Title:	Direct Engagement of TLR9 Ligand with T Helper Cells Leads to Cell Proliferation & Up-regulation of Cytokines
Authors:	Kumar, Rajendra (/jspui/browse?type=author&value=Kumar%2C+Rajendra)
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Abstract:	Purpose: Toll like receptor (TLR) engagement is primarily a function of the innate immune cells. The purpose of the study was to assess direct uptake of ODN 2216 in T helper cells and effects on cell proliferation and cytokine expression. Methods: We isolated CD4+ CD25- T helper cells by magnetic sorting and studied the uptake of ODN 2216 using flow cytometry and confocal microscopy. We then studied the effect of ODN 2216 engagement on cell proliferation and cytokine expression using flow cytometry and gene expression of TLR9 signaling genes using real time RT-PCR. Results: We made a chance observation that purified T helper cells from healthy individuals consistently bind to the TLR9 ligand ODN 2216. In PBMCs, on the other hand, 98% of monocytes preferentially bound to ODN 2216 FITC, indicating that they competed with the lymphocytes. We confirmed intracellular localization of ODN 2216 FITC as well as intracellular expression of TLR9 in Thelper cells. Furthermore, ODN 2216 FITC was also co-localized with the lysosomal membrane associated protein 1. The uptake of TLR9 ligand culminated in cellular proliferation, up-regulation of cytokines and increased mRNA expression of TLR9 and IRF7 in T helper cells, in the absence of antigen presenting cells. ODN 2216 uptake was inhibited by promethazine as well as by TLR9 antagonist. Conclusions: Our results show a direct engagement of TLR9 ligand in T helper cells and suggest involvement of TLR9 signalling in CD4+T cells, which may envisage novel targets for TLR inhibitors.
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