Therapeutic Targeting of B Cells and T Cells in Autoimmune Diabetes

Is It a Solution?

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Insulin-dependent type 1 diabetes (T1D) is attributable to the destruction of pancreatic β-cells by autoreactive T lymphocytes (T cells). Antibodies to various β-cell autoantigens also are detected in T1D that are not pathogenic but represent excellent markers of β-cell destruction and are widely used to identify high-risk individuals (1,2). This by no means excludes a major role for B lymphocytes (B cells) in the development and progression of T1D. Serreze et al. (3) first showed that disease-prone nonobese diabetic (NOD) mice lacking B cells were completely protected from disease, a finding explained by the capacity of B cells to present autoantigens (4). Furthermore, B cells are essential for "antigen/epitope spreading," a central mechanism underlying the chronicity of autoimmunity that is the progressive diversification of the autoimmune response from one epitope to another, expressed by the same or by a distinct autoantigen (i.e., intramolecular spreading compared with intermolecular spreading) (5,6). All this explains the therapeutic potential of monoclonal antibodies targeting B cells or their growth factors to halt progression of T1D in NOD mice (7–10). Thus, depleting antibodies directed to the CD20 B-cell antigen efficiently prevented disease when administered to prediabetic NOD mice and, although in a low proportion of animals, also temporarily reversed T1D once hyperglycemia was established (8). Importantly, a randomized placebo-controlled phase II study conducted established that in recent-onset T1D patients a single course of rituximab, a humanized CD20 monoclonal antibody, significantly preserved endogenous insulin secretion as compared with placebo (11). However, the effect was transient and the therapeutic benefit waned as B-cell numbers recovered, implying that "operational" immune tolerance, intended as the specific inhibition of the autoimmune response in absence of chronic immunosuppression, had not been induced (11). However, this is the real challenge the diabetology community is facing today, namely, to avoid immunosuppression-based strategies and to establish instead tolerance-based treatments that may provide a real and sustained cure for the disease (12).

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To improve along the path of tolerance the effect of CD20 antibodies, in this issue Hu et al. (13) tackle the problem head-on, opting for an original combination therapy associating the B-cell–depleting agent with the oral administration of a CD3 monoclonal antibody (Fig. 1). It has been well-established in NOD mice that parenteral administration of CD3 antibodies possesses the remarkable capacity to induce long-standing remission of T1D through restoration of self-tolerance (14,15). These results were successfully transferred to the clinic and, based on data recovered from phase II and III trials, the antibody is in pharmaceutical development (16–18). However, it is fair to admit that clinical use of CD3 antibodies was confronted with the problem of carefully adapting dosages to avoid side effects linked to T-cell activation (19), hence the interest for the pioneering data from Weiner and colleagues (20–22) showing that oral administration of CD3 antibodies is devoid of side effects and appears effective in various autoimmune conditions.

Hu et al. (13) report that in hyperglycemic NOD mice, combining intravenous CD20 antibody with oral CD3 antibody treatment afforded remission of T1D in 66% of mice, lasting in most animals for 1 month, whereas single treatments were poorly effective. Interestingly, the effect was far more obvious in overtly diabetic compared with prediabetic mice, in which CD20 antibody alone already showed a significant effect. The proposed mechanistic cornerstone sustaining the therapeutic benefit is a boost in "immune regulation" through, first, an improvement in the suppressive capacity of $CD4^+FoxP3^+$ regulatory T cells (Tregs) and, second, the local induction in the intestine of $CD4^+FoxP3^-$ interleukin (IL)-10–producing Tregs closely resembling Tr1 cells (13). Interestingly, a similar local induction of Tregs was observed in the models described by Weiner and colleagues (20–22), which, however, were transforming growth factor-β (TGF-β)–dependent, stained positive for the latency-associated peptide (normally bound to TGF-β), and were assimilated to formerly described Th3 cells.

What are the positive aspects of these results and what are the issues that give rise to some perplexity? It is reassuring that an efficient synergistic effect ensues from combined targeting of key pathophysiological actors of the disease, i.e., B cells that present the autoantigen and adaptive or induced Tregs that are well-known actors in the control of disease progression. It is also important to note the benefit from the tolerance-inducing effect of CD3 antibodies and that their oral delivery is totally safe, both in mice and humans (20–23).

It is encouraging that the therapeutic effect of the combination is more clear-cut during advanced disease

