



# The SAgA of Antigen-Specific Immunotherapy for Type 1 Diabetes

Roberto Mallone<sup>1,2</sup> and Sylvaine You<sup>1</sup>

*Diabetes* 2021;70:1247–1249 | <https://doi.org/10.2337/dbi21-0011>

Islet antigen-specific strategies are the holy grail of immunotherapy for type 1 diabetes (T1D) (1), as they selectively target the autoimmune responses involved in  $\beta$ -cell destruction (2). The idea is to induce a response opposite to that of a conventional vaccine. The administration of antigen(s) in the absence of inflammation (e.g., adjuvants) should potentiate the outcomes of physiological immune homeostasis, i.e., anergy, regulatory polarization, and, to a lesser extent, deletion. Such antigens can be delivered as proteins, peptides, bacteria engineered to secrete these products, DNA plasmids, or nanoparticles coated with antigens (either alone or preloaded on MHC molecules). Peptides are easy to synthesize by amino acid chemistry, but they are variably water-soluble and short-lived in vivo (in the order of minutes), which makes it difficult to achieve the steady concentrations more suitable for tolerance induction. Nonetheless, peptide-based immunotherapies have proven safe and, in some cases, documented encouraging immune and clinical outcomes (3).

In this issue of *Diabetes*, Firdessa-Fite et al. (4) give peptides a boost by mounting them on a hyaluronan backbone to obtain a soluble antigen array (SAgA). First, this backbone accounts for their superior solubility. The linker used was either stable (“click” chemistry, cSAgA) or hydrolysable (hSAgA), to facilitate peptide release upon uptake by antigen-presenting cells (APCs). Second, SAgAs deliver multiple peptide copies to individual APCs, thus providing a multivalent (high avidity) engagement of T cells that is more efficient for signal transduction. This may be critical for the low-affinity T cells involved in autoimmunity. A gradient of biodistribution was observed in draining lymph nodes proximal or distal to the inter-scapular subcutaneous immunization site, which raises the question of whether sites closer to pancreatic lymph nodes (5) may achieve superior effects. Thymic delivery may also deserve attention (6,7).

SAgAs were loaded with either the hybrid peptide 2.5HIP, which is the natural antigen ligand of diabeto-

genic BDC2.5 CD4<sup>+</sup> T cells (8), or with its more potent p79 mimotope. Contrary to free peptides, these SAgAs efficiently prevented diabetes in NOD mice only when used in combination. While this may reflect the need to achieve a critical quantitative threshold of T cells targeted, a synergistic functional effect is plausible. Indeed, 2.5HIP-reactive and p79-reactive T cells, which were, surprisingly, distinct populations, underwent different outcomes upon treatment with their cognate SAgA (Fig. 1). IL-10<sup>+</sup> T regulatory (Tr)1 cells were induced by the more potent p79 ligand, while Foxp3<sup>+</sup> Tregs were amplified, but not de novo induced, by the weaker (and less soluble) 2.5HIP. These effects were dose-dependent, as SAgAs induced Tr1 cells at higher dose and Foxp3<sup>+</sup> Tregs at lower dose. SAgAs promoted more effective and persistent engagement and expansion of autoreactive T cells than free peptides in vivo, with no deletional effect observed. Critically, SAgAs were stronger inducers of tolerance-associated markers, including decreased CD44 expression and increased CD73/FR4, Lag-3, PD-1, and KLRG-1, suggestive of anergic/regulatory/exhausted phenotypes. The cSAgA formulation proved superior to hSAgA at inducing CD73/FR4, PD-1, and IL-10, at preventing diabetes, and at avoiding anaphylaxis (likely as a result of decreased shedding of free peptides). Although NOD mice are particularly prone to peptide-induced anaphylaxis (9), this issue is critical in the allergy setting but probably less so in human T1D. Originally described after insulin B9-23 peptide immunization in NOD mice (10), such reactions were not observed, despite Th2 polarization, in a small-scale human trial with a similar peptide (11), and in other peptide-based intervention trials.

Some questions are open for further investigation, including whether the therapeutic outcome is IL-10-dependent, the role of B-cell and CD8<sup>+</sup> T-cell tolerance, the need for continuous treatment, the efficacy at inducing diabetes remission, and human T-cell studies in vitro and/or in humanized mice. Although the peptides to load

