Human dendritic cell maturation induced by amorphous silica nanoparticles is modulated by pulmonary surfactant.

Eléonore Guillet[1], Alexia Feray[1], Natacha Szely[1], François-Xavier Legrand[2], Emilie Brun[3], Marc Pallardy[1] and Armelle Biola-Vidamment[1].

[1] Université Paris-Saclay, Inserm, Inflammation, Microbiome and Immunosurveillance, 92290, Châtenay- Malabry, France
[2] Université Paris-Saclay, CNRS, Institut Galien Paris Sud, 92296, Châtenay-Malabry, France
[3] Université Paris-Saclay, CNRS, Institut de Chimie Physique, 91405, Orsay, France.

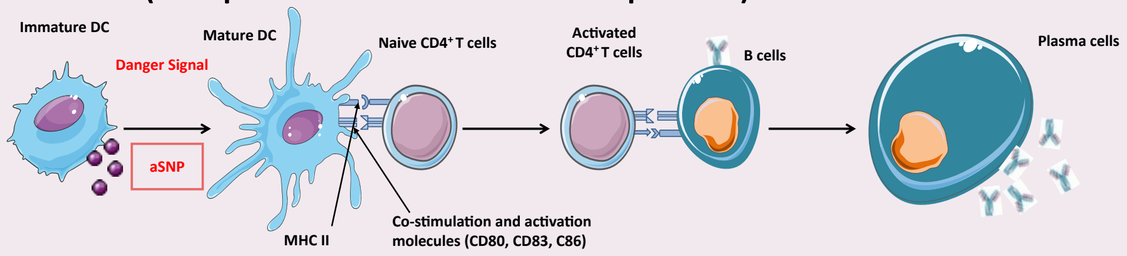
eleonore.guillet@universite-paris-saclay.fr

Introduction

Synthetic amorphous silica (SAS) nanoparticles (NPs) are widely used in cosmetics, food additives and in the biomedical and construction industries.

Workers are mostly exposed *via* respiratory route and one of the main challenge to evaluate SAS effects is to simulate the complexity of the *in vivo* situation through optimized *in vitro* models. Indeed, in the deep lung, airborne NPs first encounter alveolar lining fluids such as the **pulmonary surfactant** (PS) before innate immune cells contact. The dynamic interaction of PS, mainly composed of lipids and proteins, with inhaled particles may influence subsequent biological outcomes by forming a ring called corona around them.

Dendritic cells (DCs) are professional antigen presenting cells which ensure a constant sampling of their environment. Pulmonary DCs capture and, depending on the cellular microenvironment, present sampled antigens in an immunogenic or tolerogenic way. In the presence of "danger signals", DCs undergo a maturation process resulting in their migration to regional lymph nodes where they activate naïve T lymphocytes. **Our hypothesis is that nanomaterials could be considered as danger signals and therefore called NAMPs (nanoparticle-associated molecular patterns).**



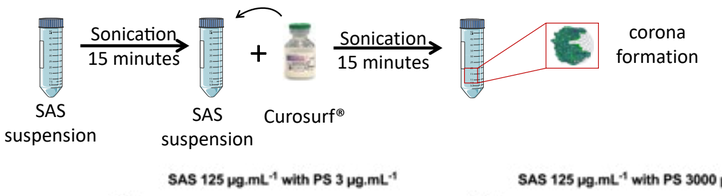
Aim of the study

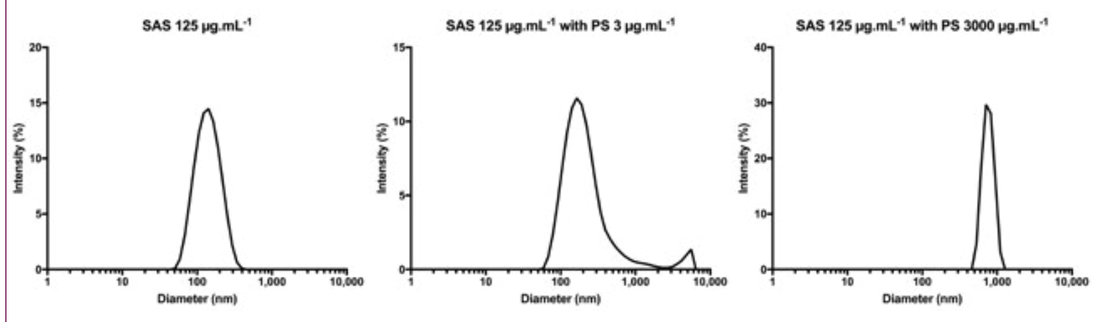
Our preliminary results showed that pyrolytic SAS induce a marked phenotypic activation of DCs.

The aim of this work was to determine whether **PS could affect DC response to SAS**.

