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Idiopathic nephrotic syndrome (INS) defines a group of rare glomerular diseases characterized by massive proteinuria and hypoalbuminemia in the absence of glomerular inflammatory lesions or immunoglobulin deposits. INS includes two main entities based on kidney biopsy findings: minimal-change nephrotic syndrome (MCNS) and focal segmental glomerulosclerosis (FSGS). The treatment of INS relies on steroids and/or immunosuppressive drugs (calcineurin inhibitors, cyclophosphamide, mycophenolate mofetil, rituximab). Distinct clinical patterns can be observed such as steroid sensitive with frequent relapses (in 70-80% of cases), and primary or secondary steroid resistant forms. INS is considered as a chronic medical condition interfering in childhood as in adulthood with well-being and health-related quality of life.

While genetic studies have elucidated rare forms of inherited nephrotic syndrome, they failed to identify a genetic defect in INS with relapse, except an association with some variants of class II-MHC antigens (1). Studies on genetic polymorphisms in the variable region of the T-cell receptor (TCR) ß-chain have revealed a selective recruitment of some variable β gene families in peripheral $CD8^+$ T cells from nephrotic patients with frequent relapses (2).

The hypothesis that INS results from a systemic disorder of immune system is supported by clinical observations such as its sensitivity to steroid therapy and immunosuppressive drugs, as well as the rapid occurrence of relapses upon antigen challenge (infections or vaccination) particularly in children.

Several disorders of T-cell subpopulations have been reported during relapses (3). The frequency of Treg is consistently reduced (4), and is restored after response to therapy (3).

The downregulation of Treg cells in relapse is correlated with a significant decrease in IL-2, which is crucial to the development and maintenance of Treg cells. Experimental evidence suggests that Treg cells contribute to control humoral response by limiting expansion of T follicular helper (TFH) and B cells. Depletion of Treg cells increases switched B cells, while adoptive transfer of Treg cells suppresses in *vivo* B-cell response. Interestingly, Treg cells prevent expansion of autoreactive B cells and induce their apoptosis (5), which suggests that downregulation of Treg may lead to escapement of autoreactive, potentially switched memory B, which may contribute to the development of autoimmune diseases. Indeed, relapses are found to be associated with more rapid reconstitution of switched memory B cells (6).

The finding that Rituximab, a B-cell-depleting agent, maintains long-lasting INS remission points out a potential role for B lymphocyte in the mechanisms of INS pathogenesis. In a multicenter, double-blind, randomized, placebo *vs* Rituximab-controlled trial, we have analyzed the modifications on T-cell subsets induced by B-cell depletion (3). Our results suggest that Rituximab does not interfere with $CD4^+$ T-cell, $CD8^+$ T-cell or $CD45RO^+$ T-cell frequencies, while relapses are associated with downregulation of $CD8^+$ T-cell subset and an increase in the frequency of $CD4^+CD45RO^+CD30^+$ circulating memory T-cell subset, which are involved in recall memory antibody responses. On the other hand, Rituximab reduces specifically the frequency of TFH cells. Interestingly, recent studies showed an expansion of TFH in relapses, associated with a defect in immunoglobulin switching, accounting for the increase in IgM class and low production of IgG affecting some subclasses, according to unknown mechanisms (7, 8). In another study, it has been shown that T cells of patients who respond to Rituximab display a weaker response to PMA/ionomycin activation, characterized by a lower percentage of $CD3^+CD4^+CD154^+$, $IFN\gamma^+CD3^+$ and $IL\text{-}2^+CD3^+$ T-cell subsets compared with non-responders, suggesting that hyporesponsiveness to T-cell stimulation could be used to identify patients susceptible to respond to Rituximab (9).

