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Enhanced lymph node retention of subcutaneously injected IgG1-PEG$_{2000}$-liposomes through pentameric IgM antibody-mediated vesicular aggregation

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Abstract

An efficient strategy for enhancing the lymph node deposition of rapidly drained liposomes from the interstitial injection site is described. Subcutaneously injected small-sized immuno-poly(ethyleneglycol)-liposomes (immuno-PEG-liposomes), containing 10 mol% mPEG$_{350}$–phospholipid and 1 mol% PEG$_{2000}$–phospholipid in their bilayer and where IgG1 is coupled to the distal end of PEG$_{2000}$, not only drain rapidly from the interstitial spaces into the initial lymphatic system, but also accumulate efficiently among the lymph nodes draining the region when compared with non-PEG-bearing immunoliposomes where IgG is directly coupled to the phospholipid. Liposome deposition among the draining lymph nodes, however, was further enhanced dramatically following an adjacent subcutaneous injection of a pentameric IgM against the surface attached IgG molecules (IgM:IgG, 10:1) without compromising vesicle drainage from the interstitium. This is suggested to arise either as a result of formation of large immuno-aggregates within the lymphatic vessels with subsequent transport to and trapping among the regional lymph nodes and/or following IgM binding to Fc receptors of the lymph node sinus macrophages forming a platform for subsequent trapping of drained IgG-coupled liposomes. This lymph node targeting approach may be amenable for the design and surface engineering of any rapidly drained nanoparticulate system bearing peptides and proteins that can be aggregated with a desired monoclonal pentameric IgM.

Keywords: IgM; Immunoliposome; Lymph node; Lymphatic system; Macrophage; Targeting

1. Introduction

Under normal physiological conditions interstitially injected liposomes are drained into the initial lymphatic system through patent junctions in the lymphatic capillaries and are then conveyed to the regional lymph nodes via the afferent lymph [1]. Within the lymph nodes the drained vesicles are susceptible to extraction by macrophages of the medullary sinuses and paracortex; however, littoral cells and polymorphonuclear granulocytes also play some role in liposome clearance [2]. Such means of vesicle transportation from interstitial sites and clearance by lymph node scavenger cells has numerous medical applications to include lymphoscintigraphic tracing, lymph node mapping, antimicrobial and antigen delivery, and immune modulation [1,3,4]. Among the key physicochemical factors controlling the kinetics of liposome drainage through the ground substance of the interstitium into the initial lymphatic system and subsequent macrophage capture are vesicular size, morphology, and surface characteristics (e.g., electric charge, hydrophilicity/hydrophobicity, and ligand expression and density) [1,5–8]. For example, in rats although up to 50% of the injected dose (footpad injection) of anionic unilamellar liposomes (size range 90–120 nm) is usually drained into the lymphatic system within 6–10 h, liposome capture by resident phagocytic cells of the primary draining lymph node rarely exceeds 2–3% of this fraction [6]. Liposome clearance by macrophages of the secondary and tertiary nodes is even less efficient. Noncaptured vesicles subsequently gain access into the systemic circulation via thoracic duct and are cleared by hepatic and splenic macrophages.

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Rational strategies are therefore required to dramatically increase the retention and localization of interstitially injected liposomes among the regional draining nodes. Recently, work from this laboratory demonstrated that the rate of drainage and lymphatic distribution (macrophage capture) of interstitially injected liposomes in rats can be improved dramatically by simultaneous attachment of a targeting ligand, immunoglobulin-G (IgG), and inclusion of appropriate methoxypoly(ethyleneglycol)–phospholipid (mPEG-PL) conjugates into the liposomal bilayer [9]. However, the extent of both liposome drainage and macrophage targeting was dependent on the mode of IgG coupling and surface poly(ethyleneglycol) configuration. For instance, the lymph node retention (both primary and secondary nodes) of rapidly drained liposomes of 100–120 nm in size was not only increased following conjugation of a non-specific IgG to the distal end of a functionalized PEG_{2000}-PL, but adjusting the molecular architecture of surface exposed PEG_{2000} chains to a "nearly overlapped mushroom/mushroom–brush transition" regime, yielded vesicles with optimal target-binding capability [9]. The latter was achieved by inclusion of 10 mol% of $mPEG_{350}$–phospholipid conjugates into the bilayer of IgG-PEG_{2000}-liposomes. This paper demonstrates a further step towards improving the lymph node retention of IgG-PEG_{2000}-liposomes through *in vivo* vesicular aggregation in the lymphatic vessels with a pentameric IgM (*in vivo* conversion of "small" to "big"; Fig. 1) thus enhancing liposome clearance by resident macrophages via Fc receptors. The principle of this approach was inspired by a recent observation of Phillips et al. [10] where it was demonstrated that the retention of interstitially injected biotin-coated liposomes in draining lymph nodes could be increased dramatically following an adjacent avidin injection.

