Defining an exhaustion-like program that restrains autoreactive CD8+ T cells

Intra-islet CD8+ T cells from a mouse model of type 1 diabetes exhibit an exhausted phenotype that differs from the canonical T cell exhaustion observed in cancer and chronic viral infection. Deletion of the inhibitory receptor LAG3 in these cells accelerated diabetes incidence and partially reversed this restrained phenotype.

This is a summary of:
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The question

Exhaustion is a differentiation state in which CD8+ T cells become predominantly unresponsive to stimulation. The conditions that drive exhaustion — chronic antigen exposure and T cell receptor stimulation[1] — occur in cancer, chronic infection and autoimmunity; however, in autoimmune diseases, the existence of exhausted T cells and their effect on disease outcomes is highly debated. Blocking inhibitory receptors (IRs), which are key to the prevention of excessive immune responses, partially reverses the exhausted phenotype and has become therapeutically relevant in the treatment of cancer. By contrast, inducing CD8+ T cell exhaustion could be a therapeutic strategy for controlling autoimmunity[2]. Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by immune infiltration into the pancreatic islets and the subsequent destruction of insulin-producing β-cells. Within the islets, autoreactive CD8+ T cells are chronically stimulated, yet disease still occurs. We investigated whether autoreactive T cells in T1D exhibit any hallmarks of exhaustion, what consequences exhaustion has on disease outcome, and what mechanisms restrain T cell pathogenicity to facilitate a chronic condition.

The observation

High-dimensional flow cytometry of islets from non-obese diabetic (NOD) mice, a model of T1D, revealed minimal differences in the composition and proportion of CD8+ T cell populations between non-draining lymph nodes and pancreatic lymph nodes, but considerable heterogeneity within the islets (Fig. 1a). Of particular interest were the expression patterns of the crucial modulators and hallmarks of the exhaustion program, TOX (thymocyte selection associated high mobility group box protein) and TCF1 (T cell–specific transcription factor 1; also known as transcription factor 7), as terminally exhausted CD8+ T cells have high expression of TOX and low expression of TCF1[1]. Intra-islet CD8+ T cells clearly had a population of TOX+IR+ cells that were either TCF1+ or TCF1− (Fig. 1a, clusters 1–3). Of note, the expression pattern of the IR LAG3 (lymphocyte activation gene 3) was unique relative to that of other IRs, as LAG3 was expressed only in more terminal TCF1− clusters (Fig. 1a, clusters 1 and 3), which indicated that LAG3 is a key marker for more terminally restrained intra-islet CD8+ T cells. Follow-up functional, metabolic, transcriptional and epigenetic analyses revealed similarities as well as distinct differences between functionally 'restrained' intra-islet CD8+ T cells and canonically exhausted CD8+ T cells (Fig. 1b).

In a NOD model in which LAG3 was selectively deleted from the surface of CD8+ T cells (*Lag3*$^{\Delta TM}$), the restrained phenotype of the CD8+ T cells was partially reversed, which resulted in a highly accelerated incidence of diabetes in both female mice (in which incidence is typically higher) and male mice (Fig. 1c). Furthermore, phenotypical, functional and transcriptional analyses revealed that LAG3 limited the trafficking of CD8+ T cells to the islets; T cell effector function, survival and proliferation; polyfunctionality; and possibly epitope spreading. In conclusion, intra-islet restrained CD8+ T cells were present in the NOD model of T1D and clearly inhibited disease onset, as indicated by the accelerated disease when these cells were perturbed by *Lag3* deletion.

Future directions

These studies expand the understanding of CD8+ T cells in autoimmunity and the features that restrain their function, showing that restrained T cells in an autoimmune disease differ from canonically exhausted T cells, and that these restrained CD8+ T cells can delay disease onset, which provides a rationale for targeting T cell IRs in autoimmunity.

It remains to be determined whether this unique 'exhausted' phenotype is found in other models of autoimmunity and in human disease. We hypothesize that progression to T1D onset would be slower in people with enrichment for restrained CD8+ T cells within the pancreatic islets, although such studies are limited in that they would have to detect restrained CD8+ T cells in circulation in the blood, and in-depth longitudinal studies are yet to be performed. Additional studies are needed to determine whether our observations also apply to CD4+ T cells, which are major drivers of many autoimmune diseases.

Further research is needed to assess the mechanisms by which CD8+ T cells contribute to the destruction of β-cells despite their restrained phenotype, and what molecular and cellular mechanisms specifically drive and/or enhance it. The development of agonist antibodies that promote LAG3 function could have substantial therapeutic potential in autoimmune diseases, and perhaps also in other inflammatory diseases.

