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ORIGINAL ARTICLE

A global antiB cell strategy combining obinutuzumab and daratumumab in severe pediatric nephrotic syndrome

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Abstract

Background Steroid-sensitive nephrotic syndrome (SSNS) is, in most patients, a chronic disease with 80% experiencing at least one relapse after first flare. B cell depletion using rituximab is effective in preventing relapse in steroid-dependent (SDNS) patients but fails to maintain long-term remission following B cell recovery, possibly due to development of autoreactive long-lived plasma cells. We investigated sequential combination of antiCD20 antibody targeting all B cell subsets, and antiCD38 antibody with high plasma cell cytotoxicity in patients with uncontrolled SDNS after failure of one or several attempts at B cell depletion.

Methods Fourteen patients with median disease duration 7.8 years received 1000 mg/1.73 m^2 obinutuzumab followed by 1000 mg/1.73 m^2 daratumumab 2 weeks later. Oral immunosuppression was discontinued within 6 weeks, and biological monitoring performed monthly until B cell recovery.

Results Median age at treatment was 11.0 [IQR 10.4–14.4] years. B cell depletion was achieved in all patients, and B cell reconstitution occurred in all at median 9.5 months after obinutuzumab injection. After median follow-up 20.3 months (IQR 11.5–22.6), 5/14 patients relapsed including 4 within 100 days following B cell repletion. Relapse-free survival was 60% at 24 months from obinutuzumab infusion. Mild infusion reactions were reported in 3/14 patients during obinutuzumab and 4/14 during daratumumab infusions. Mild transient neutropenia (500–1000/mm^3) occurred in 2/14 patients. Intravenous immunoglobulins were given to 12/14 patients due to hypogammaglobulinemia. Low IgA and IgM levels were noted in 8 and 14 patients, respectively. No severe infection was reported.

Conclusion Global antiB cell strategy combining obinutuzumab and daratumumab induces prolonged peripheral B cell depletion and remission in children with difficult-to-treat SDNS.

Keywords Children · Steroid-dependent nephrotic syndrome · Rituximab · Relapse · Obinutuzumab · Daratumumab · B cell recovery

Background

Although the majority of children with idiopathic nephrotic syndrome (INS) are steroid sensitive (SSNS), 80% of them will experience relapses and a chronic evolution of the disease [1]. A significant proportion of frequent relapsers (FRNS) and steroid dependent (SDNS) will have a protracted course exceeding 10 years and be exposed to oral prednisone and

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immunosuppressive drugs beyond adult age [2]. The endless succession of relapses and the many complications of both the disease and the exposure to immunosuppressive drugs explain the morbidity of INS and impose major constraints on the patients.

Immunosuppressive drugs have been used to reduce exposure to steroids and their toxicity in FR/SDNS. Levamisole, cyclosporine, mycophenolate mofetil, cyclophosphamide, and more recently monoclonal antibodies specifically targeting B cells, like rituximab, have demonstrated their ability to prevent relapses [3]. All these drugs are known to alter B cell immunity. Among them, cyclophosphamide and rituximab deserve special attention since they have a remnant effect, leading to a stable remission far beyond the withdrawal of the treatment in some patients [4–7]. Cyclophosphamide mainly targets naïve B cells and immature short-lived plasma cells, but not memory B cells [8, 9]. By contrast, rituximab affects the survival of naïve and memory B cells, but does not affect long-lived plasma cells [10]. Consistently, the failure of rituximab to control autoimmune diseases has been attributed to the development and/or survival of autoreactive long-lived plasma cells. The production of these plasma cells is paradoxically increased in the context of B cell depletion [11] and associated to the chronicity of autoimmune diseases. Of note, measles has been historically associated with temporary or definitive remission of childhood nephrosis [12, 13], in that it induces the depletion of both memory T and B cells, and also plasma cells [14].

This retrospective study reports the outcomes of difficult-to-treat SDNS patients, who relapsed after rituximab and were treated with a global antiB cell strategy targeting both B cells and plasma cells. The treatment sequentially combined obinutuzumab (OBI), a second generation antiCD20 monoclonal antibody targeting naïve and memory B cells, and daratumumab (DAR), an antiCD38 monoclonal antibody targeting plasma cells. This combination aimed to control the development of autoreactive long-lived plasma cells in patients where B cell depletion alone failed to provide sustained remission.

