Influence of Surface Properties on the Inflammatory Response to Polymeric Nanoparticles

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INTRODUCTION

Inflammatory response is the most relevant non-specific defense mechanism in front of tissue injury, infection and chemical or physical trauma. The following local reaction is accompanied by a large number of systemic and metabolic changes, referred to the so-called acute phase response. During the inflammatory process, a complex series of reactions are executed by the host to ongoing tissue damage, leading to the isolation and the destruction of the foreign organism. A major process that characterizes inflammation is the increase in the concentration of plasma glycoproteins, synthesized by the hepatocytes and known as acute phase proteins (1).

Foreign particles such as hydrophobic polymer nanoparticles are taken up very quickly by the cells of the Mononuclear Phagocyte System (MPS) after intravenous (IV) injection. Particles removal from the blood stream is the result of the adsorption of blood components on their surface (opsonization) that makes them recognizable by the phagocytic cells. Phagocytic stimuli induce macrophages to secrete a large number of substances, including eicosanoids, active oxygen metabolites from the respiratory burst, and cytokines that may have a strong influence on the inflammatory response (2). These substances are required for the elimination of pathogenic organisms, but are unnecessary and potentially deleterious when the ingested particles are inert and non-pathogenic. The acute phase response may result in impaired defense against infections, hypersensitivity reactions and other unknown undesirable effects (3). We have previously shown that single IV administration of polymeric nanoparticles led to an acute inflammatory response characterized by the increase of serum α1-acid glycoprotein (AGP) levels, an acute phase protein in rats (4).

Strategies used to avoid MPS capture are based on the reduction of serum opsonization by coating, simply by adsorption, polystyrene (PS) nanoparticles with hydrophilic surfactants such as block copolymers of poloxamers (polyoxyethylene-polyoxypropylene copolymers), or poloxamines (polyoxyethylene-polyoxypropylene-ethylenediamine copolymer) series or with polyethylene glycol (PEG). Coating layer provides an uncharged, extremely hydrophilic surface that reduces adsorption of blood proteins, together with an effective steric barrier that temporarily prevents phagocytosis by the MPS (5). Modifying the surface characteristics of biodegradable poly(D,L-lactic) acid (PLA) nanoparticles by adsorption of poloxamers was not efficient in overcoming the extensive capture by the MPS (6). However, PLA-PEG nanoparticles were developed from preformed diblock amphiphilic copolymers and showed to temporarily avoid liver phagocytosis after IV administration (7).

The aim of this work was to investigate the influence of the nanoparticles surface properties on the induction of inflammatory responses, in order to better understand the mechanisms involved. This approach can be potentially useful for the development of biocompatible particles for drug targeting.

MATERIALS AND METHODS

Preparation of Nanoparticles

Surfactant-free PS nanoparticles (Polysciences, Inc.) were coated with non-ionic surfactants, Poloxamer 338 (Synperonic PE/F108, ICI) and 407 (Synperonic PE/F127, ICI) and Poloxamine 908 (Synperonic T/908, ICI) by incubation in a 2% (w/v) surfactant solution for 2 days. The excess of surfactant was discarded with the supernatant after centrifugation of the nanoparticles suspension (120,000*g*, 30 min). PLA and PLA-PEG nanoparticles were prepared as described by Fessi *et al.* (8). Briefly, 100 mg of PLA polymer (MW 88,000; Boehringer Ingelheim) or PLA-PEG copolymer (MW 50,000, offered by Fessi) were dissolved in 20 ml of acetone. The solution was mixed with 40 ml of an aqueous phase containing 75 mg of Poloxamer 188 (Synperonic PE/F68, ICI), under magnetic stirring, for 10 min. Acetone and water were then evaporated under vacuum to a final volume of 10 ml.

Nanoparticles Characterization

Nanoparticles diameter was determined by Photon Correlation Spectroscopy (PCS), using a nanosizer (model N4MD, Coulter®). The surfactant coating layer thickness on PS nanoparticles can be measured by comparing the diameter of coated and noncoated nanoparticles. Surface particles charges were indirectly determined by the measurement of zeta potential, using a zetameter (model Zetasizer 4, Malvern Instruments). Zeta potential values were determined in 20 mM phosphate buffer, at room temperature.

Evaluation of Changes in Serum Proteins *in Vivo*

Male Sprague-Dawley rats (300–350 g) from IFFA-

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Abbreviations used: AGP, α1-acid glycoprotein; IL, Interleukin; MPS, Mononuclear Phagocyte System; PEG, polyethylene glycols; PLA, poly(D,L-lactic) acid; PS, polystyrene.

