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Back From the Brink: The Uses of Targeting the CXCL10:CXCR3 Axis in Type 1 Diabetes

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COMMENTARY

According to a popular quip, fittingly though incorrectly attributed to the great dyspeptic metaphysicist Arthur Schopenhauer, all truth passes through three stages: first, it is ridiculed; second, it is violently opposed; third, it is accepted as self-evident (1). In the iconoclastic world of biomedical research, however, that is so eager to challenge the old and propose novel paradigms, the evolution of truth may at times take a different path, traveling the route to knowledge almost in the opposite direction: first, a discovery is excitedly embraced; second, it is readily confirmed and expanded upon; and third, it is effectively contested and quietly slips into oblivion. That, or so it seemed, was to be the fate of the chemokine CXCL10 and its receptor CXCR3 as promising therapeutic targets in type 1 diabetes (T1D). Yet in this issue of *Diabetes*, Lasch et al. (2) pull that concept back from the brink by defining a specific context for effective CXCL10 neutralization, namely, as an adjunct treatment to achieve durable aCD3-induced T1D remission.

In the early 2000s, buoyed by a burgeoning interest in the potential contribution of individual chemokine/receptors to diabetes development, two independent groups studying the virus-induced RIP-LCMV-GP model suggested that the CXCL10:CXCR3 axis might constitute a key determinant for T1D pathogenesis (3,4). Transgenic RIP-LCMV-GP mice, in which the rat insulin promoter (RIP) drives expression of the lymphocytic choriomeningitis virus (LCMV) glycoprotein (GP) specifically in β-cells, are phenotypically normal but readily develop T1D within ~2 weeks after LCMV infection and the generation of a potent LCMV-GP–specific $CD8^+$T-cell response. Based on mRNA and protein expression screens of murine islets or pancreata obtained from infected RIP-LCMV-GP mice, CXCL10 was identified as a particularly prominent chemokine (3,4), and corresponding in vivo LCMV challenge experiments demonstrated a substantial delay and/or prevention of T1D in RIP-LCMV-GP mice lacking *Cxcr3* (~50% delay, ~50% prevention [3]) or after anti-CXCL10 antibody (aCXCL10) treatment of regular RIP-LCMV-GP mice (~70% prevention [4]). CXCL10:CXCR3–guided pancreatic T-cell trafficking as an essential component for the natural history of T1D and as a suitable therapeutic target proved an attractive concept that was further elaborated in subsequent years, notably by clinical studies of individuals with recent-onset diabetes documenting elevated CXCL10 serum levels (reviewed in [5]) and direct histopathological evidence for islet-associated CXCL10 and CXCR3 expression (summarized in [6]). Indeed, as in the mouse models, CXCL10 appeared to be the major pancreas-expressed chemokine in early human T1D (6).

At the same time, however, several publications began to question the precise extent to which CXCL10:CXCR3–dependent T-cell trafficking contributes to T1D. CXCL10 may directly compromise β-cell survival and proliferation possibly through the noncognate Toll-like receptor 4 (7,8), and transgenic overexpression of CXCL10 in β-cells promoted lymphocyte infiltration but not clinical disease (9). After LCMV infection, *Cxcl10*-transgenic RIP-LCMV-GP mice exhibited normal T1D onset kinetics, although accelerated disease development was observed in the related "slow-onset" RIP-LCMV-NP strain (9). These findings were mirrored in work with a small molecule CXCR3 antagonist that did not prevent T1D in RIP-LCMV-GP mice and only slightly delayed diabetes in the RIP-LCMV-NP model (10). In 2013, prompted by the realization that the CXCL10:CXCR3 model in its original conception failed to account for these disparate results, von Herrath and colleagues (11) revisited the foundational studies performed a decade earlier. Using combinations of $Cxcr3^{-/-}$ and $Cxcl10^{-/-}$ RIP-LCMV-GP mice, different LCMV isolates and challenge protocols, antibody-mediated CXCL10 blockade and a virus-free T1D induction system, and different strains of RIP-LCMV-GP mice, the authors demonstrated, in contradistinction to the earlier reports, at best a minimal contribution of the CXCL10:CXCR3 pathway to T1D pathogenesis (11). Even more troubling, *Cxcr3*-deficiency in the NOD

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See accompanying article, p. 4198.

mouse was neither protective nor inconsequential and, in fact, accelerated spontaneous T1D onset (12).