Methods

This is a monocentric retrospective study including SDNS patients who relapsed after rituximab and were treated with a sequential combination of obinutuzumab and daratumumab (referred to below as the OD sequence) at Robert-Debré Hospital, APHP, Paris, from June 2018 to July 2019.

A single intravenous dose of obinutuzumab 1000 mg/1.73 m^2 (a reduced dose of 300 mg/1.73 m^2 in one patient) over 4 h was injected at day 0. At day 14, patients received an injection of daratumumab 1000 mg/1.73 m^2 over 4 h. Before each infusion, patients received systematic premedication with acetaminophen (15 mg/kgBW), methylprednisolone (1 mg/kgBW) and dexchlorpheniramine (2.5 mg below 30 kgBW and 5 mg over 30 kgBW). In addition, oral prednisone at a dose of 20 mg/day was given for 2 days after daratumumab infusion. Oral immunosuppression withdrawal was scheduled as follows: prednisone was maintained at 60 mg/m^2 every other day for 2 weeks after obinutuzumab injection then tapered down to 30 mg/m^2 every other day for 2 weeks then stopped; tacrolimus was maintained at the same dose 4 to 6 weeks after obinutuzumab injection and stopped. All patients received sulfamethoxazole (800 mg × 3/week) during B cell depletion. A monthly follow-up of proteinuria, serum creatinine and albumin, white blood cells, platelets, and CD19 counts, as well as plasma levels of immunoglobulins G, A, and M, was scheduled.

All data were collected from routine clinical and biological reports. Off-label prescription of the OD sequence has been approved by our institution's "Drug Committee". Parents as well as patients over 18 years signed an informed consent of care.

Patients' medical history and characteristics at baseline are individually reported in Tables 1 and 2, using median and interquartile range (IQR) when mentioning the whole series. Time to B cell reconstitution and relapse-free survival were studied using the Kaplan–Meier method, and the evolution of concentrations of immunoglobulins over time was studied using the weighted least square method.

Results

Patient history

Fourteen patients with SDNS were treated (Table 1) including 13 males and one female. Median age at the first flare was 3.2 years (interquartile range IQR 1.9–5.4). Steroid sensitivity was defined at first flare by a proteinuria < 0.02 g/mmol of urine creatinine after 4 weeks of oral prednisone (60 mg/m^2/day) in 11 patients and after 3 additional pulses of intravenous methylprednisolone (1000 mg/1.73 m^2/pulse) in 3 patients (#4, #5, #11). All patients had a history of steroid dependency (relapses under 5–60 mg of alternate day regimen of prednisone). The course of 4 patients (#4, #5, #10, #14) after the first flare was also complicated with one episode of secondary steroid resistance, but all those patients demonstrated steroid sensitivity during later relapses prior to obinutuzumab injection. Of note, 4 patients experienced an extended duration of the disease with persistent relapses later than 10 years after INS onset (#4, #6, #7, #13). All patients were previously treated with oral treatments including levamisole in 8 patients, cyclophosphamide in 3, and mycophenolate mofetil in 12, and either cyclosporine or tacrolimus in 13. Medical adherence, assessed by the patients' physicians, was considered poor in 1 patient, partial in 2 and appropriate in 11. All patients

Table 1 History of patients and previous treatments prior to the injection of obinutuzumab

# Patients	Gender	Age at INS onset (years)	IVMP at 1st flare (*n*)	Number of relapses (*n*)	Level of steroid dependence mg/48 h	Late steroid resistance	History of IS drugs	B cell depleting agents	B cell depletion (number of attempts)	Cumulative B cell depletion (months)	Total duration of disease (years)
1	M	2.9	0	5	35	No	CI	RTX	2	2.1	3.1
2	M	6.9	0	7	30	No	CYC,MMF,CI,LEV	RTX	1	3.1	3.9
3	M	2.9	0	15	40	No	MMF,CI	RTX	1	4.8	6.5
4	M	1.6	3	8	60	Yes	MMF,CI,LEV	RTX,OFAT	5	27.3	12.1
5	M	6.8	3	7	60	Yes	MMF,CI	RTX	2	6.5	4.2
6	F	3.5	0	12	5	No	CYC,MMF,CI,LEV	RTX	3	11.7	11.2
7	M	1.5	0	12	25	No	CYC,MMF,CI,LEV	RTX	1	6.9	17.2
8	M	1.9	0	16	50	No	MMF,CI,LEV	RTX	2	24.6	8.4
9	M	5.6	0	9	30	No	MMF,CI,LEV	RTX	2	7.1	9.0
10	M	3.4	0	10	60	Yes	MMF,CI	RTX,OFAT	4	42.1	7.1
11	M	4.7	3	6	30	No	MMF,LEV	RTX	2	13.3	6.5
12	M	1.9	0	16	30	No	MMF,CI,LEV	RTX,OFAT	4	33.3	9.1
13	M	1.7	0	9	15	No	MMF,CI	RTX	1	7.8	14.1
14	M	6.4	0	6	60	Yes	CI	RTX,OBI	3	13.9	2.7

