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Uptake of biodegradable poly(γ-glutamic acid) nanoparticles and antigen presentation by dendritic cells in vivo

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ABSTRACT

Poly(γ-glutamic acid) (γ-PGA) nanoparticles (NPs) carrying antigens have been shown to induce potent antigen-specific immune responses. However, in vivo delivery of γ-PGA NPs to dendritic cells (DCs), a key regulator of immune responses, still remains unclear. In this study, γ-PGA NPs were examined for their uptake by DCs and subsequent migration from the skin to the regional lymph nodes (LNs) in mice. After subcutaneous injection of fluorescein 5-isothiocyanate (FITC)-labeled NPs or FITC-ovalbumin (OVA)-carrying NPs (FITC-OVA-NPs), DCs migrated from the skin to the LNs and maturated, resulting in the upregulation of the costimulatory molecules CD80 and CD86 and the chemokine receptor CCR7. However, the migrated DCs were not detected in the spleen. FITC-OVA-NPs were found to be taken up by skin-derived $CD103^+$ DCs, and the processed antigen peptides were cross-presented by the major histocompatibility complex (MHC) class I molecule of DCs. Furthermore, significant activation of antigen-specific $CD8^+$ T cells was observed in mice immunized with OVA-carrying NPs (OVA-NPs) but not with OVA alone or OVA with an aluminum adjuvant. The antigen-specific $CD8^+$ T cells were induced within 7 days after immunization with OVA-NPs. Thus, γ-PGA NPs carrying various antigens may have great potential as an antigen-delivery system and vaccine adjuvant in vivo.

1. Introduction

Vaccines are an effective tool for modulating host immune responses to specific antigens, especially microbial pathogen-associated antigens. However, immunization with a protein or peptide antigen alone in the absence of adjuvants often fails to induce effective immune responses [1]. Adjuvants enhance the immunogenicity of vaccines by different mechanisms [2]. Novel vaccine adjuvants and particle-based antigen delivery systems are being evaluated in a variety of vaccines, expecting potent adaptive immune responses [3,4]. Cellular immunity is required to remove intracellular pathogens such as tuberculosis and malaria, while humoral immunity plays a central role in neutralizing extracellular microorganisms [4,5]. Aluminum-based compounds are used as adjuvants in humans because of their safety. Aluminum-based adjuvants can elicit antigen-specific humoral immunity, yet they hardly induce cellular immunity [6]. Thus, the activation of cellular immune responses is an important subject for the next generation of vaccines.

Dendritic cells (DCs) are antigen-presenting cells (APCs) characterized by a unique capacity to stimulate naïve T cells and initiate innate immune responses into adaptive immune responses [7,8]. Immature DCs develop into mature DCs with the upregulation of major histocompatibility complex (MHC) and costimulatory molecule expression and inflammatory cytokine secretion, when the cells are exposed to antigens. DCs can present the uptake antigens along with the MHC class I molecule to $CD8^+$ T cells through the antigen cross-presentation pathway [9]. Immature DCs recognize pathogen-associated molecules via a series of pattern-recognition receptors, including the Toll-like receptors and inflammatory components [7,10]. Immature DCs reside in peripheral tissues, where they capture and process antigens. Subsequently, DCs migrate to the secondary lymphoid organs, present the processed antigen peptides to naïve T cells, and induce adaptive immune responses [11]. Thus, the stimulation of DCs through antigen uptake is important for inducing effective immune responses.

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Biodegradable nanoparticles (NPs) composed of poly(γ-glutamic acid) (γ-PGA) with L-phenylalanine ethyl ester have been shown to generate antigen-specific cellular and humoral immune responses after immunization with various antigens [12,13]. γ-PGA is water-soluble, biodegradable, edible, and non-toxic polyamino acids produced by certain strains of bacilli. γ-PGA NPs have a capacity of immobilizing various proteins, peptides, and chemicals onto their surfaces and/or encapsulating these substances into the particles [14]. γ-PGA NPs could induce a significantly higher level of antigen-specific adaptive immune responses in mice and monkeys than antigens alone or antigens with other common adjuvants [15–17]. In addition, recent studies demonstrated that antigen-carrying γ-PGA NPs generated protective antiviral and anticancer immunity, suggesting that they are promising vaccine candidates [18,19]. However, their immunological kinetics in vivo remains unknown. In this study, we have investigated the antigen delivery kinetics of γ-PGA NPs in mice after subcutaneous injection and found that DCs capturing γ-PGA NPs migrate to the regional lymph nodes (LNs) and strongly induce cellular immune responses.

