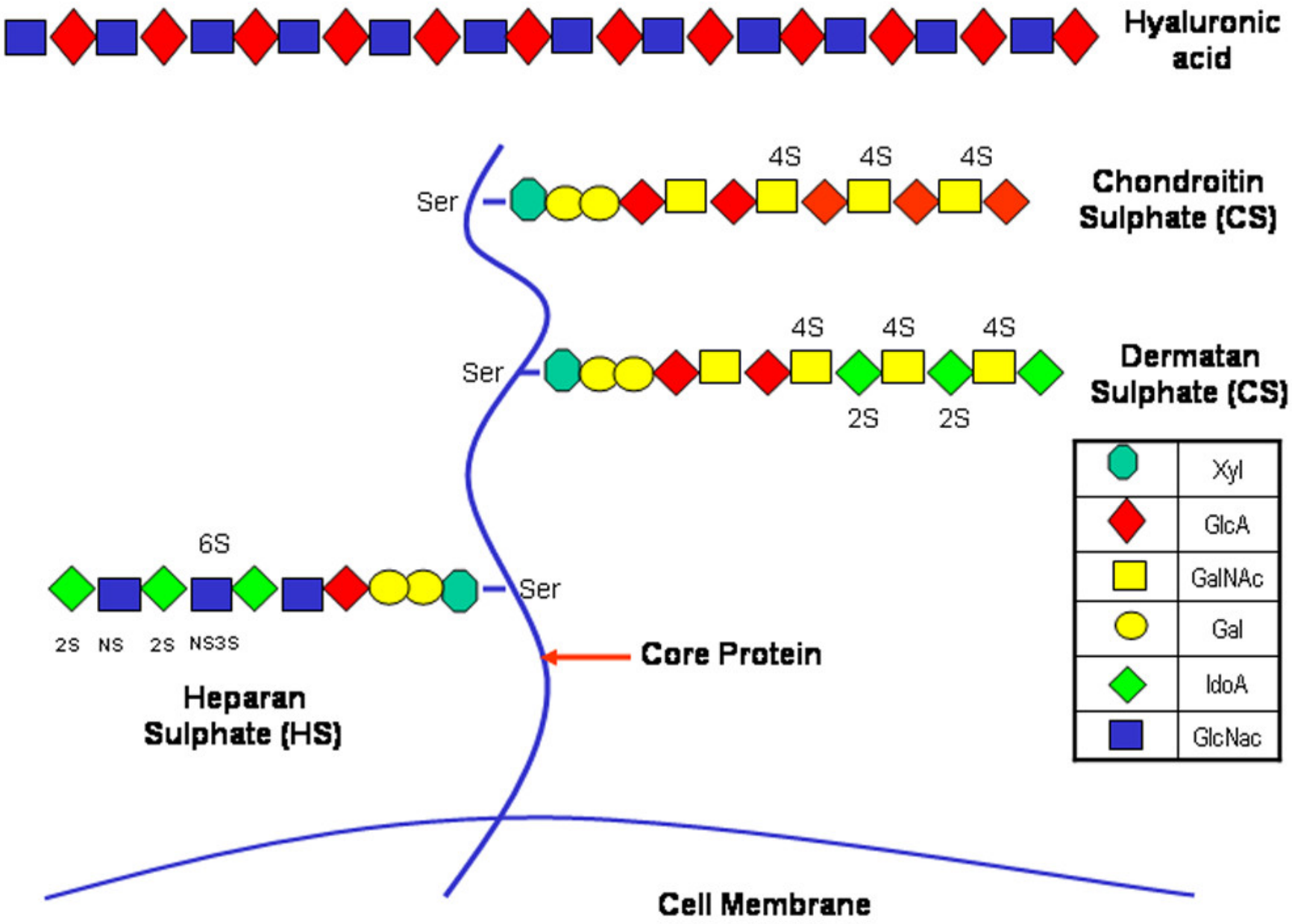
Los proteoglicanos son moléculas heterogéneas compuestas por un eje proteico al cual se unen glicosaminoglycanos de carga negativa.
En la MBG, el heparán sulfato(proteoglicano) es abundante y el responsable de la carga aniónica de la MBG.
Inicialmente fue considerado importante para la función de barrera de filtrado, ya que la administración iv de heparanasas resultó en un aumento de la permeabilidad Glomerular a la ferritina.
Estos hallazgos han sido cuestionados ahora.

Ratones transgénicos que carecen de heparán sulfato o De alguno de sus componentes no desarrollan proteinuria.



PROTEOGLICANO



LAMININA

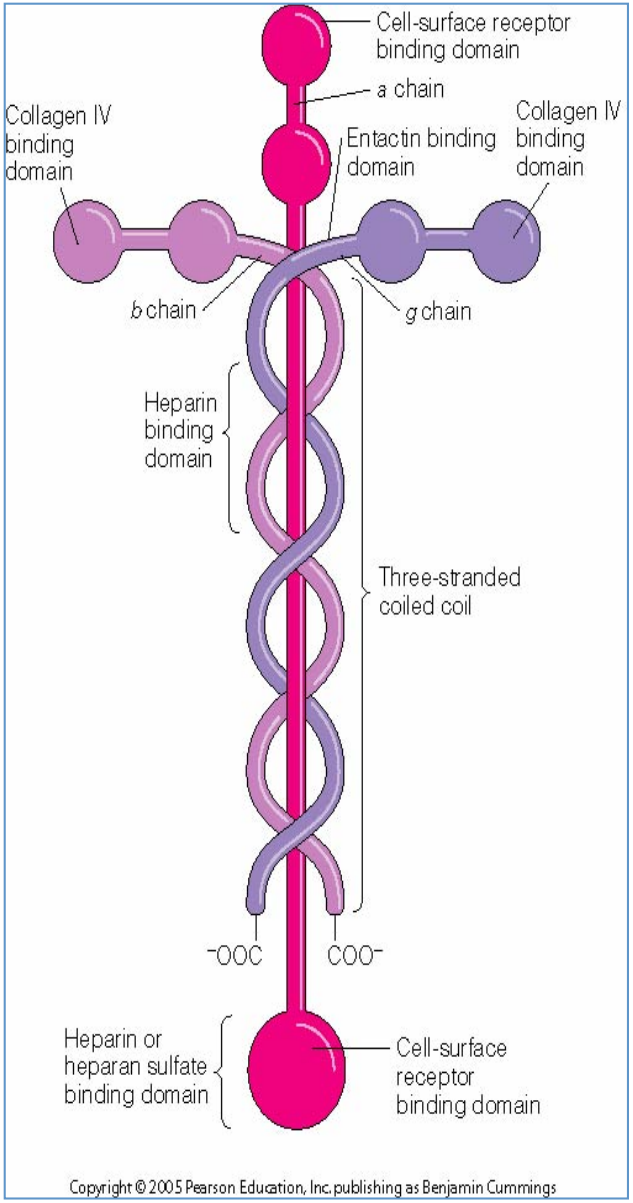
Las lamininas son proteínas heterotriméricas
que se auto-organizan en mallas en la MBG.

La laminina principal es la laminina-521,
crucial como barrera de filtración.

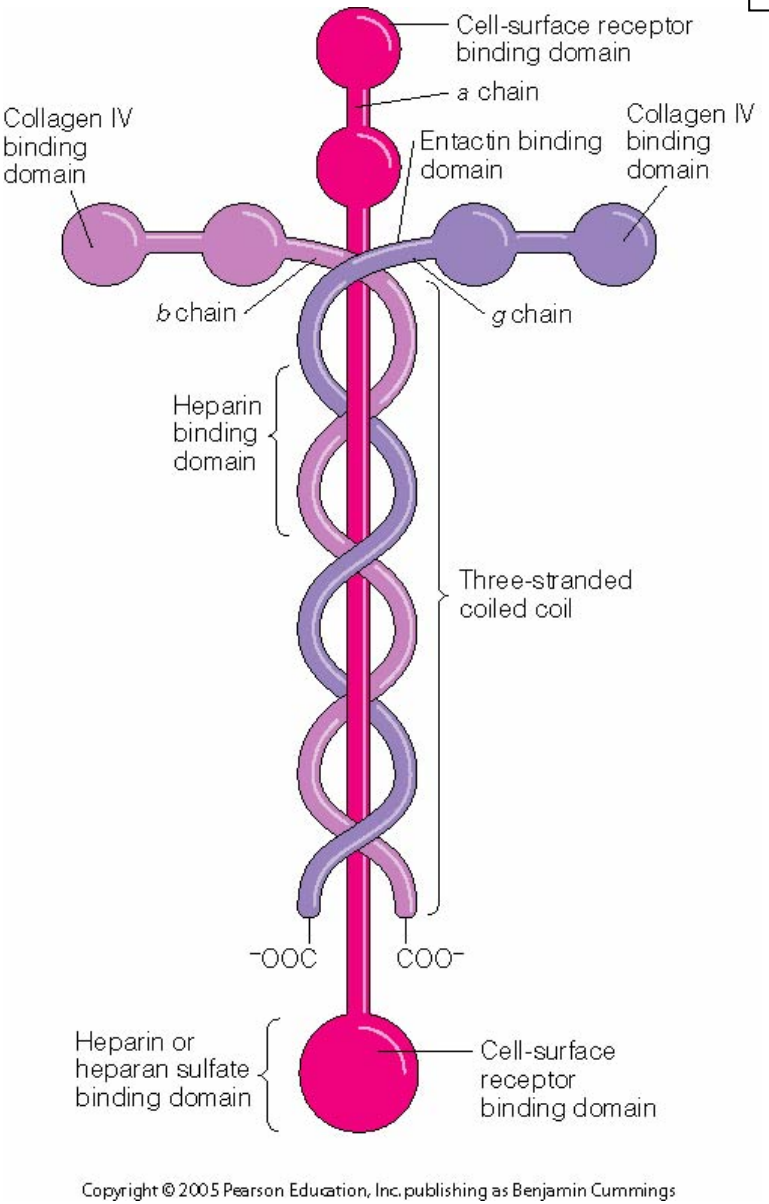
Los ratones que carecen de la cadena β2
Presentan proteinuria y mueren en el período perinatal.

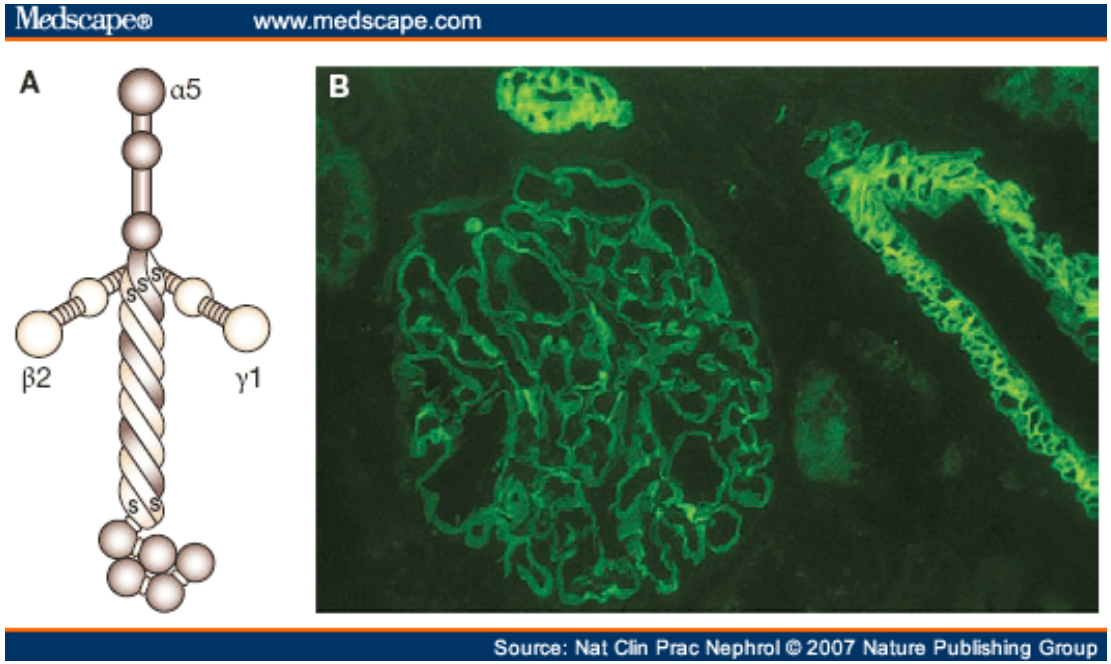
En humanos, mutaciones en el gen β2 causan
el sindrome de Pierson's syndrome,
un sindrome nefrótico asociado a anomalías oculares

En estos casos hay desorganización de la MBG y luego
proteinuria, pero los podocitos, los pedicelos y los
Diafragmas se ven normales.



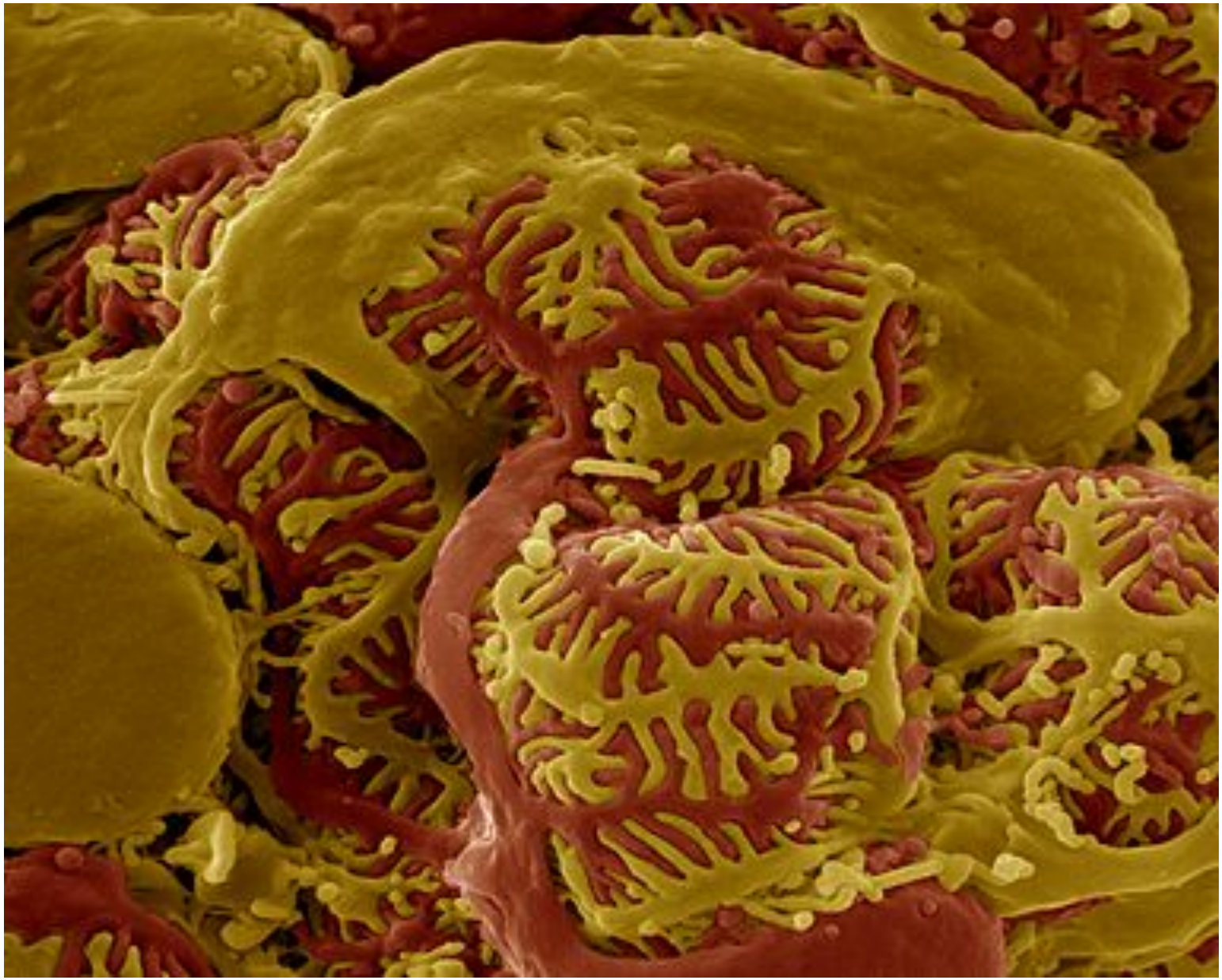
LAMININA





3

PODOCITO



El podocito posee un rol central en el desarrollo de la proteinuria y del sindrome nefrótico

La retracción y desdibujamiento de los pedicelos es un rasgo común
de las enfermedades que cursan con proteinuria.

Este desdibujamiento está asociado con el reemplazo de las hendiduras
de los diafragmas por uniones anormales célula-célula.

Cómo estas típicas alteraciones histopatológicas están involucradas
en la patogenia de la proteinuria es una pregunta clave que aún
no ha sido respondida,

y la correlación de este desdibujamiento pedicelar con el desarrollo de proteinuria
no está del todo claro.

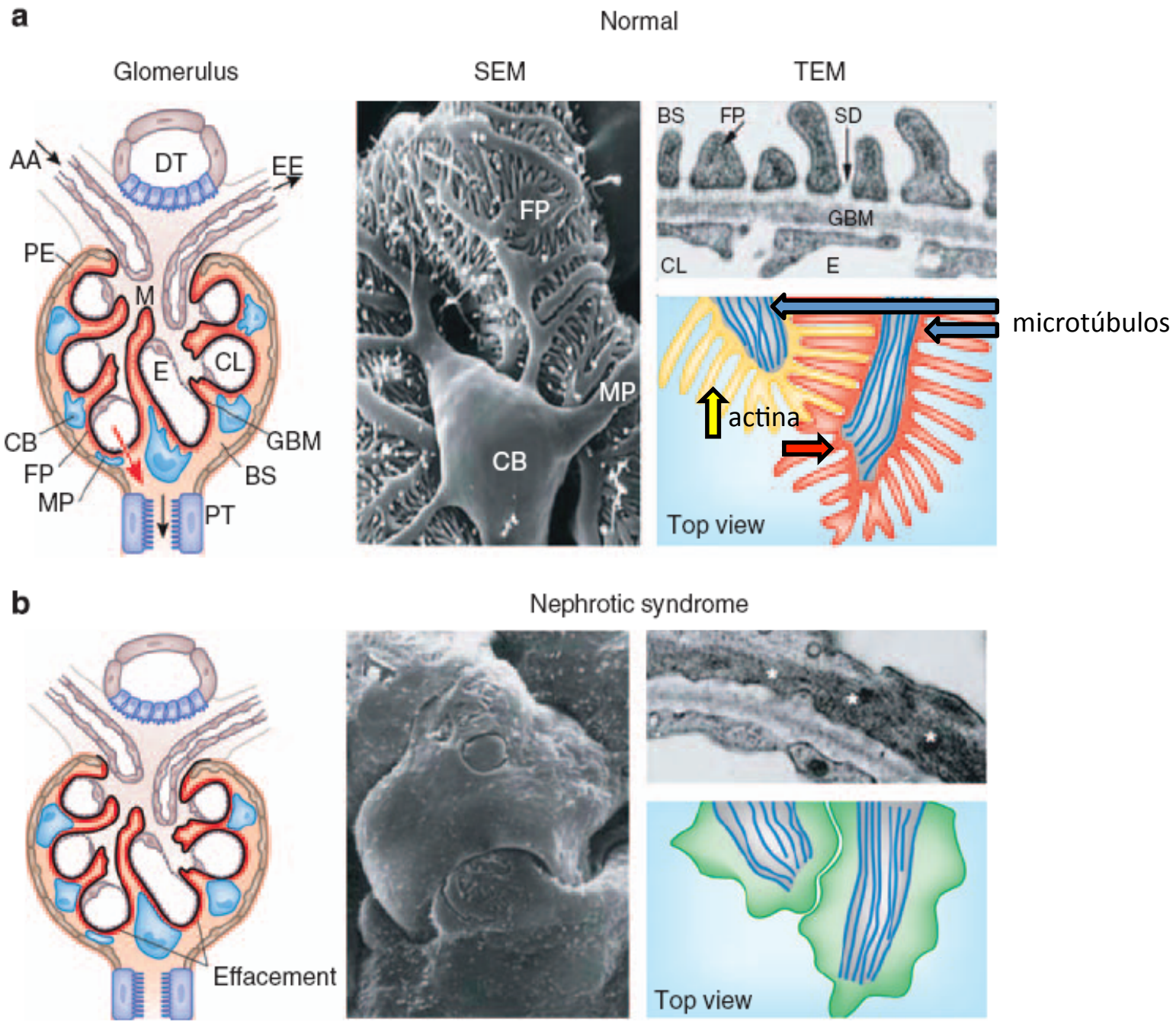
LOS PODOCITOS SON CÉLULAS TIPO PERICITOS CON UN APARATO CONTRÁCTIL BASADO EN LA ACTINA

Los podocitos diferenciados son células mesenquimáticas que provienen de precursores epiteliales durante la ontogenia.

Al igual que los pericitos, los podocitos nunca llegan a abrazar por completo un capilar

Consisten de 3 regiones morfológica y fincionalmente diferentes:
Un cuerpo celular, procesos mayores y pedicelos.

Del cuerpo se extienden los procesos que con su citoesqueleto rico en actina se unen a la membrana basal glomerular y se interdigitan con procesos y pedicelos de podocitos vecinos, conectados por las hendiduras diafragmáticas.


a
Normal
Glomerulus
SEM
TEM
AA
DT
EE
PE
M
E
CL
CB
GBM
FP
BS
MP
PT
FP
MP
CB
BS
FP
SD
GBM
CL
E
microtúbulos
actina
Top view
b
Nephrotic syndrome
Effacement
Top view

La función de los podocitos

Está basada en su compleja arquitectura celular, sobre todo dada por los altamente organizados haces paralelos de actina.

Los pedicelos tienen 3 dominios funcionales:

Dominio apical
Dominio diafragmático
Dominio basal

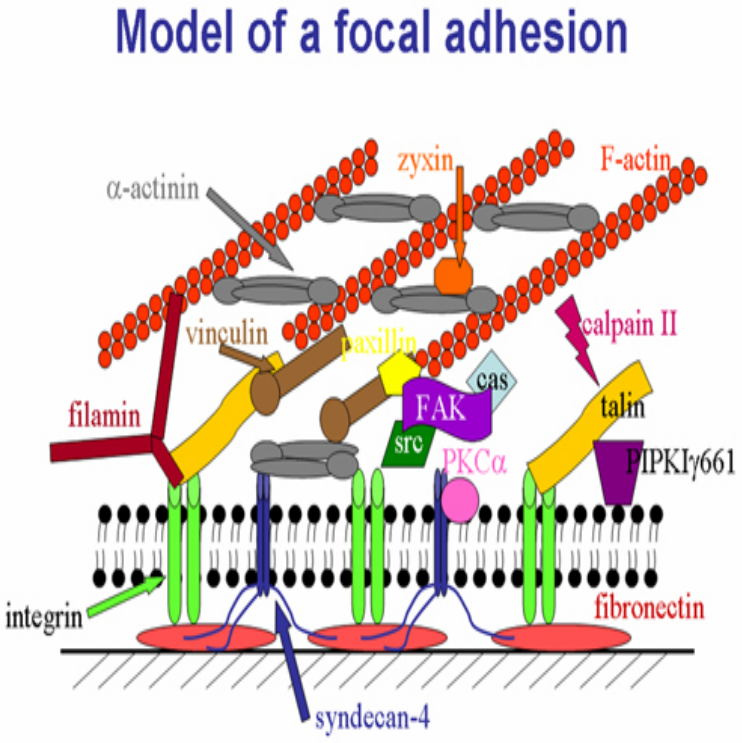
Los 3 dominios están física y funcionalmente ligados al citoesqueleto de actina.

Las proteínas que regulan la plasticidad de la actina son críticas para el funcionamiento del filtro glomerular.

Interfase podocito-MBG

Los podocitos están anclados a la MBG por receptores celulares transmembrana, como los distroglicanos y las integrinas.

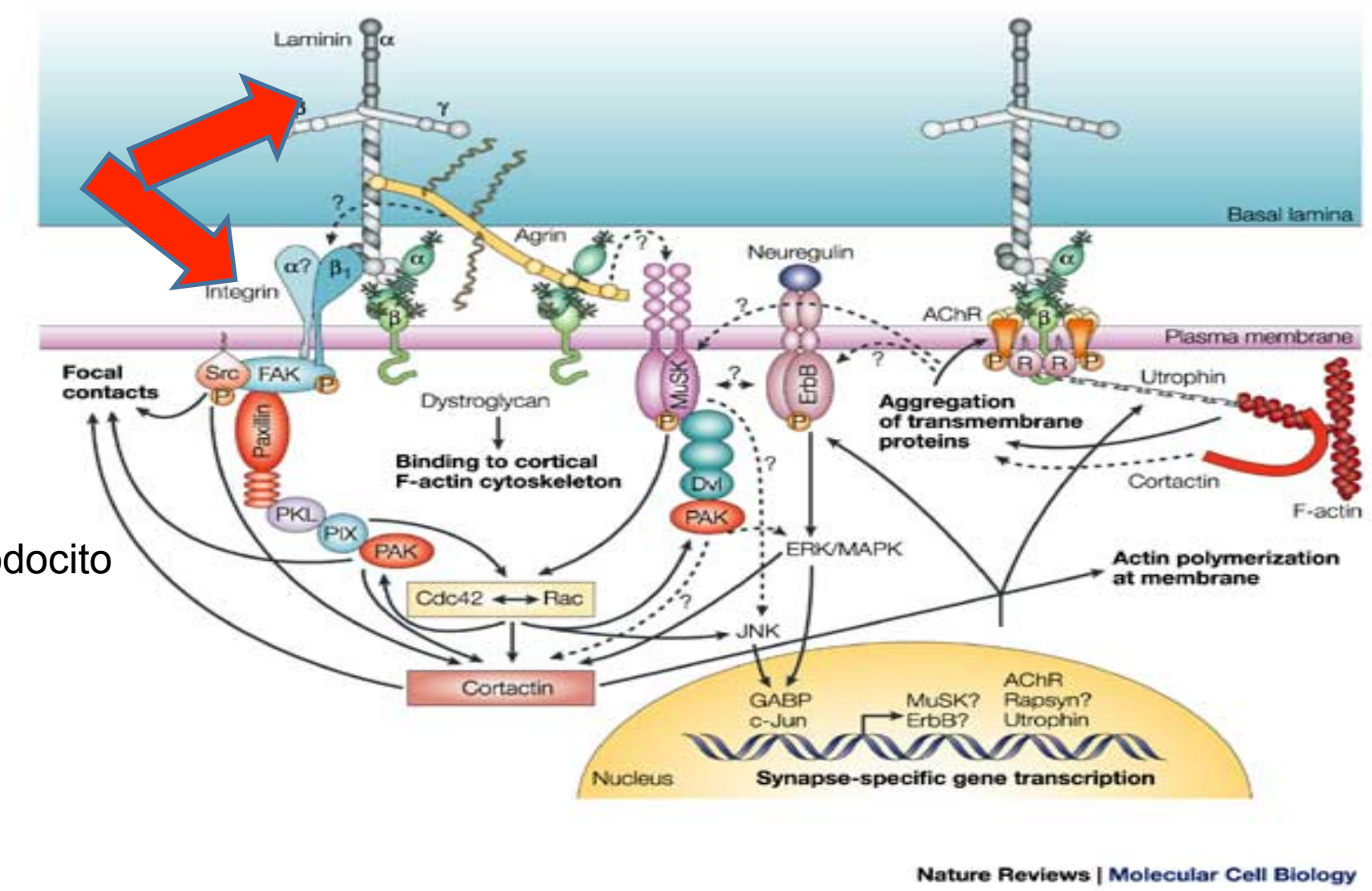
Las integrinas son proteínas αβ heterodiméricas responsables de conectar las células epiteliales a las MB.