F female; *M* male; *n* number; *IVMP* intravenous methylprednisolone; *IS* immunosuppressive (drugs); *CI* calcineurin inhibitors (cyclosporine or tacrolimus); *CYC* cyclophosphamide; *MMF* mycophenolate mofetil; *LEV* levamisole; *RTX* rituximab; *OFAT* ofatumumab; *OBI* obinutuzumab

previously received at least one course of B cell depletion using a monoclonal antiCD20 IgG antibody (Table 1 and supplemental Table S1). Overall, 34 attempts at B cell depletion were undertaken in the 14 patients reported here: 2 patients had a single attempt at B cell depletion but displayed immediate relapse following B cell repletion (#2, #3), 2 patients had a single attempt at B cell depletion and delayed relapses 11 and 13 months after B cell repletion, respectively (#7, #13), 4 patients had 2 attempts at B cell depletion that failed to maintain remission (#1, #5, #9, #11), and 6 patients had multiple unsuccessful attempts of B cell depletion with a cumulative duration exceeding 12 months (#4, #6, #8, #10, #12, #14).

Table 2 Characteristics of the patients at treatment by obinutuzumab and daratumumab

#	Age at treatment (years)	Oral IS drugs at treatment	Weight (kg)	Height (cm)	Body surface (m^2)	Dose OBI (mg)	Dose DAR (mg)	Time to oral IS withdrawal (weeks)	Duration of B cell depletion (months)	Status at last follow-up	Duration of remission after OBI (months)
1	6.0	FK	20	112	0.79	445	445	6.4	8.1	Remission	24.6
2	10.9	PRED	41	147	1.29	700	700	5.4	9.3	Relapse	21.7
3	9.5	PRED,NEO	42	137	1.27	750	800	9.7	9.2	Remission	23.5
4	13.7	FK	47	167	1.46	820	820	17.1	13.4	Remission	23.3
5	10.9	PRED	40	148	1.28	750	750	4.6	11.6	Remission	23.2
6	14.7	PRED	50	160	1.49	870	870	4.4	14.9	Remission	22.4
7	18.8	PRED	60	175	1.70	1000	1000	6.1	13.6	Remission	21.6
8	10.3	FK	39	149	1.27	700	700	3.0	9.1	Relapse	10.1
9	14.7	FK	69	156	1.75	900	900	4.7	10.8	Remission	19.8
10	10.5	PRED,FK	37	131	1.17	700	680	4.1	9.8	Relapse	11.1
11	11.2	PRED,FK	23	134	0.91	600	600	5.7	7.6	Relapse	10.8
12	11.0	PRED,FK	44	147	1.34	230	770	5.9	8.2	Remission	13.9
13	15.7	PRED	44	164	1.40	750	750	4.7	9.7	Remission	13.0
14	9.1	PRED,FK	30	135	1.06	580	580	14.1	8.7	Relapse	10.0

IS Immunosuppressive (drugs); *FK* tacrolimus; *NEO* cyclosporine; *PRED* prednisone; *OBI* obinutuzumab; *DAR* daratumumab

Median age at treatment was 11.0 years (IQR 10.4–14.4), and median duration since NS onset was 7.8 years (IQR 4.8–17.0). Detectable EBV replication was observed in 4 patients (#1, #9, #11, #14) and CMV in one (#8). PCR for BK and JC viruses were negative at start of the OD sequence in all patients.