2. Materials and methods

2.1. Mice

The wild-type C57BL/6 mice were obtained from Charles River Japan (Yokohama, Japan). The mice were bred under specific pathogen-free conditions in the breeding facilities of Kagoshima University. All experiments were approved by Kagoshima University and conducted in accordance with its guideline for animal experimentation.

2.2. Preparation of γ-PGA NPs

γ-PGA (average molecular weight: 380,000) was kindly provided by Meiji Seika (Tokyo, Japan). The synthesis of γ-PGA NPs, fluorescein 5-isothiocyanate (FITC)-labeled γ-PGA NPs (FITC-NPs), ovalbumin (OVA)-carrying γ-PGA NPs (OVA-NPs), and FITC-labeled OVA (FITC-OVA)-carrying γ-PGA NPs (FITC-OVA-NPs) has been described in our previous report [14]. The sizes of γ-PGA NPs and OVA-NPs in aqueous solution were measured by a dynamic light scattering method. The mean diameters of γ-PGA NPs and OVA-NPs were 210 ± 67 and 252 ± 92 nm, respectively. The contamination with bacterial endotoxin in γ-PGA NPs was determined by Limulus amoebocyte lysate assay (Seikagaku, Tokyo, Japan) and found to be less than 10 endotoxin units/ml.

2.3. Uptake of γ-PGA NPs by DCs in vivo

To examine the uptake of γ-PGA NPs by DCs in vivo, FITC-NPs [1.0 mg of γ-PGA NPs in 100 μl of phosphate-buffered saline (PBS)] or FITC-OVA-NPs (0.1 mg of FITC-OVA and 1.0 mg of γ-PGA NPs in 100 μl of PBS) were subcutaneously injected to mice. The regional LNs and spleen were collected from the mice and dissociated into single-cell suspensions. The cell-associated fluorescence was measured by flow cytometry (FACSCalibur™; BD Biosciences, San Jose, CA), and cytometric data were analyzed by CellQuestPro (BD Biosciences). To characterize DCs capturing γ-PGA NPs, FITC-OVA-NPs (0.1 mg of FITC-OVA and 1.0 mg of γ-PGA NPs in 100 μl of PBS) were subcutaneously injected to mice. On day 3 after injection, the LNs were collected and stained with an allophycocyanin-conjugated anti-CD11c monoclonal antibody (mAb) and phycoerythrin (PE)-conjugated anti-CD80, anti-CD86, anti-CCR7, and anti-CD40 mAbs (all from BD Biosciences), or a mAb recognizing the OVA-derived peptide (SIINFEKL) bound to the MHC class I molecule H-2K^b (eBioscience, San Diego, CA) for 30 min at 4 °C. After washing with PBS, the cells were analyzed by flow cytometry. To examine the uptake of various forms of OVA-NPs by DCs in vivo, FITC-OVA (0.1 mg of FITC-OVA in 100 μl of PBS), FITC-OVA-NPs (0.1 mg of FITC-OVA and 1.0 mg of γ-PGA NPs in 100 μl of PBS), or FITC-OVA + aluminum (Sigma, St. Louis, MO) (0.1 mg of FITC-OVA and 1.0 mg of aluminum in 100 μl of PBS) were subcutaneously injected to mice. The regional LNs and spleen were collected from the mice and dissociated into single-cell suspensions. The cells were analyzed by flow cytometry. To detect CD11c$^+$ CD103$^+$ DCs capturing γ-PGA NPs, FITC-OVA-NPs (0.1 mg of FITC-OVA and 1.0 mg of γ-PGA NPs in 100 μl of PBS) were subcutaneously injected to mice. On day 3 after injection, the LNs were collected and stained with PE-conjugated anti-CD11c and allophycocyanin-conjugated anti-CD103 mAb (both from BD Bioscience) for 30 min at 4 °C. After washing with PBS, the cells were analyzed by flow cytometry.