Undaunted by this dilemma, Lasch et al. (2) focus their most recent efforts on an area of investigation both more circumscribed and challenging and ultimately of greater clinical relevance: the reversal of established T1D. Here, a brief 3-day treatment of diabetic RIP-LCMV-GP mice with a deliberately suboptimal dosage of aCD3 is followed by a ~2-week aCXCL10 course; a similar treatment regimen is also used for diabetic NOD mice. In both models, combination therapy (CT) is significantly more effective than the respective monotherapies and durable T1D remission is achieved in more than half of the treated animals observed for up to ~6 months. At 3 weeks after treatment initiation, CT-mediated T1D reversal in both mouse strains is associated with a greater reduction of insulitis and a more pronounced increase of the $CD4^+T_{REG}$/islet antigen–specific $CD8^+$T-cell ratio in the periphery (spleen) and target organs (pancreas or pancreas-draining lymph node) (Fig. 1). Substituting the aCXCL10 treatment arm with gene deficiency, the authors find that virtually all diabetic *Cxcl10*$^{-/-}$ RIP-LCMV-GP mice could be rendered normoglycemic by suboptimal aCD3 treatment. Altogether, these findings provide compelling support for a therapeutic strategy that builds on the carefully tuned capacity of aCD3 treatment for rapid T-cell depletion and functional modulation by a subsequent disruption of pancreatic T-cell trafficking that effectively amplifies and "locks in" the beneficial therapeutic effects (Fig. 1).

The relevance of additional observations pertaining to effective CT, such as altered functional profiles of pancreatic LCMV-GP–specific $CD8^+$T cells in RIP-LCMV-GP mice or the emergence of $FoxP3^+$ $CD8^+$T cells in the NOD model, remains to be determined (2). Similarly, the potential role of in situ $CD8^+$T-cell priming by β-cell–expressed LCMV-GP, as suggested by Lasch et al. (2), will require

