Size Dependent Differential Immune Response with Poly-ε-caprolactone Nanoparticles-An *in vitro* study

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Abstract

The present study aimed at developing poly-ε-caprolactone nanoparticles (PCL NP) in two size ranges viz 60nm and 450 nm, entrapping the antigens mycobacterial early secreted antigenic target 6 (ESAT 6) and tetanus toxoid (TT) separately in them and studying the effect of the size variation of PCL NP after internalization by human blood monocyte derived macrophages (hmoM) on antigen presentation by them to autologous CD4+ T cells and CD8+ T cells respectively. Macrophages belong to the mononuclear phagocytic system and function as antigen presenting cells. Targeting them with cell-based vaccination strategies using antigens entrapped in nanoparticles may provide mechanisms whereby modulation of the type of immune response elicited through subsequent interactions with the adaptive immune system can be controlled. The development of an effective immune response thus depends on effective presentation of antigenic peptides on MHC class I and MHC class II molecules which in turn dictate mucosal, humoral and cell-mediated immunity which may be required together or separately to deal with a particular infection depending on its nature.

The aim of many vaccine development programs has been to generate a strong T cell response which requires the presentation of antigenic peptides on MHC molecules for T- cell stimulation. Therefore the challenge for an effective vaccine is to induce long-lived central memory CD8+ T cells as well as CD4+ helper T cells [1].

PCL NP were successfully synthesized in the two size ranges of 60nm and 450nm and the sizes were determined using transmission electron microscopy and DLS. Zeta potential of 60nm particles was -3mv and that of 450nm was -14mv. They were entrapped with antigens of interest viz ESAT 6 and TT. The particles with highest entrapment efficiency were taken for antigen presentation assays. Cell viability assay done on human monocytic cell line, THP1 with MTT assay showed that the synthesized PCL NP were perfectly biocompatible and they also did not create significant ROS generation as determined by H_2DCFDDA assay.

The results of in vitro antigen presentation assays using human monocyte derived macrophages (hmoM) indicated that irrespective of whether the pure antigen alone generates a Th1 or Th2 type of response, 60 nm PCL NP cause M1 polarization of the macrophages and generate a Th1 type immune response. 450nm PCL NP on the other hand, cause M2 polarisation of the macrophages and skews the T cell polarization towards Th2 type. This is clearly indicated by the ELISA results. When void 60nm PCL NP are incubated with hmoM for 72h and the supernatant is assayed for cytokines, we found significant amounts of IL12 and small amounts of IL 10 ($p<.001$). When the macrophages were incubated with void 450nm PCL NP, they caused copious amounts of IL 10 secretion with non-significant amounts of IL12 ($p<.05$).

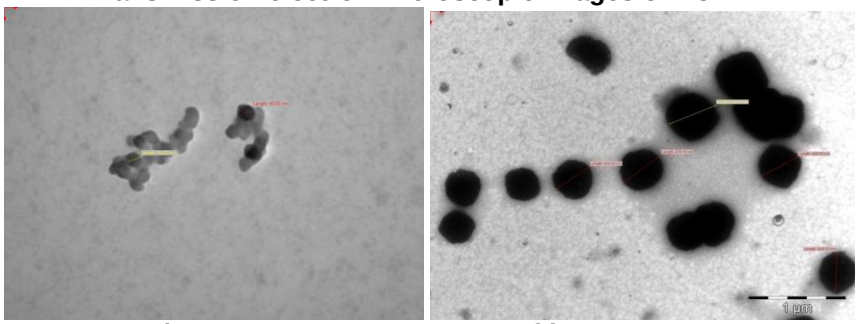
When either ESAT 6 or TT entrapped 60nm PCL NP are used in the antigen presentation assays, CD4+ T cells produce IFN gamma in significant amounts ($p<0.001$). When 450nm antigen entrapped PCL NP are used, CD4+T cells produce significant amounts of IL 4 and IL 10 ($p<0.05$). When compared to the antigen entrapped particles, pure ESAT 6 causes IFN gamma secretion while TT and alum adsorbed TT produce IL4 and IL 10 respectively during antigen presentation assays.

The results are important in the light of modulating immune response to antigens with implications in vaccine design. Here, the study indicates that sub-nanometer PCL NP of about 60 nm diameter efficiently induce Th1 polarisation and cross-presentation of antigens. This may be important when a strong cell mediated immunity (CMI) may be needed against the antigen of interest. Sub-micron size PCL NP of about 450nm, on the other hand, cause Th2 polarisation and boost humoral immune responses, much like alum. Thus we observe that the nature of material used and the size of the particles formed affect the adjuvant properties of particulate delivery systems for vaccination.