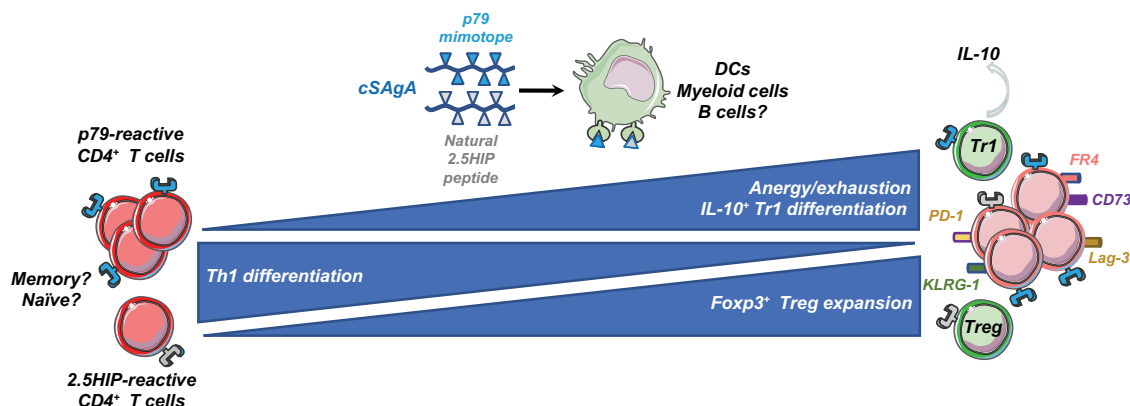
<sup>1</sup>Université de Paris, Institut Cochin, CNRS, INSERM, Paris, France

<sup>2</sup>Assistance Publique Hôpitaux de Paris, Cochin Hospital, Service de Diabétologie et Immunologie Clinique, Paris, France

Corresponding author: Roberto Mallone, [roberto.mallone@inserm.fr](mailto:roberto.mallone@inserm.fr)

© 2021 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at <https://www.diabetesjournals.org/content/license>.

See accompanying article, p. 1334.



**Figure 1**—Tolerogenic effects of p79 and 2.5HIP cSAGAs on their cognate CD4<sup>+</sup> T cells. DCs, dendritic cells.

on SAGAs will need to be adapted to the HLA haplotype of a given individual, the overrepresentation of HLA-DQ2/DR3 and -DQ8/DR4 in patients with T1D eases the burden of this task. The use of longer peptides requiring some intermediate processing by APCs may also be considered to increase epitope, and possibly HLA, coverage. It will also be interesting to define the critical features of the “ideal” peptides. Although peptide solubility may limit the choice (e.g., leading to the exclusion of insulin B9-23 in this report), the list of options is expanding (12–14) and now includes several neo-epitopes similar to the 2.5HIP used here (8). In this respect, the Tr1 induction achieved by SAGAs through endogenous APCs is reminiscent of that induced through artificial APCs (peptide-MHC class II-coated nanoparticles) (15). Any  $\beta$ -cell peptide was effective with this strategy, but only T cells that had already been antigen-primed during the disease process could differentiate into Tr1 (CD49b<sup>+</sup>Lag-3<sup>+</sup>IL-10<sup>+</sup>) cells. This point remains to be addressed for SAGAs, but the earlier timing of treatment (8-week-old prediabetic stage) might suggest different requirements compared with nanoparticles (administered at diabetes onset). A head-to-head comparison with nanoparticles (which were shown to revert disease), as well as with SAGAs loaded with other  $\beta$ -cell antigens or with irrelevant peptides, would be critical to strengthen the therapeutic potential and specificity of SAGAs. Nanoparticles also drove differentiation of B regulatory cells, and previous experience with SAGa treatment in experimental autoimmune encephalomyelitis (16) suggests that this may be another therapeutic mechanism for tolerance spreading. Upregulation of the ectonucleotidase CD73 may further contribute to this bystander effects by promoting a local immunosuppressive environment via adenosine. Indeed, SAGAs, in particular cSAGAs, induced antigen-reactive CD4<sup>+</sup> T cells exhibiting an anergic (CD73<sup>high</sup>FR4<sup>high</sup>) and/or exhausted (PD-1<sup>+</sup>, KLRG-1<sup>+</sup>) phenotype. Similar phenotypes have also been described in CD4<sup>+</sup> (anergy) and CD8<sup>+</sup> T cells (anergy and exhaustion) following anti-CD3 or LFA3-Ig treatment in the context of human T1D (17,18) or murine islet

transplantation (19,20). Thus, states of T-cell unresponsiveness may be a common feature of tolerance restoration and, possibly, of different degrees of “benign,” quiescent autoimmunity in healthy individuals (21) and in patients with different rates of disease progression (22,23). The work of Firdessa-Fite et al. provides a novel therapeutic tool to attempt restoring this state of benign autoimmunity.

**Funding.** Work in the laboratory of R.M. and S.Y. is supported by grants from The Leona M. and Harry B. Helmsley Charitable Trust (1901-03689), the Fondation pour la Recherche Médicale (EQU20193007831), the Agence Nationale de la Recherche (ANR-17-CE17-0004, ANR-19-CE15-0014-01), the European Foundation for the Study of Diabetes EFSDF/JDRF/Lilly European Programme in Type 1 Diabetes Research 2019, and the EU Innovative Medicines Initiative 2 Joint Undertaking under grant agreements 115797 INNODIA and 945268 INNODIA HARVEST. These joint undertakings receive support from the European Union’s Horizon 2020 research and innovation programme, European Federation of Pharmaceutical Industries Associations, JDRF, and The Leona M. and Harry B. Helmsley Charitable Trust.