En los podocitos, la integrina α3β1 es la más abundante y la cadenaα3 chain es necesaria para el desarrollo del ovillo capilar glomerular:
Los ratones deficientes en integrin-α3exhiben defectos en la ramificación capilar glomerular
y no hay desarrollo pedicelar,
cursando con proteinuria masiva proteinuria

La importancia de la adhesión mediada por la dupla α3β1 está también demostrada por podocitos que carecen del gen β1.

Se especula que la disrupción del complejo integrina–laminina resulta en un debilitamiento de la interacción podocito–MBG y en un despegamiento progresivo de podocitos, el cual se asocia a proteinuria.



podocito

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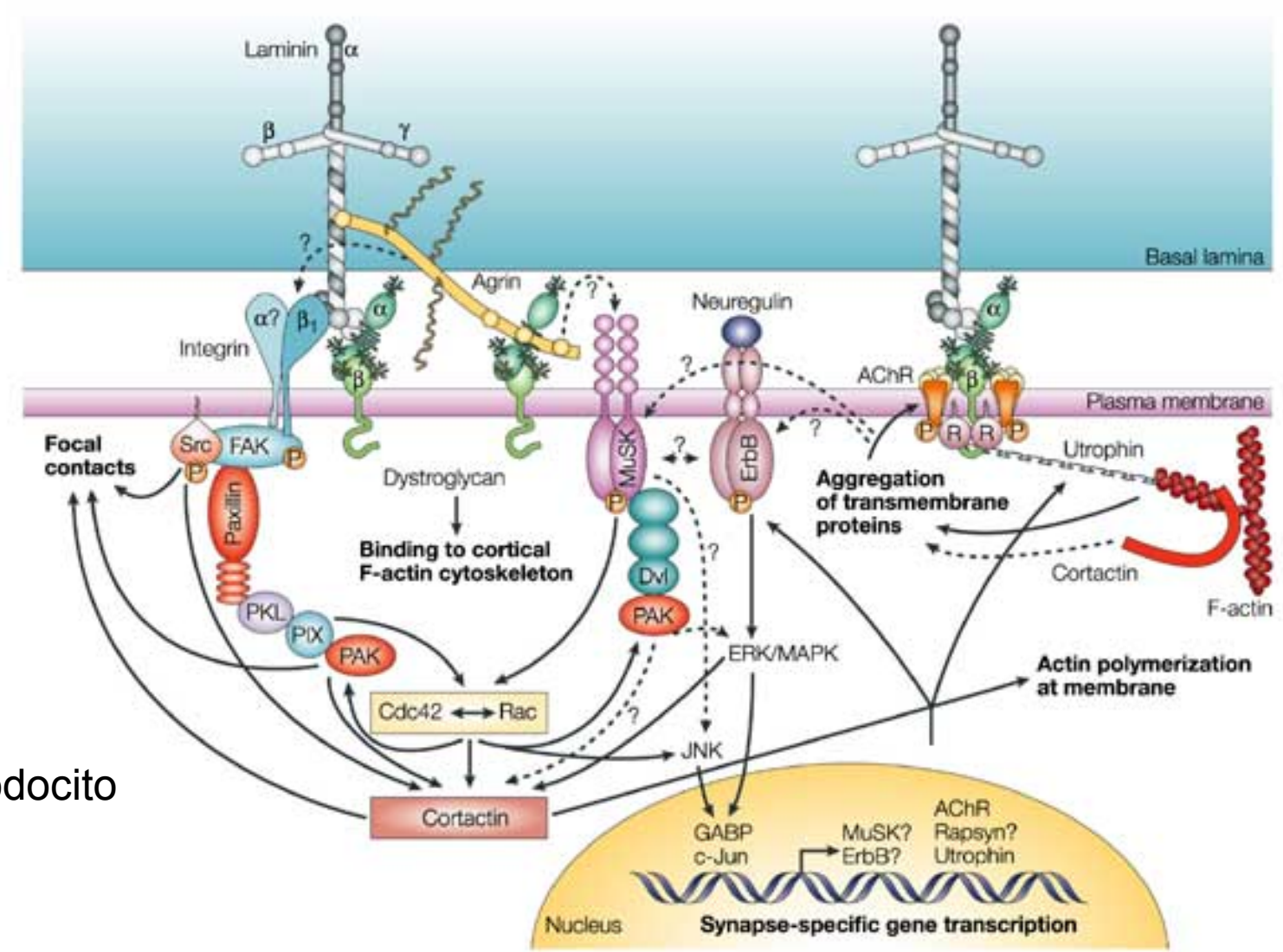
podocito



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La kinasa ligada a la integrina (ilK) es esencial en la barrera de la MBG.
Su inactivación específica lleva a proteinuria progresiva y a esclerosis focal y segmentaria.
Al comienzo hay es engrosamiento de la MBG, seguido de una distribución anormal de las integrinas α3.

La ilK forma un complejo con la nefrina y α-actinina-4, y es crítica para el funcionamiento normal del podocito.
La ilK podría participar en la señalización podocitaria tanto basal como lateral
(en los diafragmas).

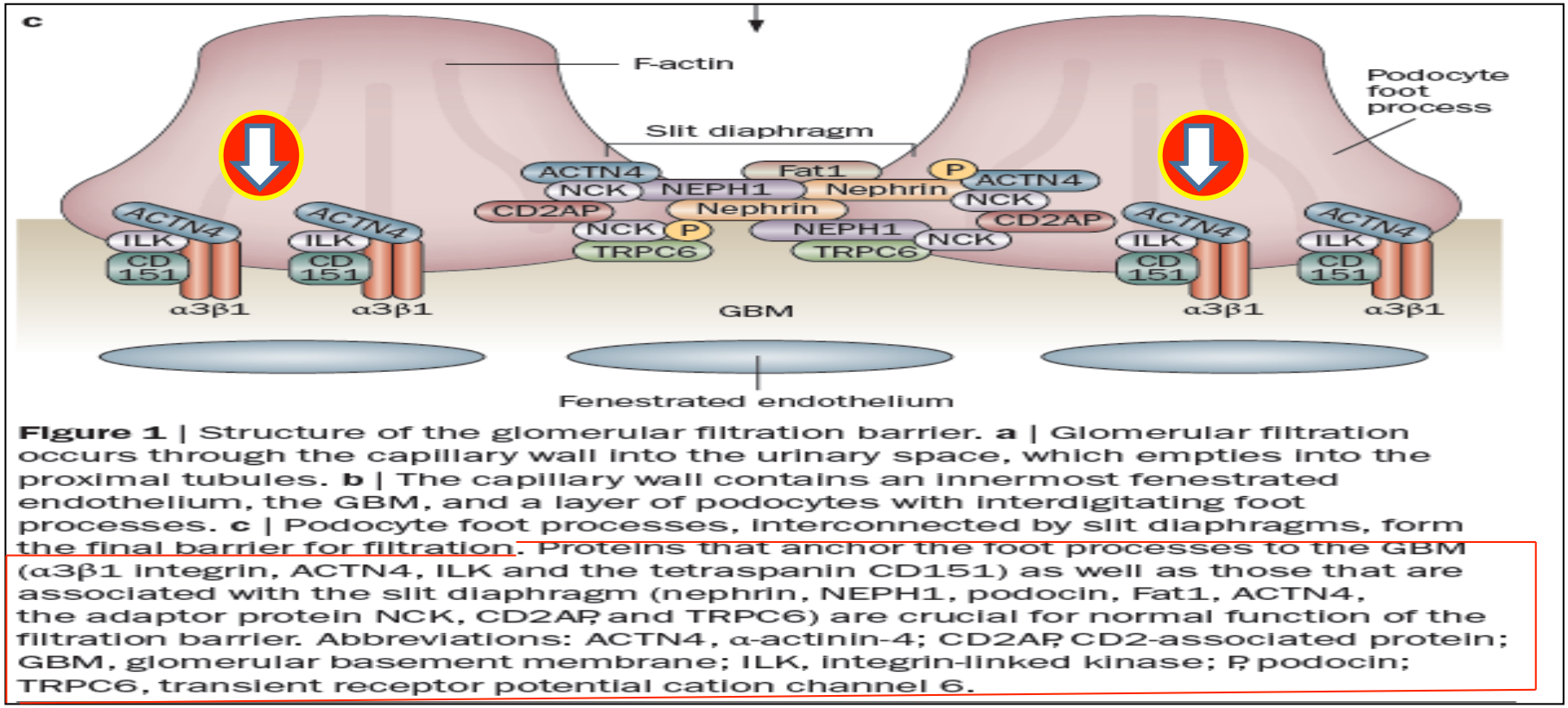
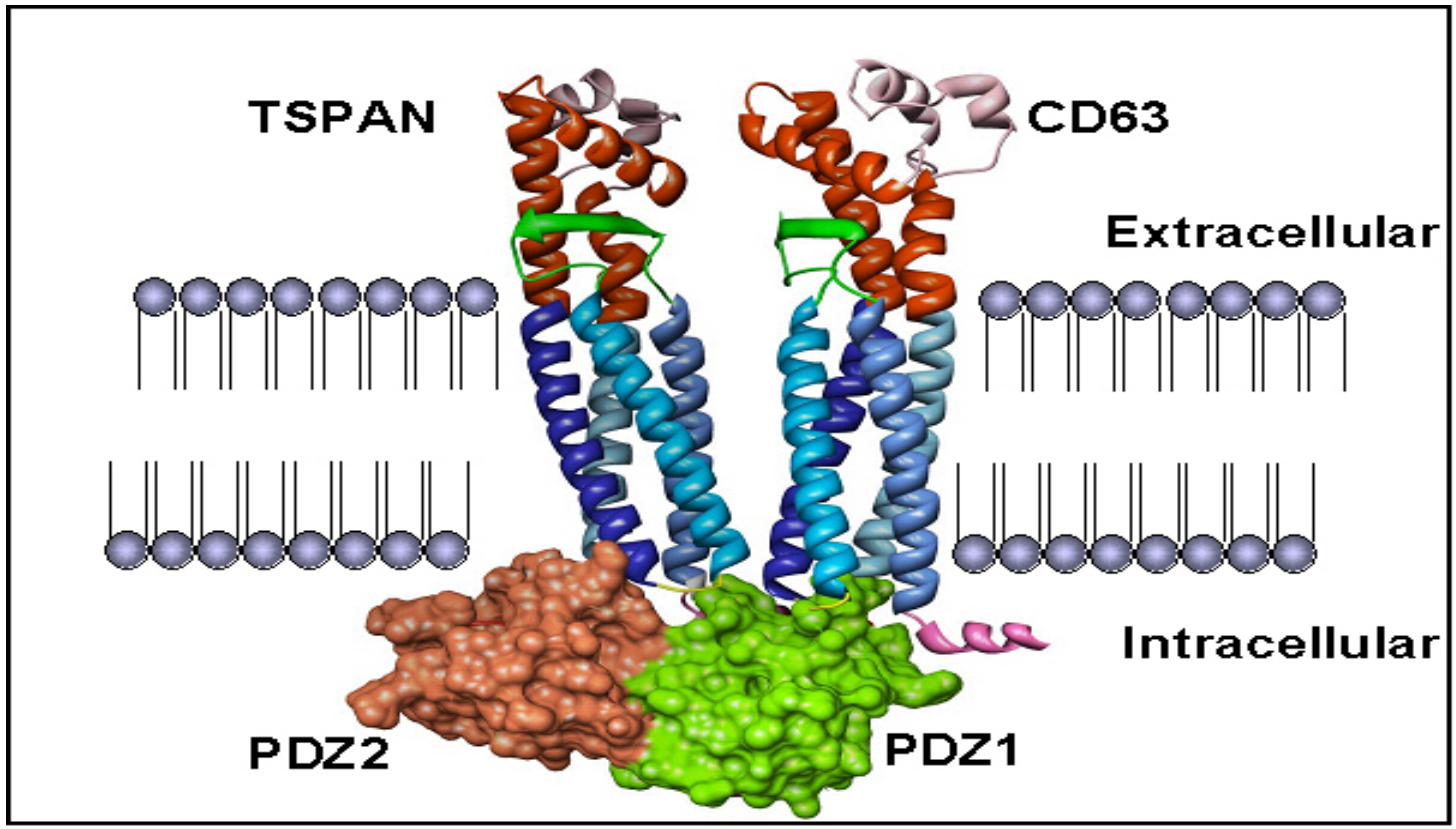


Figure 1 | Structure of the glomerular filtration barrier. **a** | Glomerular filtration occurs through the capillary wall into the urinary space, which empties into the proximal tubules. **b** | The capillary wall contains an innermost fenestrated endothelium, the GBM, and a layer of podocytes with interdigitating foot processes. **c** | Podocyte foot processes, interconnected by slit diaphragms, form the final barrier for filtration. Proteins that anchor the foot processes to the GBM (α3β1 integrin, ACTN4, ILK and the tetraspanin CD151) as well as those that are associated with the slit diaphragm (nephrin, NEPH1, podocin, Fat1, ACTN4, the adaptor protein NCK, CD2AP, and TRPC6) are crucial for normal function of the filtration barrier. Abbreviations: ACTN4, α-actinin-4; CD2AP, CD2-associated protein; GBM, glomerular basement membrane; ILK, integrin-linked kinase; P, podocin; TRPC6, transient receptor potential cation channel 6.

La interfase podocito–MBG también involucra a las tetraspaninas,
proteínas de transmembrana presentes en virtualmente todos los tipos celulares.
Las tetraspaninas se oligomerizan en microdominios que se asocian con las integrinas.

Por ejemplo, la tetraspanina CD151 tiene una fuerte interacción lateral con la integrina α3β1. En los podocitos, esta interacción es importante para la adhesión a la MBG, ya que los ratones CD151 knock-out desarrollan proteinuria, laminación y espigas en la MBG y desdibujamiento de los pedicelos.

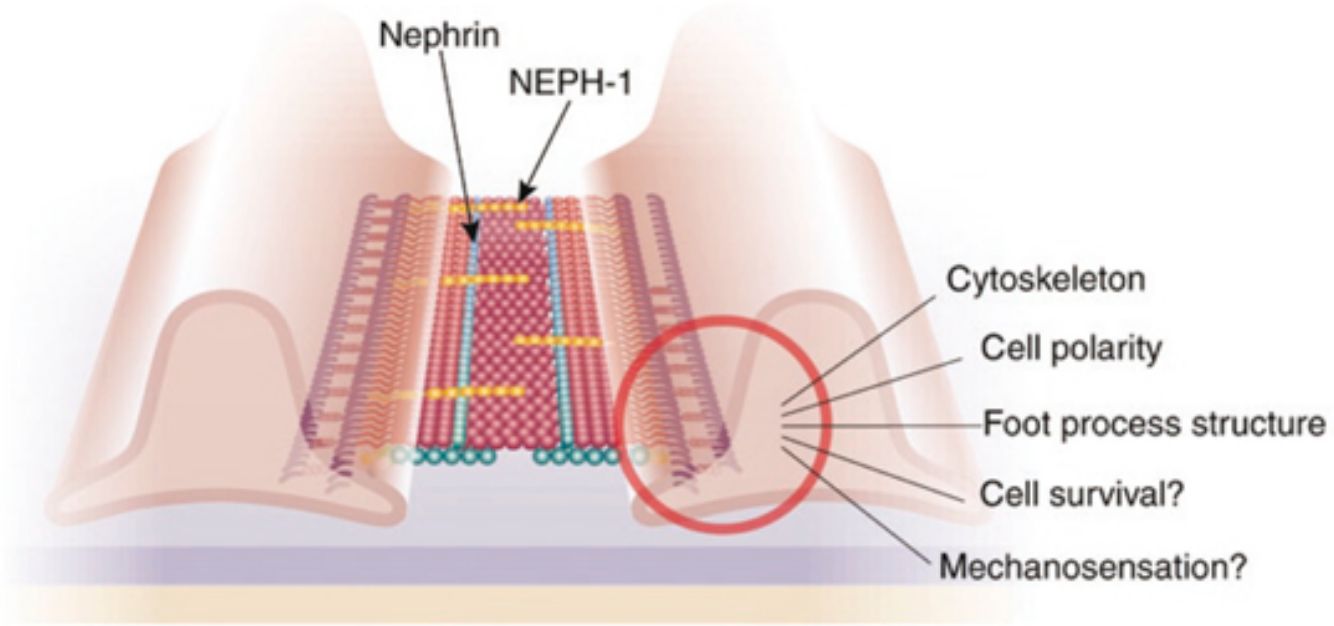


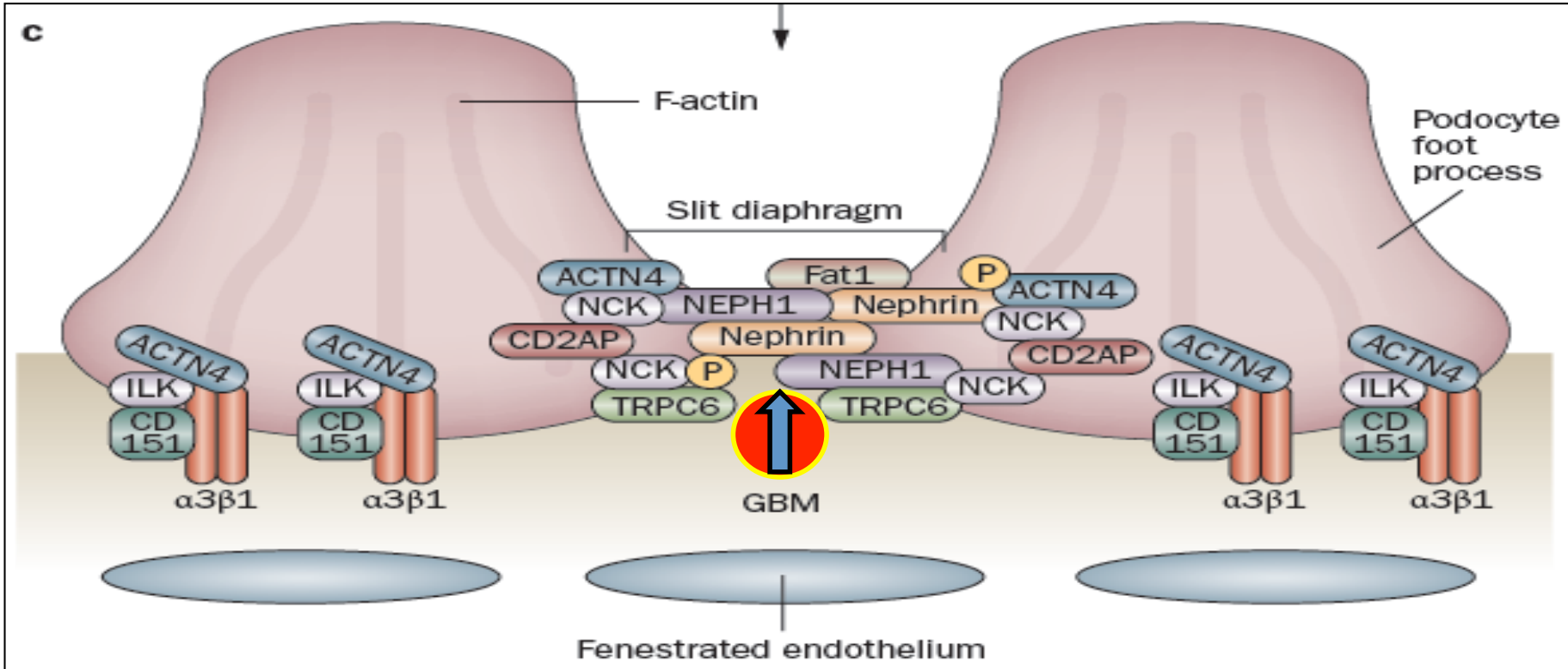
El Diafragma

El diafragma conecta pedicelos adyacentes y forma el último paso en la barrera final de filtración, con un ancho de 30 a 50 nm.
Tiene forma de cierre y son de un tamaño similar al de la albúmina.
Está compuesto por un complejo de proteínas de membrana:
nefrina, nePH1–3, podocina, Fat1, ve-cadherina, y P-cadherina.

La nefrina, nePH1, podocina, y Fat1 son necesarias para la formación de la barrera normal de filtración, no así la P-cadherina.