SAS characterization

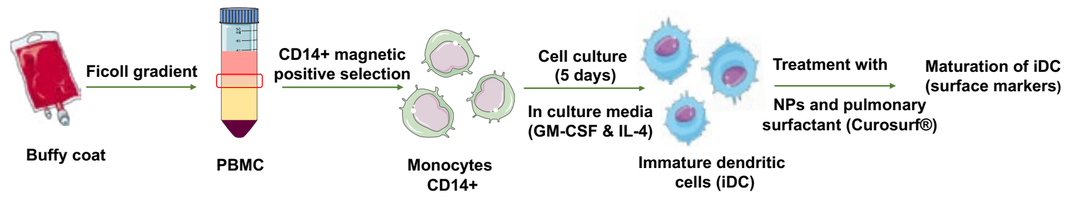
Dynamic Light Scattering





Fumed silica, with an approximate 14-nm diameter primary particle size (S5505 Sigma®), were pre-incubated in RPMI culture medium (without fœtal calf serum) in the presence or not of a well-characterized commercially available PS substitute (Curosurf®) at different concentrations of 3, 30, 300, 3000 and 9000 µg.mL^{-1} and sonicated for 15 minutes. The NPs/PS ratios used for the DLS are identical to those used in cell culture.

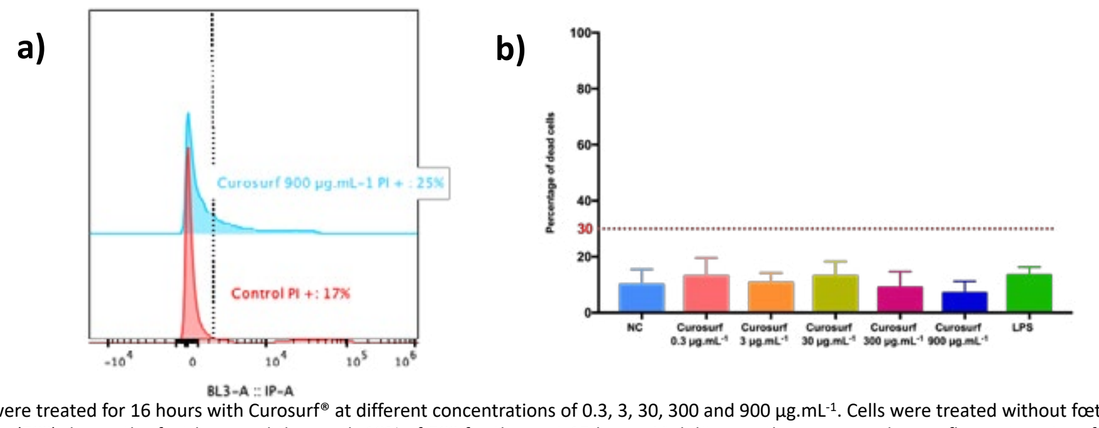
In vitro model



Immature human monocyte-derived DCs (iDC) were exposed for 16 hours to final concentrations of 12.5 and 25 µg.mL^{-1} of fumed silica NPs, in the presence or not of a PS substitute (Curosurf®). NP were pre-coated with five increasing concentrations of Curosurf® to reach realistic concentrations and sonicated before addition in the cell culture. Cell viability and phenotypical changes were measured by flow cytometry.

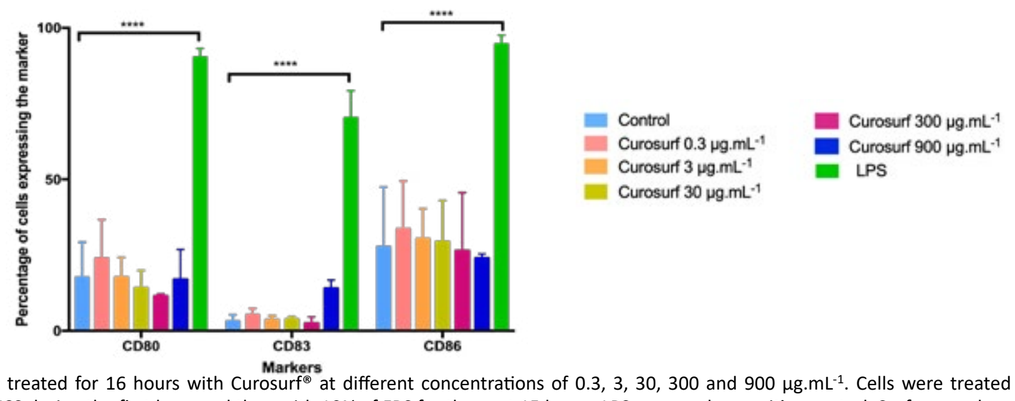
Effects of pulmonary surfactant on SAS-induced dendritic cell activation

