TOLERANCE INDUCTION WITH QUANTUM DOTS DISPLAYING TUNABLE DENSITIES OF SELF-ANTIGEN

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During autoimmune diseases like type 1 diabetes or multiple sclerosis (MS), the immune system mistakenly recognizes and attacks healthy tissues in the body. In MS, myelin, which surrounds and protects the axons of neurons, is attacked by inflammatory cells leading to neurodegeneration. The current standard of care for MS patients is regular injection of immunosuppressive drugs that non-specifically suppress immune function, leaving patients immunocompromised and open to opportunistic infection. New investigations aim to address this problem with immunotherapy-based strategies that promote myelin-specific tolerance. Recent reports reveal that the development of inflammation or tolerance against certain molecules is influenced by the concentration and form of self-antigen presented to immune cells (i.e. free, particle). Strategies that allow tunable delivery of self-antigen are therefore of great interest to further probe these connections. Quantum dots (QDs) were chosen as the nanomaterial to investigate these questions because they can be conjugated with a large and controllable number of biomolecules. Additionally, their size facilitates rapid drainage through lymphatics to lymph nodes (LNs), where they accumulate and can be visualized by deep-tissue imaging due to their intrinsic fluorescence. QDs could be decorated with up to 130 myelin oligodendrocyte glycoprotein (MOG) peptides, a known self-antigen of MS (**Fig 1A**). In a mouse model of MS (EAE), we treated groups with two different doses of self-antigen conjugated to QDs. Treatment resulted in a significant reduction in paralysis that was dependent on MOG dose. At a time point just before EAE symptoms arise (day 9), it was discovered that the draining inguinal LN in MOG-QD treated mice had a significantly higher number of regulatory T cells (T_{REGS}) than those of untreated mice (**Fig 1B**). This response was also dose dependent, as mice receiving a higher dose of self-antigen on QDs (52:1) had more T_{REGS} than those injected with a lower dose (17:1). This cell population can induce tolerance to self-antigens by controlling inflammatory cells. We next tested if the level of tolerance induced was dependent on peptide dose alone or if ligand density had an effect. We treated groups with the same dose of MOG spread out over different amounts of QDs and found a trend of decreasing disease severity with decreasing ligand density (**Fig 1C**). This result inspired us to further investigate the trafficking of peptide decorated QDs and the effect that these nanomaterials have on the LNs they accumulate in. Initial studies indicate that we can track the biodistribution of MOG-QDs in naïve mice over time by flow cytometry. Strong QD signal is visible just one day post injection (*p.i.*), and peaks in the draining left inguinal LN on day 3 (**Fig 1D**). While this signal then wanes over the next 4 days, it sustains at a high level in the left axillary LN up to 1 week after injection. Interestingly, a much lower level of QD signal is seen in the right inguinal (non-draining) LN. We also quantified changes in cell populations over time following MOG-QD injection and discovered a spike in macrophages in the draining inguinal LN on day 3 *p.i.*, corresponding with peak MOG-QD signal. This is especially interesting because macrophages can engulf and present antigens to naïve T cells, which then become polarized towards regulatory or inflammatory phenotypes. Another antigen-presenting cell type, $CD11b^+$ dendritic cells, saw a steady increase over the one week study. Interestingly, a sustained drop in T cell frequency and increase in B cell frequency was seen in the draining LN beginning 3 days after injection. In future experiments, we will use this tool to investigate how antigen density and sequence affect biodistribution and immune functionality in both healthy and diseased mice. We also plan to exploit the theranostic capabilities of QDs by performing live, *in vivo* imaging experiments.

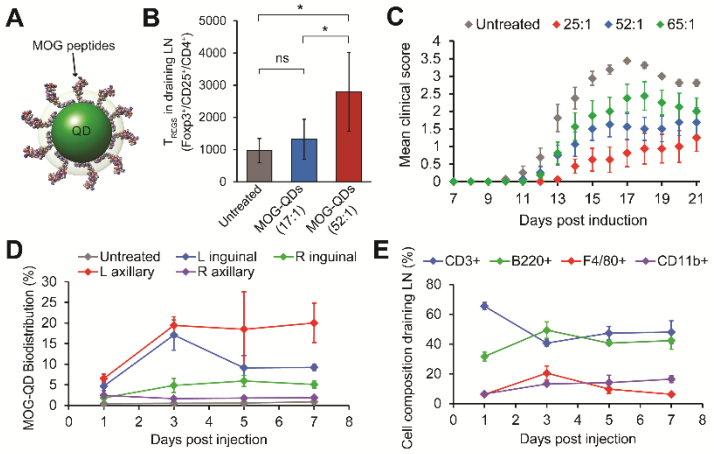

Figure 1 – MOG-decorated QDs (A) induce T_{REG} polarization in a self-antigen dose-dependent manner (B). Tolerance in EAE is correlated to MOG ligand density (C). Biodistribution of MOG-QDs (D) and the change in LN composition can be quantified (E).