Las funciones de la ve-cadherina y de la nePH2–3 en el glomérulo se desconocen





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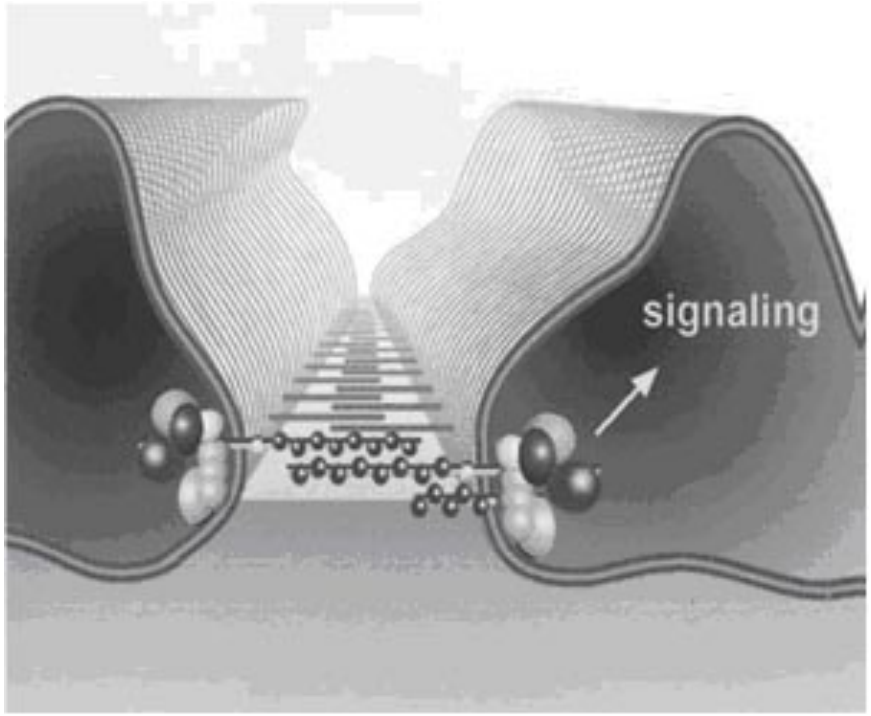
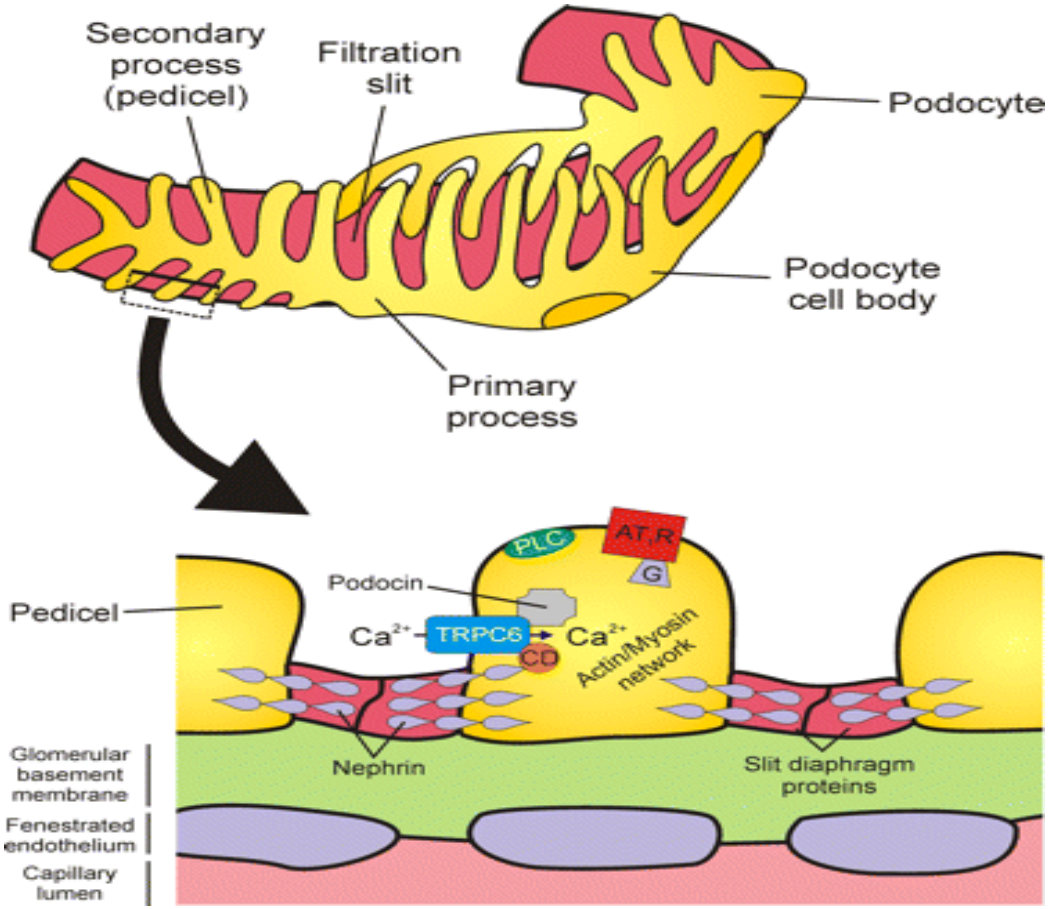
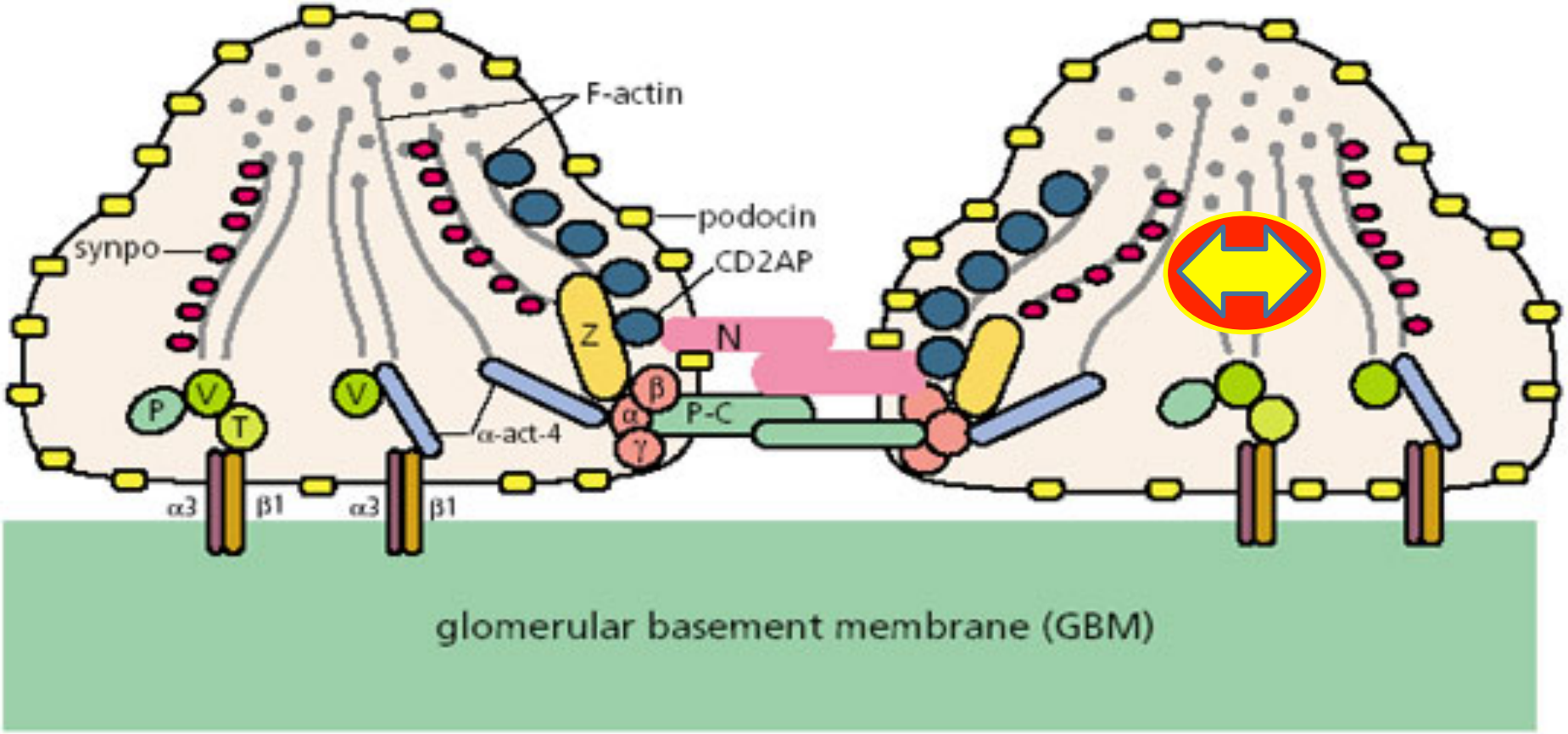
La hendidura del diafragma
está conectada al citoesqueleto
de actina por proteínas conectoras,
incluyendo a laCD2aP y a la nCK.







Alteraciones en la CD2aP y en la proteína asociada a la actina llamada sinaptopodina resulta en proteinuria nefrótica.

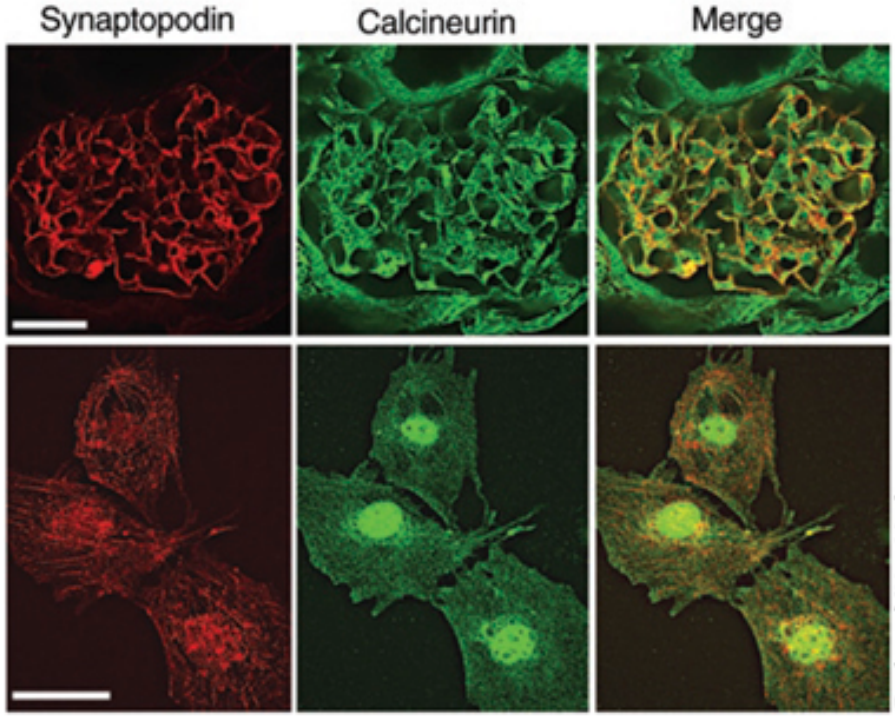
El efecto antiproteinúrico de la ciclosporina está mediado por la estabilización de la sinaptopodina.

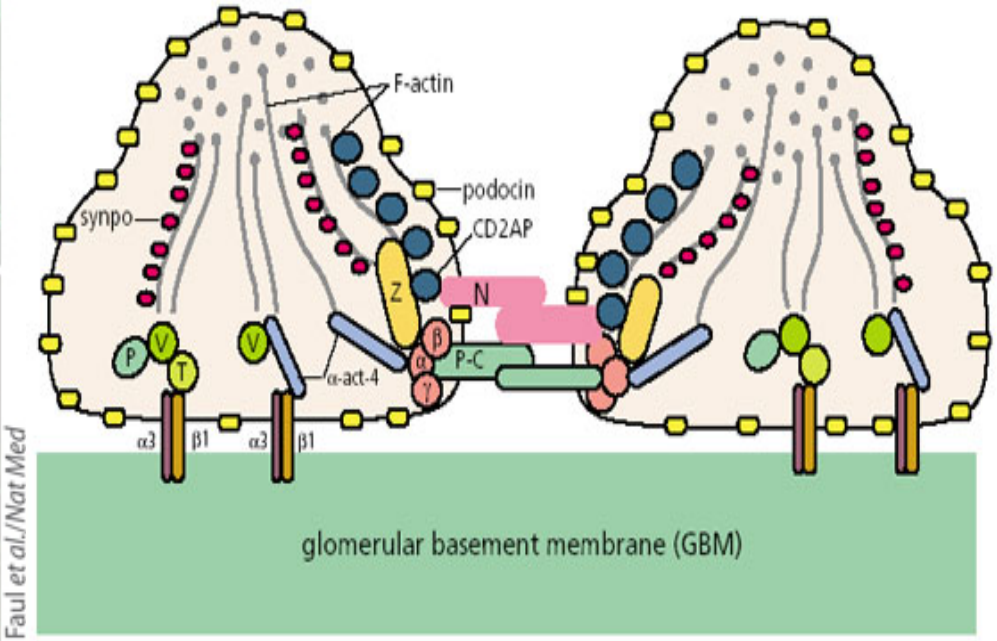
CICLOSPORINA

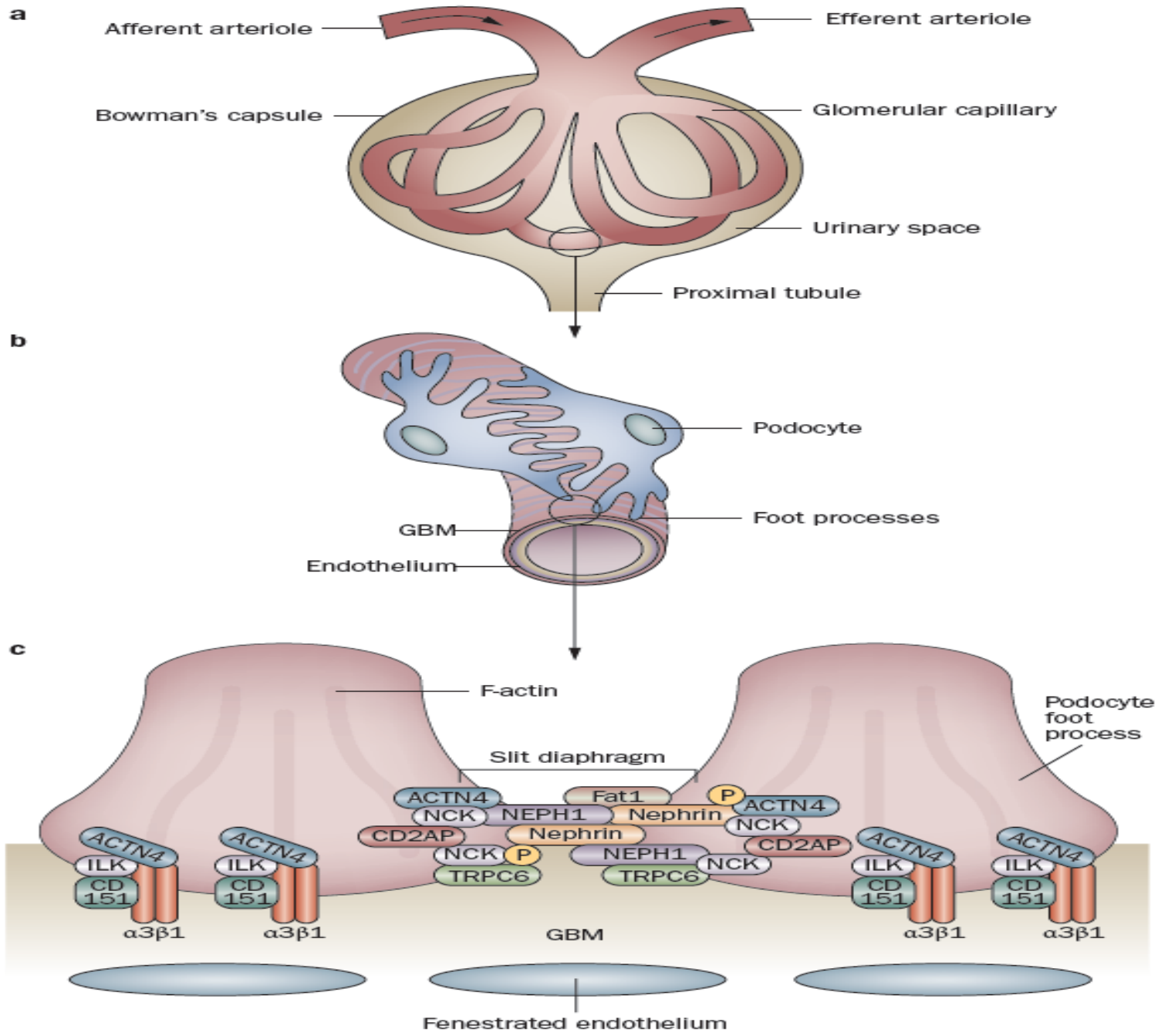
La CsA bloquea la defosforilación de la sinaptopodina, una proteína organizadora de la actina del podocito. Este bloqueo inhibe la proteólisis de la sinaptopodina, estabilizando las hendiduras diafragmáticas y la contracción-relajación normal del podocito.

Este efecto es independiente de la acción sobre las células B y T.

Interesante: La expresión de calcineurinas en el podocito resulta en la degradación de la sinaptopodina y el desarrollo de proteinuria.






a
Afferent arteriole
Efferent arteriole
Bowman's capsule
Glomerular capillary
Urinary space
Proximal tubule
b
Podocyte
Foot processes
GBM
Endothelium
c
F-actin
Podocyte foot process
Slit diaphragm
ACTN4
Fat1
P
ACTN4
NCK
NEPH1
Nephrin
NCK
CD2AP
Nephrin
CD2AP
NCK
P
NEPH1
NCK
TRPC6
TRPC6
ACTN4
ILK
CD 151
α3β1
GBM
Fenestrated endothelium

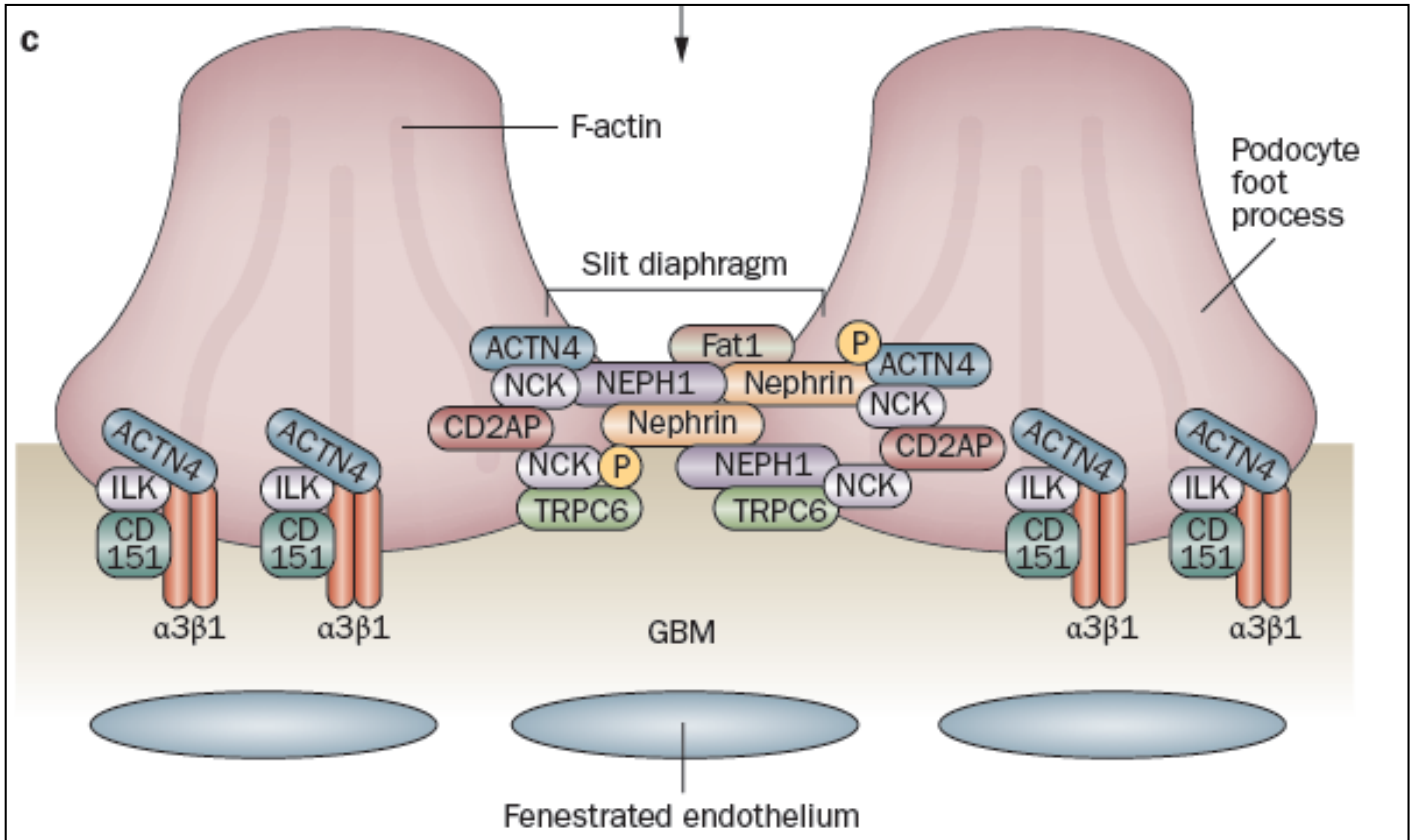


Figure 1 | Structure of the glomerular filtration barrier. **a** | Glomerular filtration occurs through the capillary wall into the urinary space, which empties into the proximal tubules. **b** | The capillary wall contains an innermost fenestrated endothelium, the GBM, and a layer of podocytes with interdigitating foot processes. **c** | Podocyte foot processes, interconnected by slit diaphragms, form the final barrier for filtration. Proteins that anchor the foot processes to the GBM (α3β1 integrin, ACTN4, ILK and the tetraspanin CD151) as well as those that are associated with the slit diaphragm (nephrin, NEPH1, podocin, Fat1, ACTN4, the adaptor protein NCK, CD2AP, and TRPC6) are crucial for normal function of the filtration barrier. Abbreviations: ACTN4, α-actinin-4; CD2AP, CD2-associated protein; GBM, glomerular basement membrane; ILK, integrin-linked kinase; P, podocin; TRPC6, transient receptor potential cation channel 6.

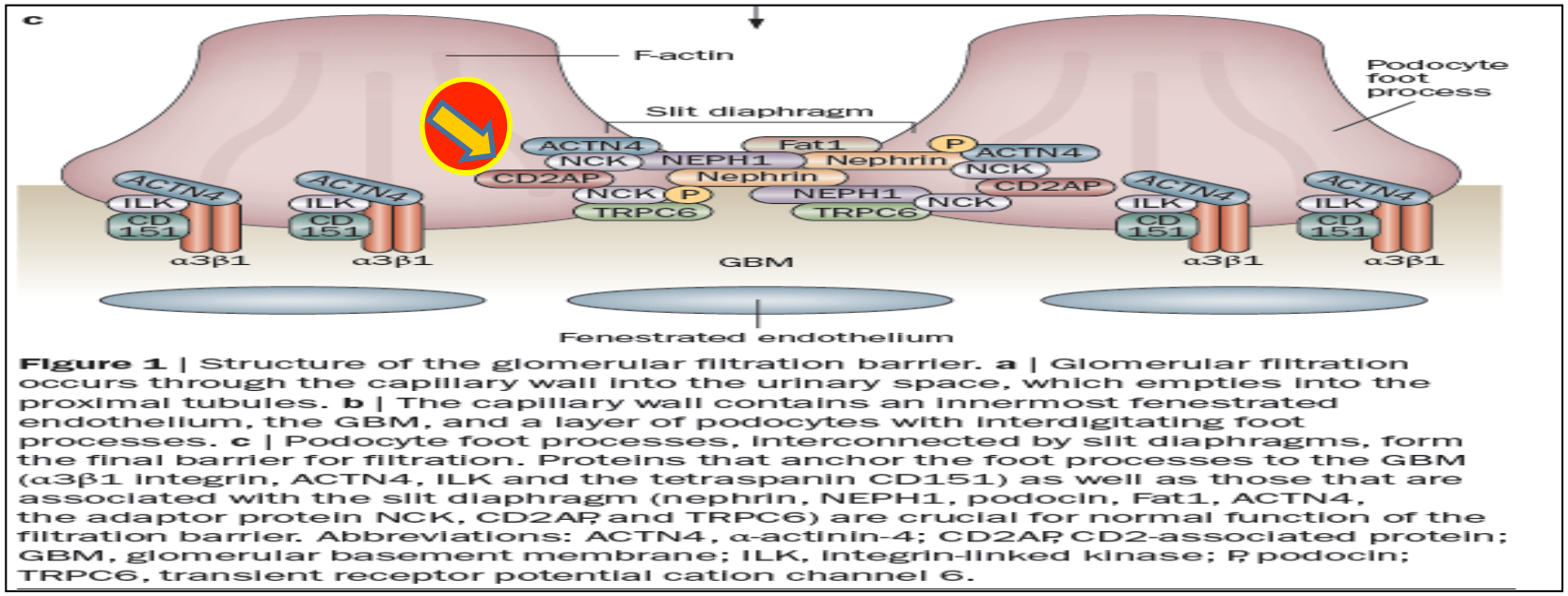
La subfamilia nCK de proteínas adaptadoras posee 2 miembros:—nCK1 and nCK2.

Estas proteínas nCK interactúan con residuos de fosfotirosina y reclutan proteínas involucradas en la regulación del ensamblaje de actina.

Las proteínas *nCK* sirven como conectores cruciales entre la nefrina y la actina en los podocitos.

La fosforilación de residuos de tirosina en la nefrina le permiten asociarse a las proteínas nCK, fundamental para una filtración normal.

Por lo tanto, las proteínas nCK median la polimerización de la actina y la reorganización del citoesqueleto del pedicelo tanto en el desarrollo coomo en la reparación celular.



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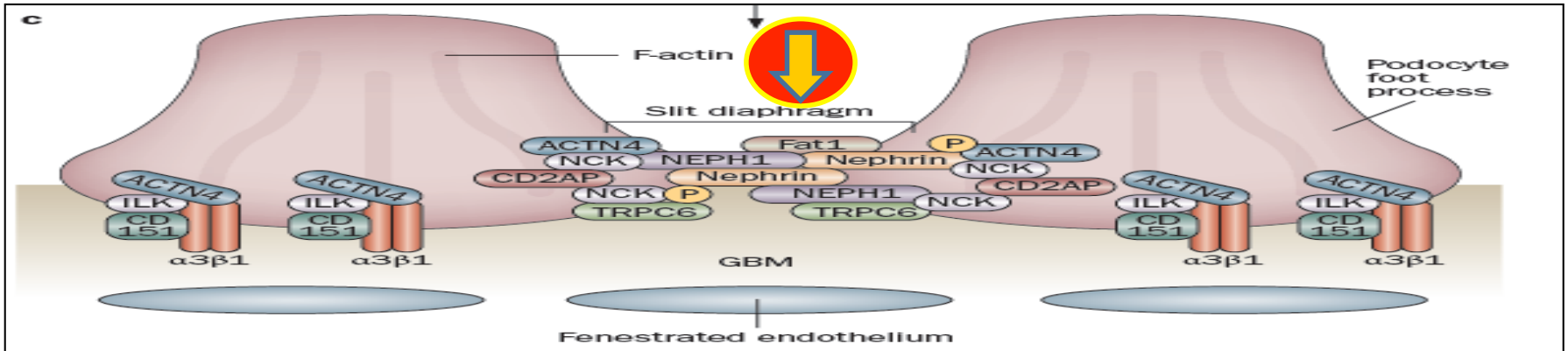
Nephrin-interacting protein *nePH1*

Esta proteína también conecta al diafragma a la actina.

Su fosforilación aumenta la polimerización de la actina luego de la fosforilación de la nefrina.

Tanto las proteínas CD2aP y nCK son fundamentales en la conexión funcional de la actina al diafragma.

La interacción con CD2aP predominaría en estados estables, mientras que con las proteínas nCK lo sería en el desarrollo y en la injuria podocitaria.



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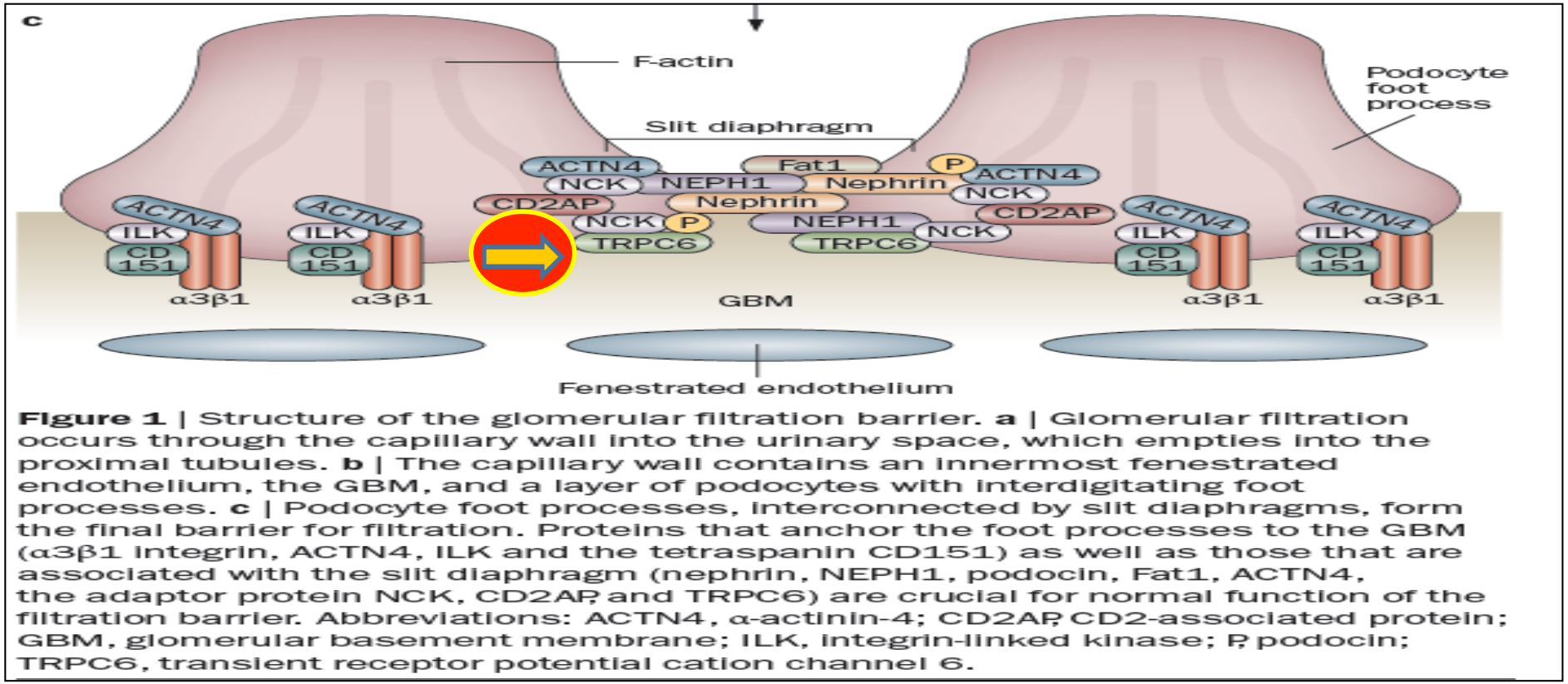
Rol del TRPC6 Transient receptor potential cation channel 6 (trPC6)

Sobreexpresada en familias con FsGs autosómica-dominante.
Estos canales regulan la entrada de calcio intracelular.