The role of B cells in INS pathogenesis remains to be clarified. The frequency of B-cell subpopulations before and after Rituximab therapy has been investigated in pediatric patients, showing that memory B cell recovery is faster in patients who relapse compared to non-relapsers (6). Rituximab is thought to erase germinal center B cells in human lymph nodes, without affecting the TFH cell population (10). This raises the possibility that the delayed reconstitution of switched memory B cells could result from qualitative alterations of TFH cells induced by B-cell depletion. Indeed, expansion of switched memory B cells requires cognate TFH cells since it is abrogated in the absence of IL-21 receptor or CD40 ligand (11). Remission following B-cell depletion suggests that glomerular disease could be induced by some B lymphocyte subsets such as autoreactive B-cells but some arguments advocate a more intricate mechanism: (i) immunofluorescence studies on renal biopsies consistently show the absence of Ig deposits in INS relapse and (ii) remission can be maintained despite complete recovery of peripheral B-cell compartment, while relapses can occur in presence of sustained B cell^{-} depletion (12). Beside their role in antibody-mediated mechanisms, B cells are also involved in antigen presentation, T-cell activation/regulation and production of cytokines and growth factors. Thus, B cells may facilitate disease activity by sustaining pathogenic T-cell responses through antibody-independent mechanisms. Therefore, although Rituximab may be considered as an innovative therapeutic agent in frequent relapsing, steroid-dependent INS, the mechanisms by which it interferes with T-cell disorders remain unclear.

CMIP (CMaf-inducing protein) is a poorly described gene, initially identified in T cells of patients with INS (13). In normal lymphoid tissue, CMIP is found selectively expressed into lymphoid follicles, mainly at the interface of B-cell and T-cell zones (14), while in peripheral T cells CMIP is scarcely detected in resting conditions (13), which suggests that in basic conditions, CMIP acts primarily in secondary lymphoid organs. On the other hand, CMIP was found highly expressed by subset of as yet uncharacterized T cells. Selective expression of

CMIP in peripheral T cells by targeted transgenesis in mice results in T cell dysfunction. CMIP interferes with the early events of T-cell signaling by inhibiting lipid raft clustering, the activation of Src kinases (Fyn and Lck) and ZAP-70 (Figure). Transgenic T-cells exhibit a lower proliferative capacity and are less prone to producing cytokines, notably IL2, after stimulation (13). Moreover, CMIP binds to the P85 subunit of PI3 kinase and prevents its dissociation from the p110 catalytic subunit, which results in inhibition of PI3-kinase activation and downstream signaling molecules, notably Akt. It is interesting to note that Akt activation is inhibited *in vitro* and *in vivo* in T cells and podocytes overexpressing CMIP, as well as in INS disease ((15) and manuscript in preparation). These observations may account for the lower reactivity of T lymphocytes to mitogens and the decrease of delayed hypersensitivity reported in patients with INS. However, a clear identification of CMIP function in the immune system is mandatory for a better understanding of its role in INS pathogenesis.

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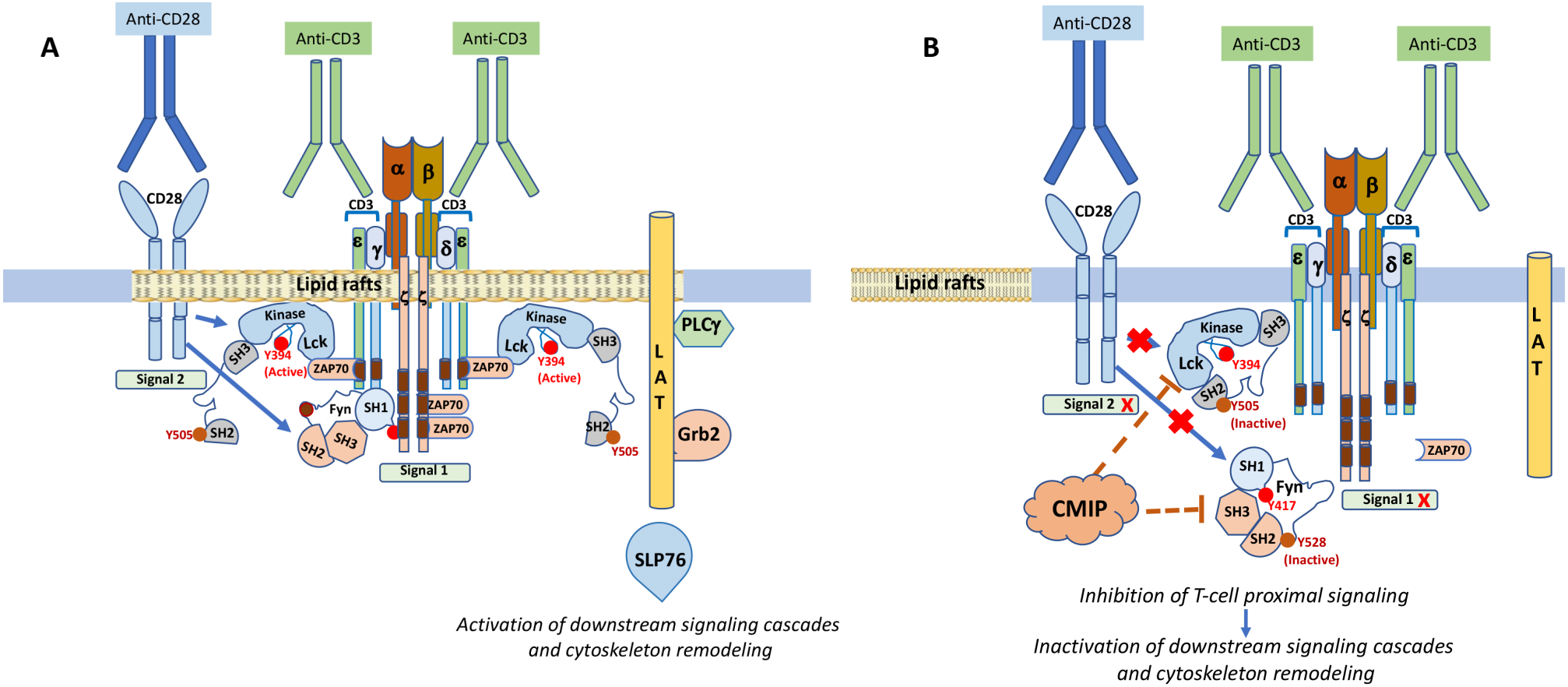
Legend of the figure. CMIP inhibits T-cell proximal signaling