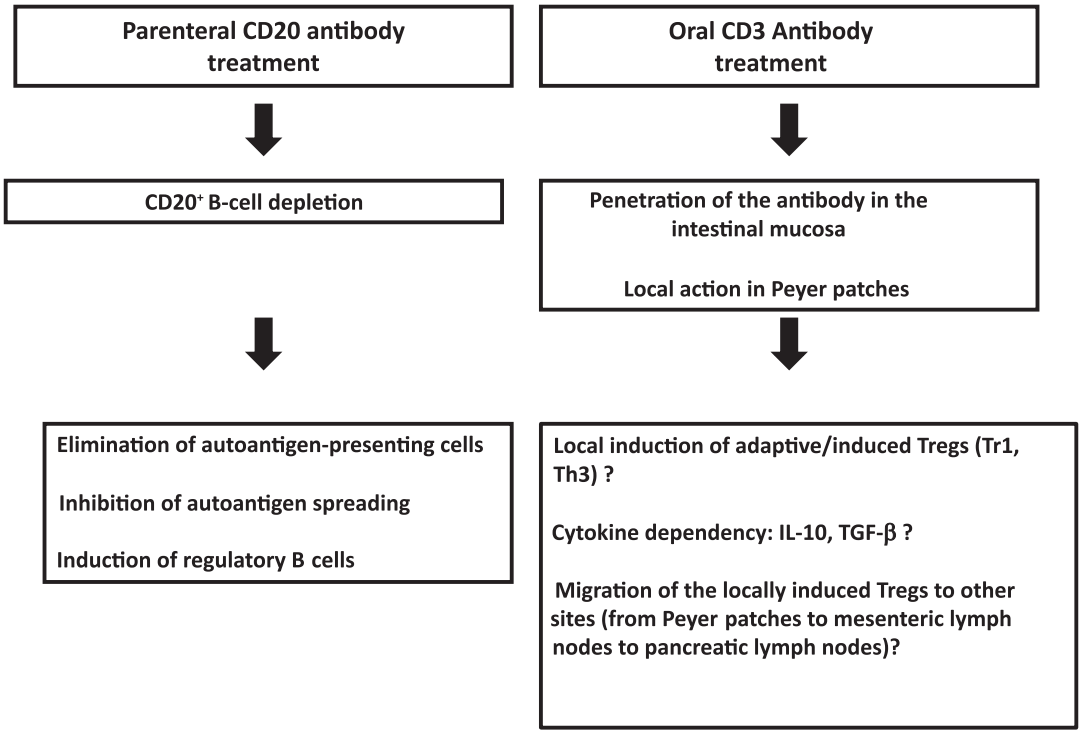

FIG. 1. Schematic summary of the biological mechanisms observed after parenteral CD20 and oral CD3 antibody administration. Parenteral CD20 administration leads to massive depletion of B cells expressing the surface antigen. The consequences of this depletion are a deprivation of autoantigen-presenting B cells and an inhibition of autoantigen spreading. Another described consequence is the induction of regulatory B cells (8). After oral delivery, CD3 antibody reaches the intestine and reported data show uptake in the intestinal mucosa and Peyer patches (20). Local stimulation of adaptive/induced Tregs that express distinct phenotypes, depending on the model, is observed. In the report by Hu et al. (13), the cells were $CD4^+FoxP3^-$ IL-10–producing, thereby closely resembling Tr1 cells. In the work by Weiner and colleagues (20–22). in the experimental allergic encephalomyelitis model, in the collagen-induced arthritis model, and in the streptozotocin-induced diabetes model, the cells were $CD4^+FoxP3^-$TGF-β/LAP^+TGF-β–dependent. In further studies it will be important to elucidate the pattern of migration of these induced Tregs; does the pattern go from Peyer patches to mesenteric lymph nodes and then to pancreatic lymph nodes or to the bloodstream? It also will be important to define the relationship between these "polyclonal" Tregs induced in the intestine and $CD4^+FoxP3^+$ Tregs, which probably include antigen-specific Tregs, present in the target tissue and that have a suppressive function that appears upregulated by the combined treatment (13).

stages, when there is already a massive activation of pathogenic T cells, which favorably prejudges the potential clinical application.

Points that give rise to some perplexity are the pieces presently missing from the puzzle that will certainly allow, once satisfactorily addressed, a better approach to the mechanisms underlying the therapeutic effect and, most importantly, the synergy between the two biological products. An insulitis that does not change, or that changes very little, while β-cell destruction has stopped is an observation certainly complementary of the data showing the enrichment induced by the treatment of $CD4^+FoxP3^+$ Tregs within islet infiltrates and pancreatic lymph nodes. One also may question whether local changes do not also affect pathogenic effector T cells.

Is it possible that by better-adapting the dose of oral CD3 antibody one may obtain longer remission? In fact, the dose used by Hu et al. (13) was 0.5 μg/kg, which is ~0.01–0.02 μg per mouse for 5 days, a much lower dose than that used by Ochi et al. (20) in the experimental allergic encephalomyelitis model (e.g., 5 μg/day for 5 days).

Is the major role of IL-10 and of IL-10–producing T cells determined from these results a causal element or just an epiphenomenon? Is there any role left for regulatory B cells that were initially described as important players to explain the therapeutic effect of the CD20 antibody when administered alone (8)?

For now, T1D is an important disease that affects a growing number of patients. Epidemiological studies predict that T1D incidence will increase significantly in the coming decade and that the disease will predominantly affect children younger than 5 years of age (24). It is essential in this context to have effective treatments addressing the cause of the disease, namely, the autoimmune process.

As with all complex diseases, combination therapy and all the benefits ensuing from synergistic effects are the logical choice (12,19). Last, but not least, CD20 monoclonal antibodies already are available for clinical use. One would hope that CD3 antibodies may be available soon. Given that safety concerns appear to be met with this type of combination, why not give it a try?

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