2.4. Cross-presentation assay in vitro

Bone marrow (BM)-derived DCs (BMDCs) were prepared by culturing bone marrow cells in the presence of 20 ng/ml granulocyte/macrophage colony stimulating factor (PeproTech, Rocky Hill, NJ), as previously described [12]. BMDCs (1×10^6/ml) were stimulated with either PBS, OVA (10 μg/ml), OVA-NPs (10 μg/ml OVA and 100 μg/ml γ-PGA NPs), or OVA + lipopolysaccharide (LPS) (10 μg/ml OVA and 1 μg/ml LPS) for 24 h. The cells were harvested and washed with PBS. Antigen-specific CD8$^+$ T cells were obtained by the negative selection of splenic mononuclear cells in immunized mice with OVA-NPs using mAbs to Ly-76, B-220, Ly-6G, I-A/I-E, and CD4 (all from eBioscience) and anti-rat IgG antibody-conjugated immunomagnetic beads (Life Technologies, Grand Island, NY). The cells were found to be more than 90% pure, as determined by flow cytometry after staining with an FITC-conjugated anti-CD8 mAb (BD Biosciences). CD8$^+$ T cells and DCs were incubated in a flat-bottomed 96-well plate. After incubation for 4 days, the proliferation of CD8$^+$ T cells was determined by ^{3}H-thymidine incorporation.

2.5. Induction of antigen-specific CD8$^+$ T cells in vivo

The mice were anesthetized by an intraperitoneal injection of sodium pento-barbital and immunized with OVA and various adjuvants. Mice were immunized once with PBS, OVA alone (10 μg), OVA-NPs (100 μg of NPs carrying 10 μg of OVA), or OVA + aluminum (10 μg of OVA and 100 μg of aluminum) by a subcutaneous route. On day 14 after immunization, spleen cells were collected. A PE-labeled tetramer recognizing the immunodominant CD8$^+$ T-cell peptide (SIINFEKL) of OVA bound to the C57BL/6 MHC I allele H-2K^b (Medical & Biological Laboratories, Nagoya, Japan) was used for experiments. To detect OVA-specific CD8$^+$ T cells, spleen cells were stained with the tetramer and an FITC-conjugated anti-CD8 mAb (Beckman Coulter, Brea, CA). The cells were washed with PBS containing 0.1% sodium azide and 0.1% bovine serum albumin and fixed with PBS containing 2.5% formaldehyde (Wako, Osaka, Japan). The cell-associated fluorescence was measured by flow cytometry. To examine the kinetics of OVA-specific CD8$^+$ T cells, mice were immunized once with OVA-NPs (100 μg of NPs carrying 10 μg of OVA) by a subcutaneous route. On days 1, 3, 5, 7, 10, and 15 after immunization, spleen cells were collected, stained, and analyzed by flow cytometry.

2.6. Statistical analysis

Data were analyzed for their statistical significance by the Student's *t*-test.