(A), costimulation by anti-CD3/CD28 mimics T-cell activation induced by ligation of the TCR by a peptide bound to a major histocompatibility complex (MHC) class II-antigen. In both cases, signal 1 (dependent on TCR ligation) and signal 2 (CD28 costimulation), trigger rapid Src kinases-mediated phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM), a conserved domain of signal transducing chains of the TCR complex. Phosphorylated ITAMs serve as binding sites for ZAP-70 kinase, which is activated by phosphorylation by the Src kinase Lck resulting in stability of the ITAM-ZAP-70 interaction. The clustering of active TCR and the recruitment of Lck/Fyn and ZAP-70 occurs in lipid rafts (LR), which are plasma membrane microdomains enriched in cholesterol and glycosphingolipids and serve as signaling platforms. Activation of ZAP-70 induces phosphorylation at multiple tyrosine residues of the transmembrane adapter molecule LAT (linker for activation of T-cells) and a leukocyte phosphoprotein of 76 kDa (SLP-76) contributing to generate through protein-protein or protein-lipid interactions a complex-signaling within LR, leading to cytoskeletal reorganization, formation of immunological synapse and efficient signal transmission from rafts to downstream

signaling cascades, ultimately resulting in activation of transcription factors such as NF-kB, NFAT, and AP-1. **(B),** overexpression of CMIP inhibits the activation of Src kinases and the activation of ZAP-70, suggesting that CMIP interferes with the T-cell proximal signaling and prevents the clustering of lipids rafts, the cytoskeleton reorganization and the activation of downstream signaling cascades.


A
Anti-CD28
Anti-CD3
Anti-CD3
CD28
CD3
CD3
α
β
ε
γ
δ
ε
ζ
ζ
Lipid rafts
Kinase
Lck
SH3
Y394
(Active)
ZAP70
Signal 2
Y505
SH2
Fyn
SH1
SH3
SH2
ZAP70
ZAP70
ZAP70
Signal 1
Kinase
Lck
Y394
(Active)
SH3
SH2
Y505
LAT
PLCγ
Grb2
SLP76
Activation of downstream signaling cascades
and cytoskeleton remodeling
B
Anti-CD28
Anti-CD3
Anti-CD3
CD28
CD3
CD3
α
β
ε
γ
δ
ε
ζ
ζ
Lipid rafts
LAT
Kinase
SH3
Lck
Y394
SH2
Y505
(Inactive)
Signal 2 X
ZAP70
SH1
Fyn
Y417
CMIP
SH3
SH2
Y528
(Inactive)
Signal 1 X
Inhibition of T-cell proximal signaling
Inactivation of downstream signaling cascades
and cytoskeleton remodeling