TLR 9 ligand ODN 2216 binds to the effector T cells from healthy controls leading to proliferation of CD4 cells & up-regulation of cytokines

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ABSTRACT

Toll like receptor (TLR) engagement is primarily a function of innate immune cells yet recent reports indicate that TLR9 may be expressed on certain subsets of T lymphocytes but their precise role in T effector (Teff) cells has not been elucidated. We made a chance observation that purified Teff cells from healthy individuals consistently bind to the TLR9 ligand ODN 2216. We confirmed intracellular localization of ODN 2216 FITC as well as intracellular expression of TLR9 in Teff cells. Furthermore, ODN 2216 FITC was also co localized with the lysosomal membrane associated protein 1 (LAMP1). In the whole blood, on the other hand, 98% of monocytes showed binding to ODN 2216 FITC indicating that the monocytes compete with the lymphocytes for ligand binding. The uptake of TLR ligand culminated in cellular proliferation, up-regulation of cytokines and increased mRNA expression of TLR9 and IRF7 in T effector cells. ODN uptake by Teff cells was inhibited by an endocytosis inhibitor, promethazine, as well as by TLR9 antagonist. Our results show a direct engagement of TLR9 ligand in Teff cells giving new insight into the role of TLR9 signalling and novel mechanisms of action of TLR inhibitors.

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INTRODUCTION

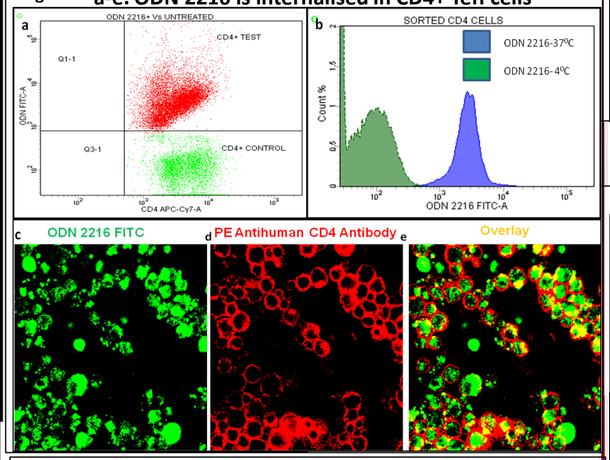
TLR expression is primarily a prerogative of cells of the innate immune system. However, stray reports indicate TLR expression on certain subsets of T cells and B cells. Here we document intracellular TLR9 expression and the mechanism of ODN uptake and sequence of events in purified T effector cells (Teff cells).

METHODS AND MATERIALS

Teff cells were purified from peripheral blood mononuclear cells (PBMCs) using magnetic bead based cell isolation kit and subjected to flow cytometry and confocal microscopy using ODN 2216 FITC and CD4-PE antibody. Anti LAMP1 antibody was used for localisation of LAMP1 and ODN 2216. PBMCs were separated on a ficoll hypaque gradient and subjected to flowcytometry using appropriately labelled CD3, CD4,CD14 and CD56 antibodies and relative binding of ODN 2216 on different cells was evaluated. Teff cells were subjected to proliferation assays after challenge with ODN 2216. Cellular mRNA was isolated and subjected to real time PCR for evaluating gene expression of TLR9, IRF 7 IRAK4 and NFkB genes after ODN 2216 stimulation of Teff cells.

RESULTS

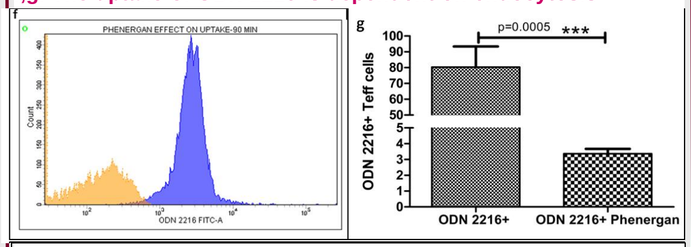
Figure 1 a-e. ODN 2216 is internalised in CD4+ Teff cells



(a) More than 98% of Teff cells showed uptake of ODN 2216 FITC (red) as compared to control (green). (b)The uptake at 37 °C resulted in clear shift in fluorescence as compared to cells at 4 °C (control). (c,d,e) Intracellularly located ODN 2216 (green) was observed in CD4+ (red) cells.