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EXPERT OPINION

"Using single cell–based and population-based sequencing approaches and various other sophisticated methods, the authors demonstrated that *Lag3* genetic deletion in CD8⁺ T cells results in alteration of T cell receptor clones recruited to the islet through epitope spreading. Thus, the adaptation of CD8⁺ T cells to chronic antigen encounter in autoimmunity is distinct from tumor or viral infection contexts and is predominantly mediated by LAG3, which could be therapeutically targeted." **An anonymous reviewer.**

FIGURE

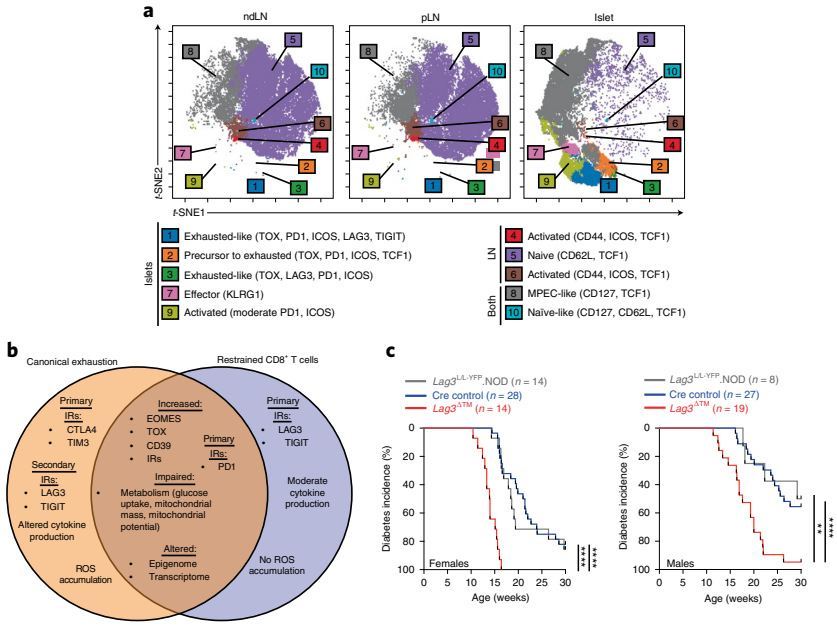

Fig. 1 | The phenotype of intra-islet CD8⁺ T cells. a, Phenotypes of CD8⁺ T cells in non-draining lymph nodes (ndLN), pancreatic lymph nodes (pLN) and islets, based on the expression of various markers (key). MPEC, memory precursor effector cells; *t*-SNE, *t*-distributed stochastic neighbor embedding. **b**, Comparison of canonical exhaustion to what is observed in NOD mouse islets. ROS, reactive oxygen species. **c**, Diabetes incidence in female and male NOD control mice (expressing Cre or yellow fluorescent protein (YFP)-tagged *Lag3* (*Lag3*$^{L/L\text{-}YFP}$.NOD)) and *Lag3*$^{\Delta TM}$ NOD mice. $^{**}P < 0.01$ and $^{****}P < 0.0001$.

BEHIND THE PAPER

We have been studying LAG3 for over 20 years and have been very interested in understanding its role in regulating autoimmune disease. Global loss of LAG3 led to substantially accelerated disease; therefore, we wanted to investigate the role of LAG3 in different T cell subsets[3,4]. We were surprised to see the importance of LAG3 in CD8⁺ T cells, especially as single deletion of LAG3 has a limited effect in cancer and chronic viral infection models, and CD4⁺ T cells have a more predominant role in autoimmunity[5]. As we started to probe the phenotypes of wild-type and LAG3-deficient CD8⁺ T cells in NOD mice, we realized these cells exhibited many, but not all, of the hallmarks of exhaustion. We feel this study yielded several important results and has opened multiple new avenues to pursue. **D.A.A.V.**

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FROM THE EDITOR

"This paper stood out to me, as little is known about T cell exhaustion in autoimmunity. The data show accumulation of LAG3⁺ exhausted CD8⁺ T cells in the islets, and deletion of *Lag3* in these cells exacerbates diabetes pathology. Given the clinical interest in modulating IR function in cancer and chronic viral infection, this study might point to similar strategies for autoimmune diseases." **Nick Bernard, Senior Editor, *Nature Immunology***