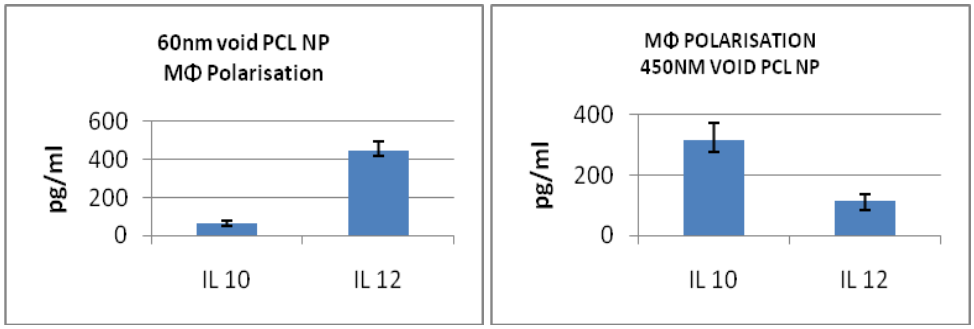
References

[1] Berzofsky, J. A.; Ahlers, J. D.; Janik, J.; Morris, J.; Oh, S.; Terabe, M.; Belyakov, I. M., JCI **114(4)**, (2004) 450 - 462

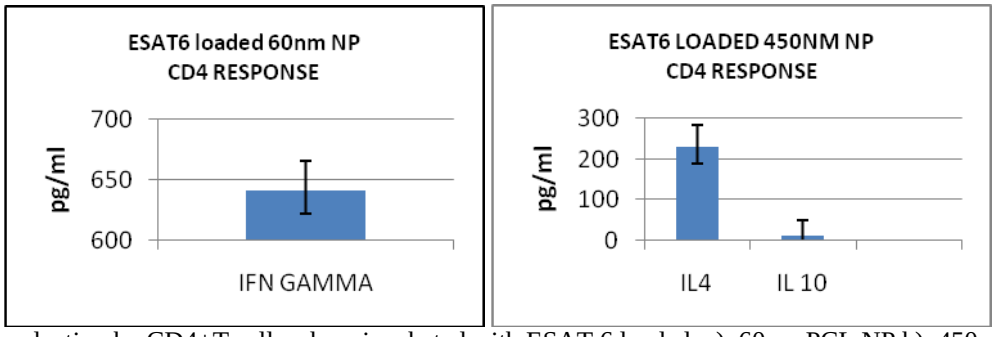
Transmission electron microscopic images of PCL NP



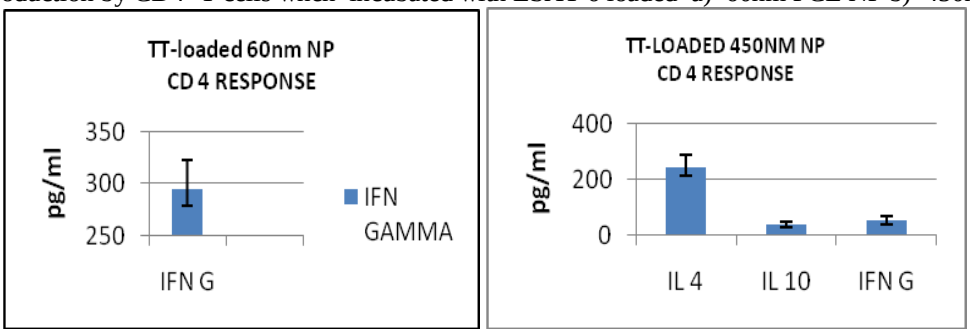
a) 60nm PCL NP **b) 450nm PCL NP**



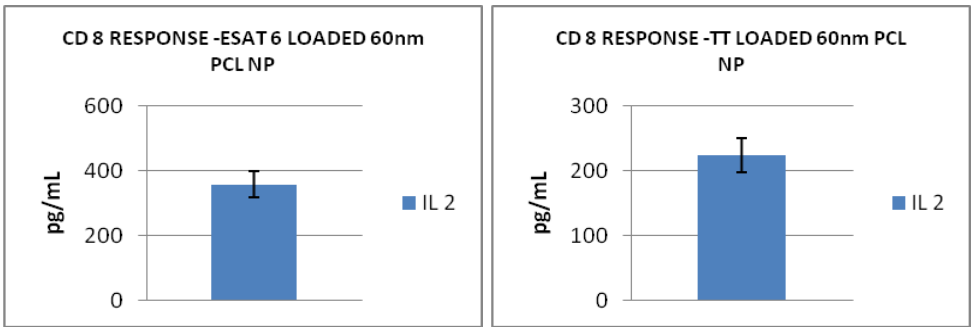
Cytokine production by macrophages when incubated with a) void 60nm PCL NP b) void 450nm PCL NP



Cytokine production by CD4+T cells when incubated with ESAT 6 loaded a) 60nm PCL NP b) 450nm PCL NP



Cytokine production byCD4+ T cells when incubated with TT loaded a) 60nm PCL NP b) 450nm PCL NP



Cytokine production by CD8+ T cells when incubated with ESAT 6 or TT loaded 60nm PCL NP