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Of Bugs and Men: Antigen-Fortified *Lactoccoccus lactis* for Type 1 Diabetes Immunotherapy

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One of the hurdles encountered by immunotherapy attempts for type 1 diabetes (T1D) is the delicate ethical issue of minimizing risks for a disease with a normal life expectancy in many patients. For this reason, antigen (Ag)-specific immunotherapies are attractive in view of their selectivity and favorable safety profile (1). However, they have not met expectations in therapeutic trials using insulin (2–4) and GAD (5). These trials face several technical difficulties: the need to produce and administer proteins of suitable quality and quantity; bioavailability issues, as prolonged delivery of small Ag amounts may be more effective at restoring long-term immune tolerance; routes of administration, with the noninvasive oral route being more tolerogenic but suffering considerable Ag degradation; and Ag delivery in a noninflammatory environment to avoid unwanted immunogenic outcomes. The most important issue is the timing of immune intervention, with a panoply of agents effective at preventing disease even in the reductionist NOD mouse model, but only a handful working at reversing established disease, which is the preferred setting for human trials.

In this issue, Robert et al. (6) bring Ag-specific therapies into a new dimension by letting genetically modified *Lactococcus lactis* do the job. *L. lactis* is a nonpathogenic, noncolonizing, commensal bacterium with a long safety record in the dairy industry. Biological containment of genetically modified strains to avoid environmental propagation can be obtained by removing genes coding for enzymes essential for their growth, thus impeding their long-term survival (7). This safety profile has been reassuring in a phase 1 trial using interleukin (IL)-10–expressing *L. lactis* in Crohn patients (8) and is increasingly inviting consideration for oral delivery of different therapeutics (7).

Building on their previous report using proinsulin (PINS) (9), the authors produced *L. lactis* strains secreting the β-cell Ag GAD along with the regulatory cytokine IL-10. Oral administration of this strain to newly diabetic NOD mice led to diabetes reversal in 67% of treated animals, an effect that persisted after treatment discontinuation. Importantly, combination with a low-dose 5-day course of systemic anti-CD3 antibody (combi-GAD therapy) was needed to obtain significant diabetes reversal. This therapeutic effect was accompanied by preservation of β-cell function, with islet infiltrates found less abundant and less inflammatory. No deletion or anergy of pathogenic T cells was induced, as ex vivo proliferative responses to GAD were similar in combi-GAD–cured versus diabetic animals and as regulatory T-cell (Treg)–depleted splenocytes from different treatment arms were equally efficient at transferring diabetes. Interestingly, combi-GAD and anti-CD3 therapy led to Treg accumulation at different locations, namely the draining lymph nodes and the pancreas, respectively. A fraction of these Tregs boosted by combi-GAD therapy was GAD reactive, showing that the therapeutic effect was Ag-specific both in vitro and in vivo (i.e., no effect with an *L. lactis* strain secreting an irrelevant Ag).

Notwithstanding all the caveats that NOD mouse studies raise once translated into human trials, this is one of the few immune interventions effective at reversing diabetes together with anti-CD3 (10) and rituximab (11). Major strengths of this approach are that it bypasses the need for synthesizing recombinant Ags and avoids their degradation in the stomach, thus achieving a steady, low-dose delivery of both Ag and IL-10 through repeated *L. lactis* administration, which survive in the gut

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COMMENTARY

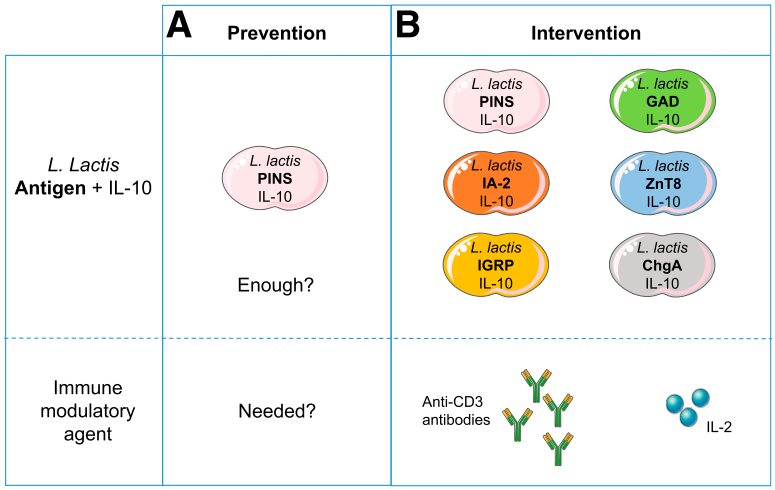

Figure 1—A possible spectrum of *L. lactis*–based combination therapies for T1D prevention and intervention. *A*: Prevention therapies may obviate the need for anti-CD3 conditioning and allow targeting of a more restricted Ag panel. *B*: Intervention therapies in new-onset T1D patients may require targeting a wide array of β-cell Ags. Combination with other immunomodulatory agents such as IL-2 could also be envisioned.

for 8–48 h. This pharmacokinetic profile probably explains the boosting effect on Tregs rather than blunting of pathogenic T cells, although a causal relationship between Treg induction and diabetes reversal was not formally proven. These results are reminiscent of a previous report by Bresson et al. (12) in which intranasal PINS administration in combination with systemic anti-CD3 $F(ab')_2$ treatment induced diabetes reversal associated with the emergence of PINS-responsive Tregs.

Another key point is that combi-GAD was more effective than the previous combi-PINS therapy, leading to cure of 67% versus 59% of diabetic NOD mice—including a higher efficacy in mice that were overly hyperglycemic at diagnosis. This may reflect the fact that PINS is the earliest and initiating Ag for pathogenic T cells in the NOD mouse (13,14). Therapeutic targeting of PINS at the time of diabetes onset may thus fall relatively late, while inducing tolerance to Ags such as GAD that lie downstream of the epitope spreading cascade may prove more effective at the clinical stage of disease.

Combination therapies are advocated in the T1D community (15), and combining immunomodulatory antibodies and Ag vaccination is particularly attractive. In the case of *L. lactis*, it would minimize the risk of side effects by reducing the dose of anti-CD3 antibody, whose therapeutic efficacy in clinical trials is limited by its narrow window of safe dosing (16). At the same time, combination therapies can exploit the synergy among different therapeutic mechanisms. In the case of combi-GAD and combi-PINS treatments, the effect of anti-CD3 conditioning may be to promote Treg migration to the gut (17), thus maximizing exposure to locally released Ag and IL-10. More important, anti-CD3 may exert a reset effect before reprogramming T cells toward β-cell–specific tolerance. The implication of this putative conditioning effect is that combination with anti-CD3 may be dispensable when *L. lactis* treatment is initiated before disease onset, thus improving its safety profile and inviting its testing for diabetes prevention (Fig. 1*A*). In light of the early autoimmune targeting of PINS in the NOD mouse, we may speculate that *L. lactis* delivery of IL-10 and PINS as an only Ag may be sufficient for preventing diabetes. This however may not be the case in humans, where prevention trials are usually offered at a more advanced stage of autoimmune progression, as witnessed by the presence of multiple autoantibodies (18). The same principle may apply a fortiori for intervention trials in new-onset T1D patients (Fig. 1*B*), where a cocktail of *L. lactis* strains producing different β-cell Ags together with IL-10 may induce more complete tolerance. A combination with the low-dose IL-2 protocols currently under testing (19) could provide another potential association should IL-2 regimens prove therapeutically more ductile than anti-CD3. The final message is to keep combining, as successful follow-up studies in NOD mice could warrant expedite translation into clinical trials.

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