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See related article, "Ergocalciferol Supplementation in Hemodialysis Patients With Vitamin D Deficiency: A Randomized Clinical Trial," on pages 1801–1810.

## Busy Bs

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In clinical medicine, there is a two-way street with heavy traffic between pathophysiology and treatment. In an idealized world, advances in our fundamental understanding of mechanisms of disease are leveraged to guide discovery of new therapeutic agents. However, there are times when a beneficial treatment emerges unexpectedly, leading to reassessment of traditional views about the biologic basis of disease. The use of rituximab to treat idiopathic nephrotic syndrome falls in this second category.

The first report of successful use of rituximab in the treatment of nephrotic syndrome involved the unique case of a 7-year-old boy who received a kidney transplant for FSGS. He developed recurrent disease in the allograft within 2 weeks of surgery and was treated with corticosteroids and plasmapheresis. However, it was not until he received rituximab to treat post-transplantation lymphoproliferative disease that developed 5 months after transplantation that he achieved remission of the glomerular disease.<sup>1</sup> Interestingly, despite this sentinel case, rituximab was not immediately applied to treat FSGS. Instead, it was tried on a case by case basis in patients with hard-to-manage nephrotic syndrome (*i.e.*, frequently relapsing or steroid-dependent minimal change disease [MCD]). Over the next several years, numerous reports were published that documented the efficacy of rituximab under these circumstances in single patients or case series involving  $\geq 33$  children.<sup>2–5</sup> In an effort to minimize the risk of serious adverse events and limit the cost of this novel treatment, it was shown that a single dose of the antibody was able to induce a long-term remission in these patients.<sup>6</sup> Finally, in the last 2 years, three randomized

clinical trials have been performed documenting that compared with varying conventional immunosuppressive regimens, administration of rituximab is associated with overall prolongation of disease remission, reduction in proteinuria, and improvement in health outcomes, such as linear growth in children with frequently relapsing or steroid-dependent nephrotic syndrome.<sup>7–9</sup> Although there is some heterogeneity in the patient cohorts, with a mixture of MCD and FSGS, and variation in the treatment protocols and standard therapy arms, these results indicate that rituximab should no longer be considered experimental and that it is a valid treatment option for children with MCD who are suffering from intolerable steroid burden.

One of the key questions is the relationship between B cell depletion and maintenance of remission of proteinuria. Clinically, it has been shown that there is no clear relationship between the duration of the response to rituximab and the timing of recovery of B cells. It is this gap in knowledge that Colucci *et al.*<sup>10</sup> address in their important paper in this issue of the *Journal of the American Society of Nephrology*. Colucci *et al.*<sup>10</sup> studied 28 children with presumed MCD on the basis of a history of frequently relapsing or steroid-dependent disease. In line with standard clinical practice in pediatric nephrology, the diagnosis of MCD was made on the basis of responsiveness to steroids and not on the basis of a kidney biopsy. Because none of the patients had steroid-resistant disease, it is highly unlikely that this series included children with FSGS. The patients received one to two doses of rituximab (375 mg/m<sup>2</sup>) and achieved complete B cell depletion within 1–2 weeks. Colucci *et al.*<sup>10</sup> analyzed blood samples obtained before administration of rituximab and 1, 3, 6, 9, and 12 months after infusion of the antibody. They performed detailed flow cytometry analyses of B and T cell subsets and retrospectively assessed the clinical course in relationship to the temporal course of recovery of the different subsets.<sup>10</sup>

For those of us who think that immunologists may be overdoing it with superscripts and subscripts, this article is a welcome relief, because the cell subsets are described with plain sense names rather than strings of positive and negative antibody staining patterns. What first emerged from this careful protocol was that no clinical or routine laboratory test distinguished 14 patients who relapsed within 24 months from 14 patients who had a sustained remission for the entire follow-up period. B cell recovery generally began at approximately 6 months. Moreover, the timing of recovery of total and mature B cells was similar at 1 year. In contrast, by multivariate analysis, reconstitution of switched memory B cells, cells that have undergone isotypic conversion from IgM to IgG antibody production, was the most delayed. This is consistent with the kinetics of B cell development, in which the sequence is transitional followed by mature, culminating in memory B cells. Reappearance of this subgroup of B cells was the best predictor of subsequent relapse of the nephrotic syndrome. It is worth noting that, in univariate analysis, the number of immunosuppressive drugs prescribed and tacrolimus dose in the last 4 months of follow-up were asso-

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ciated with an increased risk of relapse, whereas there was a trend for a lower risk of relapse in patients who received mycophenolate mofetil during the last year before rituximab use ( $P=0.08$ ). The later observation is consistent with uncontrolled studies that suggest that use of mycophenolate mofetil as maintenance therapy prolongs the beneficial effect of rituximab.<sup>11</sup>

