Understanding 42)42 2.4.2 delivery of the antigens

Understanding the molecular mechanism of engineered nanoparticles would help in the developing the new nanoparticles. The immune activity of the nanoparticles may be attributed to following mechanisms: 1) Depot affect; 2) activation of the NLR pyrin domain containing 3 (NLRP3) inflammasome; 3) delivery of antigens to draining lymph nodes; 4) pattern presenting receptor (PPR) dependent immune activation; 5) repetitive antigen presentation; T cell differentiation. However often it's difficult to isolate one mechanism as each nanoparticle might act as adjuvant using multiple mechanisms. 2. 4. 1 Depot affect: The nanoparticles can persist at the site of injection for long period of time thus enhancing the immune response this effect is called as ' Depot effect'. Zhang et. al42 studied the persistence of the soluble antigen (OVA), antigen-loaded PLGA nanoparticles, antigen and nanoparticles physical mixtures and the combination of antigen-loaded and physically mixed nanoparticles.

The showed that the OVA-loaded PLGA had highest depot effect as compared to the soluble protein. They further relate it to the immune response and suggested that the high immune response in the PLGA loaded nanoparticles as compared to soluble OVA was a result of the depot effect. Figure 2. 3: Depot formation of encapsulated OVA when OVA was encapsulated in PLGA. (Reproduced with permission from reference 42)42 2. 4. 2 Delivery of the antigens into the draining lymph node: The major site for the most of the immune cells and interactions is the lymph node. The targeting of the lymph node has been a major mechanism by which most of the small nanoparticles enhance the immune response.

For example, Mueller and co-authors synthesised different size of NPs using print technology and targeted the draining lymph nodes using various molecular weight polyethene glycol. In order to enhance the immune response and OVA was used as an antigen. They showed that 80X180 nm nanoparticle with 500 kDa PEG showed the highest targeting to draining lymph nodes as compared to the soluble OVA and also showed the highest antibody titres.

43 2. 4. 3 NLRP3 inflammasome activation NLRP3 is a main determinant in the activation of the innate immune response. NLRP-3 triggers the high dose of ATP, which leads to down activation of IL-1?. Nanoparticles like silica, titanium dioxide have shown to have excellent adjuvant response through the NLRP-3 pathway. 44 2. 4. 4 Pattern recognition receptors (PRR) agonist: PRRs play a crucial role in the innate immune response.

They recognise the conserved pathogen-associated molecular patterns (PAMPs) which are distinctive to each pathogen. PRRs can detect various microbial pathogens like bacteria, viruses, parasites, fungi, and protozoa. PRRs are not only expressed by antigen-presenting macrophage and dendritic cells but also by other cells (both immune and non-immune cells).

The PRRs are localized on the cell surface, within the endosomes and cellular matrix. They are involved in activating pro-inflammatory signalling pathways, stimulating phagocytic responses (macrophages, neutrophils and dendritic cells) or binding to micro-organisms as secreted proteins. There are various types of PRRs depending on the localisation: 2.

4. 4. 1 Membrane bound PRRs 2. 4. 4. 1. 1 Toll like receptors (TLRs)2. 4.

4. 1. 2 TLRs are transmembrane receptors which detect various types of infecting pathogens. The TLRs located on the surface of the cell-like TLR-1, 2, 4, 5, 6 and 11 while TLR3, TLR4, TLR7, TLR8, and TLR9 are expressed on endosomal or lysosomal compartments and endoplasmic reticulum (ER). TLR3, 7 and 8 recognize viral RNA, TLR9 recognizes bacterial DNA, and TLR5 and 10 recognize bacterial or parasite proteins. After the first detection of the pathogens, the TLR further initiates the activation of signalling pathways such as NF-kappa B pathway that helps in secretion of inflammatory signals like cytokines. 45 Figure 2.

4: Localisation of TLR. (Reproduced with permission from reference 45). 452. 4. 4.

1. 3 C- type lectin receptors (CLR) CLR are carbohydrate-binding receptors through carbohydrate recognition domains (CRDs). There are 17 groups of CLRs.

The pathogen recognised by CLRs are summarised in table no. 1. 46 2. 4.

4. 2 Cytoplasmic PRRs 2. 4. 4. 2.

1 Nucleotide oligomerization (NOD) like receptors (NLR): The NODs (NOD1 and NOD2) can recognize bacterial peptidoglycan motifs and NALPs which also recognize microbial pathogens. These NLRs are involved in the regulation of inflammation and apoptosis. Table 2. 1: Type of CLRs and its ligands (Reproduced with permission from reference 46). 46 CLR Ligand Ligand origin References Group II: Calcium-dependent CRD Dectin-2 ?-mannans O-linked mannobiose- rich glycoprotein S. mansoni C. albicans M.

tuberculosis 47-50 Group VI: Calcium-dependent multiple CRD Mannose Receptor (MR) High mannose Omega-1 ManLam schistosoma egg antigen Trichuris muris Mesocestoides corti 51-52 DEC-205 (CD205) plasminogen activator Y.

pestis 53 Group V: Calcium-independent non-CRD Dectin-1 ?-glucans L. infantum C. albicans Mycobacterium spp. 54-55 2. 4.

4. 2. 2 Retinoic acid-inducible gene I (RIG-I) like receptors (RLR) RIG-1, melanoma differentiation-associated gene 5 (MDA5) and DDX3 help recognize viral RNA. 2. 4. 4. 3 Secreted PRRs: Peptidoglycan recognition proteins (PGRs) and the leucine-rich repeat receptor (LRR) can recognise the invading micro-organisms directly.

Taking advantage of these receptors researchers are developing new nanoparticles with could enhance the receptor activity by strategies like mannose receptor targeting on dextran particles. 56Gausse and co-authors synthesised muramyl dipeptide tagged particles, which by itself could enhance the NOD2 receptor and loaded CPG an agonist for TLR9. They showed that these particles induced both adaptive and innate immune response. 38