Oral drugs

At the time of obinutuzumab infusion, all patients had been recently treated for a relapse, and were in remission under oral drugs: 5 under prednisone alone, 4 under tacrolimus alone, and 5 under prednisone and tacrolimus (Table 2). We treated 4 patients with tacrolimus alone at the time of relapse to maintain statural growth in 3 patients (#4, #8, #9) and because of a history of severe psychiatric issues under high dose of prednisone in one (#1). Median time to oral drugs withdrawal was 5.6 weeks (IQR 4.6–6.4) after obinutuzumab infusion.

Relapse-free survival

All 14 patients experienced prolonged B cell depletion with a median time to B cell recovery of 9.5 months (IQR 8.9–12.1) (Supplemental Fig. S1). The 2-year relapse-free survival was 60% after the OD sequence (Fig. 1). No significant differences in relapse-free survival rates were found between patients with or without an episode of secondary steroid resistance (Supplemental Fig. S2). Similarly, treatment of the last relapse with tacrolimus alone had no impact (Supplemental Fig. S3). Nine out of 14 patients remain in remission with a median follow-up time of 22.5 months (IQR 19.9–23.3) from the injection of obinutuzumab (Fig. 1) and a median time of 9.1 (IQR 8.0–11.7) months after B cell repletion (Supplemental Fig. S4). These 9 patients maintain stable remission despite the withdrawal of all oral immunosuppression for a median time of 20.2 (IQR 18.8–21.4) months (Supplemental Fig. S5). A relapse occurred in 5 patients (#2, #8, #10, #11, #14), 4 relapsed early within 100 days following B cell recovery while one relapse later within the first year.

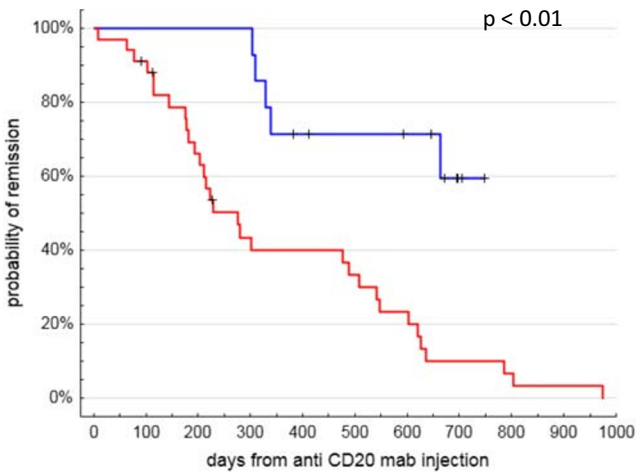

Fig. 1 Probability of remission following anti CD20 monoclonal antibodies in 34 previous attempts of B cell depletion (red line) vs. after obinutuzumab injection in the 14 patients (blue line)

We compared the outcomes following the OD sequence with the ones following the 34 previous attempts at B cell depletion (Fig. 1 and Supplemental Figs. S1, S4, S5). The median time of continuous B cell depletion was 4.0 months [IQR 3.3–6.7] following the 34 previous B cell depletions and 9.5 [IQR 8.9–12.1] ($p < 0.05$) after the OD sequence. Consistently, the probability of remission at month 6, month 12, and month 24 after OD sequence and anti-CD20 alone were 100.0 vs. 53.5%, 78.6 vs. 40.5%, and 59.5 vs. 10.1%, respectively (Fig. 1). Moreover, the probability of remission after B cell repletion after OD sequence and antiCD20 alone at month 6, month 12, and month 18 was 69.6 vs. 23.6%, 69.6 vs. 10.1%, and 49.7 vs. 6.7%, respectively (Supplemental Fig. S4). In terms of direct patient benefit, the median time without oral immunosuppression was 4.4 months [IQR 1.0–11.0] in the previous B cell depletions and 19.1 months [IQR 10.6–21.1] in the OD sequence ($p < 0.001$).

Tolerance

Mild infusion–related reactions were reported in 3 patients during obinutuzumab infusion: vomiting (#4, #10) and urticaria (#3), and in 4 patients during daratumumab infusion: mild transient bronchospasm, throat itching and labial edema (#2, #12, #13, #14).