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

## References

- Culina S, Boitard C, Mallone R. Antigen-based immune therapeutics for type 1 diabetes: magic bullets or ordinary blanks? *Clin Dev Immunol* 2011; 2011:286248
- Carré A, Richardson SJ, Larger E, Mallone R. Presumption of guilt for T cells in type 1 diabetes: lead culprits or partners in crime depending on age of onset? *Diabetologia* 2021;64:15–25
- Alhadj Ali M, Liu YF, Arif S, et al. Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes. *Sci Transl Med* 2017;9:eaaaf7779
- Firdessa-Fite R, Johnson SN, Leon MA, et al. Soluble antigen arrays efficiently deliver peptides and arrest spontaneous autoimmune diabetes. *Diabetes* 2021;70:1334–1346
- Phillips B, Nylander K, Harnaha J, et al. A microsphere-based vaccine prevents and reverses new-onset autoimmune diabetes. *Diabetes* 2008;57: 1544–1555
- Zelenay S, Bergman ML, Paiva RS, et al. Cutting edge: intrathymic differentiation of adaptive Foxp3<sup>+</sup> regulatory T cells upon peripheral proinflammatory immunization. *J Immunol* 2010;185:3829–3833

7. Culina S, Gupta N, Boisgard R, et al. Materno-fetal transfer of preproinsulin through the neonatal Fc receptor protects from autoimmune diabetes. *Diabetes* 2015;64:3532–3542
8. DeLong T, Wiles TA, Baker RL, et al. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. *Science* 2016;351:711–714
9. Overbergh L, Decallonne B, Branisteanu DD, et al. Acute shock induced by antigen vaccination in NOD mice. *Diabetes* 2003;52:335–341
10. Liu E, Moriyama H, Abiru N, et al. Anti-peptide autoantibodies and fatal anaphylaxis in NOD mice in response to insulin self-peptides B:9-23 and B:13-23. *J Clin Invest* 2002;110:1021–1027
11. Alleva DG, Maki RA, Putnam AL, et al. Immunomodulation in type 1 diabetes by NBI-6024, an altered peptide ligand of the insulin B epitope. *Scand J Immunol* 2006;63:59–69
12. Gonzalez-Duque S, Azoury ME, Colli ML, et al. Conventional and neo-antigenic peptides presented by  $\beta$  cells are targeted by circulating naive CD8<sup>+</sup> T cells in type 1 diabetic and healthy donors. *Cell Metab* 2018;28:946–960.e6
13. Azoury ME, Tarayrah M, Afonso G, et al. Peptides derived from insulin granule proteins are targeted by CD8<sup>+</sup> T cells across MHC class I restrictions in humans and NOD mice. *Diabetes* 2020;69:2678–2690
14. James EA, Mallone R, Kent SC, DiLorenzo TP. T-Cell epitopes and neo-epitopes in type 1 diabetes: a comprehensive update and reappraisal. *Diabetes* 2020;69:1311–1335
15. Clemente-Casares X, Blanco J, Ambalavanan P, et al. Expanding antigen-specific regulatory networks to treat autoimmunity. *Nature* 2016;530:434–440
16. Hartwell BL, Pickens CJ, Leon M, et al. Soluble antigen arrays disarm antigen-specific B cells to promote lasting immune tolerance in experimental autoimmune encephalomyelitis. *J Autoimmun* 2018;93:76–88
17. Long SA, Thorpe J, DeBerg HA, et al. Partial exhaustion of CD8 T cells and clinical response to teplizumab in new-onset type 1 diabetes. *Sci Immunol* 2016;1:eaai7793
18. Diggins KE, Serti E, Muir V, et al. Exhausted-like CD8<sup>+</sup> T cell phenotypes linked to C-peptide preservation in alefacept-treated T1D subjects. *JCI Insight* 2021;6:142680
19. Baas M, Besançon A, Goncalves T, et al. TGF $\beta$ -dependent expression of PD-1 and PD-L1 controls CD8<sup>+</sup> T cell anergy in transplant tolerance. *eLife* 2016;5:e08133
20. Besançon A, Baas M, Goncalves T, et al. The induction and maintenance of transplant tolerance engages both regulatory and anergic CD4<sup>+</sup> T cells. *Front Immunol* 2017;8:218
21. Culina S, Lalanne AI, Afonso G, et al.; ImMaDiab Study Group. Islet-reactive CD8<sup>+</sup> T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. *Sci Immunol* 2018;3:eaao4013
22. Wiedeman AE, Muir VS, Rosasco MG, et al. Autoreactive CD8<sup>+</sup> T cell exhaustion distinguishes subjects with slow type 1 diabetes progression. *J Clin Invest* 2020;130:480–490
23. McKinney EF, Lee JC, Jayne DR, Lyons PA, Smith KG. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. *Nature* 2015;523:612–616