What are memory B cells, and why would they be detrimental in childhood nephrotic syndrome? They are a subtype formed within lymphoid germinal centers after primary exposure to an antigen. They then enter the circulation and manifest an accelerated and more robust immune response after re-exposure to the antigen in a secondary immune response. Presumably, they represent the population of cells committed to production of antibody in response to a specific immunogen. The number of these cells at baseline was similar in the children with nephrotic syndrome and age-matched healthy controls. However, delayed recovery of switched memory B cells with maintenance of remission implies that the immune response to a single antigen or group of antigens is responsible for recurrence of proteinuria and resumption of a relapsing course after receiving rituximab. Does this mean that exposure to these inciting molecules is responsible for triggering the first relapse after receiving rituximab? Is this inevitable, or is it possible to achieve permanent remission after receiving the biologic agent? Without knowing the immunogens or the specificity of the switched memory B cells, it is not possible to answer these questions. However, this finding does suggest the possibility that control of environmental factors such as viral infections, which are the most common trigger for relapses,<sup>12</sup> could favorably affect the course of childhood nephrotic syndrome. It is also consistent with reports of children with MCD whose relapses are linked to exposure to food antigens, including gluten, and in whom elimination of the suspected allergen from the diet has resulted in marked improvement in disease course.<sup>13</sup> Alternatively, in those children who do not relapse, the effect of rituximab may be to reboot the immune system to eliminate pathogenic memory B cells and induce a permanent cure of MCD.

The sample size in this study is modest, and it is surprising how robust the data are, despite this limitation. Although the patient cohort (Italian children) raises questions about the external validity of the findings, there have been no reports indicating a variation in efficacy of rituximab therapy in distinct patient populations. This suggests that this analytic approach to B cell monitoring after rituximab treatment may have broad application. It is worth noting that the number of switched memory B cells ( $1-2/\mu\text{L}$ ) that was used as a threshold to predict relapsing from nonrelapsing children is very low. For this reason alone, the findings warrant verification. Flow cytometry methods are routinely available and relatively inexpensive. If recovery of switched memory B cells is linked to prediction of relapse, this measurement could be easily incorporated into clinical practice without modifying the schedule

of clinic visits. It is important to note that Colucci *et al.*<sup>10</sup> do not provide information about the short- or long-term adverse effects of rituximab. In addition, their observations do not answer the question regarding the appropriate place of rituximab in the treatment of children with MCD.<sup>10</sup>

Returning to the point of departure, what does the success of rituximab in childhood nephrotic syndrome tell us about this disease? For nearly 60 years, the prevailing hypothesis was that MCD was a T cell disorder. This notion was enshrined in a classic article by Shalhoub<sup>14</sup> that was published in *The Lancet* in 1974. However, times change. We now recognize that there is extensive interaction between T and B cells, and this may contribute to the pathogenesis of MCD. For example, in patients with lupus nephritis, B cell depletion with rituximab is associated with an increase in the number of circulating regulatory T cells.<sup>15</sup> This result may correct an abnormal T helper 17 cell-to-regulatory T-cell ratio that has been documented in adults with MCD.<sup>16</sup> The linkage of steroid-responsive nephrotic syndrome to genetic variants in the HLA-DQA1 locus implicates antibody production in this condition.<sup>17</sup> The pathogenesis of childhood nephrotic syndrome involves several independent disease-causing pathways—intrinsic defects in the podocyte or increased production of circulating factors that increase glomerular permeability to albumin.<sup>18</sup> The efficacy of rituximab in MCD does not enable discrimination between these two possibilities. It may exert its action on B cells by binding to CD20 on the cell surface and diminishing the production of permeability factors by immunoeffector cells. Alternatively, rituximab may bind to sphingomyelin phosphodiesterase acid-like 3b and directly alter podocyte structure and function.<sup>19</sup> The findings of Colucci *et al.*<sup>10</sup> highlight the peripheral effects of rituximab outside the kidney. Rituximab is much less effective in patients with FSGS compared with those with MCD. Without addressing the vexing topic of the relationship between MCD and FSGS, correlating the response to rituximab with changes in the number of circulating switched memory B cells may help clarify the contribution of extrarenal immune mechanisms in the pathogenesis of FSGS. Regardless of how rituximab works, the exciting work performed by this group will help refine the monitoring of patients with MCD who receive rituximab and ensure the most effective use of this potent but expensive agent.<sup>10</sup> Hopefully, it will also help us understand the pathogenesis of childhood nephrotic syndrome, something that would be very welcome among pediatric nephrologists and their patients.

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## DISCLOSURES

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