En los podocitos, el trPC6 se localiza en la hendidura del diafragma, y participa en la señalización.

Su sobreexpresión resulta en proteinuria.



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LA VIA NOTCH

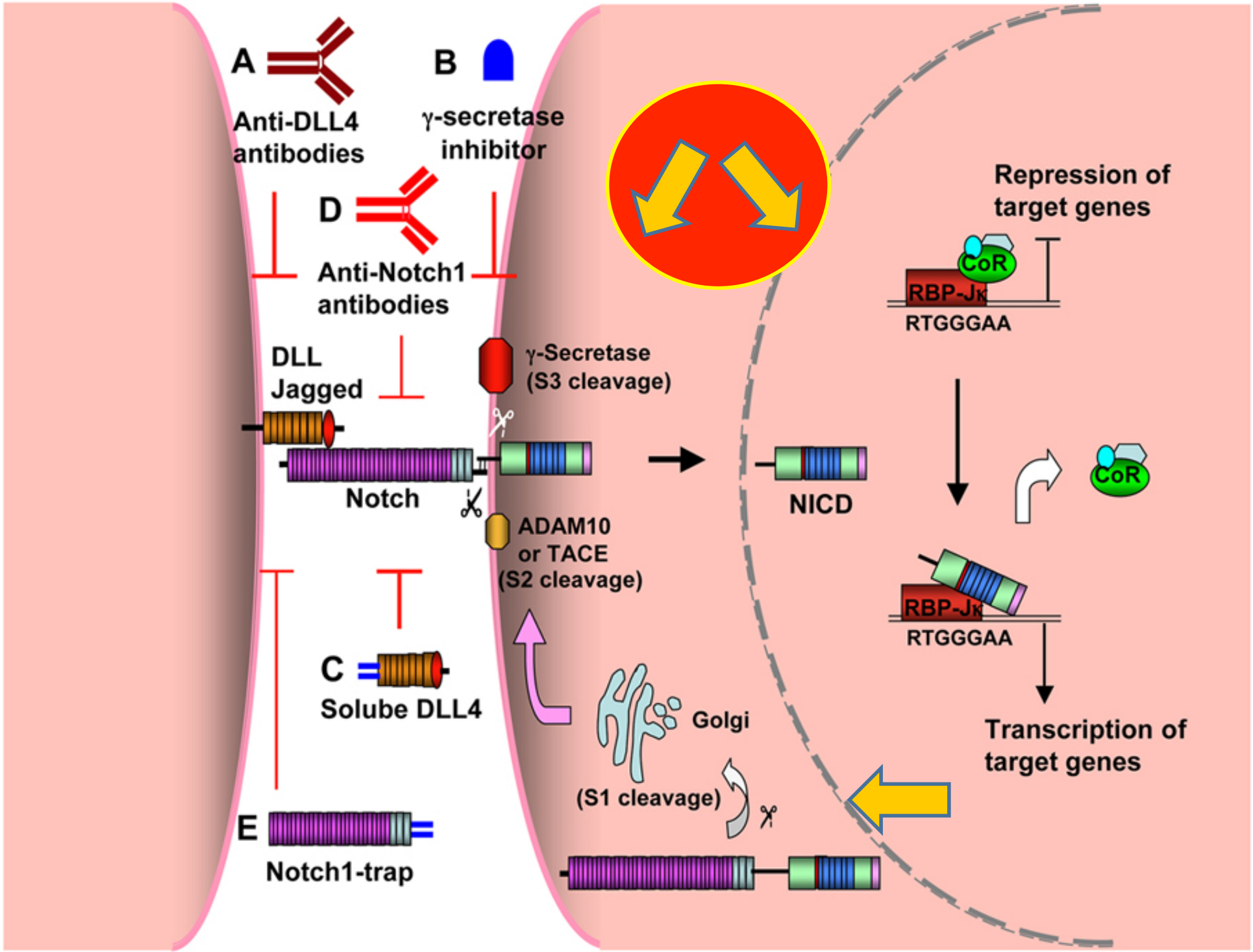
La vía notch ha sido involucrada en la patogenenia de la proteinuria.

Las moléculas notch son proteínas de transmembrana que al activarse por ligandos extracelulares, sufren clivaje proteolítico y liberan el dominio notch intracelular.

Este dominio luego se transloca al núcleo, donde estimula la transcripción de diversos genes.

Su activación se ve en podocitos dañados, y la expresión de notch1 resulta en apoptosis podocitaria, albuminuria, y glomeruloesclerosis.

La supresión de la vía notch atenúa la proteinuria.


A
Anti-DLL4
antibodies
B
γ-secretase
inhibitor
D
Anti-Notch1
antibodies
DLL
Jagged
γ-Secretase
(S3 cleavage)
Notch
ADAM10
or TACE
(S2 cleavage)
NICD
C
Solube DLL4
Golgi
(S1 cleavage)
E
Notch1-trap
Repression of
target genes
CoR
RBP-Jκ
RTGGGAA
CoR
RBP-Jκ
RTGGGAA
Transcription of
target genes

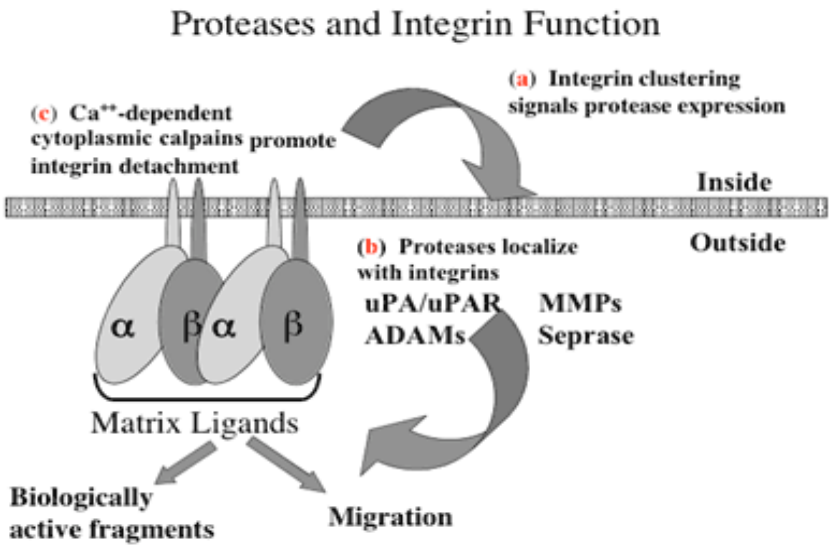
Receptor de Urokinasa

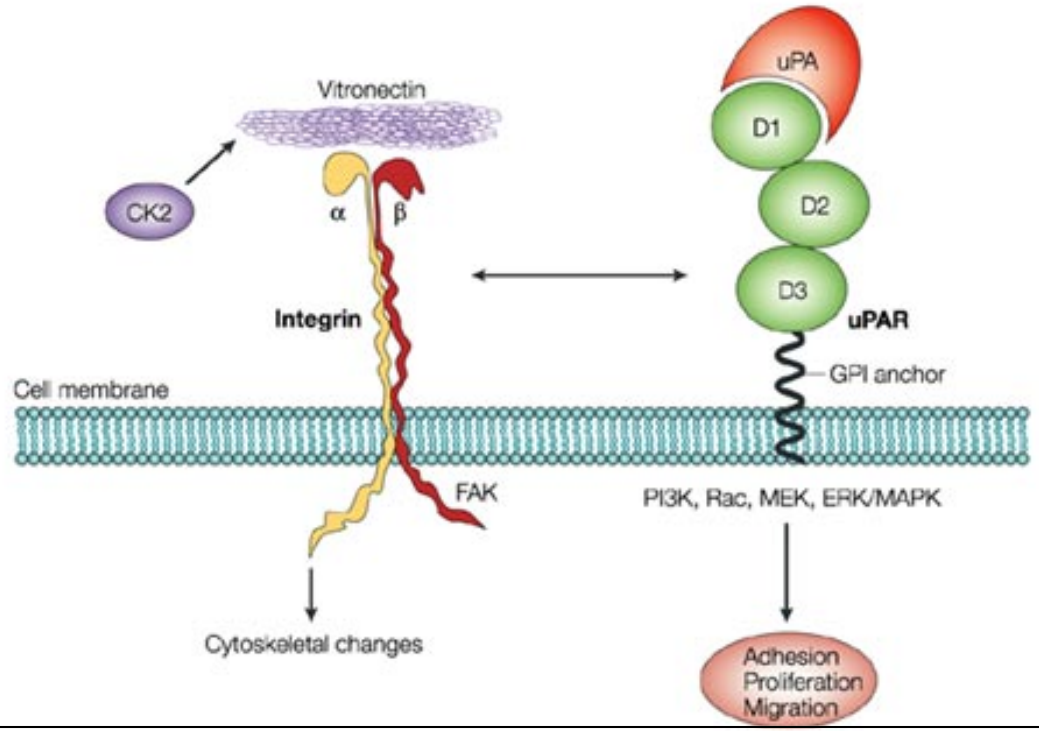
Este receptor ha sido implicado en la patogenia de la proteinuria.

Es una proteinasa, pero también presenta interacciones con otras proteínas de membrana como las integrinas.

Durante la injuria podocitaria, el receptor (uPar) promueve el desdibujamiento de los pedicelos por su interacción con la integrina αvβ3.

La expresión de la vitronectina, el ligando extracelular de la integrina αvβ3, está estimulado en la proteinuria.





El uPAR se une tanto a la urokinasa (uPA) como a la vitronectina, que es a su vez el receptor del PAI-1.

LaProtein kinasa CK2 fosforila a la vitronectina y regula la adhesión celular uPA-dependiente a la vitronectina.

El uPAR carece de un dominio citosólico pero transmite señales intracelulares por su asociación con las integrinas de transmembrana.

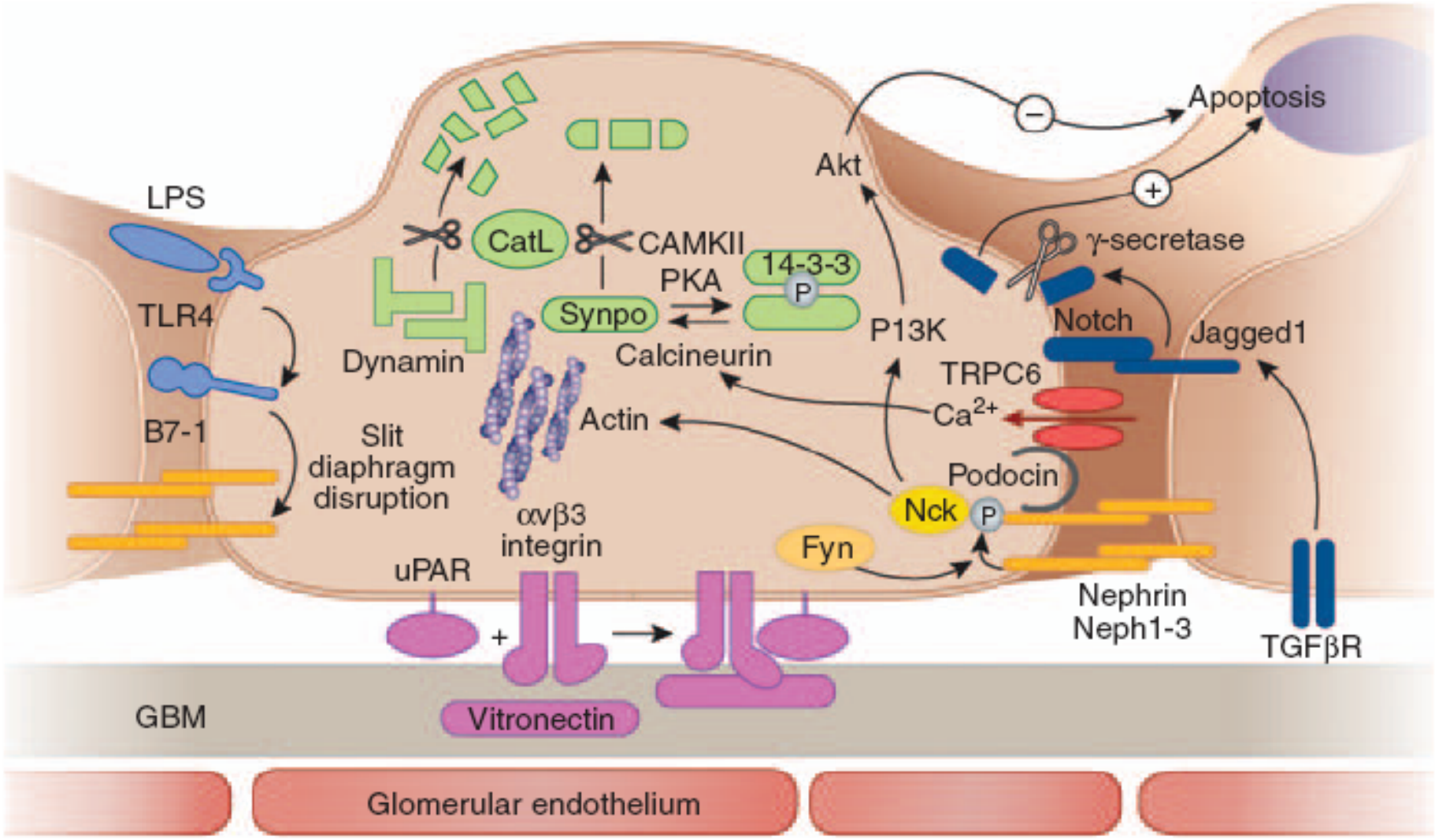
ERK, extracellular-signal-regulated kinase FAK, focal adhesion kinase MAPK, mitogen-activated protein kinase.

Table 1 | Pathways involved in acquired glomerular diseases

Molecular pathways	Human diseases
Transmembrane receptors	
Nephrin	DNP, MCD
B7-1	LN
uPAR	FSGS, DNP
Notch	FSGS, DNP
PLA_2R	MN
Ion channels	
TRPC6	MCD, MN
Growth factors	
VEGF-A	Preeclampsia
TGF-β	DNP
Proteases	
Cathepsin L → dynamin, synaptopodin	MN, FSGS, DNP

Abbreviations: DNP, diabetic nephropathy; FSGS, focal segmental glomerulosclerosis; LN, lupus nephritis; MCD, minimal change disease; MN, membranous nephropathy; TGF-β, transforming growth factor-β; TRPC6, transient receptor potential cation channel-6; uPAR, urokinase plasminogen-activator receptor; VEGF-A, vascular endothelial growth factor A.

Only those pathways that have been shown to be involved in acquired human glomerular diseases are listed.


LPS
TLR4
B7-1
Slit
diaphragm
disruption
CatL
Dynamin
CAMKII
PKA
14-3-3
P
Synpo
Calcineurin
Actin
αvβ3
integrin
uPAR
Vitronectin
GBM
Akt
P13K
Apoptosis
γ-secretase
Notch
Jagged1
TRPC6
Ca2+
Podocin
Nck
P
Fyn
Nephrin
Neph1-3
TGFβR
Glomerular endothelium

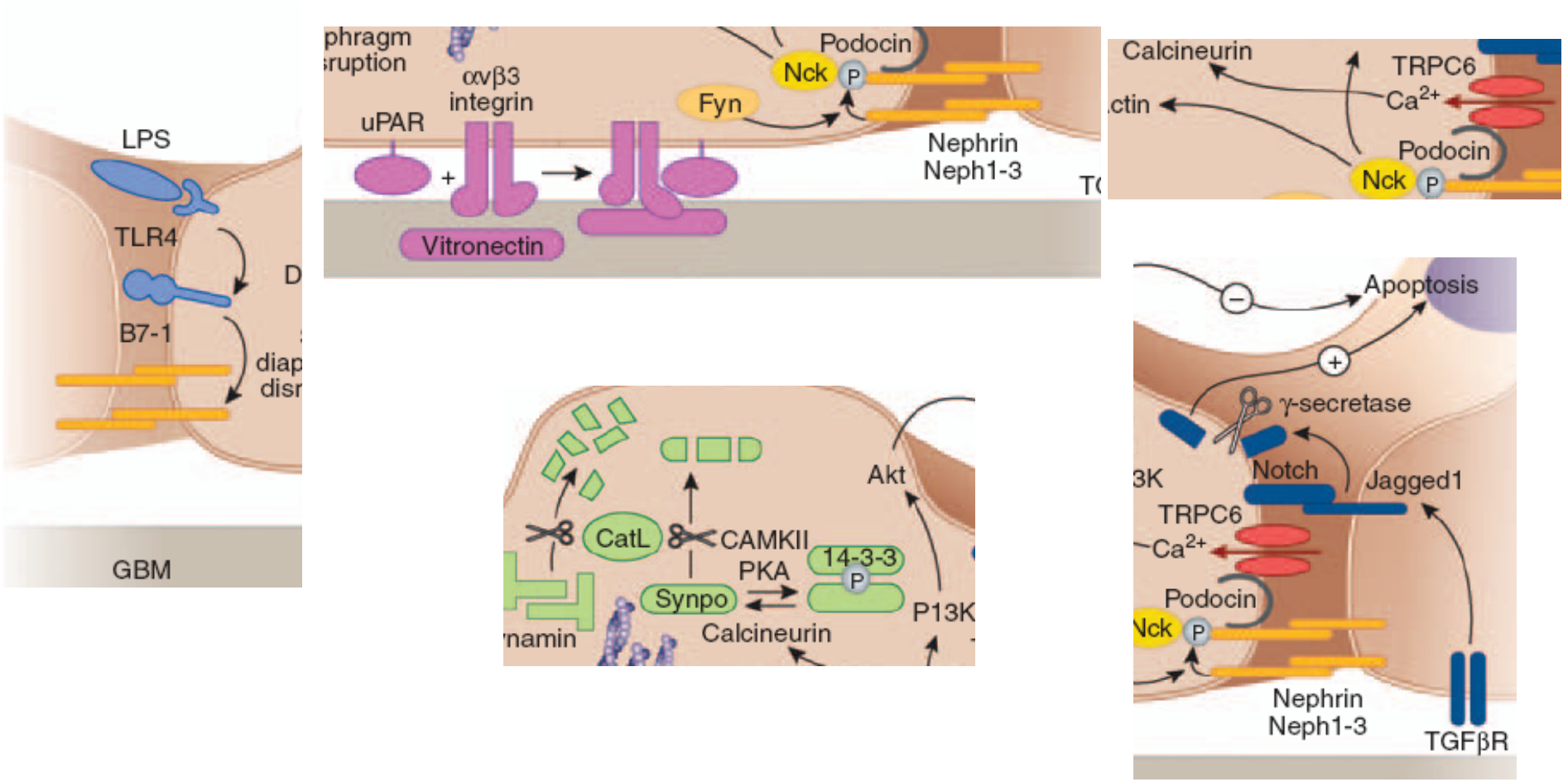


Figure 1 | Pathways involved in acquired glomerular diseases representing targets for podocyte-specific drugs. A schematic cross-section of a podocyte foot process with the corresponding cell body, the glomerular basement membrane, and the glomerular endothelium is shown. Pathways involved in podocyte injury that may be drug-targeted are depicted in different colors. Sustaining nephrin expression and phosphorylation (yellow) might contribute to both antiapoptotic signaling and actin polymerization. The B7-1 pathway (light blue) may be targeted by (1) toll-like receptor-4 antagonists or (2) blocking the binding of B7-1 to slit diaphragm structure proteins. Urokinase plasminogen-activator receptor (uPAR)-induced podocyte motility (violet) could be inhibited by (1) interfering with binding of uPAR to αvβ3 integrin, (2) inhibiting β3 integrin activation, or (3) inhibiting binding of αvβ3 integrin to vitronectin. The notch pathway (dark blue) can be targeted by (1) interfering with its upstream activation by blocking the TGF-β1 effect, (2) inhibiting γ-secretase, which is required for proteolytic receptor activation, or (3) interfering with target gene transcription. TRPC6 channels (red) may be targeted by (1) channel blockers or (2) inhibiting their expression. The CatL pathway (green) could be targeted by (1) specifically inhibiting CatL expression or activity, (2) shifting the equilibrium of synaptopodin toward the phosphorylated form by inhibiting calcineurin-mediated dephosphorylation or enhancing PKA or CAMKII-mediated phosphorylation, (3) protecting synaptopodin and dynamin by compounds that bind to the CatL cleavage site, or (4) delivering cleavage-resistant synaptopodin or dynamin mutants.

La identificación de causas monogénicas, el empleo de ratones transgénicos, y la aplicación de otros métodos genéticos *in vivo* han dado nueva información detallada sobre la fisiología y la patología glomerular.

La transcripción glomerular por análsis de microarrays ha dado también sus frutos.

En el futuro, el perfil molecular pueda quizá hasta superar a la histología para categorizar las glomerulopatías.

El valor práctico de la enorme y nueva información está recién emergiendo, pero varias moléculas importantes, vías de señalización patológicas e interacciones proteína-proteína han sido identificadas y pueden servir como blanco para la intervención farmacológica.

Si bien los estudios disponibles destacan la importancia de las 3 capas glomerulares en el mantenimiento de la barrera de filtración
en mi opinión, el podocito ha pasado a ser el blanco más adecuado en las enfermedades que cursan con proteinuria.

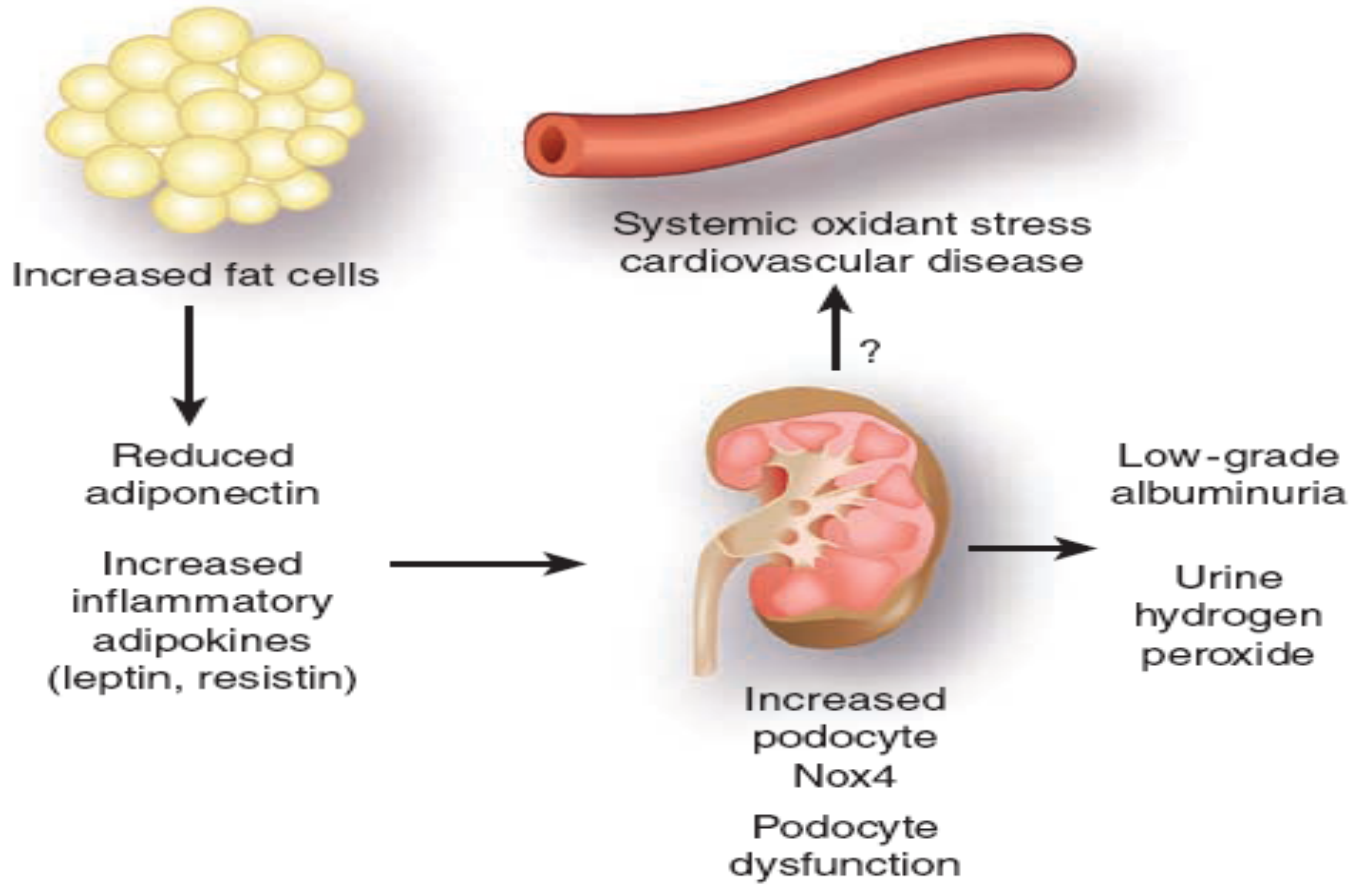


Figure 1 | Potential links between adiposity, kidney response, and cardiovascular disease. With increasing fat mass, visceral adipocytes decrease the production of circulating adiponectin and increase the production of adipokines that enhance insulin resistance. The lower adiponectin levels lead to impaired podocyte function, possibly because of increased podocyte NADPH oxidase. Podocyte dysfunction will lead to albuminuria and increased levels of hydrogen peroxide in the urine. The increased production of hydrogen peroxide from renal NADPH oxidase could potentially cause hydrogen peroxide to enter the circulation, contributing to the systemic inflammation that accompanies low-grade albuminuria.

