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Reduced immune response to polymeric micelles coating sialic acids

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ABSTRACT

Effects of sialic acid coatings on polymeric micelle consisting of poly(sarcosine)-*block*-poly(L-lactic acid) (Lactosome) in the aim of prevention of the accelerated blood clearance (ABC) phenomenon are studied. Two kinds of the sialic acid-presenting Lactosomes targeting the immunosuppressive receptors of Siglec-G and CD22 have been successfully prepared. Lactosome presenting 5-*N*-acetylneuraminic acid or 5-*N*-acetylneuraminyl-α(2 → 6)-galactosyl-β(1 → 4)-*N*-acetylglucosamine at the nanocarrier surface diminished the ABC phenomenon due to the reduction of the anti-poly(sarcosine) IgM production. Further, the sialic acid moieties could interact possibly with Siglec-E on immune cell to suppress phagocytosis of the opsonized nanocarriers.

Nanocarriers are currently explored extensively as a vehicle of various medicinal agents in biomedical fields including targeted drug delivery systems (T-DDS), medical imaging, and diagnosis.[1,2] Particularly in regard of cancer, nanocarriers are believed to become one of the most powerful tools to challenge this tricky disease.[3] Current cancer treatments include chemotherapy by anticancer agents, radiation therapy, surgical removal of tumor tissues, and in most cases, combination of these treatments and the immune checkpoint antibodies under some circumstances. However, these treatments often cause serious damages to healthy cells in the patients. In order to avoid such side effects, it is desirable to develop cancerous cells-specific T-DDS. Nanocarriers are highly potential to transport a variety of substances with their loading in nanocarriers. Particularly in cancer therapies, nanocarriers can be accumulated in tumor sites due to the characteristic features around the tumor region, i.e., the enhanced permeability and retention (EPR) effect that is explained by extravasation through leaky vessels and poor lymphatic drainage in tumors.[4] Furthermore, the long-circulating ability (the 'stealth' property) of nanocarriers also assists feasibility for the cancer therapies, which can be attainable by surface modification of nanocarriers with the highly hydrated and dense polymers such as poly(ethyleneglycol) (PEG),[5] poly[*N*-(2-hydroxypropyl)methacrylamide] (HPMA),[6] poly (hydroxyethyl-L-asparagine) (PHEA),[7] chitosan,[8] etc. Normally, the intravenously administered nanocarriers without the stealth functionalization are rapidly cleared by the mononuclear phagocyte system (MPS) within several minutes. Thus the stealth nanocarriers are the first choice in DDS.

The stealth nanocarriers, however, have been pointed out to lose the stealth property upon multiple administrations. For example, the stealth PEGylated liposomes become 'visible' for the MPS at the second administration and after that. This change has been known as the accelerated blood clearance (ABC) phenomenon.[9,10] The ABC phenomenon was also observed with the PEGylated nanocarrier, which was found to be recognized by B lymphocyte through the PEG chains as the T cell-independent type 2 (TI-2) antigen,[11] leading to production of anti-PEG IgM.[12,13] Many efforts have been made in order to avoid the ABC phenomenon, but it is still challenging to date.[14]

Previously, we have established the novel stealth nanocarrier consisting of amphiphilic block copolymers of poly(sarcosine)-*block*-poly(L-lactic acid) (PSar-PLLA, **1**) named as 'Lactosome' (Fig. 1a and b).[15] Poly(sarcosine) is a hydrophilic polypeptide, which provides the hydrophobic core of poly(L-lactic acid) in Lactosome with the sterically hindered hydrophilic shell. Lactosome is very effective as a vehicle for delivery to various solid tumors.[16,17] However, the ABC phenomenon was observed similarly to the PEGylated liposome.[18] The intensive studies revealed that the ABC phenomenon induced by Lactosome was due to the

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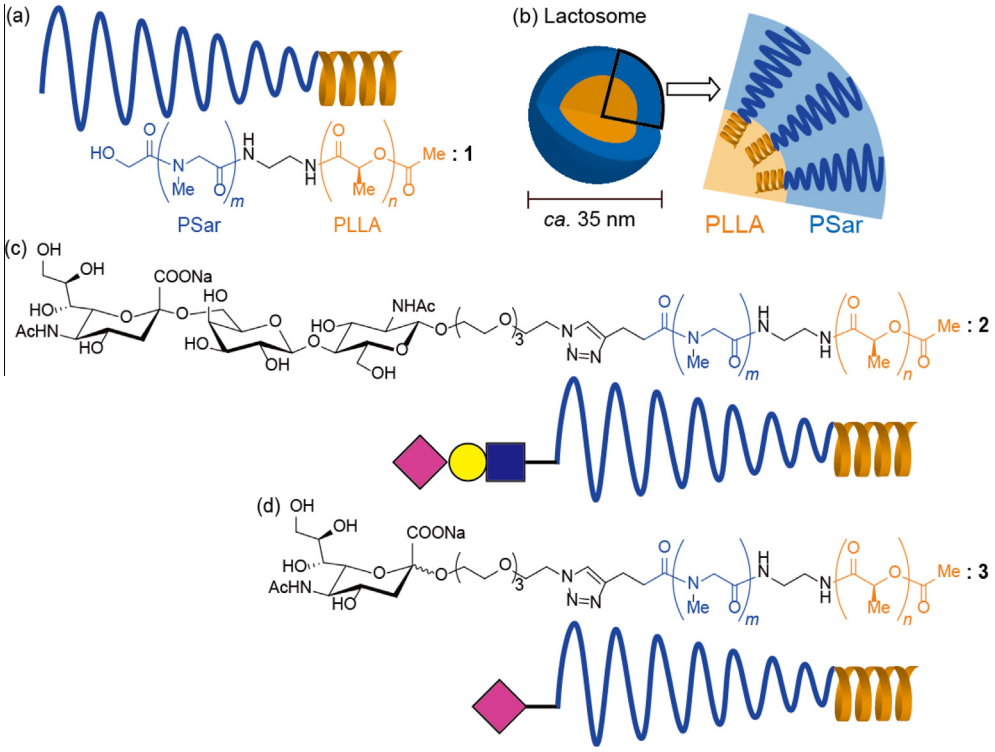