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CREDO (Arbresle, France) were treated with a single IV injection (20 mg/kg) of uncoated and coated PLA or PS nanoparticles prepared as previously described. Serum was sampled at different times after injection for 10 days. Serum AGP levels were determined by a sandwich-type immunoenzymatic assay, ELISA (9). Results were expressed as the mean (± standard deviation) of 4 assays. Data were statistically compared by the Mann-Whitney test. The criteria of significance was 0.05.

RESULTS AND DISCUSSION

Opsonization of foreign particles is a necessary step for their recognition and uptake by macrophages of the MPS. It was shown that coating PS particles with poloxamers and poloxamine (5) or covalent linkage of PEG onto PLA nanoparticles (7) lead to a reduction in their opsonisation, thus decreasing their uptake by liver and spleen after IV injection. Poloxamer 407- and poloxamer 338-coated PS nanoparticles accumulated in the bone marrow, whereas poloxamine 908-coated nanoparticles circulated in blood for prolonged periods (5).

In this work, nanoparticles' mean diameters were comprised in the range of 100–140 nm (Table I). Coating the PS nanoparticles with poloxamers and poloxamine lead to a reduction of surface charge (Table I). This reduction was also observed with PLA-PEG nanoparticles compared to PLA nanoparticles (Table I). The protective effect of surfactants is expected to lead to the reduction of serum opsonization (5). Indeed, our results clearly showed that the increase of serum AGP concentration, following the administration of PS nanoparticles, was avoided by coating them with poloxamers and poloxamine (Figure 1). Although coating particles reduced their uptake by the liver and by the spleen, some particles are supposed to be still distributed in these organs. This liver accumulation, although poor, may be responsible for the slight AGP increase observed 24 h after the treatment (Figure 1). In addition, AGP increased 9 days after the treatment with poloxamine 908-coated PS nanoparticles. These particles circulating in the blood stream are supposed to partially lose their coating layer, thus becoming more susceptible to the MPS uptake. Concerning PLA nanoparticles, it was observed that the inflammatory response was more evident than with PS nanoparticles (Figure 2). This strong inflammation did not take place in the case of PLA-PEG nanoparticles. These particles even induced a chronically reduction of AGP serum levels. This effect could be due to an inhibition of AGP synthesis or to a stimulation of AGP degradation. PEG did not play any role in the inflammatory response since the injection of 4 mg/kg did not change the AGP levels (data not shown).

Table I. Nanoparticles Mean Diameters and Zeta Potential Values

Nanoparticles	Diameter (nm)	Zeta potential (mV)
PS	103 ± 24	−53.4
PS-Poloxamer 407	126 ± 30	−11.5
PS-Poloxamer 338	121 ± 31	−9.0
PS-Poloxamer 908	126 ± 30	−6.8
PLA	134 ± 58	−13.5
PLA-PEG	137 ± 85	−6.0

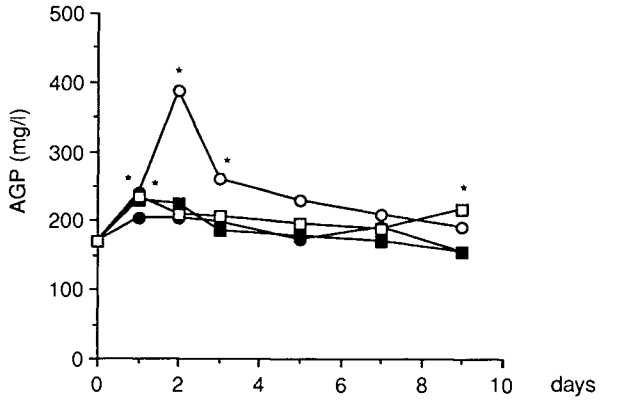

Fig. 1. Serum AGP levels after treatment with a single injection of 20 mg/kg of PS nanoparticles (open circle), poloxamer 338-coated nanoparticles (closed circle), poloxamer 407-coated nanoparticles (closed square) and poloxamine 908-coated PS nanoparticles (open square). * Statistical analysis. Significant differences from control values (time zero); $p < 0.05$.