Monoclonal antibodies for podocytopathies

The podocytopathies, including minimal-change nephropathy, focal segmental glomerulosclerosis,
collapsing glomerulopathy, and diffuse mesangial sclerosis, involve diverse types of injury to podocytes.
These injuries can have genetic causes, or can be caused by viral infection, mechanical stress, medication
or—probably—immunologic injury.

several lines of evidence—including the immunosuppressive effects of standard therapies—suggest a role for immunologic injury in some cases, but the precise pathologic mechanisms are far from clear.

newly available biologic therapies that target immune
cells and cytokines have been used to treat a number of patients with different podocytopathies. Of these
therapies, the greatest experience has been gained with rituximab. The data on all such therapies remain too
fragmentary to provide firm conclusions, but further clinical research with such agents might help to define
pathogenetic pathways and could potentially contribute to new therapies.

Podocytes have a critical role in glomerular architecture
and function, providing a barrier to the transit of
protein into the Bowman space. Podocyte damage leads
to impairment of the glomerular filtration barrier and
proteinuria

minimal-change nephropathy (mCn),
idiopathic primary focal segmental glomerulo sclerosis
(FsGs), idiopathic collapsing glomerulopathy and
diffuse mesangial sclerosis constitute the podocytopathies,
diseases in which the pathology arises from podo cyte damage or dysfunction.3,4 each of the syndromes
has a distinct podocyte phenotype. mCn
is associated with reversible podocyte injury, FsGs
with podocyte depletion, collapsing glomerulopathy with
podocyte prolifera tion and diffuse mesangial sclerosis
with podocyte maturation arrest

Podocytopathies can have genetic etiologies, reactive etiologies (for example, infections, medicationassociated disorders and systemic disorders) or they can be idiopathic

although an increased number of specific causes of podocytopathies have been identified over the past decade, the majority of cases remain idiopathic.4 in 1974, shalhoub hypothesized that mCn represents the renal manifestation of a systemic immunologic abnormality. 5 in particular, he suggested that the pathogenesis of mCn might involve dysregulation of t cells, which results in the secretion of a soluble mediator that causes nephrotic syndrome.5

He offered several lines of clinical evidence to support his hypothesis: the remission of mCn induced by measles (the measles virus inhibits cell-mediated immunity), the association of mCn with Hodgkin disease (which was ascribed, in 2008, to defective regulatory t-cell activity6), the absence of immune complexes in glomeruli in mCn, and the therapeutic benefits of glucocorticoid and cyclophosphamide therapy on mCn.5

in the decades since shalhoub's hypothesis, additional evidence that links the immune system with podocyte injury has emerged. Podocytes express a variety of cytokine and chemokine receptors, and produce inflamma tory mediators such as inter leukin (il)-1, il-6, il-8 and transforming growth factor (tGF)-β.2,7 in 2004, mundel and colleagues demonstrated that podocytes express the injury marker and co-stimulatory molecule B7-1, which is upregulated in various experimental models of nephrotic syndrome.8 B7-1 and B7-2 molecules are a family of membrane proteins expressed on antigen-presenting cells that bind to CD28 or CD152 (Ctla-4) on t cells and provide a co-stimulatory signal that can enhance or reduce t-cell responses.9

in a murine
model of lipopolysaccharide-induced transient nephrotic syndrome that resembles human mCn, proteinuria was associated with overexpression of B7-1 in podocytes and disruption of the actin cytoskeleton.8 these effects were absent in B7-1-null mice (which confirmed the role of B7-1), and present in mice with severe combined immunodeficiency (sCiD), which demonstrated that the effects occur independently of t cells and B cells.8 Further evidence of a link between immune system dysregulation and podocyte injury came from a study by lai *et al., which showed that il-13-transfected rats* developed nephrotic-range proteinuria and podocyte foot-process effacement.10

Role of immune cells in podocytopathies

each of the four podocytopathies clearly represents a clinicopathologic syndrome with diverse etiologies.
nevertheless, immune cells could plausibly be involved in at least some cases of the diseases now termed idiopathic
mCn, idiopathic FsGs, and idiopathic collapsing glomerulopathy.

T cells

shalhoub's hypothesis that cell-mediated immunity has a role in the development of idiopathic nephrotic syndrome has gained further support. several lines of evidence have been developed to support the hypothesis but a conclusive demonstration of causality is lacking.

one line of evidence that supports the hypothesis is that the onset or relapse of mCn has been associated with immunogenic stimuli, particularly those that involve t-cell activation, such as viral infections, recent vaccina tions, allergic reactions, atopic illness, and lymphoid malignancies.3,5,7 Furthermore, thymomas are associated with various glomerular diseases, including mCn and FsGs, which suggests that t cells or t-cell precursors might contribute to podocyte injury.11

another piece of evidence in support of the hypothesis is that several studies have suggested an abnormal distribution of t-cell subsets and their soluble products in patients with mCn.3,7,14 idiopathic nephrotic syndrome has been associated with elevated levels of il-2, il-4, il-8, il-13, tumor necrosis factor (tnF) and interferon γ.3,14 increased plasma levels of il-4 and il-13, together with the link between mCn and atopic illness, have led to suggestions that mCn is associated with an increased t-helper type 2 (tH2) response.2,14 levamisole enhances tH1 responses and diminishes tH2 responses, and pilot studies of this agent in idiopathic nephrotic syndrome have shown a remission rate of around 50% in steroid- dependent disease.3,7

Furthermore, transplantation of a normal kidney into a proteinuric Buffalo/mna rat is associated with the induction of proteinuria in the transplanted kidney, which is reminiscent of the recurrent FsGs that occurs after transplantation in human patients, and provides further evidence that a circulating cell or soluble factor is involved in the pathogenesis of FsGs.19 this finding is also consistent with the findings from ali *et al.,* who described the successful transplantation of kidneys from a patient with active mCn, which resulted in the absence of proteinuria and the reversal of foot process changes in the recipients.21

Circulating factors

the aforementioned observations, which support a link between t cells and soluble factors, lead to a consideration of the long-standing efforts to identify soluble factors that are associated with immune dysregulation and/or podocyte injury and that might or might not represent immune cell products.

in the 1980s, an active search took place for a soluble immune response suppressor in nephrotic syndrome, but the molecular source of the activity could not be identified.3

B cells
only limited evidence links B cells with podocyte injury.
mean serum ige levels are significantly higher in patients with mCn than in those with other nephropathies.3

Furthermore, idiopathic
nephrotic syndrome could be accompanied by B-cell activation, as suggested by the substantial elevation of serum sCD23 (a soluble B-cell stimulation marker)

Perhaps the strongest evidence for
a role of B cells in podocytopathies is that rituximab therapy is able to induce remission in some patients with steroid-dependent and multirelapsing idiopathic nephrotic syndrome, as will be discussed.

natural killer cells

natural killer (nK) cells have important roles in innate immunity, but their role in autoimmunity is still uncertain.[28] nK cells seem to prevent autoimmune responses in some settings, while in other settings they have a permissive role in autoimmunity.[28] Bagga *et al. and Daniel et al. studied children with steroid-sensitive idiopathic* nephrotic syndrome and found that nK cell numbers were significantly higher in patients with active disease than in healthy controls.[29,30] the number of nK cells decreased after remission of disease and increased signifi cantly again during relapse.[29,30] By contrast, lapillone *et al. found that nK cell number was increased* after steroid-induced remission compared with during relapse and compared with controls.[13] the reasons for these divergent results are far from clear, but they could relate to differences in the therapies used and the timing of measurements

Macrophages

although macrophages probably do not represent the initial players in the pathogenesis of podocyte injury, infiltrating macrophages are thought to have a pivotal role in progression to glomerulosclerosis.31 moreover, increased production of macrophage-associated cytokines, particularly tnF, was found in the kidneys of Buffalo/mna rats before the onset of proteinuria.20 of particular clinical relevance, serum levels of il-12, a pleiotropic cytokine produced primarily by macrophages, correlates with disease activity in patients with mCn.32

hematopoietic stem cells
the idea that FsGs arises from a disorder of stem cells
was first suggested in 1994 by nishimura *et al. in osaka,*
on the basis of the observation that bone marrow transplantation
from a mouse strain that spontaneously
develops FsGs to control mice transfers disease, and
transplantation from control mice to FsGs mice ameliorates
disease

Despite the limitations of this mouse model of FsGs,
experimental work in humans and experimental animal
models has lent support to the intriguing hypothesis
that CD34+ stem cells have a role in podocyte injury.

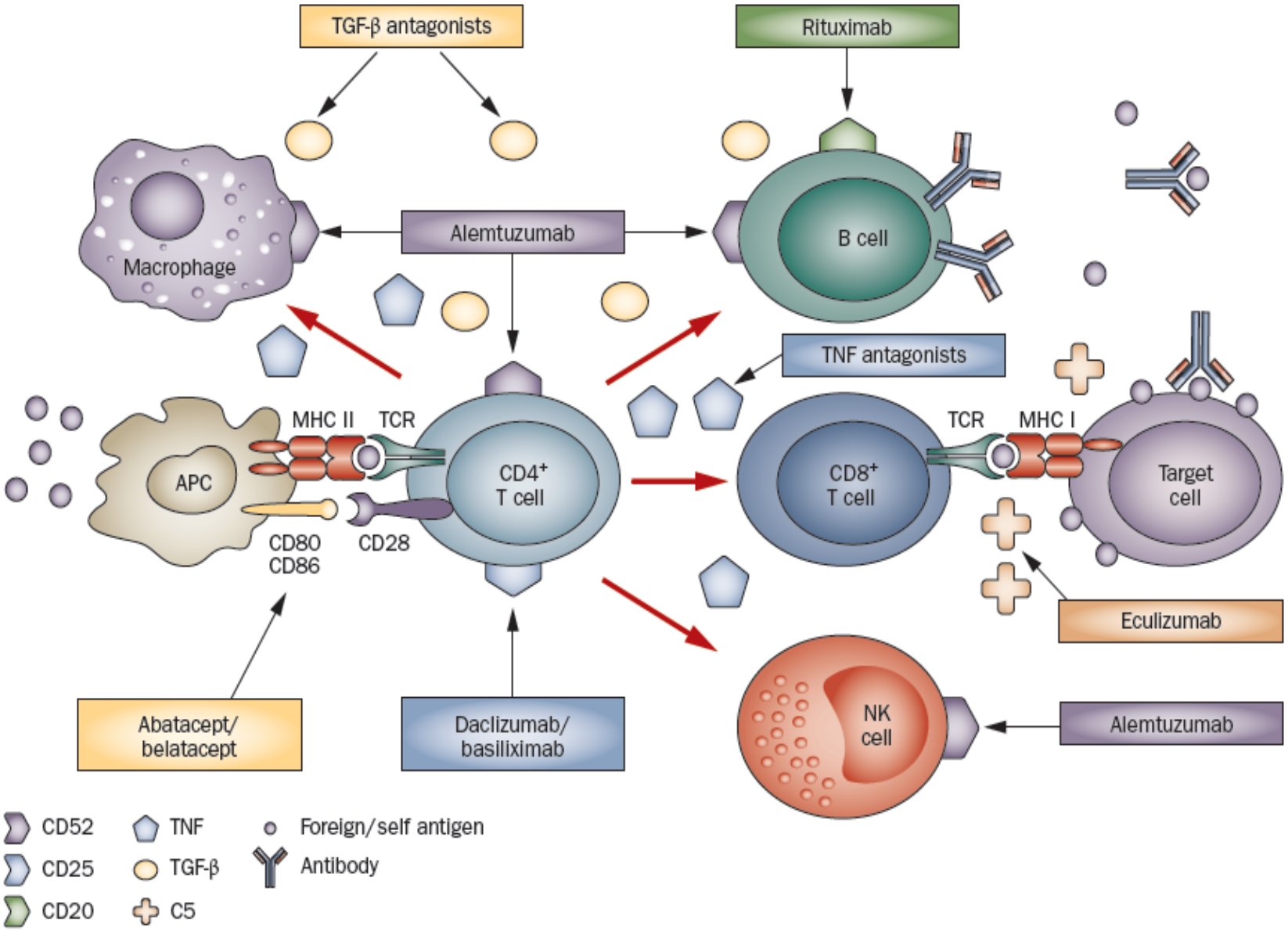
lapillone *et al. quantified t-cell subsets in the peripheral* blood of patients with steroid-sensitive idiopathic nephrotic syndrome during relapse and remission.13 only CD34+ stem cells were significantly higher during relapse than during remission or in control individuals. 13 Furthermore, during relapse, no differences were observed in B, t or nK cells, which are all derived from CD34+ cells.13 the appearance of mCn and FsGs after hematopoietic cell transplantation might indicate a similar role for CD34+ stem cells.35–37 Patients who undergo peripheral blood stem cell transplantation have a higher likelihood of developing nephrotic syndrome (including membranous nephropathy and mCn) than those who undergo bone marrow transplantation.37

Monoclonal antibodies and biologic therapies
new therapies for autoimmune diseases include monoclonal antibodies (mabs), generally directed against immune cell surface ligands, and biologic agents that target soluble complement components and cytokines (Figure 1). mabs might deplete, or affect the activity of, specific subsets of immune cells. these agents hold the promise of precisely targeted action, with increased potency and decreased toxic effects, compared with classic immunosuppressive agents. moreover, in line with the recently described direct antiproteinuric effect of ciclosporin, some of these agents possibly also act by exerting a stabilizing effect on podocytes; however, this process would require the target antigens to be expressed on podocytes. a useful summary of adverse events for all FDa-approved medication is available.39 long-term safety data from these antibodies are, however, still lacking. Furthermore, since podocyte injury might involve multiple, distinct pathways, it might be unrealistic to expect a single, highly selective agent to have widespread success.

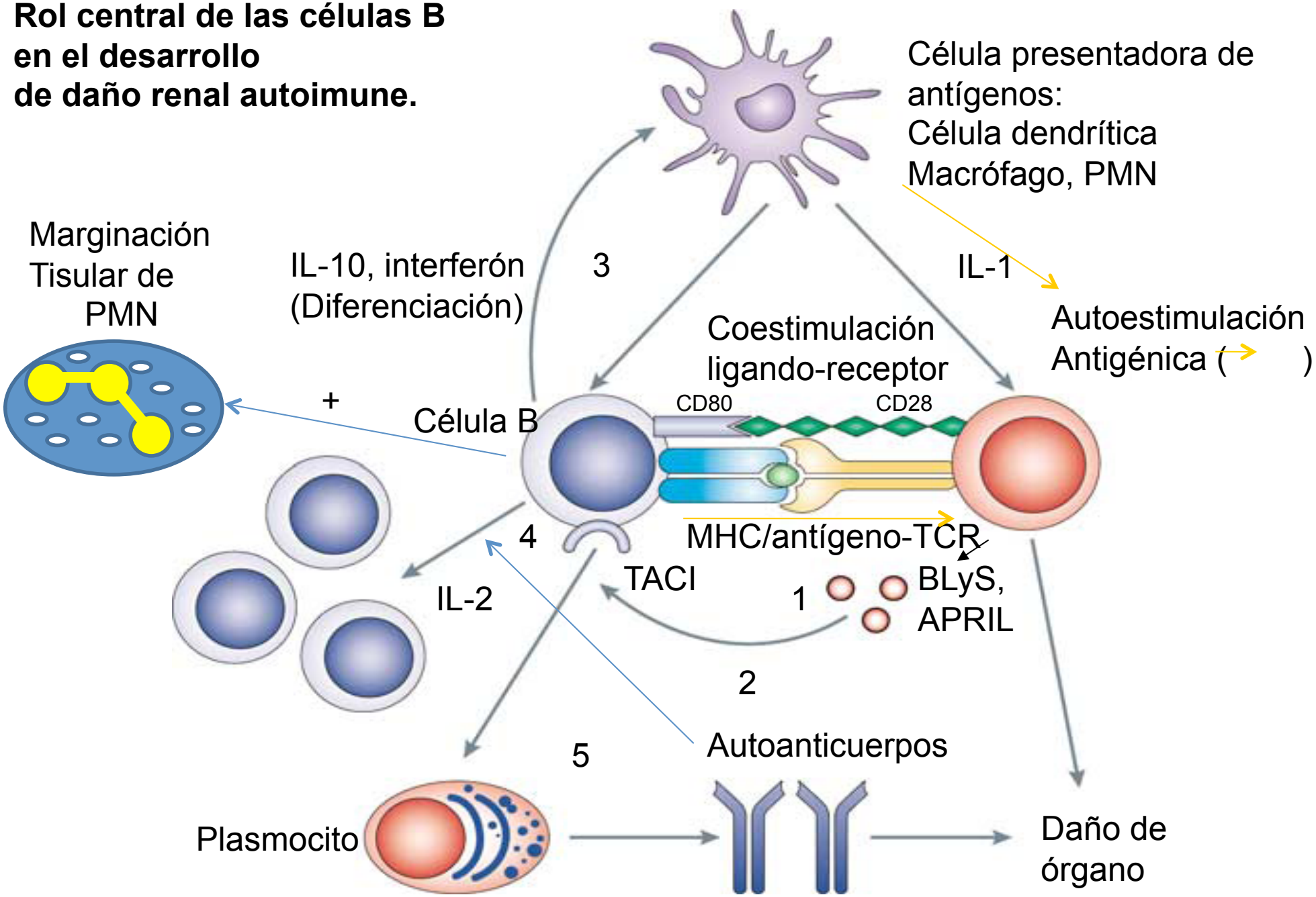
Table 1 | Naming system for monoclonal antibodies developed by the International Nonproprietary Names program

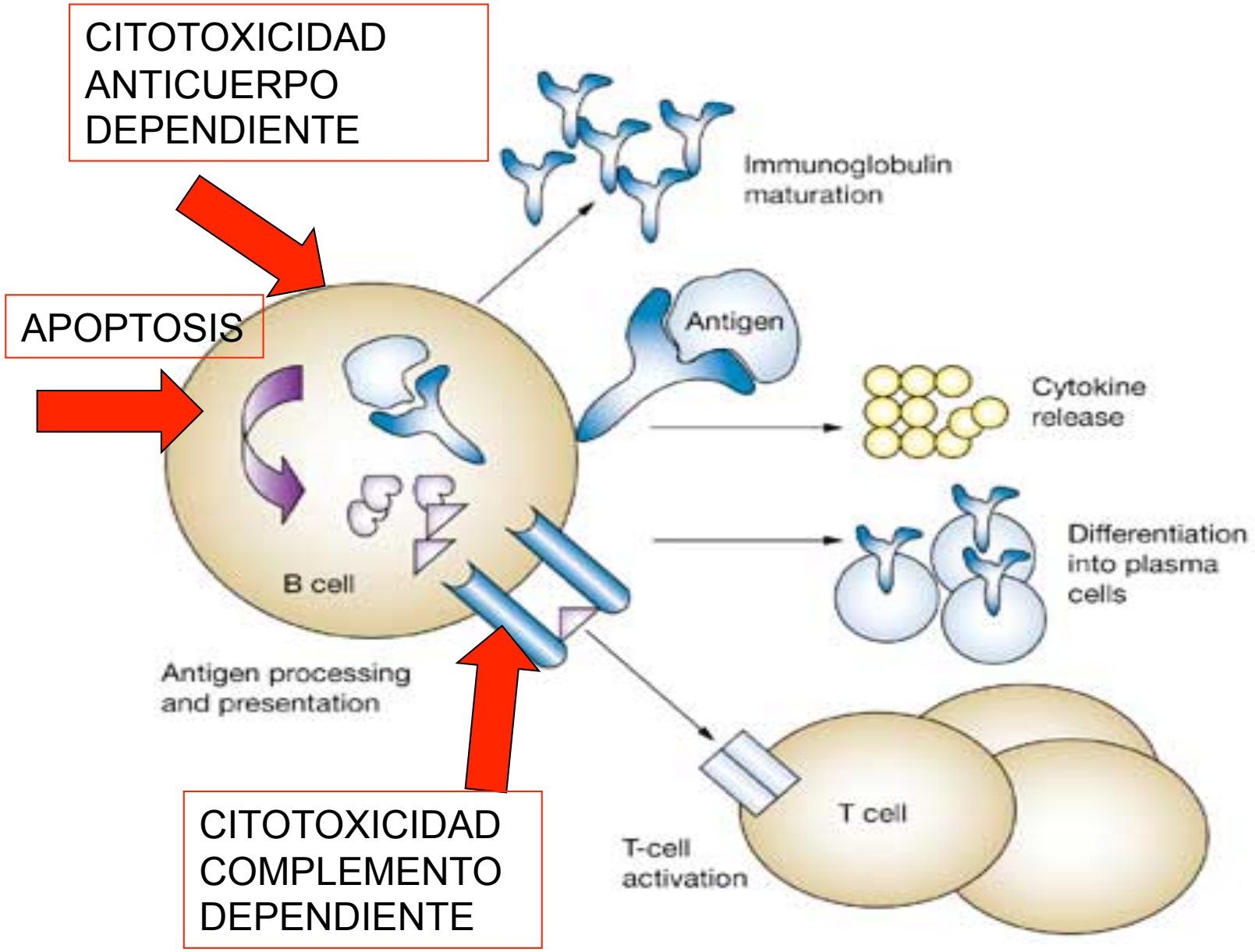
Prefix	First infix[a] = target	Second infix = source of the product	Suffix = class
Varies	-ki(n)- = interleukin	-o- = mouse	-mab = monoclonal antibody
	-li(m)- = lymphocyte, immunomodulator	-axo- = mouse–rat hybrid	
	-tu- = tumor (miscellaneous)	-xi- = chimeras	
		-zu- = humanized	
		-u- = human	

[a]Final letter of the first infix can be deleted when pronunciation is difficult. Each monoclonal antibody name consists of a unique prefix, a first infix related to the molecular target, a second infix that describes the source species of the product, and the suffix '-mab'. Names of other biologic therapies include the suffixes -atacept (CTLA-4 antagonist) and -nercept (tumor necrosis factor antagonist).[40]


TGF-β antagonists
Rituximab
Macrophage
Alemtuzumab
B cell
TNF antagonists
MHC II
TCR
APC
CD4+
T cell
CD8+
T cell
TCR
MHC I
Target
cell
CD80
CD86
CD28
Eculizumab
Abatacept/
belatacept
Daclizumab/
basiliximab
NK
cell
Alemtuzumab
CD52
CD25
CD20
TNF
TGF-β
C5
Foreign/self antigen
Antibody

Rol central de las células B en el desarrollo de daño renal autoimune.





Salama AD and Pusey CD (2006) Drug Insight: rituximab in renal disease and transplantation
Nat Clin Pract Neprol **2:** 221–230 doi:10.1038/ncpneph0133

PODOCITO

La podocalyxina tiene múltiples sitios glicosilados.

La sección externa permite repelerse con moléculas semejantes de otros podocitos y así mantener los diafragmas abiertos para la filtración

Su sección interna interacciona con la ezrina

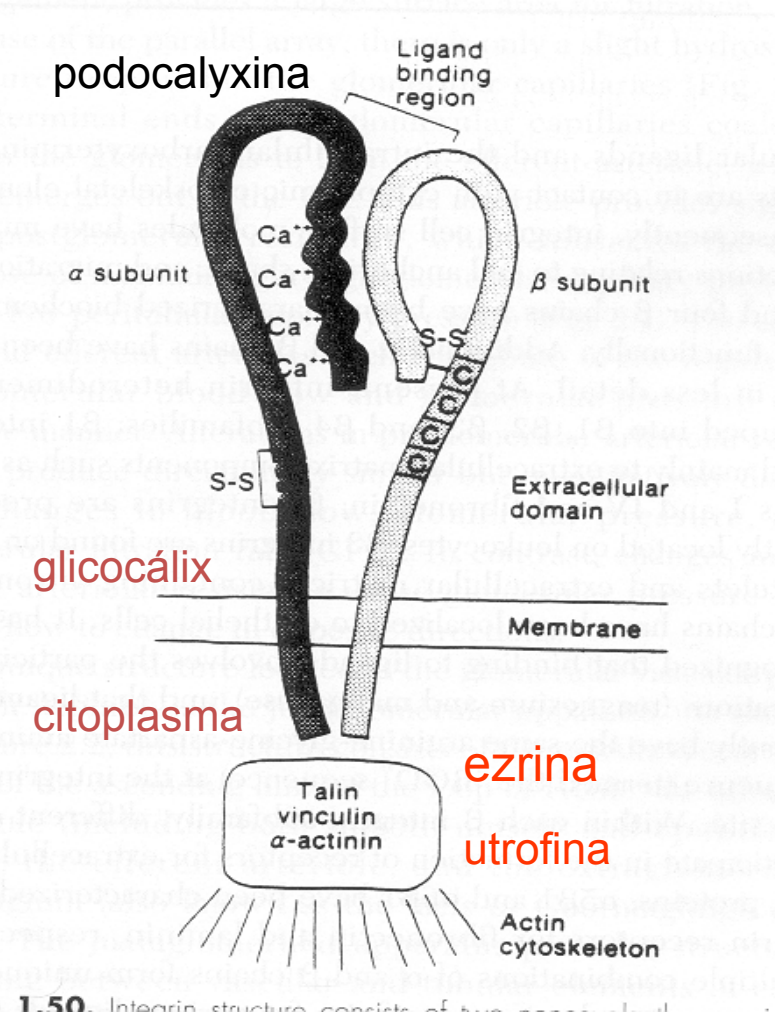


Figure 1 | Biologic therapies and the immune system. CD4+ T cells circulate in a resting state until they recognize foreign or self antigens through the binding of TCrs to MHC ii molecules on APCs. Abatacept and belatacept inhibit the co-stimulatory signal provided by the interaction between CD28 on the T cell and CD80 and/or CD86 on the APC. Antigen recognition triggers a cascade of signals that leads to activation of effector T cells, cytokine secretion, and interactions with B cells, CD8+ T cells, macrophages, NK cells and APCs. eventually these interactions result in cell death and tissue damage through migration of these cells to target tissue. These cells express antigens that are targets of specific mAbs (for example, daclizumab, basiliximab, rituximab and alemtuzumab). CD8+ cytotoxic T cells induce lysis of target cells following interaction of the TCr with target antigen through MHC i and adhesion molecules. Macrophages and NK cells are part of the innate immune system but are also involved in the cascade of events triggered by antigen recognition. These events are regulated by cytokines and circulating mediators such as TNF and TGF-β, which can be targeted by specific antagonists.