Asymptomatic neutropenia below 1500/mm^3 was transiently observed by months 1, 4, and 12 in 2 patients (#3, #9) and did not require any specific treatment. However, a decrease of neutrophils was detected in 7 additional patients with a nadir ranging from 1760 to 2310/mm^3. By contrast, an expansion of the CD8 T cells was observed after the OD sequence, while CD4 T cells were unaffected (Supplemental Figs. S4 and S5). Eighteen months after the OD sequence and 6 months after complete B cell repletion, patient #5 developed Covid-19 along with his parents and all siblings. He displayed isolated fever during 36 h and fully recovered without any respiratory signs. No other infections were reported.

Hypogammaglobulinemia

At INS onset, all patients had normal plasma concentrations of immunoglobulins when in remission. By contrast, as a result of previous immunosuppression and B cell depletions, 2 patients displayed a decrease in plasma immunoglobulin G (IgG) levels (#7, #10), 4 a significant decrease of plasma immunoglobulin A (IgG) (#4, #7, #8, #9), and one had undetectable plasma IgA (#13) prior to the OD sequence. One patient with hypoIgA had an increased plasma level of IgG (#4). Another patient had an isolated increased level of plasma

IgA (#5). The immunoglobulin profile was normal in the other 7 patients.

As expected, all patients experienced a decrease in the level of circulating immunoglobulins following the OD sequence (Fig. 2a–c) including a complete disappearance of immunoglobulin M (IgM) in 9 patients. Circulating IgM reappeared within 1 to 8 months in all patients but one, who had undetectable circulating levels. Similarly, IgA was undetectable in 3 patients (2 with previously low plasma level and one with a complete lack of IgA at start of the OD sequence). IgA remained undetectable in these 3 patients at last follow-up.

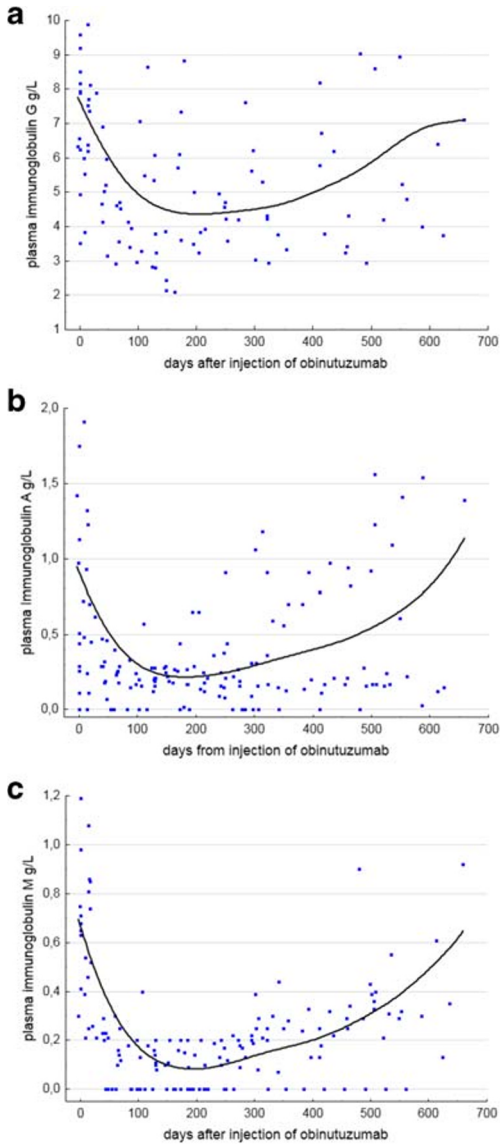

Fig. 2 Time course of the plasma level of immunoglobulin G (IgG) (graph a; $n = 101$), immunoglobulin A (IgA) (graph b; $n = 150$), immunoglobulin M (IgM) (graph c; $n = 150$) from obinutuzumab injection. Obviously, 49 points of plasma IgG obtained within 10 weeks from intravenous polyclonal IgG infusion have been discarded in order to analyze the spontaneous course of the level of plasma IgG. The regression lines have been obtained by the weighted least squares method

The other eleven patients with normal plasma IgA at the start experienced a decrease of plasma IgA below the normal value in the months following the OD sequence and five had persistent low plasma IgA levels at last follow-up, while 6 fully recovered (Fig. 2b).

