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# Size-dependent immune-modulating effect of amorphous nanosilica particles

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The immune-modulating effect following intradermal injection of various-sized amorphous silica particles was analyzed in terms of induction of ovalbumin-specific CD8$^+$ T cells *in vivo*. IFN-γ ELISPOT assays revealed that only nanosilica particles with a diameter of less than 100 nm significantly enhanced CD8$^+$ T cell responses against ovalbumin. These results indicate that the size of nanomaterials is a critical determinant in terms of their safe use.

Recently, practical uses of nanomaterials (NMs) have rapidly spread to a wide variety of fields, such as cosmetics, foods, and medicines (Bowman et al. 2010; Liu and Gu 2009; Salata 2004). However, potential harmful effects of NMs on humans are raising concerns with regard to their safety, because NMs may possess novel properties different from micro-sized materials (Nel et al. 2006). Despite intensive research, the relationship between the biological response and physicochemical properties of NMs is not well understood. Specifically, it is important to clarify the relationship between particle-size and cellular distribution/associated biological effects, thereby facilitating a more accurate risk assessment of NMs.

Currently, amorphous nanosilica particles (nSPs) are among the most widely used NMs in a variety of different applications (Napierska et al. 2010). Here, we evaluated the relationship between the *in vivo* distributions of nSPs with their biological effect. Recently, we revealed that the nSPs with a diameter of 70 nm (nSP70) can penetrate the skin barrier and reach various tissues such as the lymph node, liver, and brain (Nabeshi et al. 2011). In a separate study, we also revealed that amorphous silica particles have size-dependent toxicity to murine Langerhans cell line XS52 (Nabeshi et al. 2010). Both the lymph nodes and Langerhans cells play a critical role in the immune response. Here, we wanted to clarify whether applications of nSPs induce an immune-modulating effect. Therefore, we investigated the induction of CD8$^+$ T cells in response to an exogenous antigen in the presence/absence of nSPs.

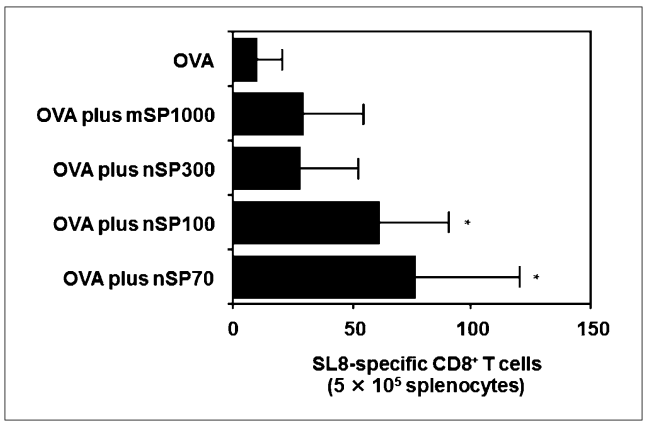


Fig.: Analysis of SL8 specific CD8$^+$ T cell response *in vivo*. C57BL/6 mice were intradermally immunized with OVA alone or OVA plus various sized silica particles. Six days after the immunization, splenocytes from each group were cultured with 5 μg/ml $OVA_{257–264}$ (SL8) peptide. The levels of SL8-specific CD8$^+$ T cells were examined using an IFN-γ ELISPOT assay. Data are presented as means ± SD of 3–4 animals per group. * $P < 0.05$ vs. OVA alone group.

Female C57BL/6 mice were intradermally immunized with ovalbumin (OVA) alone or OVA plus various sized silica particles. Six days after the immunization, splenocytes from each group were cultured with $OVA_{257–264}$ peptide (SL8), which corresponds to an immunodominant H-2K$^b$-restricted CD8$^+$ T cell-epitope of OVA. The levels of SL8-specific CD8$^+$ T cells were then examined using an IFN-γ ELISPOT assay. Our results show that induction of SL8-specific CD8$^+$ T cells was not enhanced in the submicron-sized silica particle immunized group. However, significant induction of SL8-specific CD8$^+$ T cells was observed in the group of mice immunized with OVA plus nSPs of diameter below 100 nm (Fig.). Moreover, the corresponding level of induction increased as the diameter of nSPs was reduced. Thus, nSPs can affect immune response against exogenous antigen.

Our results suggest that the intradermal injection of nSPs can affect CD8$^+$ T cell responses against exogenous antigen. Further studies are required to analyze the immune-modulating effect of nSPs using topical applications of nSPs, which is a more likely mode of exposure to NMs. We believed that a robust quantitative analysis of the results of exposure to nSPs will provide critical information to ensure the safety of nSPs. This study indicates that the sizes of NMs as well as the target cell type are critical determinants for the design of safer NMs. We believe that our study will provide useful information for developing safer NMs in the future.

## Experimental

### *1. Silica particles*

Amorphous silica particles with a diameter of 1000, 300, 100, 70 nm (designated mSP1000, nSP300, nSP100, nSP70, respectively; Micromod Partikeltechnologie GmbH, Rostock, Germany) were used in this study. The silica particles were suspended in phosphate-buffered saline (PBS; pH7.4). The suspensions of silica particles were sonicated for 5 min and then vortexed for 1 min immediately prior to use.

### *2. Evaluation of $OVA_{257–264}$ (SL8) -specific T cell responses in vivo*

Female C57BL/6 mice (H-2K$^b$) were purchased from Japan SLC (Shizuoka, Japan) and used at 10 weeks of age. All of the animal experimental procedures were performed in accordance with the institutional ethical guidelines for animal experiments of Osaka University and National Institute of Biomedical Innovation. Mice were intradermally injected with either a mixture of 625 μg silicia particles and 100 μg OVA (Sigma Chemical Co., St.

Louis, MO) in the 50 µl PBS, or 100 µg OVA (50 µl/mouse) in the 50 µl PBS. Six days after immunization, splenocytes were prepared from these mice. The splenocytes were cultured *in vitro* ($5 \times 10^5$ cells) and stimulated with $OVA_{257–264}$ peptide (SL8) (5 µg/ml), which corresponds to an immunodominant H-$2K^b$-restricted $CD8^+$ T cell-epitope of OVA (MBL, Nagoya, Japan). Cells were incubated for 24 h and the number of SL8 specific $CD8^+$ T cell was determined using an IFN-γ ELISPOT assay kit (BD Biosciences, San Diego, CA) according to manufacturer's protocol.

### *3. Statistical analysis*

All data are reported as the mean ± SD. The significance of variation among different groups was determined by one-way ANOVA. Differences between the experimental group and control group were determined by Williams' test. $P < 0.05$ was considered significant.

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