Abbreviations: APC, antigen-presenting cell; C5, C5 complement component; mAb, monoclonal antibody; MHC i, major histocompatibility complex class i; MHC ii, major histocompatibility complex class ii; NK, natural killer; TCr, T-cell receptor; TGF-β, transforming growth factor β; TNF, tumor necrosis factor.

under normal circumstances,
moderate amounts of albumin (perhaps 3–6 g
per day) and smaller amounts of intact igG (molecular weight 150 kDa) are filtered through the glomerulus; most of these molecules are taken up by proximal tubular
epithelial cells.3 in podocyte disease, increased passage of macromolecules across the GBm occurs; therefore, molecules the size of these antibodies and biologic agents
should have ready access to the podocyte.

Table 2 | Antibodies and biologic therapies approved by the FDA for immunosuppression

Drug	Molecular target(s)	FDA-approved indications	Serious adverse effects	Use in human podocytopathies (cases)
Daclizumab (144 kDa)	CD25 (IL-2Rα) (expressed on activated T cells)	Prophylaxis of acute rejection in renal transplantation	Hypersensitivity reactions; severe infections	None reported
Basiliximab (144 kDa)	CD25 (IL-2Rα) (expressed on activated T cells)	Prophylaxis of acute rejection in renal transplantation	Hypersensitivity reactions	Multirelapsing MCN (1 case)[44] (2007)
Rituximab (145 kDa)	CD20 (expressed on B cells)	$CD20^+$ B-cell non-Hodgkin lymphoma; moderately to severely active RA with inadequate response to other therapies	Fatal infusion reactions; tumor lysis syndrome; severe mucocutaneous reactions; PML; hepatitis B viral reactivation	Post-transplant recurrent FSGS (16 cases, 2 associated with PTLD)[48–58] Steroid-dependent or resistant primary MCN or FSGS (39 cases)[44,59–67]
Alemtuzumab (150 kDa)	CD52 (expressed on T and B cells, NK, monocytes and/or macrophages, eosinophils, and some $CD34^+$ cells)	B-cell chronic lymphocytic leukemia	Severe cytopenias; infusion reactions; serious infections	None reported

Abatacept and belatacept (92 kDa)	B7-1 (CD80) and B7-2 (CD86) (on T cells)	Abatacept: moderately to severely active RA with inadequate response to other therapies Belatacept (not FDA-approved)	Serious infections when given concurrently with TNF antagonists; hypersensitivity reactions	None reported
Etanercept (150 kDa)	TNF Lymphotoxin α	Moderately to severely active RA; moderately to severely active polyarticular JIA; psoriatic arthritis; moderate to severe chronic plaque psoriasis; active AS	Severe infections; malignancies; CNS demyelinating disorders; formation of autoantibodies or development of lupus-like syndrome; systemic and renal vasculitis; pancytopenia; hepatitis B virus reactivation	Post-transplant recurrent steroid-resistant idiopathic nephrotic syndrome (1 case)[87] (2007)
Infliximab (149 kDa)	TNF	Moderately to severely active RA; psoriatic arthritis; severe chronic plaque psoriasis; active AS; moderately to severely active CD with inadequate response to conventional therapies; fistulizing CD; moderately to severely active UC with inadequate response to conventional therapy	Severe infections; malignancies; hepatitis B virus reactivation; severe hepatotoxicity; pancytopenia; heart failure; hypersensitivity reactions; CNS demyelinating disorders; formation of autoantibodies or development of lupus-like syndrome; systemic and renal vasculitis; heart failure	Multiresistant MCN (1 case)[86] (2004)

Anti-Il-2 receptor monoclonal antibodies
Daclizumab and basiliximab are humanized
and chimeric,
respectively, nondepleting mabs that target the
CD25 antigen, the α-chain of the il-2 receptor
(il-2r)

expressed on t lymphocytes.42 these agents
block t-cell
activation and proliferation, are widely used as
induction
therapy to prevent acute rejection in solid
organ
transplantation, and have excellent safety
profiles.42,43

a 23-year-old woman with multirelapsing mCn that failed to respond to standard therapy was given a single dose of basiliximab without any beneficial effect.44 long-term remission was only obtained following treatment with four weekly doses of rituximab monotherapy.44 recently, a case report described a child with treatment-refractory nephrotic syndrome who experienced sustained remission following a single dose of basiliximab.45

of note, because of their highly selective, non depleting mechanism of action, anti-il-2r mabs have limited efficacy when used alone.43 Given the possible role of autoreactive t-cell clones in idiopathic nephrotic syndrome, these agents might be useful in combination with other immunosuppressive therapies.

rituximab

rituximab is a chimeric mab that targets the CD20 antigen on B cells and induces cell lysis through complement- dependent and complement-independent mechanisms.46 the ligand for CD20 and the function of CD20 remain unknown. rituximab has been approved for the treatment of B-cell non-Hodgkin lymphoma and rheumatoid arthritis, and has been used successfully to treat many autoimmune diseases, including idiopathic membranous nephropathy.47

as summarized in tables 3 and 4, the literature now includes reports of rituximab use in two cases of recurrent FsGs associated with lymphoproliferative disease, 14 cases of recurrent FsGs without lymphoproliferative disease, and 39 cases of idiopathic nephrotic syndrome in a native kidney.44,48–67 of note, in the prospective study by Guigonis *et al., rituximab was associated with* a significant reduction in immunosuppressive therapy in 14 of 22 patients (a mean dosage reduction of 70% for all drugs), and total withdrawal of such therapy in 5 patients.59

in most of the above-mentioned cases, disease remission was consistently associated with depletion of CD20+ B cells,44,48,52–54,59,61,63,66 but treatment failure can occur despite CD20+ B-cell depletion.55,57,59 treatment failure despite removal of CD20+ B cells from the blood might indicate a failure to deplete the central B-cell population, or might indicate that recurrent FsGs is a syndrome with multiple etiologic pathways, only one or some of which involve B cells.

in general, rituximab has shown an excellent safety profile, but cases of JC virus- associated progressive, multi focal leukoencephalo pathy in rituximab-treated patients with systemic lupus erythema tosus71 are worrying,

rituximab might act both through impairment of antibody production and through interference with antigen presentation, which affects t-cell activation and might inhibit the production of glomerular permeability factors.44,48 interestingly, work reported in 2007 in an animal model of autoimmunity suggests that rituximab might promote the expansion of il-10-secreting regulatory t cells through the contact between naive t cells and emerging B lymphocytes primed by apoptotic cells.68 regulatory t cells have immune-regulatory properties and are able to suppress activation of effector t and B lymphocytes.69 as patients affected by mCn show impaired regulatory t-cell activity, one can reasonably speculate that the effect of rituximab on proteinuria might also be a result of the re-establishment of the regulatory t-cell pool.7

Table 4 | Published experience of rituximab use in native-kidney podocytopathies

Details of patients in study	Patient's age in years; sex	First-course dosage of rituximab	Outcomes	Follow-up (months)	Reference
22 patients treated with rituximab, steroid, calcineurin inhibitor and sometimes MMF: 16 MCN, 3 FSGS, and 3 nonbiopsied; all steroid-dependent or steroid-resistant and ciclosporin-dependent	6.3–22.1; 9 F and 13 M	2–4 weekly doses of 375 mg/m²	16 patients did not have proteinuria. Among 6 nephrotic patients, 3 CRs and 3 NRs occurred. Significant decrease in dosage of immunosuppressive therapies occurred in 14 patients (mean dosage reduction for all drugs 70%; total withdrawal in 5 patients)	6–39	Guigonis *et al.* (2008)[59]
3 nephrotic cases excluded from the above study because they received rituximab alone: 2 MCN, 1 nonbiopsied; all steroid-dependent or steroid-resistant and ciclosporin-dependent	a) 15; M b) 9; F c) 18; M	2–4 weekly doses of 375 mg/m²	a) NR b) NR c) NR	a) 12 b) 14 c) 15	Guigonis *et al.* (2008)[59]
5 cases: 2 MCN and 3 FSGS, all multiresistant	a) 10.3 b) 8.6 c) 15 d) 16 e) 2.8 Sexes not available	4 weekly doses of 375 mg/m²	3 CRs 2 PRs (1 relapse)	a) 14 b) 9 c) 7 d) 2 e) 2	Bagga *et al.* (2007)[60]
2 cases of FSGS, both steroid-dependent	a) 10; F b) 12; F	Single dose of 375 mg/m²	a) CR b) CR (1 relapse)	a) 8 b) 15	Nakayama *et al.* (2008)[61]
1 FSGS (+ITP), steroid-dependent	16; M	4 weekly doses of 375 mg/m²	CR	13	Benz *et al.* (2004)[62]
1 MCN, steroid-dependent	15; F	4 weekly doses of 375 mg/m²	CR (1 relapse)	18	Gilbert *et al.* (2006)[63]
1 MCN, steroid-dependent	23; F	Single dose of 375 mg/m²	CR	28	Francois *et al.* (2007)[44]
1 MCN, steroid-dependent	20; F	2 doses of 1 g, 2 weeks apart	PR	4	Hofstra *et al.* (2007)[64]
1 MCN, multiresistant	14; M	Single dose of 375 mg/m²	CR	9	Smith *et al.* (2007)[65]
1 FSGS (+HUS), multiresistant	1; M	4 weekly doses of 375 mg/m²	CR	3	Suri *et al.* (2008)[66]
1 MCN, resistant to steroids and MMF	40; F	4 weekly doses of 375 mg/m²	CR	12	Yang *et al.* (2008)[67]

Alemtuzumab

alemtuzumab (Campath®; Genzyme Corporation, Cambridge, ma) is a humanized, CD52-targeted mab that induces sustained depletion of t cells and, to a lesser extent, of B cells and monocytes.72,73 CD52 is a cell surface marker expressed on t and B lymphocytes, monocytes and/or macrophages, eosinophils and nK cells, but not on plasma cells.72,74 alemtuzumab is approved by the FDa for the treatment of chronic lymphocytic leu kemia and is being investigated in a variety of immune diseases including cytopenias, vasculitis, myositis, and multiple sclerosis.7

During the last decade, alemtuzumab has been used as induction therapy in organ transplantation to enable

minimization of maintenance immunosuppression.75 in prospective studies that compared the safety and efficacy profiles of alemtuzumab with those of current induction therapies (for example, anti-il-2r mabs and thymoglobulin) the efficacy of alemtuzumab seems similar to that of the other agents in these studies.

75 However,
long-term, randomized controlled trials are still lacking.
intriguingly, alemtuzumab therapy allows a subset
of CD4+CD25highFoXP3+ regulatory t cells to emerge
during immune reconstitution, provided that patients
receive sirolimus but not a calcineurin inhibitor as
concomitant therapy.76 evidence exists that regulatory
t cells are instrumental in the induction of tolerance
after organ transplantation and could also prevent the
development of autoimmune diseases.69 on the other
hand, alemtuzumab-induced lymphocyte depletion has
led to the homeostatic expansion of memory t cells.74
this highly reactive t-cell subset could account for the
increased incidence of antibody-mediated adverse reactions,
including autoimmune hyperthyroidism, idiopathic
thrombocytopenic purpura, anti-GBm disease
and antibody-mediated rejection, associated with alemtuzumab
treatment.74,77 Furthermore, although the drug
is generally well tolerated, an increased incidence of
opportunistic infections has been described in patients
treated with alemtuzumab.73

Abatacept and belatacept

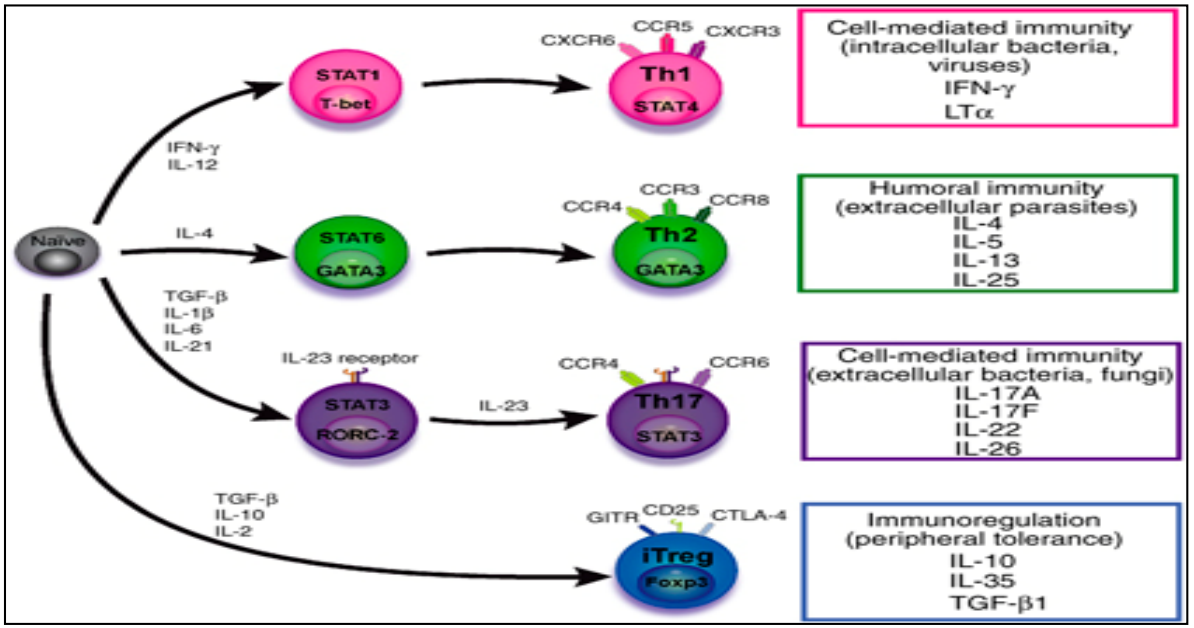
abatacept and its derivative belatacept are recombinant fusion proteins made up of the Fc fragment of igG1 fused with cytotoxic t-lymphocyte-associated antigen 4 (Ctla-4).9 Ctla-4 is a t-cell membrane protein with high affinity for B7-1 (CD80) and B7-2 (CD86) costimulatory molecules expressed on antigen-presenting cells.9 During an immune response, the interaction between B7-1 and B7-2 and the t-cell membrane protein CD28 induces t-cell proliferation and increases antibody production by B cells; by contrast, the engagement of B7 ligands with Ctla-4 suppresses t-cell activation and inhibits antibody production.9 Both abatacept and belatacept bind avidly to B7-1 and B7-2 molecules, which inhibits t-cell activation and t-cell-dependent antibody production.79 abatacept is approved by the FDa for rheumatoid arthritis and has been studied in other autoimmune diseases, including psoriasis vulgaris and systemic lupus erythematosus.9,79 Belatacept was developed to bind with greater avidity than abatacept to B7-2, to provide more potent immunosuppression and prevent acute rejection in transplant patients.79

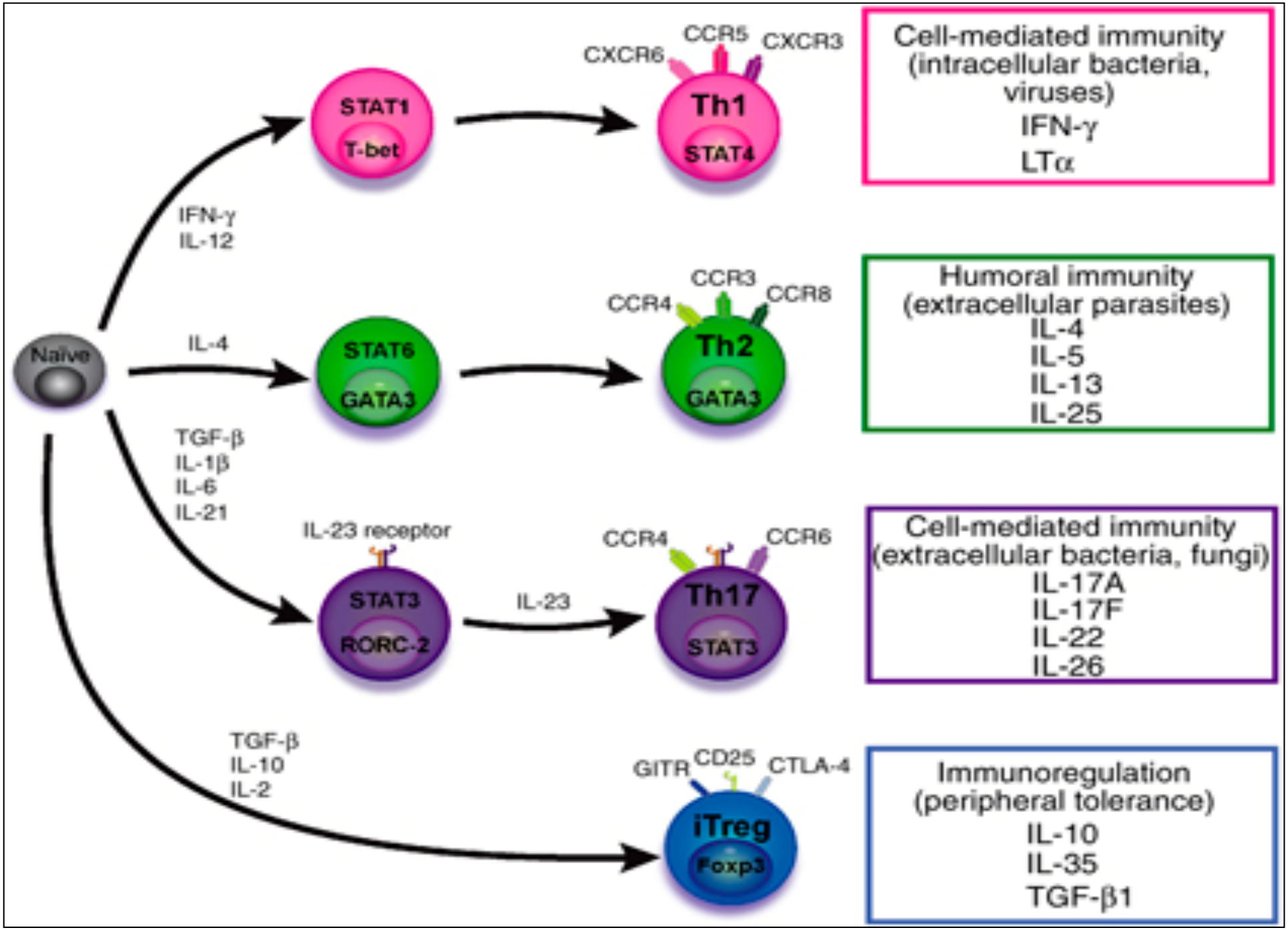
Naive CD4 T cells are activated after interaction of T cell receptors with antigen/ MHC (signal 1) and co-stimulation (signal 2).

Depending on the fine texture of the inflammatory milieu in which antigen activation takes place, these newly activated T cells commit to one of several CD4 subset phenotypes.

In addition to the classical Th1 and Th2 CD4 phenotypes, regulatory (Treg) and Th17 phenotypes have been more recently identified and characterized.

Whereas effector T cells such as the Th1,Th2, and Th17 phenotypes exert injurious,cytopathic effects on tissues, theTreg phenotype restrains or “regulates” effector T cell–mediated tissue injury.




Naive
IFN-γ
IL-12
STAT1
T-bet
CXCR6
CCR5
CXCR3
Th1
STAT4
Cell-mediated immunity
(intracellular bacteria,
viruses)
IFN-γ
LTα
IL-4
STAT6
GATA3
CCR4
CCR3
CCR8
Th2
GATA3
Humoral immunity
(extracellular parasites)
IL-4
IL-5
IL-13
IL-25
TGF-β
IL-1β
IL-6
IL-21
IL-23 receptor
STAT3
RORC-2
IL-23
CCR4
CCR6
Th17
STAT3
Cell-mediated immunity
(extracellular bacteria, fungi)
IL-17A
IL-17F
IL-22
IL-26
TGF-β
IL-10
IL-2
GITR
CD25
CTLA-4
iTreg
Foxp3
Immunoregulation
(peripheral tolerance)
IL-10
IL-35
TGF-β1

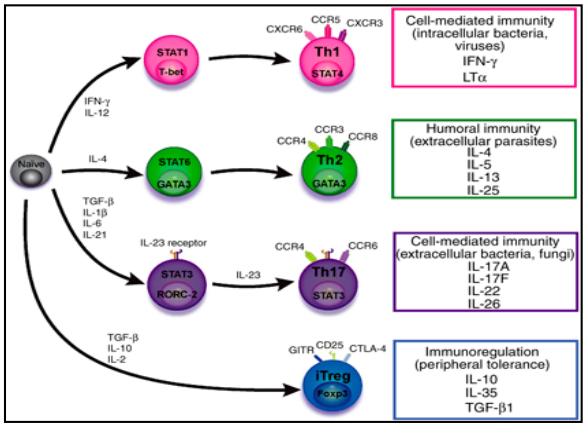
Naive CD4 cells T commit to the tissue destructive,-IFN–expressing Th1 program when signals 1 and 2 are delivered in a milieu rich in IL-12, a product of certain stimulated antigen-presenting cells.

In contrast, antigen activation conducted in an IL-4 –rich environment leads to commitment to the Th2 phenotype.

Commitment to theTh1 or Th2 phenotype rests with expression of a distinctive DNA-binding lineage specification factor by CD4 T cells.

Expression of the t-bet specification factor commits newly antigen-activated and IL-12–stimulated CD4 T cells to the Th1 phenotype.

In contrast, expression of GATA 3 commits newly antigen-activated and IL-4 –stimulated T cells to the Th2 phenotype.1 Until recently, it was thought, upon antigen activation, helper T cells became either Th1or Th2 T cells.



IL-2–producing Th1 and IL-4 –producing Th2
are considered terminally differentiated phenotypes.

Once they commit, there is no “going back.”

Th1 and Th2 cells were once held responsible for diametrically opposing functions in tissue injury.

Th1 cells were the most potent mediator and principle architects of CD4-dependent tissue-destructive reactions,
whereas Th2 cells were thought to protect antigen-bearing tissues from Th1 cells.

Although this scenario is easy to remember,
Th1 cells attack while Th2 cells protect “foreign” tissues,
it is not altogether true.

Th1 cells, IFN-gamma or IL-2 (Th1 cell products) are not required for rejection.

Rejection of MHC-mismatched allografts can be caused by T cells in the Th2 mode.

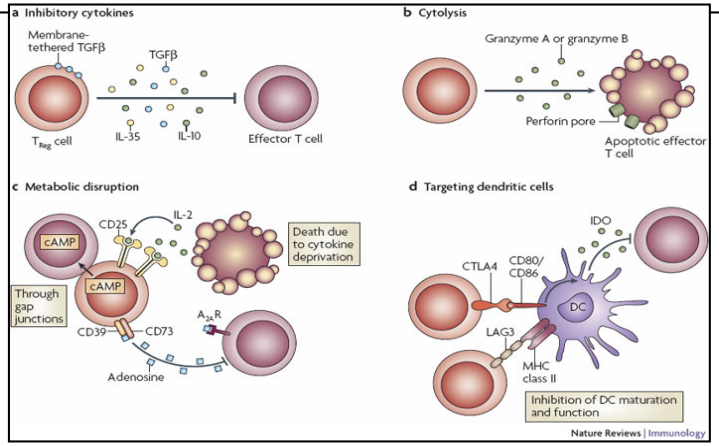
CD4 Tregs, not Th2 cells, are crucially important in restraining the destructive effects of cytopathic T cells.

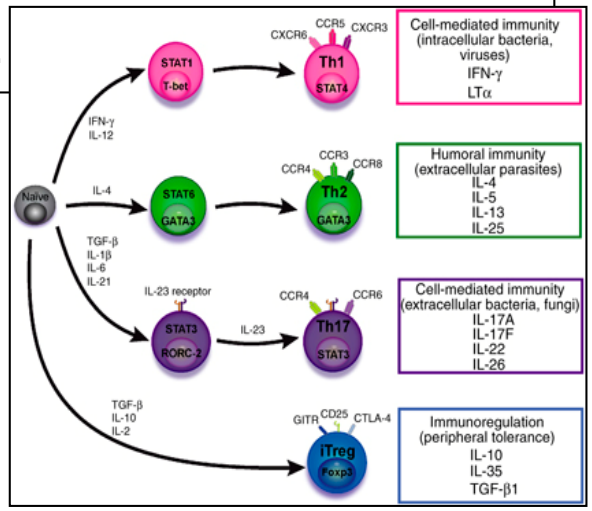
In keeping with new dogma that CD4 T cells take cues from the cytokine environment, a TGF-beta dominant environment leads naive CD4 T cells to commit to the regulatory phenotype.