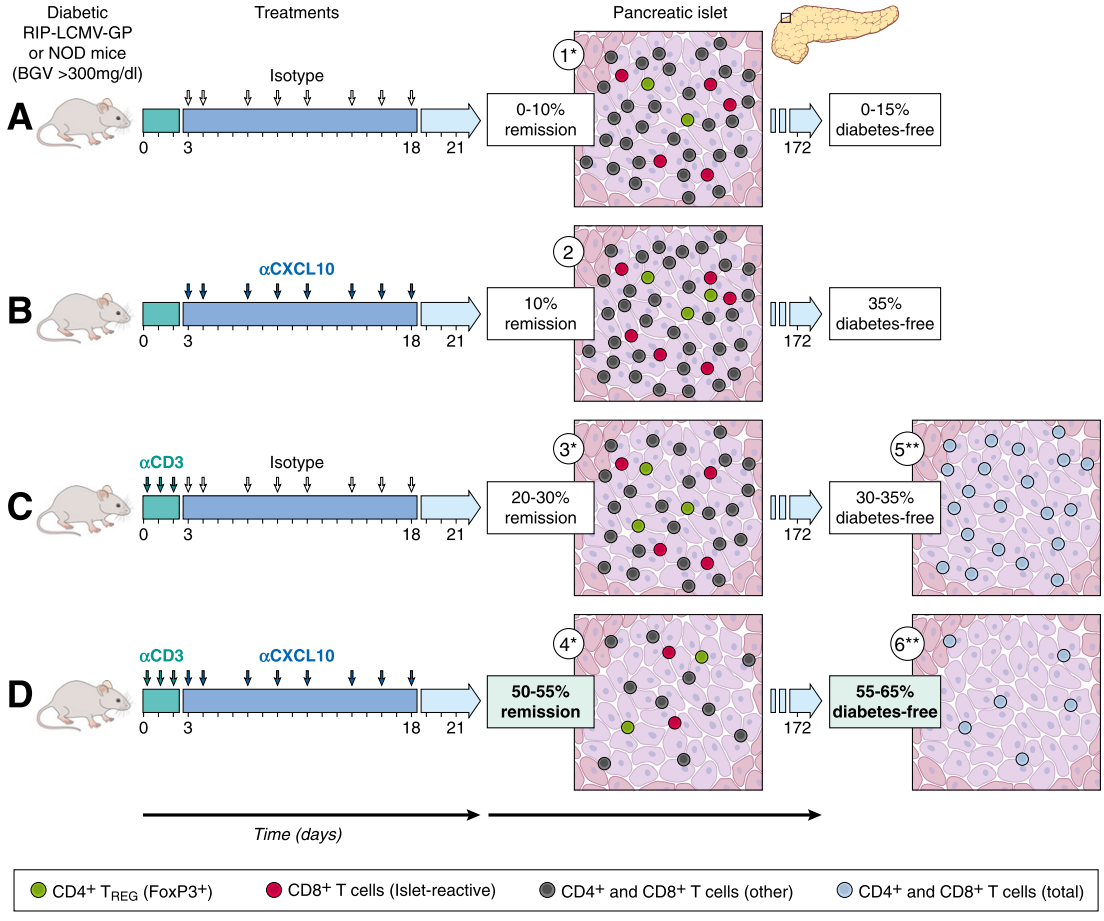

Figure 1—Durable T1D reversion by aCD3/aCXCL10 CT. Recent-onset diabetic RIP-LCMV-GP or NOD mice were treated with a 3-day course of aCD3 (3 × 3 μg or 3 × 30 μg aCD3ε clone 145-DC11 [F(ab')2 fragment], respectively), followed by 8 × 100 μg aCXCL10 (clone 1F11) administered over the subsequent 16 days. The respective aCD3 dosages are deliberately chosen to have suboptimal effects in order to model a clinical scenario that minimizes adverse effects; control treatments are performed as indicated. The figure depicts T1D status and the composition of islet infiltrates at 3 and ~25 weeks after treatment with isotype control antibodies (*A*), aCXCL10 monotherapy (*B*), aCD3 monotherapy (*C*), or aCD3/aCXCL10 CT (*D*). *Note that the composition of NOD islet infiltrates on day 21 in regards to islet-reactive $CD8^+$T cells is extrapolated from corresponding data obtained for the pancreas-draining lymph node. **The histopathological examination at ~172 days after treatment initiation is only conducted with diabetes-free RIP-LCMV-GP mice, and T cells are not stratified according to islet reactivity or regulatory function.

further exploration, as will the long-term treatment effects on the systemic $CD4^+T_{REG}$/islet antigen–specific $CD8^+$T-cell balance or on the general immunocompetence of mice rescued from T1D. Given the systemic nature of effective aCD3/aCXCL10 CT, the durability of T1D reversal is particularly striking in light of the recently reported "open configuration" of the insulitic lesion, i.e., its ready accessibility to various T-cell subsets (including naïve T cells) even after aCD3-induced disease remission (13). Is the long-term exclusion of lymphocytes from islets after CT (Fig. 1) uniquely dependent on a history of CXCL10 blockade or could the therapeutic benefits be broadened and improved on by the combined targeting of CD3 and CXCR3? Conversely, to better focus the beneficial therapeutic effects on affected tissues, further preclinical treatment refinements may consider the inclusion of an antigen-specific CT component.

All these questions can be effectively addressed in the available mouse models and any speculation about clinical translation, no matter how tantalizing, has to remain just that at the present stage, though it may draw on a cautious optimism about antigen-specific T1D trials (14) and the apparent promise of CD2-targeting as an alternative to the clinical limitations of aCD3 therapy (15). But the trajectory of CXCL10:CXCR3–focused T1D research from initial concept formation and validation to refutation and eventual, if partial, rescue also imparts another, perhaps rather obvious yet nonetheless important, lesson: the critical relevance of experimental context and detail. In an age where scientific discoveries are advertised in bullet-pointed summary bits and, should we be so lucky, in correspondingly abbreviated public media coverage, it is easy to neglect the pesky particulars buried in the methods section. Still, those details matter, and they matter greatly, and their diligent consideration may not only reconcile some apparent contradictions but also account for other unexpected turns (e.g., T1D prevention after antibody-mediated CXCR3 treatment of both RIP-LCMV-GP mice [D.H., unpublished data] and NOD mice [M. Youd, personal communication]) and, hopefully, allows us to better focus on and interpret the work that remains to be done.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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