1. Cytotoxicity of pulmonary surfactant (Curosurf®) on dendritic cells



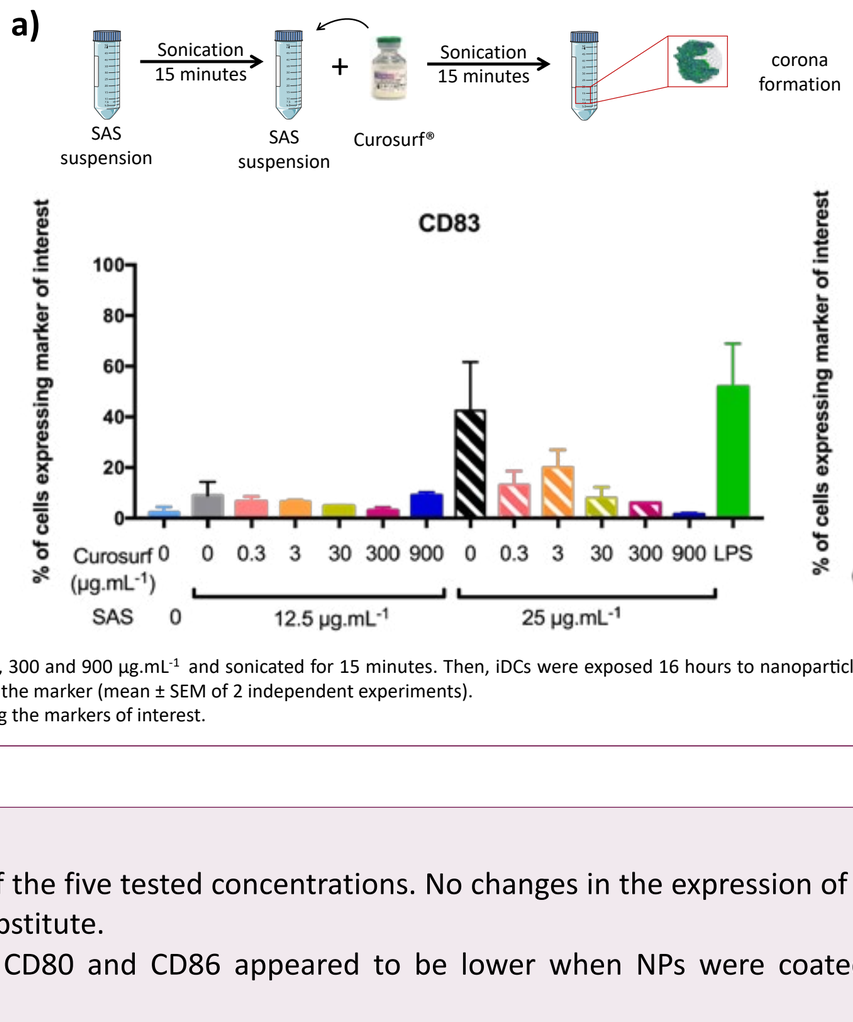
iDC were treated for 16 hours with Curosurf® at different concentrations of 0.3, 3, 30, 300 and 900 µg.mL^{-1}. Cells were treated without fœtal calf serum (FCS) during the first hour and then with 10% of FCS for the next 15 hours. Viability was then measured using flow cytometry after DC labelling with propidium iodide. Lipopolysaccharide (LPS) was used as positive control. Results are expressed as percentage of dead cells (mean ± SEM of 3 independent experiments). **a)** Representative histogram of cell viability after exposure 900 µg.mL^{-1} of Curosurf® ; **b)** Histogram of viability after exposition to different Curosurf® concentrations.

2. Dendritic cell activation in presence of pulmonary surfactant (Curosurf®)



iDC were treated for 16 hours with Curosurf® at different concentrations of 0.3, 3, 30, 300 and 900 µg.mL^{-1}. Cells were treated without FCS during the first hour and then with 10% of FBS for the next 15 hours. LPS was used as positive control. Surface markers were measured using flow cytometry. Results are expressed as percentage of cells expressing the marker (mean ± SEM of 3 independent experiments).
ANOVA test, Kruskal-Wallis test, **** : p<0,0001.

3. Dendritic cell activation in presence of pulmonary surfactant (Curosurf®) and SAS



SAS NPs were pre-incubated with the PS (Curosurf®) at different concentrations of 0.3, 3, 30, 300 and 900 µg.mL^{-1} and sonicated for 15 minutes. Then, iDCs were exposed 16 hours to nanoparticles pre-incubated with Curosurf®. LPS was used as positive control. Surface markers were measured using flow cytometry. Results are expressed as percentage of cells expressing the marker (mean ± SEM of 2 independent experiments).
a) Diagram of the SAS pre-incubation process with Curosurf®; **b)** Percentage of cells expressing the markers of interest.

Conclusions

- The PS substitute did not increase DC lethality at any of the five tested concentrations. No changes in the expression of activation and co-stimulation (CD83) markers (CD80, CD86) were observed on cells incubated with the PS substitute.
- At both NP tested concentrations, the expression of CD80 and CD86 appeared to be lower when NPs were coated with Curosurf® compared to pristine NP, in a PS concentration-dependent manner.
- **Pulmonary surfactant, and thus the NP corona composition and biological identity, may play a critical role in DCs activation in response to SAS.**