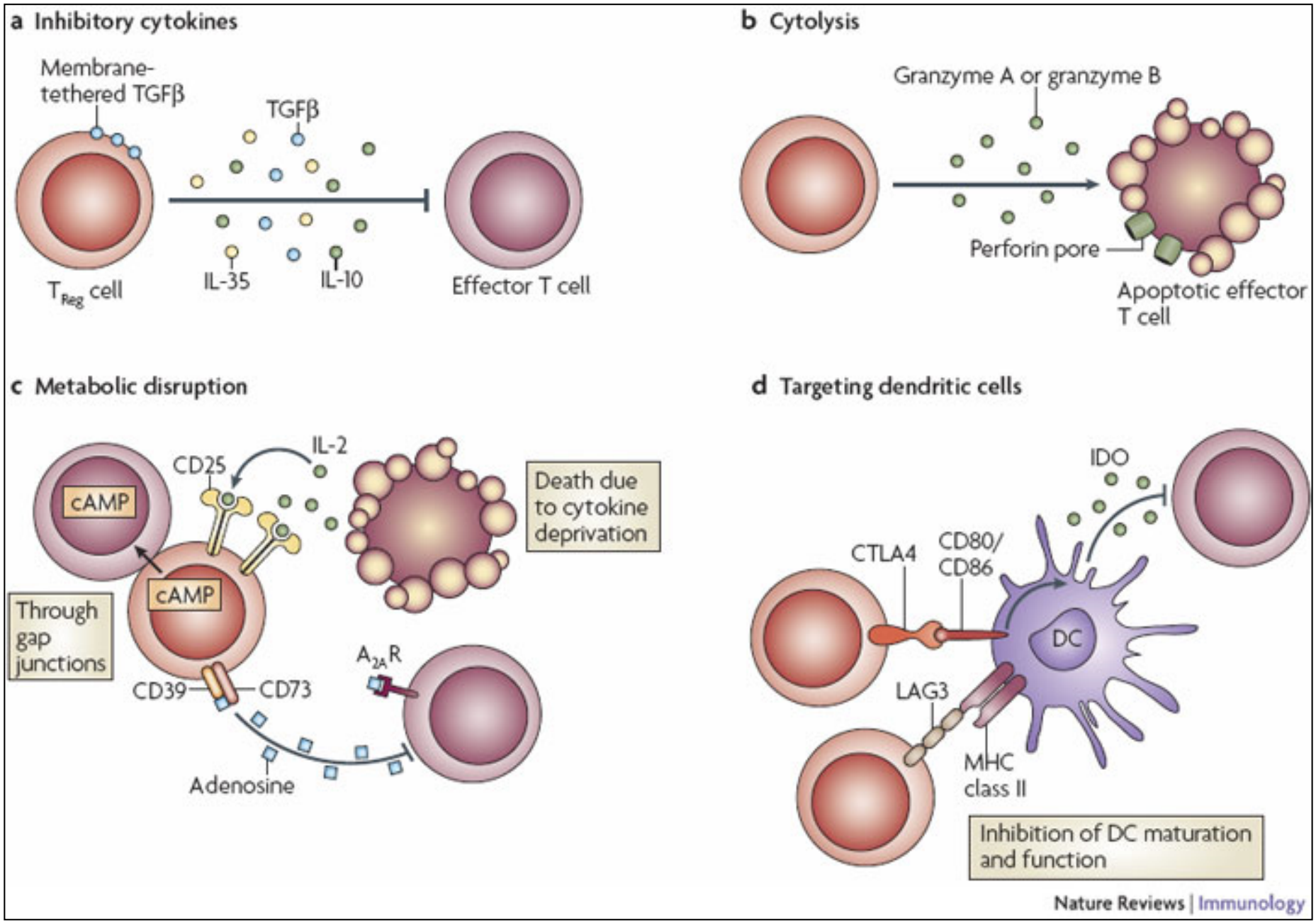
Indeed, this commitment is obtained by the TGF-beta triggered expression of the lineage-unique Foxp3 lineage specification factor.

Whereas newly antigen-activated and TGF-beta stimulated, mature, naive CD4 T cells are induced to express the Treg phenotype, a population of Foxp3 “natural” Tregs also emerge from the thymus with potent regulatory properties.

Hence, two populations, induced and natural Tregs, exist.



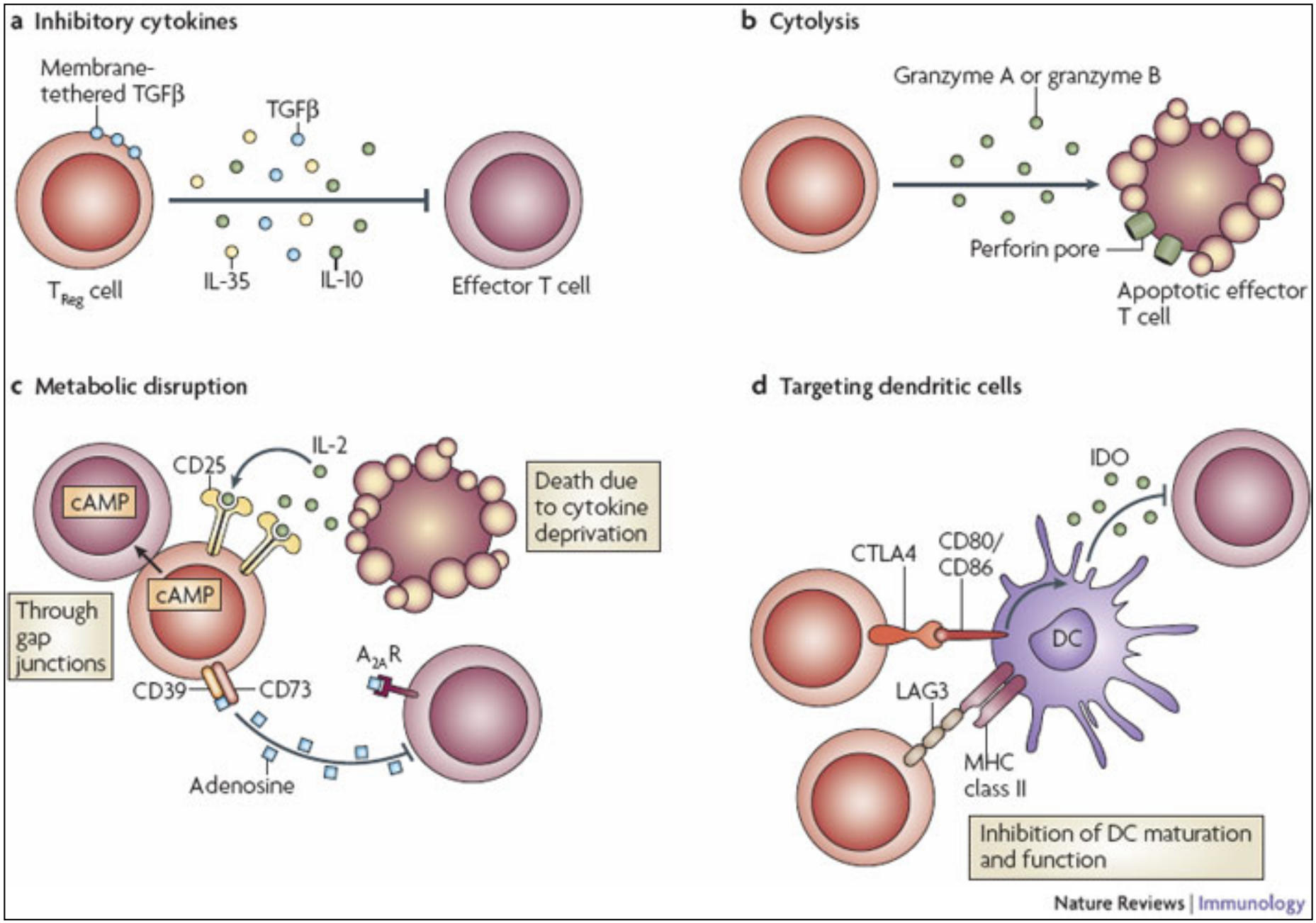



a Inhibitory cytokines
Membrane-tethered TGFβ
TGFβ
T_{Reg} cell
IL-35
IL-10
Effector T cell
b Cytolysis
Granzyme A or granzyme B
Perforin pore
Apoptotic effector T cell
c Metabolic disruption
IL-2
CD25
cAMP
cAMP
Through gap junctions
Death due to cytokine deprivation
$A_{2A}R$
CD39
CD73
Adenosine
d Targeting dendritic cells
IDO
CTLA4
CD80/CD86
DC
LAG3
MHC class II
Inhibition of DC maturation and function
Nature Reviews | Immunology

Humans born with loss-of-function or deletional mutations of Foxp3 rapidly develop devastating forms of autoimmunity.

There can be no doubt that Foxp3 Tregs are crucial to the development and maintenance of tolerance.

The means by which Tregs restrain effector T cells from destroying antigen-bearing tissue seems multifactorial and includes cell– cell interactions with both effector T cells and dendritic cells as well as release of immunosuppressive cytokines, such as TGF-beta and IL-10, and the generation of adenosine catalyzed by subset-specific expression of ectoenzymes.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Nature Reviews | Immunology

Remarkably, TGF- beta, in the presence of IL-6, IL-12, promotes commitment of naive murine and human CD4 T cells to the highly cytopathic Th17 phenotype.

In humans, other proinflammatory cytokines, including TNF- alpha and IL-1 in addition to IL-6, elicit a similar effect.

Indeed, the presence of these proinflammatory cytokines precludes commitment of naive CD4 T cells to the regulatory phenotype.

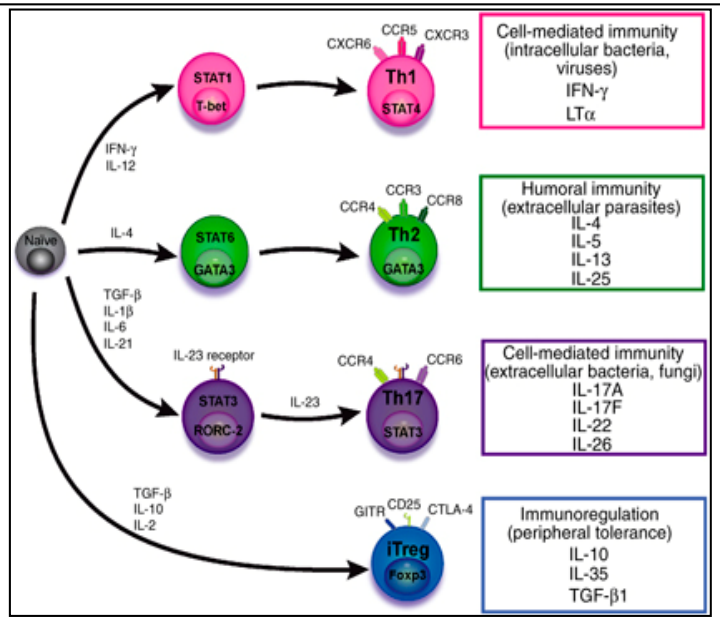
Th17 cells participate in extremely inflamed forms of T cell– dependent tissue injury.

Within these toxic environments, the ability of Foxp3 T cells to restrain effector T cells from executing tissue injury is severely compromised.

Owing to the violence of Th17-dependent tissue injury, a means to target Th17 selectively for therapy is a potentially important unmet need.

The precise role of Th17 cells in rejection is under study.

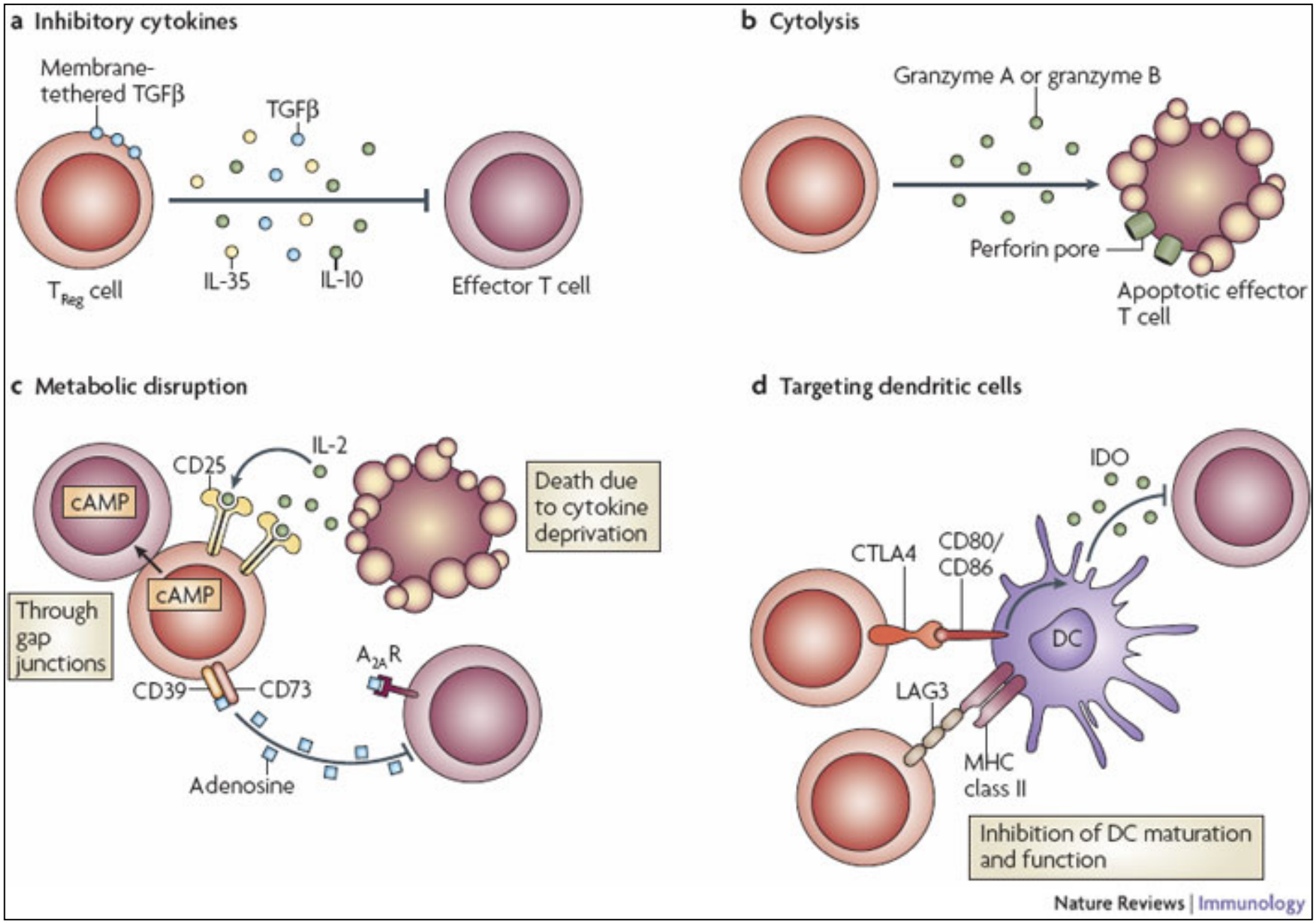
Preliminary experiments suggest, as is the case in autoimmune diseases, that Th17 cells participate in rejection.



The pivotal role of particular cytokines in dictating the precise nature of the commitments of naive T cells undergoing antigen activation is now clear for the Th17 as well as for the Treg, Th1, and Th2 phenotypes.

Thus, the role of cytokines in directing differentiation or commitment to the Th17 and Treg phenotypes is new but also classical in the sense that cytokines are widely known to influence the expression of lineage-determining specification-type transcription factors.

Unprecedented is the recent discovery that the cytokine and inflammatory milieu in which Tregs and Th17 cell function alters the molecular and functional phenotype of these committed, presumably terminally differentiated T cells.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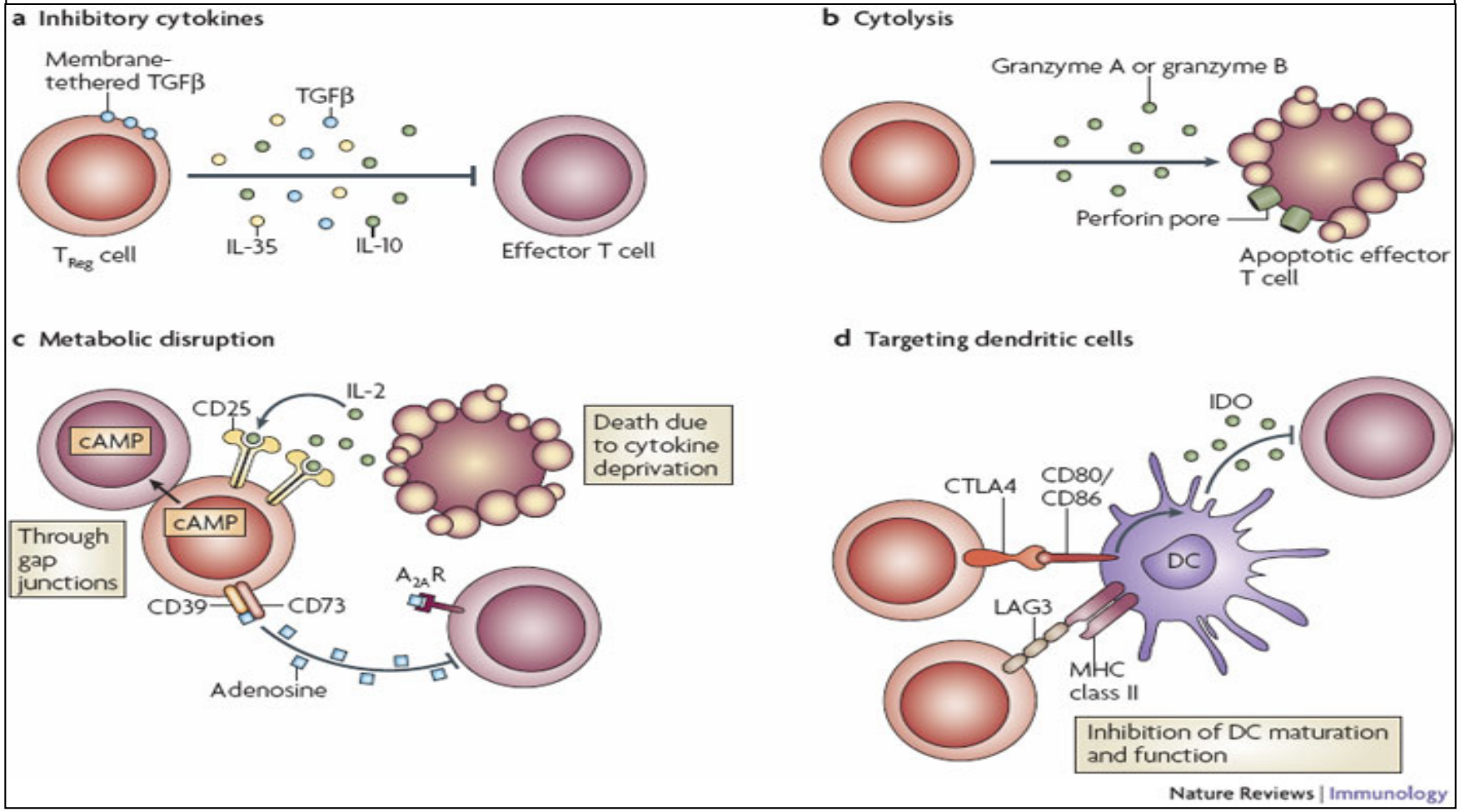
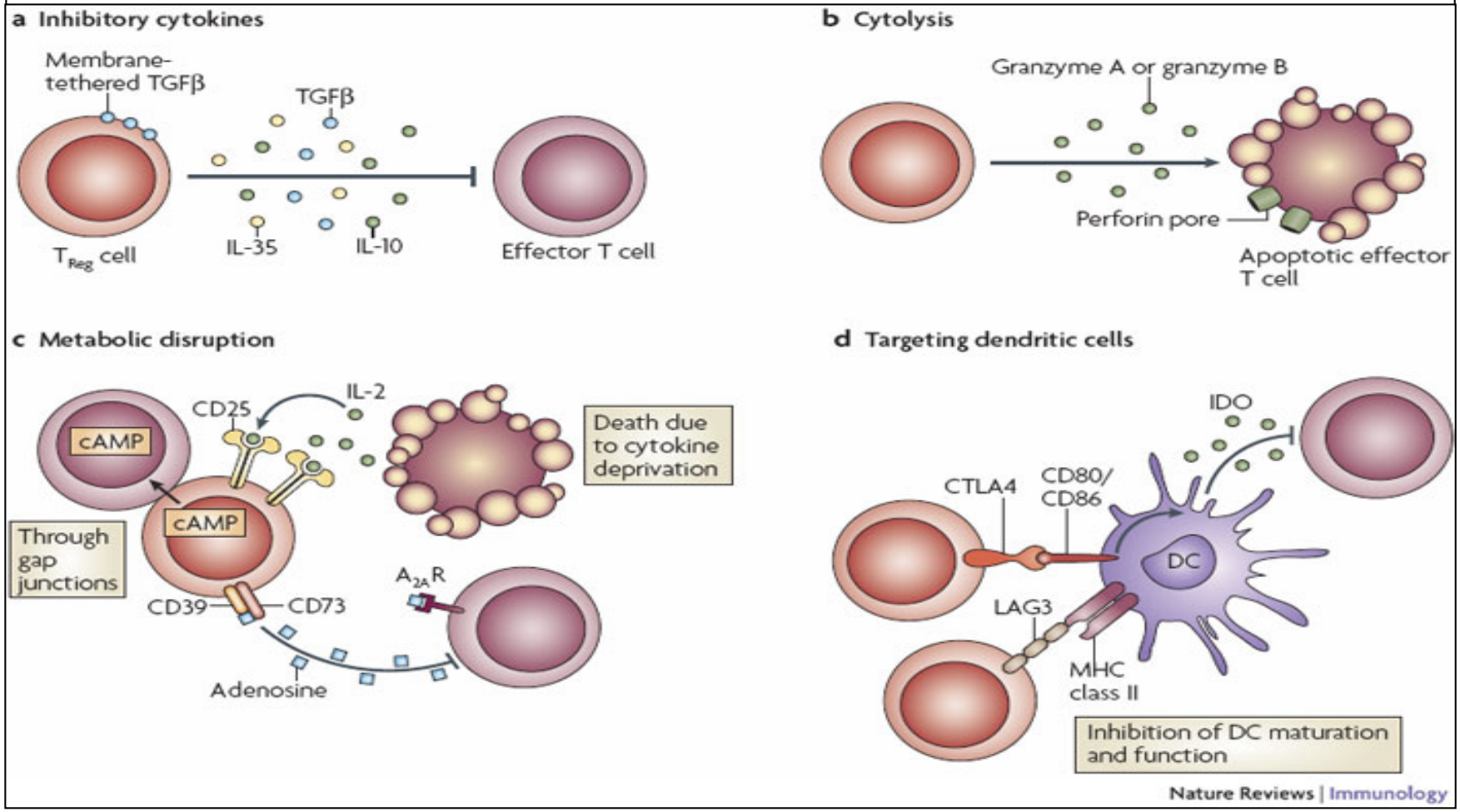
MHC class II
Inhibition of DC maturation and function
Nature Reviews | Immunology

Depiction of the various regulatory T (T_{Reg})-cell mechanisms centred around four basic modes of action.