These data clearly evidenced that particle-induced inflammatory reactions are related to their opsonization and/or phagocytosis by liver macrophages. Interactions between hepatocytes and macrophages are mediated by cytokines (10). The main mediators in the inflammatory response are interleukin (IL)-6, IL-1 and Tumor Necrosis Factor-α. These cytokines are well known to stimulate the liver cells to produce higher amounts of acute phase proteins, such as AGP (1). Hepatic macrophages could then induce the secretion of hepatic AGP through the secretion of cytokines after nanoparticle phagocytosis. The question is how do nanoparticles activate the liver macrophages. Macrophages have been shown to be activated after phagocytosis of polymeric particles (11). Their uptake stimulates the release of lysosomal enzymes and oxygen metabolites that facilitate the destruction of ingested material and have the potential to stimulate and amplify the inflammatory response (Figure 3).

On the other hand, polymer surfaces have the ability to activate the complement system (12,13). Complement activation results in the production of humoral mediators, able

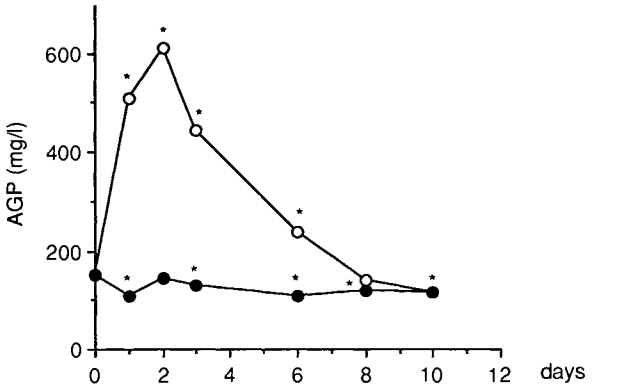

Fig. 2. Serum AGP levels after treatment with a single injection of 20 mg/kg of PLA (open circle) and PLA-PEG (closed circle) nanoparticles. * Statistical analysis. Significant differences from control values (time zero); $p < 0.05$.

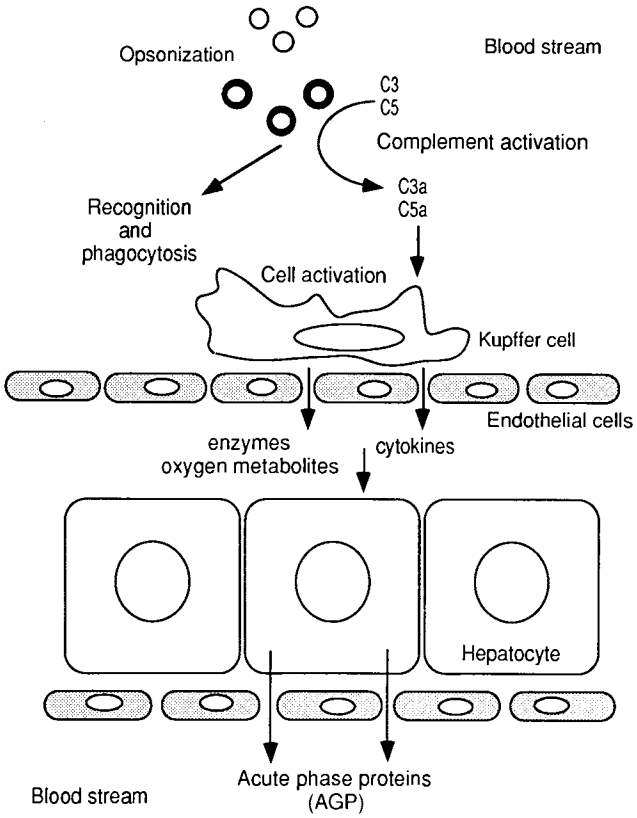

Fig. 3. Hypothetical mechanism of the inflammatory response to polymer nanoparticles.

to induce inflammatory reactions. Interaction of the anaphylatoxins C3a and C5a (released during the activation of their precursors, C3 and C5) with monocytes and macrophages, lead to cell activation which may result in an increased phagocytic activity and an inflammation due to the secretion of IL-6 and IL-1 (14, 15) (Figure 3). Opsonization is minimized in the case of poloxamers-coated PS nanoparticles or PLA-PEG nanoparticles and this is a possible mechanism that allows to avoid the secretion of inflammatory mediators.

In conclusion, inflammatory responses after IV injection of polymeric nanoparticles were avoided after the modification of their surface properties by coating with hydrophilic materials. This is an important establishment for developing biocompatible drug targeting systems.

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