In vivo MR Imaging of the Evolution of the Immune Response in Type 1 Diabetes Progression

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Background

Insulin-dependent diabetes mellitus (Type1 diabetes; T1D) results from the progressive autoimmune destruction of the insulin-producing pancreatic beta-cells. CD8+ T cells have been identified as major players in eliciting the autoimmune response and several beta-cell autoantigenic targets of these cells have been identifided. The NOD mouse represents a suitable model for the study of Type 1 diabetes progression, since the autoimmune destruction of pancreatic islets is influenced by age. Whereas insulitis begins to develop at around 3-wk of age, diabetes does not appear until at least 9 weeks later. The progression of pancreatic islet inflammation to overt diabetes in non-obese diabetic (NOD) mice is driven by the "avidity maturation" of a prevailing, pancreatic beta-cell-specific T lymphocyte population carrying the CD8+ antigen and recognizing a Kd-restricted peptide ligand (NOD-relevant V7 peptide, NRP-V7) with high avidity.

In our previous studies directed towards non-invasive imaging of infiltration of diabetogenic CD8+ T cells in the pancreas, we designed and tested an imaging probe consisting of superparamagnetic iron oxide nanoparticles (MN) conjugated to H-2Kd molecules presenting the NRP-V7 peptide (1). These nanoparticles specifically labeled T cell receptor (TCR)-transgenic NRP-V7-reactive CD8+ T cells, both in vitro and in vivo, as well as islet-associated NRP-V7-reactive CD8+ T cells in wild-type pre-diabetic NOD mice in vivo. Furthermore, these probes allow for non-invasive MR imaging of islet inflammation (1) because they are ferried to (or label autoreactive T cells directly within) pancreatic islets. The goal of this study was to investigate the evolution of the autoantigen-specific immune response in NOD mice in vivo using MN-NRPV7 to target autoreactive CD8+ T cells.

Methods and Materials

MN-NRP-V7 or unmodified control MN nanoparticles (10mg/kg Fe) were injected intravenously into 5-, 8-, 15-, or 24-wk old female NOD mice. Animals were subjected to MR imaging before and 24 h after probe injection. MR imaging was performed using a 9.4T Bruker horizontal bore scanner (Billerica, MA) equipped with ParaVision 3.0 software. The imaging protocol consisted of transverse T2 weighted spin echo (SE) pulse sequences. To produce T2 maps, the following imaging parameters were used: SE TR/TE = 3000/8, 16, 24, 32, 40, 48, 56, 64; FoV = 40x40 mm; matrix size 128x128; slice thickness = 0.5mm (total 13 slices); resolution 312x312 μm and an imaging time of 25min 18 sec. The MR imaging findings were validated by flow cytometry and ex vivo histology.

Results

Whereas in mice injected with MN-NRPV7, there was a significant drop in pancreas-associated T2 relaxation times after injection, in animals injected with unmodified parental MN nanoparticles, there was no significant difference between pre-contrast and post-contrast T2 relaxation times In addition, the changes in pancreas-associated T2 values, following MN-NRPV7 administration, reflected a tendency of age-dependence ($p = 0.002$). In 5-wk old NOD mice, there was only a 1±1.2% change in the T2 relaxivity of the pancreas, consistent with lower levels of insulitis at that age. The drop in T2 relaxation times of the pancreas was most pronounced in 8-wk old NOD mice, whereas the changes in T2, as a reflection of probe accumulation, were not significantly different between 15- and 24-wks of age, suggesting that after 15-wks of age there is an overall reduction in the rate of recruitment of NRP-reactive clones to the pancreas. These MR imaging findings correlated highly with results obtained by flow cytometry ($R^2 = 0.98$). Namely, the percentage of MN-NRPV7+ CD8+ intra-islet lymphocytes was highest in 8-wk old NOD mice.

Flow cytometry of peripheral blood leukocytes of NOD animals of different ages revealed a peak in circulating MN-NRPV7-reactive CD8+ T lymphocytes at 7-8 wks of age. These results supported the conclusion that the relative frequency of MN-NRPV7-specific CD8+ T cells in the intra-islet lymphocyte population closely matched the trend observed for circulating lymphocytes. This observation suggested that, after injection, MN-NRPV7 labels the circulating CD8+ T cell pool, and, as a result, can be used to trace its subsequent biological fate in vivo.

Summary

Based on our observations, we propose a model in which, after intravenous injection, MN-NRPV7 labels the circulating autoreactive T lymphocite pool, prevalent in type 1 diabetes. This labeling event permits the tracking of these autoreactive T cells to pancreatic lymph nodes, where they undergo selective priming and expansion. Following priming, the autoreactive T cell clones migrate into pancreatic islets, in the process of autoimmune infiltration. As a function of their labeling, the autoantigen-specific T cell pool can be visualized by MRI during both the priming and infiltration stages of the process. In addition, accumulation of MN-NRPV7 in pancreatic tissue would be effected by partial leakage of free probe through the islet vasculature, followed by interstitial retention, as a result of uptake by NRPV7-reactive T lymphocytes already recruited to the pancreas. Finally, MN-NRPV7 and related dextran-coated iron oxides represent known lymphotropic agents, for which macrophages have a high affinity. Consequently, MN-NRPV7 is also partially taken up by macrophages in a non-antigen-specific manner. This process contributes to the overall degree of probe uptake by the pancreas, as macrophages home to pancreatic lymph nodes driven by non-specific inflammatory signals.

We believe that our imaging method permits the reliable monitoring of the autoantigen-specific T lymphocyte pool, as it is recruited to the diabetic pancreas, is primed and expands in pancreatic lymph nodes, and ultimately invades pancreatic islets in the process of autoimmune attack. With these new studies, we have begun to define the fate of MN-NRPV7 after injection. It appears that the time-course of probe accumulation as a function of age in the islets and in the blood of NOD mice describes the innate evolution of the NRP-specific autoimmune response in this animal model. To our knowledge, our imaging strategy represents the first example of a noninvasive method, which would allow us to define in real time the evolution of the autoantigen-specific immune response in Type 1 diabetes.

References

1. Moore A, Grimm J, Han B, Santamaria P. Tracking the recruitment of diabetogenic CD8+ T-cells to the pancreas in real time. Diabetes 2004;53(6):1459-1466.