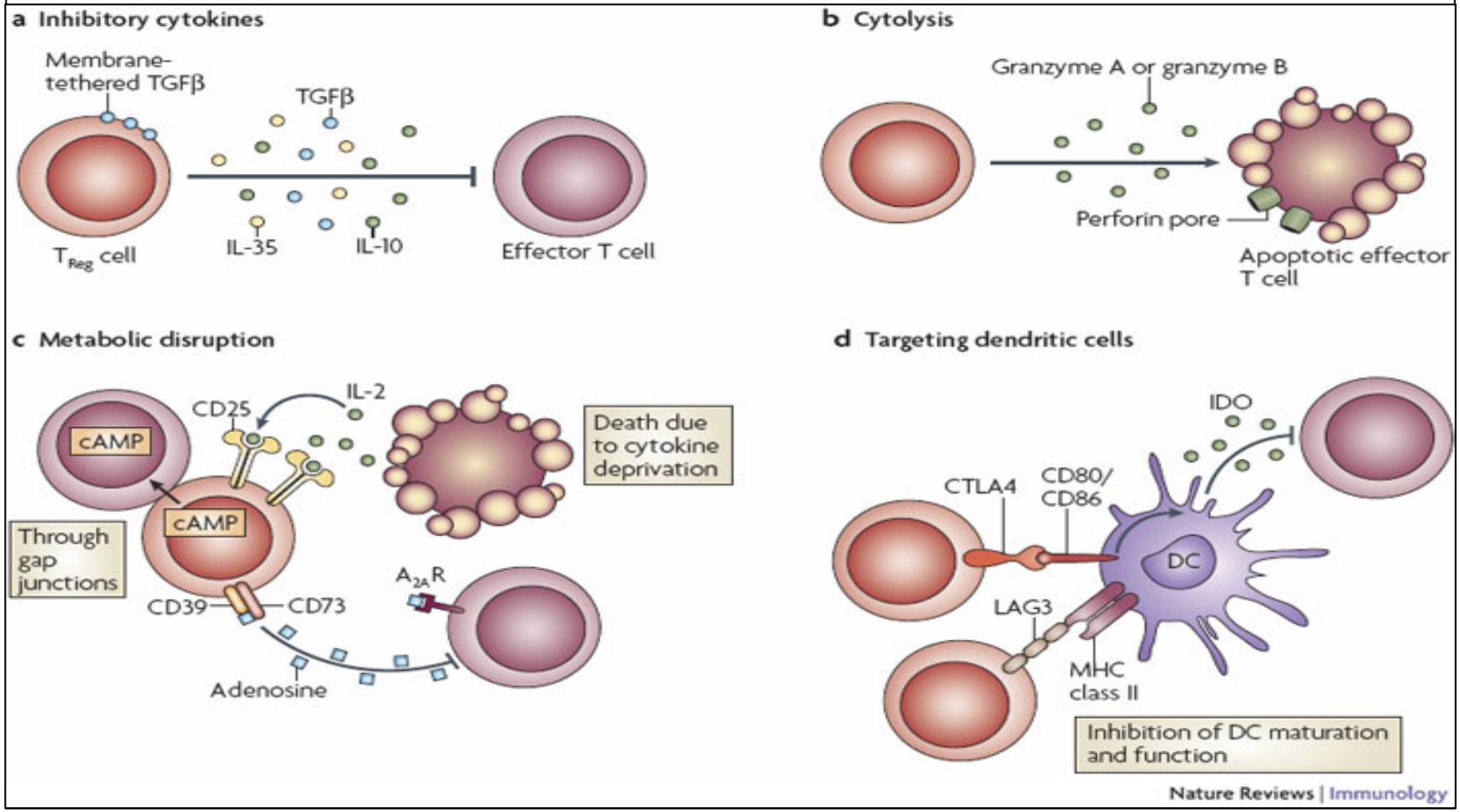
a | Inhibitory cytokines include interleukin-10 (IL-10), IL-35 and TGF-beta



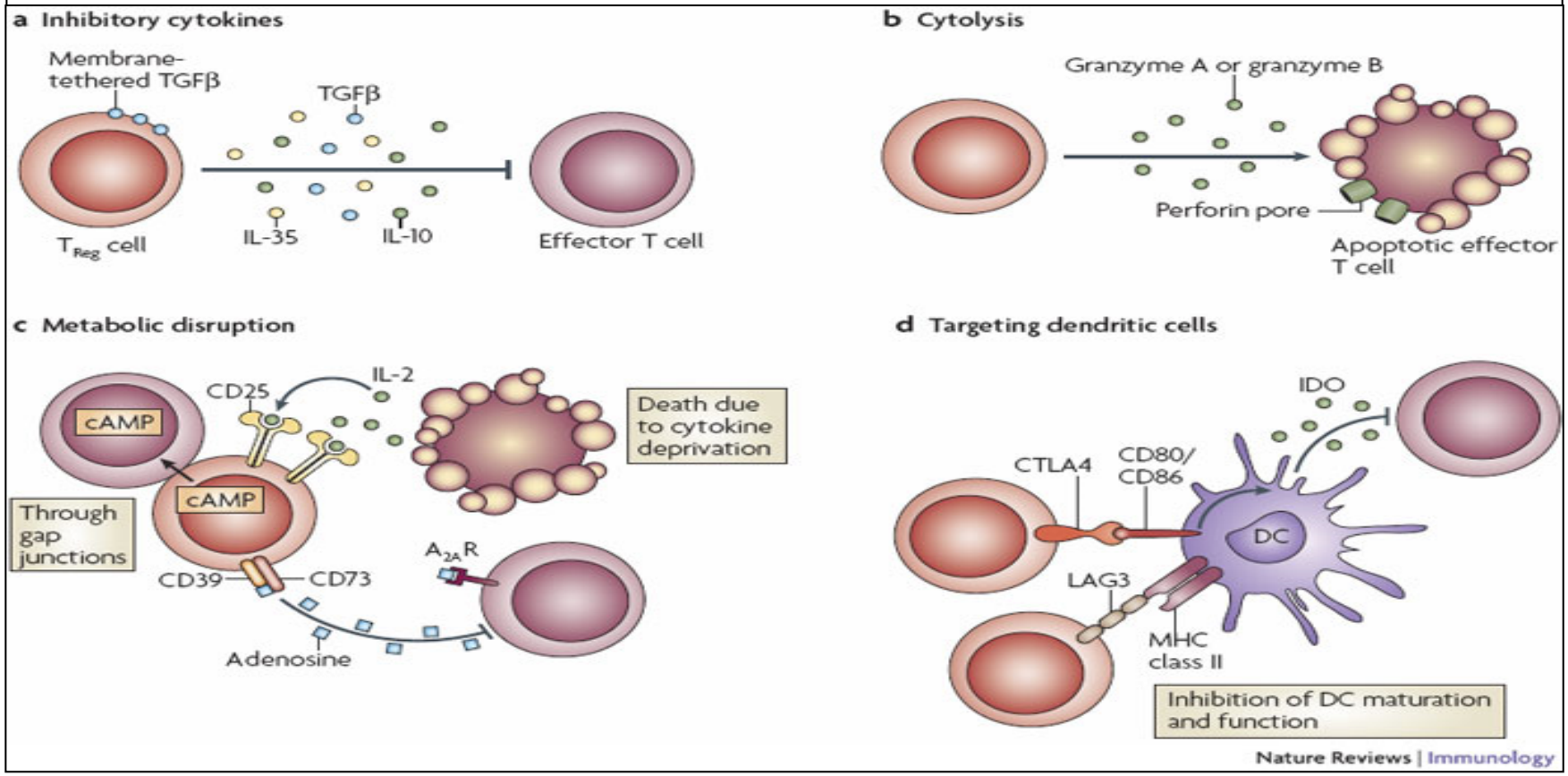
b | Cytolysis includes granzyme-A- and granzyme-B-dependent and perforin-dependent killing mechanisms.

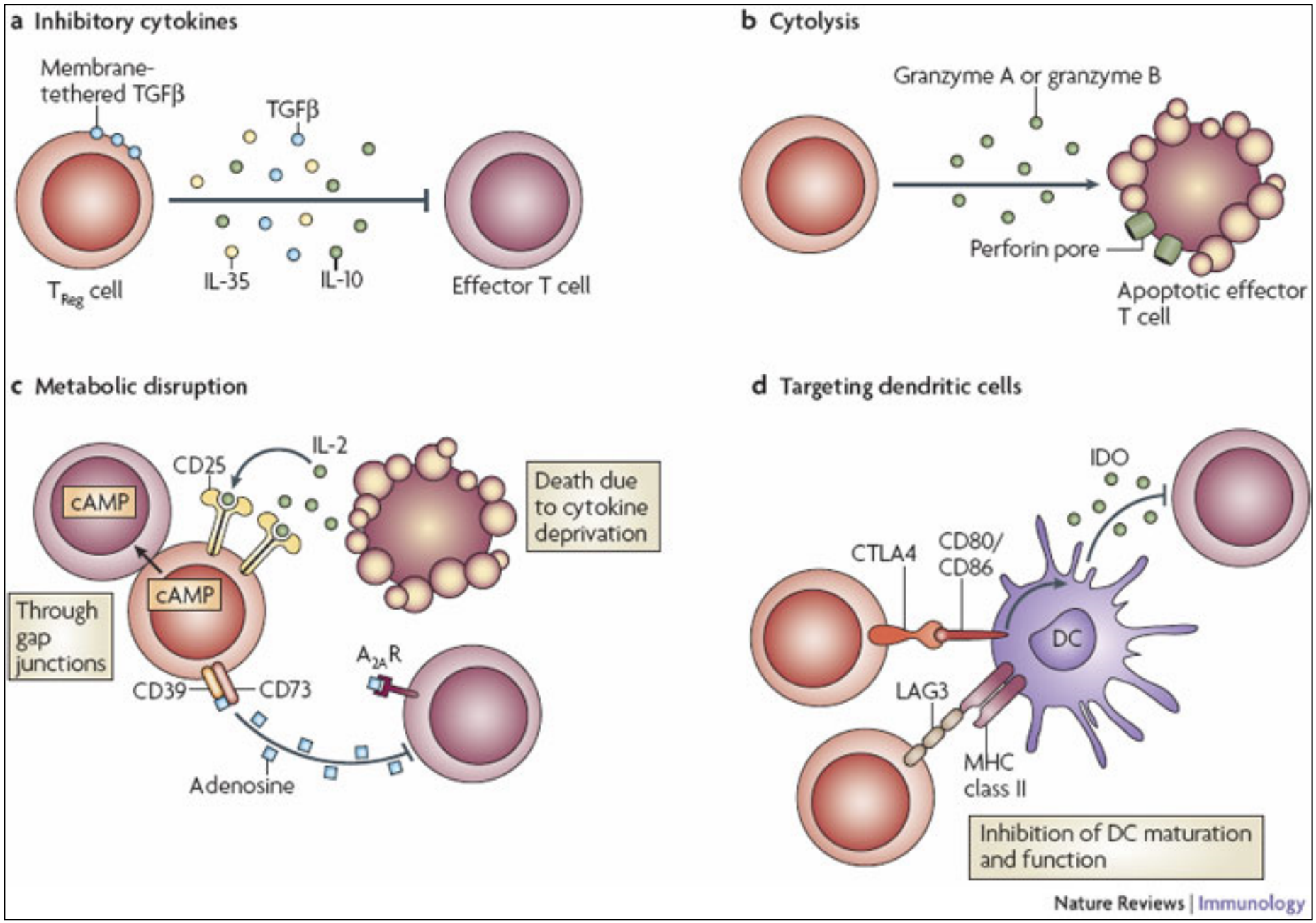


c | Metabolic disruption includes high-affinity CD25 (IL-2 receptor)-dependent cytokine-deprivation-mediated apoptosis, cAMP-mediated inhibition, and CD39- and/or CD73-generated, adenosine receptor 2A ($A_{2A}R$)-mediated immunosuppression.



d | Targeting dendritic cells (DCs) includes mechanisms that modulate DC maturation and/or function such as lymphocyte-activation gene 3 (LAG3)–MHC-class-II-mediated suppression of DC maturation, and cytotoxic T-lymphocyte antigen-4 (CTLA4)–CD80/CD86-mediated induction of indoleamine 2,3-dioxygenase (IDO),an immunosuppressive molecule made by DCs.

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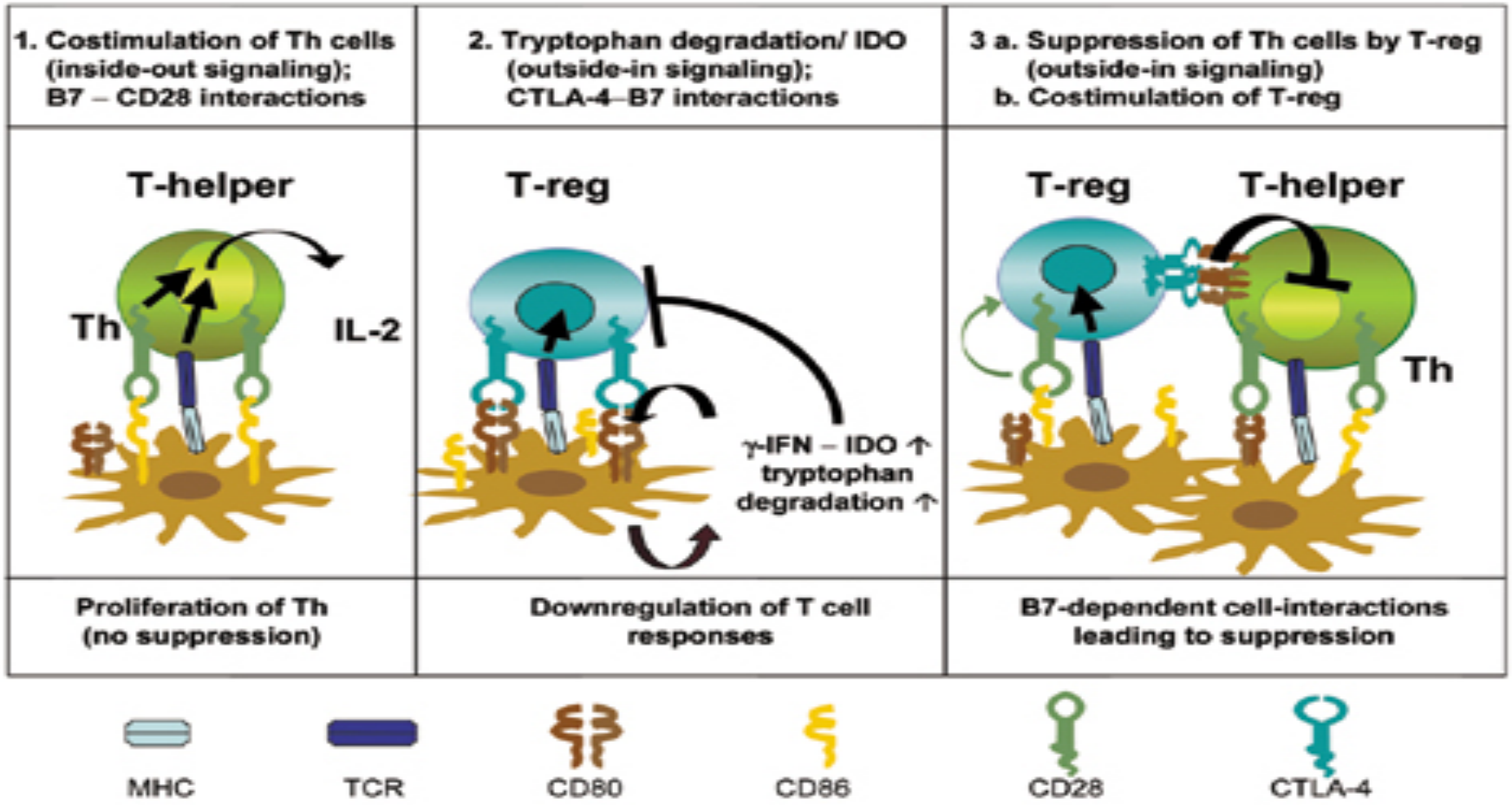
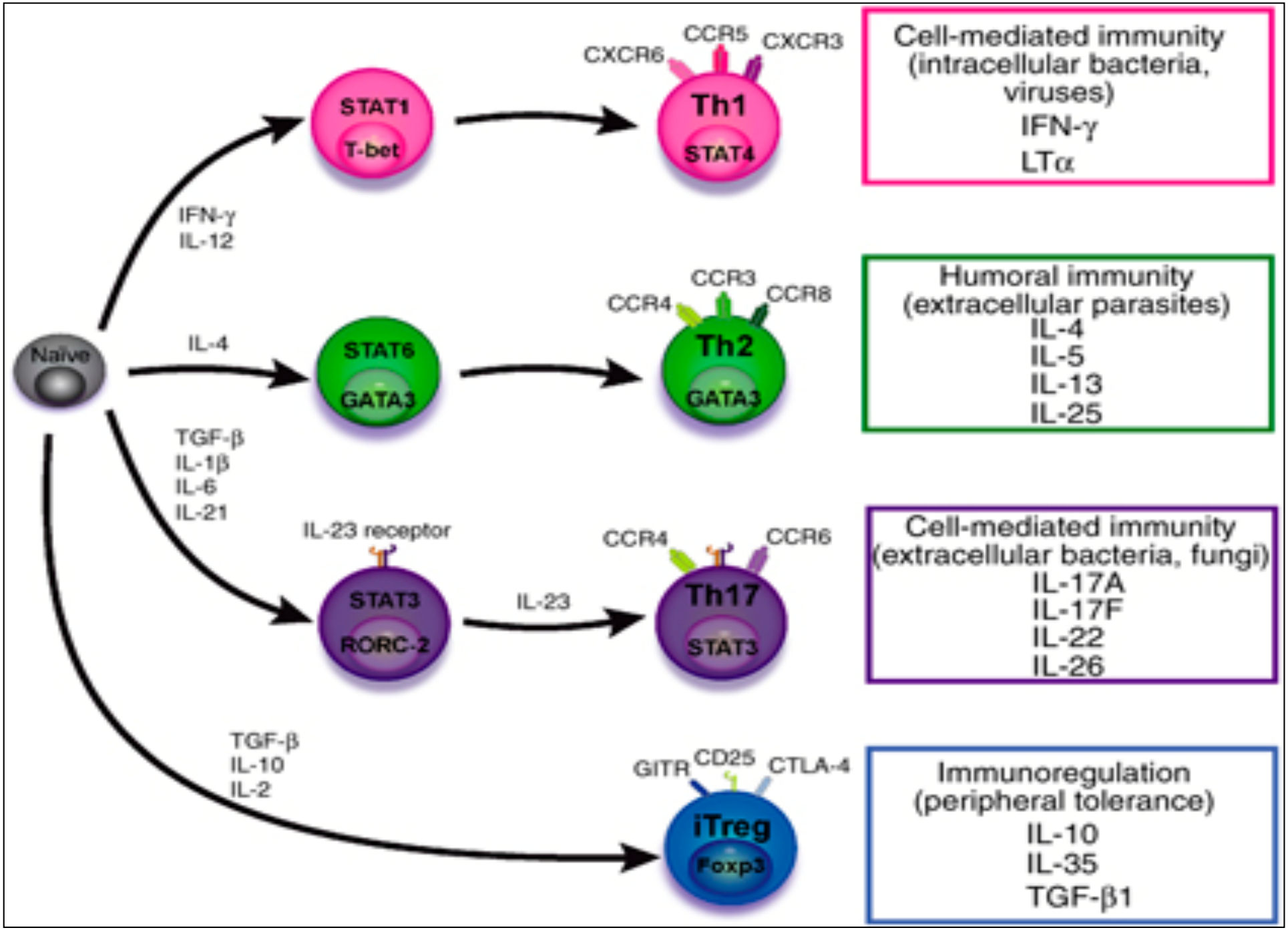
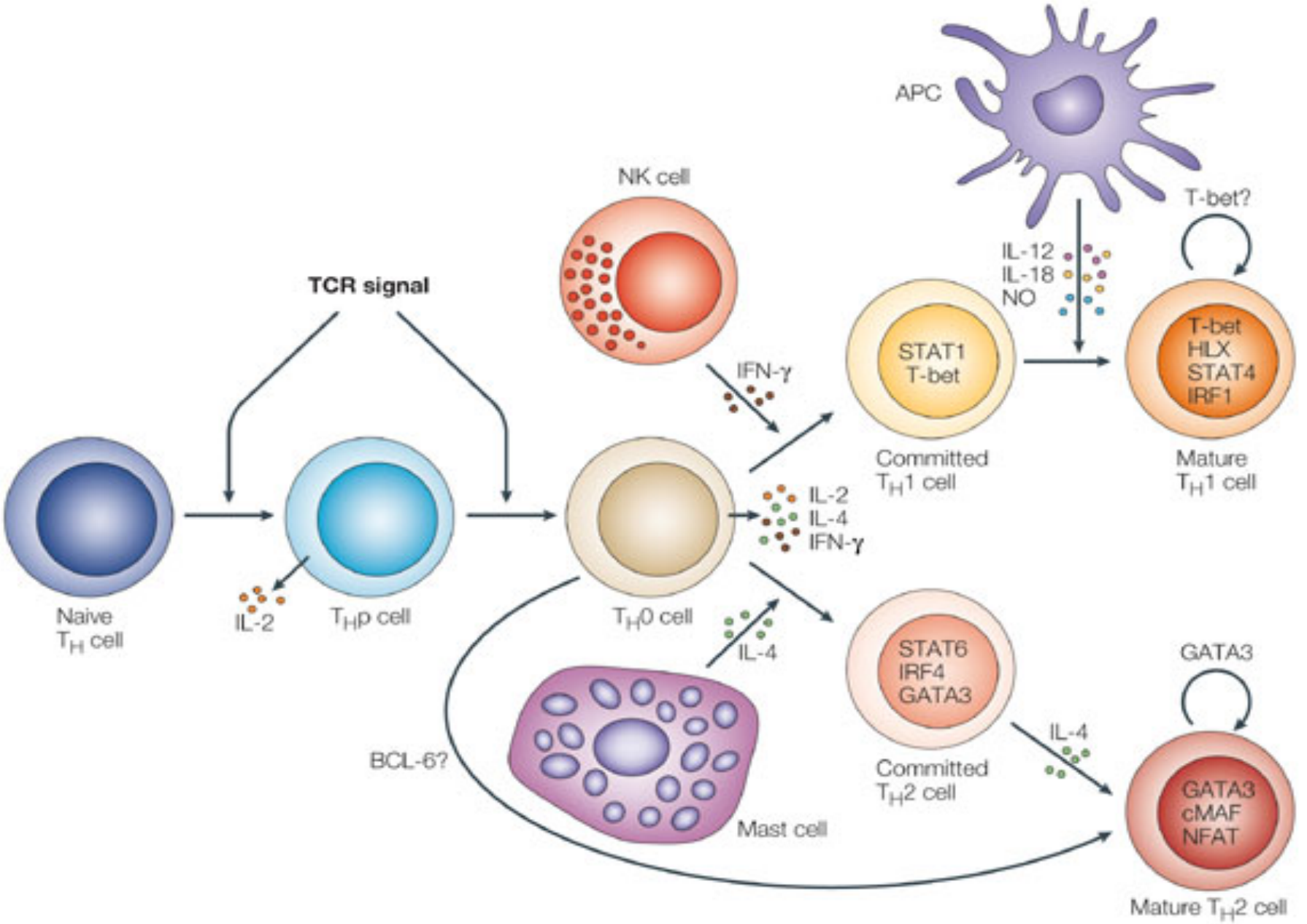
Through gap junctions
Death due to cytokine deprivation
$A_{2A}R$
CD39
CD73
Adenosine
d Targeting dendritic cells
IDO
CTLA4
CD80/CD86
DC
LAG3
MHC class II
Inhibition of DC maturation and function
Nature Reviews | Immunology



B7-mediated pathways of immune regulation. T-reg, regulatory T cells; Th, T helper; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; TCR, T cell receptor; IDO, indoleamine 2,3-dioxygenase.


Naive
IFN-γ
IL-12
STAT1
T-bet
CXCR6
CCR5
CXCR3
Th1
STAT4
Cell-mediated immunity
(intracellular bacteria,
viruses)
IFN-γ
LTα
IL-4
STAT6
GATA3
CCR4
CCR3
CCR8
Th2
GATA3
Humoral immunity
(extracellular parasites)
IL-4
IL-5
IL-13
IL-25
TGF-β
IL-1β
IL-6
IL-21
IL-23 receptor
STAT3
RORC-2
IL-23
CCR4
CCR6
Th17
STAT3
Cell-mediated immunity
(extracellular bacteria, fungi)
IL-17A
IL-17F
IL-22
IL-26
TGF-β
IL-10
IL-2
GITR
CD25
CTLA-4
iTreg
Foxp3
Immunoregulation
(peripheral tolerance)
IL-10
IL-35
TGF-β1


APC
NK cell
T-bet?
IL-12
IL-18
NO
TCR signal
IFN-γ
STAT1
T-bet
T-bet
HLX
STAT4
IRF1
Committed
T_H1 cell
Mature
T_H1 cell
IL-2
IL-4
IFN-γ
Naive
T_H cell
IL-2
T_HP cell
T_H0 cell
IL-4
STAT6
IRF4
GATA3
GATA3
IL-4
BCL-6?
Mast cell
Committed
T_H2 cell
GATA3
cMAF
NFAT
Mature T_H2 cell

Model for T helper (Th) or T regulatory (Treg) differentiation from naïve CD4+ T cells.

Th1 cells differentiate in the presence of IL-12, and require activation of the master regulator transcription factor, T-beta, through STAT1.

Fully committed Th1 cells express chemokine receptors, CXCR6, CXCR3, and CCR5, and produce IFN-gamma and lymphotoxin through STAT4.
They are involved in cell-mediated immunity against intracellular bacteria and viruses.

Th2 cells depend on the presence of IL-4, STAT6, and GATA-3, and release IL-4, IL-5, IL-13, and IL-25. Th2 cells express chemokine receptors, CCR3, CCR4, and CCR8, and are important in humoral immunity against parasites and helminthes.

Th17 cells require a combination of TGF- beta and proinflammatory cytokines (IL-1 , IL-6, and/or IL-21) to differentiate from naïve CD4+, and RORC-(variant) acts as the key transcriptional regulator.

Upregulation of the IL-23 receptor makes these cells responsive to IL-23.

Human Th17 cells produce IL-17A, IL-17F, IL-22, and IL-26, and are important in host protection against extracellular pathogens and in autoimmunity. Their surface markers include chemokine receptors, CCR4, CCR6, and CD161.

In addition to effector T cells, naïve CD4+ T cells can also differentiate into induced Treg (iTreg) in the presence of IL-2 and TGF-beta or IL-10.
iTreg produces immunosuppressive cytokines, TGF-beta, IL-10, and IL-35, and express surface markers, GITR, CD25, and CLTA-4.

Similar to thymus-derived naturally occurring Treg (nTreg), iTreg also expresses the master regulator transcription factor, Foxp3.

T_H1 cells produce IFN-gamma, IL-2 and lymphotoxin, whereas T_H2 cells produce IL-4, -5, -6, -10 and -13

T_H1 and T_H2 cells originate from precursor T_H (T_Hp) cells, which secrete IL-2 but not IL-4 or IFN-gamma; these cells then differentiate into T_H0 cells, which produce both T_H1 and T_H2 cytokines.

IL-4 drives T_H0 cells to differentiate towards the T_H2-cell phenotype by activating signal transducer and activator of transcription 6 (STAT6), which in turn upregulates the expression of GATA-binding protein 3 (GATA3)

GATA3 is crucial for chromatin changes that stabilize the T_H2-cell phenotype, and it cooperates with growth-factor independent 1 (GFI1) in triggering T_H2-cell Proliferation.

Differentiation into T_H2 cells occurs independently of IL-4 or STAT6 in mice that are deficient in B-cell lymphoma 6 (BCL-6).

Differentiation into T_H1 cells depends on IL-12-mediated activation of STAT4 which in turn supports IFN-gamma production. IFN-gamma signals induce STAT1 to activate the transcription factor T-beta, which cooperates to increase expression of IFN-gamma and the 2-subunit of the
IL-12 receptor (IL-12R 2).

T_H0 cells that are destined to become T_H2 cells downregulate expression of IL-12R .

Cytokines that cooperate with IL-12 include IL-18, -23 and -27. In a positive-feedback loop, IFN-gamma drives T_H1-cell responses independently of IL-12.
IFN-gamma supports the differentiation of human T_H cells into T_H1 cells.
As well as cytokines, nitric oxide (NO), which is produced by inducible NO synthase, promotes differentiation into T_H1 cells, by upregulating expression of IL-12R.

Also, the type of dendritic cell (DC) that is encountered by the uncommitted T_H cell is relevant: $B220^+$ plasmacytoid DCs, which produce IFN- gamma, and conventional $CD8^+$ $B220^-$ DCs, which produce IL-12, both trigger T_H1 responses. By contrast, conventional $CD8^-B220^-$ DCs prime T_H2 responses.

APC, antigen-presenting cell; IRF, IFN-regulatory factor; NK, natural killer;
TCR, T-cell receptor.