2. Materials and methods

2.1. Liposome preparation and characterization

All lipids were from Sigma (Poole, UK) with the exception of -(4′-(4″-maleimidophenyl)butyroyl)-phosphatidylethanolamine (MPB-PE), $mPEG_{350}$-distearoylphosphatidylethanolamine ($mPEG_{350}$-DSPE) and MPB-PEG_{2000}-DSPE, which were obtained from Avanti Polar Lipids (Alabaster, AL, USA). Immunoliposomes were composed of egg phosphatidylcholine (egg PC), cholesterol (Chol), dicetylphosphate (DCP) and MPB-PE in a molar ratio of 6.925:6.925:1:0.15, respectively. Immuno-PEG-liposomes were composed of

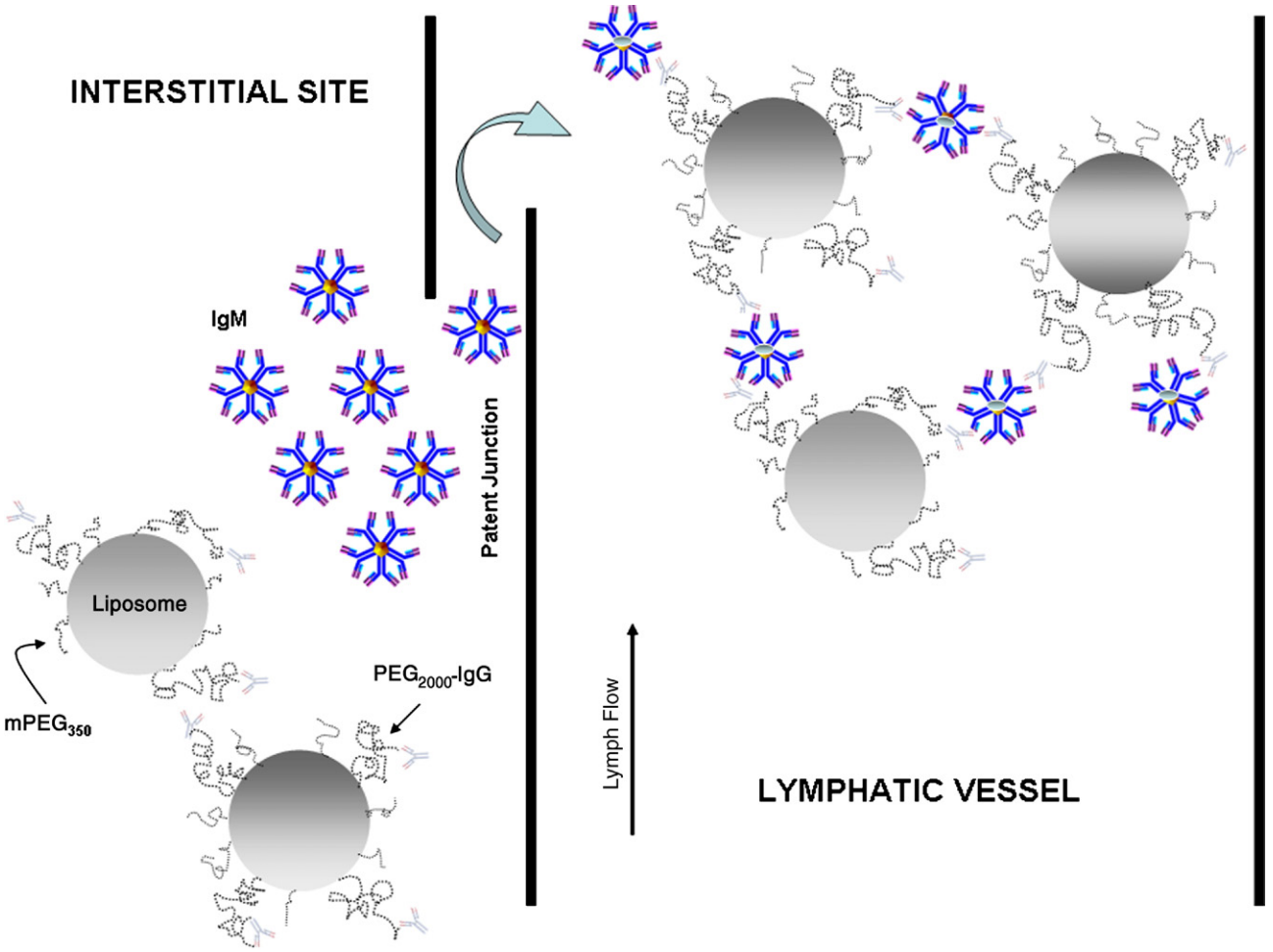

Fig. 1. Schematic representation of immuno-PEG_{2000}-liposome drainage from a subcutaneous injection site followed by IgM-mediated vesicular aggregation in the lymphatic vessel. Liposomes are injected first into the dorsal surface of rat footpad. Next, pentameric IgM molecules are injected subcutaneously proximal to the site of liposome injection. Both IgM and immuno-PEG_{2000}-liposome drain rapidly into the initial lymphatic vessels. Within the lymphatic vessels IgM molecules and liposomes encounter each other resulting in the formation of large immuno-aggregates. These entities drain into the regional lymph nodes where they become trapped.