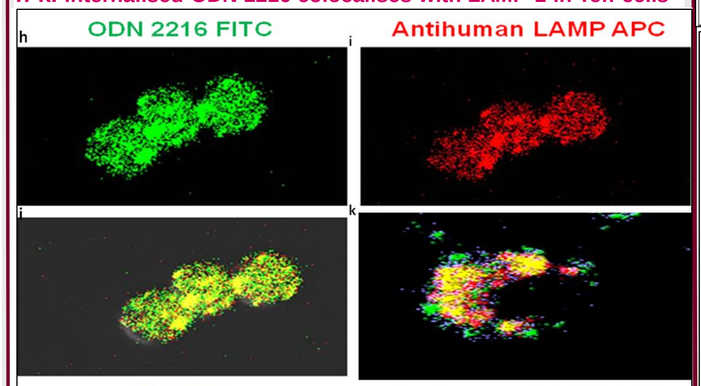
RESULTS

f,g. The uptake of ODN 2216 is dependent on endocytosis



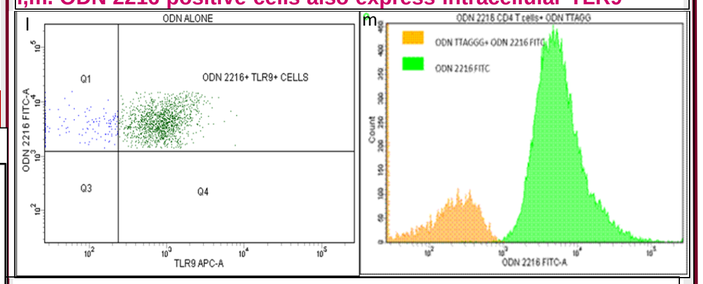
(f) Phenergan (endocytosis inhibitor) inhibited uptake of ODN 2216 in Teff cells. (g) The frequency of Teff cells showing uptake of ODN 2216 was significantly lower in Phenergan treated cells as compared to untreated cells

h-k. Internalised ODN 2216 colocalises with LAMP-1 in Teff cells



(h) Internalised ODN 2216 in Teff cells (green) localised with (i) LAMP-1 (red), resulting in (j) overlap of the two signals. (k) The distribution of LAMP-1 and ODN 2216 was different in CD14+ monocytes as compared to Teff cells.

l,m. ODN 2216 positive cells also express intracellular TLR9

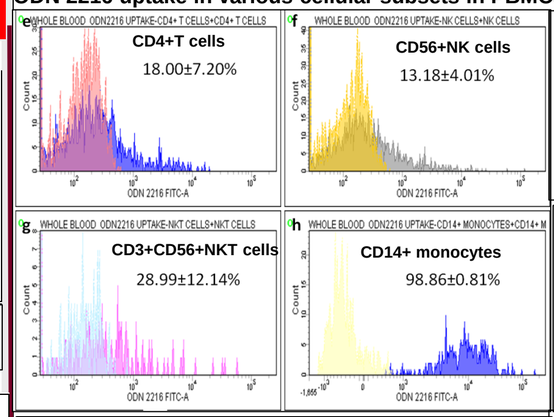


(l) Majority of ODN 2216+ CD4+ cells also expressed intracellular TLR9. (m) The inhibition of TLR9 using TLR9 antagonist ODN TTAGGG (copper) resulted in visibly lower uptake of ODN 2216 FITC in Teff cells than uninhibited cells (green).

Figure 2

The uptake of ODN 2216 was assessed in PBMCs. (a) Lymphocytes and monocytes were gated on the basis of forward and side scatter. (b) Cells from lymphocytes gate were divided into CD3+T cells, CD56+ NK cells and CD3+CD56+ NKT cells on the basis of CD3 and CD56 fluorescence. (c) CD14+ monocytes were gated from monocytes gate on the basis of CD14 fluorescence. (d) CD3+CD4+T cells and CD3+CD4- T cells were gated from T cells quadrant (green) on the basis of CD3 and CD4 fluorescence

ODN 2216 uptake in various cellular subsets in PBMCs



(e-h) The frequency of cells showing uptake of ODN 2216 FITC in CD4+T, NK, NKT and CD14+ monocytes respectively. (i) The frequency of cells showing uptake was highest in CD14+ monocytes, followed by NKT cells, CD4+T cells and NK cells respectively.

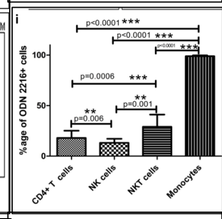
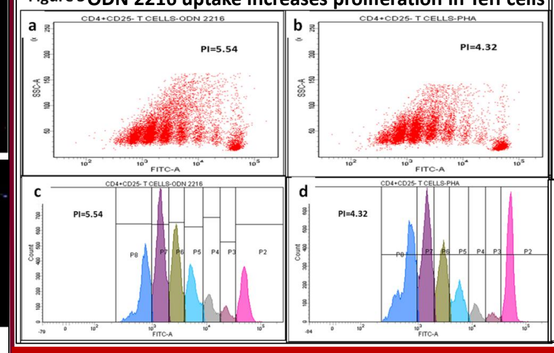


Figure 3 ODN 2216 uptake increases proliferation in Teff cells



(a,b) Dot plots show proliferation of Teff cells after stimulation with ODN 2216 and PHA-M respectively. (c,d) Figure showing proliferation peaks of the same. (e) We observed a significant increase in proliferation index after ODN 2216 stimulation versus PHA-M

Conclusions

1. Purified CD4+Teff cells endocytose ODN 2216 and co-localise with intracellular LAMP-1.
2. Majority of ODN 2216+ Teff cells also express intracellular TLR9.
3. Monocytes compete with lymphocyte subsets for ODN 2216 in whole blood.
4. ODN 2216 stimulation increases proliferation in CD4+ Teff cells ;up- regulates TLR 9 and IRF 7.
5. The observations may have far reaching implications in oncology , autoimmunity and vaccinology.

References

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