Figure 1. Schematic illustrations of Lactosome and the glycan-coated Lactosomes: (a) chemical structure of the component block copolymer PSar-PLLA, (b) formulation of Lactosome, (c) the PSar-PLLA with a Neu5Acα(2 → 6)Galβ(1 → 4)GlcNAcβ- at the terminal (**2**), (d) the PSar-PLLA with a Neu5Ac at the terminal (**3**).

production of anti-poly(sarcosine) IgM and IgG_3 by the direct activation of the peritoneal B lymphocyte.[19,20] The ABC phenomenon has been shown to be solved out by using A_3B-type ((poly(sarcosine))$_3$-*block*-poly(L-lactic acid)) polymer[21] or taking a nanosheet morphology,[22] which are based on the concept to increase the surface density of poly(sarcosine) chains on the nanocarrier surface to prevent the B lymphocyte recognition. On the other hand, it has been pointed out that the activation of the B cell subset can be inhibited by the suppressive ligand of sialic acid concurrently associating with Siglec of the B cell.[23] Siglecs are the family of sialic acid-binding immunoglobulin-like lectins found mainly on immune cells, which are believed to regulate a variety of immune cell functions. Siglec-G and CD22 on mouse B cells have been reported as the critical Siglecs responsible for suppression of the immune response to TI-2 antigens via induction of apoptosis.[24–26] We thus report here the availability of the surface modification of Lactosome with sialic acid to escape from the ABC phenomenon.

The ligands for Siglec-G and CD22 have been reported to be the sialoglycans, i.e., those having Siaα(2 → 6)Galβ(1 → 4)GlcNAcβ terminal structures, where Sia represents a 5-*N*-glycolylneuraminic acid (Neu5Gc) or a 5-*N*-acetylneuraminic acid (Neu5Ac) in mice.[23] The latter is a common carbohydrate structure in both humans and mice. We therefore chose Neu5Acα(2 → 6)Galβ(1 → 4)GlcNAcβ connecting to the terminal of the poly(sarcosine) chains (**2**) (Fig. 1c) to construct the glycan-coated Lactosome. In order to evaluate the effect of sialic acid-coatings,[27,28] the monosaccharidic Neu5Ac ligand at the poly(sarcosine) terminal (**3**) (Fig. 1d) was also prepared and studied. Retrosynthetic analysis of **2** is shown in Scheme 1. Copolymer **2** should be formed by ligation of the terminal azido functionalized carbohydrate part of **4** and the amphiphilic block copolymer part of **5** bearing an alkyne moiety via the Huisgen cycloaddition. Copolymer **5** should be prepared by condensation of pentynoic acid **6** and the copolymer **7**.[15] The carbohydrate part of **4** is formed by glycosylation of the sialic acid donor **8**[29] to the novel disaccharide derivative **9** followed by deprotections. The derivative **9** is obtained by glycosylation of the glycosyl donor **10**[30] to the commercially available azido functionalized oligoethyleneoxide alcohol **11**.

Copolymer **2** was synthesized according to the reactions outlined in Scheme 2. Compound **11** was successfully glycosylated with **10** to afford **12** in a 90% yield. All of the protecting groups in **12** were removed, then peracetylation was carried out to afford **13**. *O*-Acetyl protection in **13** was fully removed to obtain **14** in a 95% yield, and then it was treated with 4-anisaldehyde dimethylacetal and catalytic amount of 10-camphorsulfonic acid, followed by acetylation and removal of 4′,6′-(4-methoxybenzylidene) group to provide **9** in a 88% yield via 3 steps. Regioselective O-sialylation of **9** was carried out with **8** under the conditions described in 'Supplementary data' section. The products were obtained as the mixture of the diastereomers (α/β = 3/1) in a 70% yield. Fortunately the target compound of **15** (α-anomer) was separated by silica gel column chromatography in a 43% yield. All of the *O*-acetyl groups and the methyl ester in **15** were removed successively by sodium methoxide in methanol and then by 1 M aq NaOH at 0 °C, to give **4** in a 74% yield. The terminal alkyne functionalized copolymer **5** was prepared by condensation of pentynoic acid **6** and the amphiphilic block copolymer **7**[15] (m = 29, n = 30; as average numbers of monomers determined by ^{1}H NMR measurements) by using 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)]methanaminium hexafluorophosphate (COMU)[31] as a coupling reagent. This condensation reaction proceeded almost quantitatively, which was inferred by MALDI-TOF MS. Compound **4** and copolymer **5** were connected by the Huisgen cycloaddition reaction to afford **2** quantitatively.

Copolymer **3** was also prepared chemically according to the reactions outlined in Scheme 3. Compound **11** was glycosylated with **8** under the similar reaction conditions to that for **15**, which gave **16** as the mixture of α- and β-anomers (α/β = 2/1) in a 90%