Although all patients experienced a decrease in circulating IgG, the lowest level of plasma IgG was 2 g/L in one patient (#10) and ranged from 3 to 6 g/L in all other patients. Of note, all patients who had normal immunoglobulin levels before treatment fully recovered normal immunoglobulin levels after B cell repletion. Only 2 patients remained with low plasma IgG levels at last follow-up. In order to prevent severe infections, 3 patients received a systematic supplementation with intravenous polyclonal IgG every month in order to maintain the level of plasma IgG over 5 g/L. In 9 other patients, intravenous polyclonal immunoglobulin was given intermittently when the plasma level of IgG dropped below 4 g/L. Two patients did not require any immunoglobulin supplementation.

Discussion

Maintaining sustained remission in patients with SDNS remains a challenge. Here, we report the outcomes following the association of obinutuzumab and daratumumab to induce stable remission and allow withdrawal of oral immunosuppressive drugs in patients with uncontrolled SDNS despite previous treatments including B cell depletion.

Obinutuzumab is a second generation antiCD20 monoclonal antibody targeting naïve and memory B cells. It was designed to overcome resistance to rituximab in the treatment of B cell malignancies. While rituximab relocalizes CD20 to lipid rafts and induces significant complement-dependent cytotoxicity, obinutuzumab has a powerful antibody-dependent cytotoxicity and can evoke greater direct cell death by mechanisms that are largely caspase-independent. In detail, obinutuzumab is able to overcome the low affinity of certain polymorphisms of the FcGamma receptor 3A on effector cells, hence improving antibody-dependent cytotoxicity in a greater proportion of patients. Clinically, obinutuzumab induces more profound and lasting remission, with an almost twofold higher complete response rate in patients treated for indolent nonHodgkin lymphoma [15] and may also be more efficient than rituximab in autoimmune diseases [16–18].

Daratumumab is a human monoclonal antibody that targets CD38. Its multifaceted mechanisms of action include direct plasma cell cytotoxicity and an immunomodulatory component that results in depletion of immunosuppressive cells and clonal expansion of cytotoxic T cells. Although daratumumab has been designed for the therapy of multiple myeloma [19], its use has been successfully reported, combined with rituximab, in pediatric patients with refractory autoimmune

hemolytic anemia [16, 17], and in adults for HLA-desensitization prior to kidney transplantation [18].

In this small series of 14 patients, the combination of obinutuzumab and daratumumab was both effective and safe, when associated with a preventive treatment of infections with sulfamethoxazole and IgG infusion in case of hypogammaglobulinemia. A 60% relapse-free survival at 2 years in severe SDNS patients without oral immunosuppressive drugs is a very encouraging preliminary result that surpasses the results of both conventional oral treatments and rituximab alone [4, 5, 20, 21]. The specific role of each monoclonal antibody of the OD sequence will deserve further investigation. The efficacy of this treatment is likely due to the extended B cell depletion induced by a single dose of 1000 mg/1.73 m^2 of obinutuzumab since none of the patients relapsed during the phase of B cell depletion. However, this alone may not fully explain the sustained effect observed in the majority of the patients even months after B cell recovery. This finding supports the hypothesis of a beneficial effect of daratumumab that deserves further exploration.

No severe infectious events happened in our study although one patient developed the Covid-19. Yet, all patients decreased their plasma levels of one or more immunoglobulin classes. Three major observations deserve attention in this study. First, IgG never reached a level below 2 g/L, indicating the persistence of well protected plasma cells that are functional and able to produce IgG. Second, in most patients, the level of circulating immunoglobulins returned to normal within 2 years. Third, a majority of patients had transiently undetectable plasma IgM levels after treatment. This raises concerns over their ability to develop a naïve immune response if infected with a previously unmet pathogen [22] and supports the need for supplementation of IgG during at least 18 months after treatment in order to prevent severe infections, especially infection by enteroviruses [23]. Postvaccination serologies after B cell reconstitution and vaccine reimmunization are also mandatory at a distance from B cell repletion, as already suggested after any B cell depletion course in children [24]. Of note, oral immunosuppressive drugs have been shown to decrease circulating immunoglobulin levels in up to 65% of patients with INS [24]. Moreover, oral immunosuppressive drugs, especially when a combination of steroids, CNI and antimetabolites are used, impair all components of immunity, including innate and adaptive immunity, and the T cell–mediated continuous monitoring process by which invading pathogens and tumoral cells are recognized and eliminated. This explains the high prevalence of skin carcinoma in transplanted patients [25].

