Integrating into high density lipoprotein trafficking pathways to target lymph-node resident cells and enhance vaccine efficacy

*Gracia, Angus PR Johnston, Christopher JH Porter, Natalie L Trevaskis**

Monash Institute of Pharmaceutical Sciences
Monash University
381 Royal Parade
Parkville, Victoria, Australia
Natalie.Trevaskis@monash.edu

The effectiveness of therapeutic vaccines has been hindered by failures to elicit a strong cytotoxic T lymphocyte (CTL) response. A strategy to overcome this is to incorporate vaccines into biocompatible nano-sized carries that enhance the lymphatic and immune cell disposition of vaccines and vaccine adjuvants[1,2]. One such nano-carrier is high density lipoproteins (HDLs) which collect cholesterol from peripheral tissues and return to the blood circulation via lymphatic vessels and lymph nodes (LNs)[3,4]. We aimed to 1) Determine the impact of HDL physicochemical properties on lymph uptake and LN retention. 2) Evaluate how incorporation into HDLs alters uptake into lymph and resident cells, and vaccination CTL responses.

Endogenous HDLs were isolated from lymph and plasma. Synthetic HDLs were prepared with lipids and apolipoprotein A-I. All HDLs were characterized for size, shape, surface charge, composition etc. For aim 1, HDLs were radiolabelled and SC administered into the leg of thoracic lymph-cannulated rats to determine lymph and LN uptake. For aim 2, the vaccine adjuvant CpG was conjugated to cholesterol (Chol) and incorporated into HDL (HDL(Chol-CpG-Cy5), and compared with free CpG-Cy5 or Chol-CpG-Cy5. All CpG formulations were administered SC to mice and LN and tissue accumulation determined by imaging. Mice were immunised with the CpG formulations with free OVA antigen and DC uptake, DC activation, T cell activation and antigen-specific CTL response were assessed.

In aim 1, all HDLs preferentially drained into lymph (lymph:plasma concentration ratios were >100:1), and accumulated in draining LNs. Some variations in lymph uptake and LN retention were observed across the HDL particles that appeared unrelated to HDL physical properties. Instead, lymph uptake appeared related to HDL protein composition and LN retention to surface charge and composition.

In aim 2, both Chol-CpG-Cy5 and HDL(Chol-CpG-Cy5) had increased LN and DC association relative to free CpG-Cy5. Chol-CpG-Cy5 appeared to integrate into endogenous HDL pathways into lymph. Both Chol-CpG-Cy5 and HDL(Chol-CpG-Cy5) appeared to access lymphoid-resident CD8α+ DCs in the LN rather than associating with CD103+ DCs at the injection site followed by migration to LNs. For the Chol-CpG-Cy5 group, the increased LN accumulation and DC association relative also correlated with increased DC, and non-specific T cell, activation. However, for HDL(Chol-CpG-Cy5) increased LN accumulation, DC association was not accompanied by increased T cell activation, which could be due to delayed processing by DCs. All CpG formulations boosted antigen-specific CTL responses although free CpG-Cy5 and HDL(Chol-CpG-Cy5) appeared less effective.

Overall, this study demonstrates that HDL promote vaccine cargo drainage into lymph and access to LN-resident DCs, which leads to a CTL vaccination response. Delayed processing by DCs may be a limitation for HDL vaccines which may be overcome with further optimisation.

References; 1) Irvine et al, Chem. Rev. 2015, 115, 19, 11109-11146. 2) Trevaskis et al, Nat. Rev. Drug. Discov. 2015, 14, 781-803. 3) Martel et al, JCI. 2013, 123(4), 1571-1579. 4) Lim et al, Cell Metab. 2013, 17, 671-684