The rationale of the OD sequence to control difficult-to-treat steroid-dependent patients was based on the central role of B cell immunity. The pathophysiology of INS remains elusive but many clinical facts and results from translational studies support the evidence of a circulating glomerular permeability factor of immune origin. Despite its major effect on the kidney excretion of albumin, attempts to precisely identify this factor have failed. The first attempt to understand the whole complexity of the disease was undertaken by Shimada and Garin [26] who suggested a 2-hit podocyte immune disorder based on the high expression of TLR3 in podocytes. They hypothesized that the stimulation of TLR3 by viral or bacterial antigens induces an abnormal persistence of CD80 expression and a subsequent loss of podocyte integrity due to the action of specific circulating cytokines during infections. Following the demonstration of the ability of antiCD20 monoclonal antibodies to maintain remission [5], other mechanisms have been suggested to accommodate this new clinical fact. First, the effect of rituximab on nephrotic syndrome was supposed to be due to an off-target binding to sphingomyelin-phosphodiesterase-acid-like-3b expressed on podocytes [27]. The major limit of this hypothesis is that rituximab does not bind to sphingomyelin-phosphodiesterase-acid-like-3b in vivo and the binding previously reported is a fixation artifact [28]. Moreover, fixation of other antiCD20 monoclonals on glomerular substructure has never been reported so far. Because no glomerular immunoglobulin deposits are observed on biopsies of INS patients, most authors are legitimately reluctant to consider a direct effect of B cell depletion and impairment of antibody production and support the notion that the beneficial effect of rituximab is mediated by the B-T cooperation through indirect pathways affecting T cell functions [3, 29].

However, this hypothesis ignores the physiological transcellular flow of antibodies through the podocyte [30] that may be at the origin of a loss of podocyte integrity without visible deposits on immunofluorescence. Precisely, the following hypothesis is based on a 2-hit podocyte immune disorder due to EBV infection involving the antibody flow through the podocytes. Recently, the association of the first flare with EBV DNA replication [31], the fact that memory B cell reconstitution is the earliest biomarker of INS relapse and the site of EBV latency [32, 33], as well as a common genetic locus between SSNS and the genetic regulation of antiEBNA1 IgG antibodies [34], suggest a close association between EBV infection and the disease. Indeed, EBV infection may have an impact on podocytes through 2 mechanisms. First, EBV generates numerous noncoding RNAs involved in the virus cycle. All those RNAs, including EBER1 and EBER2 are powerful TLR3 agonists [35] susceptible to interact with TLR3 expressed on the podocyte and to affect podocyte integrity in a similar way as previously described by Shimada and Garin. Moreover, the transmission of proteinuria to mice by antiUCHL1 IgG antibodies purified from a relapsing patient [36], as well as the efficacy of immunoglobulin adsorption in the multiresistant forms of INS [37] are also relevant arguments to involve antibodies as the second podocyte hit that leads to massive proteinuria. It turns out that EBNA1 and

UCHL1 share two short peptide sequences located in the immunogen domain of EBNA1 and in close vicinity at the surface of UCHL1 (blast in https://web.expasy.org/sim/; reference of protein sequences: YP_401677.1 and NP_004172.2 respectively). Interestingly, UCHL1 is a deubiquitination enzyme involved in the podocyte balance of autophagy [38] and is massively overexpressed in podocytes during all glomerular diseases with massive proteinuria in humans except one, namely idiopathic nephrotic syndrome [39]. Consistently, the combination of obinutuzumab and daratumumab is not only targeting the production of IgG antibodies through the deletion of memory B cells and differentiated memory plasma cells, but is also affecting the EBV latency and lytic cycle: memory B cells are the location of EBV latency and lifelong persistence of the virus in the immune system, while plasma cells are the mandatory step for the EBV lytic cycle and virus multiplication [33] (Supplemental Fig. S8).

In conclusion, a combination of therapeutic monoclonal antibodies targeting the B cell system and the production of antibodies might be effective to disrupt the chronic forms of INS. However, a formal demonstration with a randomized control trial comparing obinutuzumab alone versus the OD sequence is mandatory prior to expanding this strategy. These results further support the first line role of B cells and antibodies in the pathophysiology of INS.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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