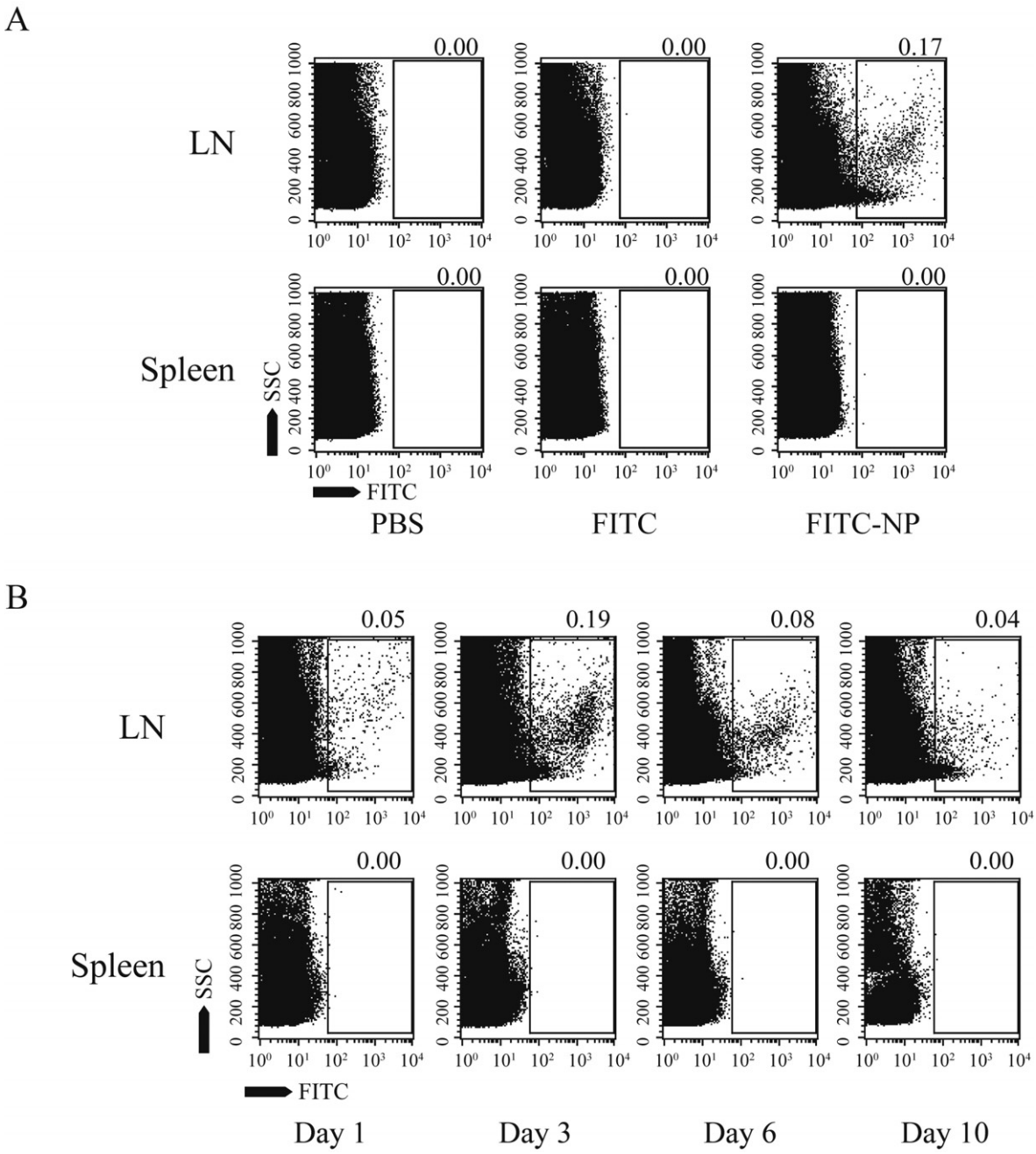

Fig. 1. Uptake of FITC-NPs by APCs in vivo. (A) Mice (n=3) were subcutaneously injected with FITC-NPs. After 3 days, cells from the regional LNs or spleen were collected and analyzed by flow cytometry. Mice injected with PBS or FITC only were used as controls. Each number represents the percentage of FITC$^+$ cells. (B) Mice (n=3) were injected subcutaneously with FITC-NPs. Cells from the LNs or spleen were collected at the indicated time points and analyzed by flow cytometry. Each number represents the percentage of FITC$^+$ cells. A representative result of three separate experiments is shown.

3. Results

3.1. Uptake of γ-PGA NPs in vivo

To examine the uptake of FITC-NPs by APCs in vivo, the regional LNs and spleen were collected from mice on day 3 after subcutaneous injection of PBS, FITC, or FITC-NPs. It is clear that the FITC$^+$ cells were observed in the LNs obtained from the mice injected with FITC-NPs (Fig. 1A). In contrast, no FITC$^+$ cells were detected in the LNs obtained from the mice injected with PBS or FITC. Furthermore, FITC$^+$ cells were not observed in the spleen obtained from the mice injected with PBS, FITC, or FITC-NPs. To examine the kinetics of FITC-NPs, mice were subcutaneously injected with FITC-NPs and the LNs and spleen were collected at various time points. FITC$^+$ cells could be found in the LNs on day 1 after injection of FITC-NPs (Fig. 1B). The strongest signal of FITC$^+$ cells was detected in the LNs on day 3. Again, FITC$^+$ cells were not observed in the spleen at any time points. These results suggest that γ-PGA NPs are efficiently taken up by APCs and transported to the regional LNs.

3.2. Uptake of antigen-carrying γ-PGA NPs in vivo

Targeting antigens to APCs is assumed to be an effective strategy for potent induction of antigen-specific effector T cells in vaccine development [7,8]. Therefore, the uptake of antigen-carrying γ-PGA

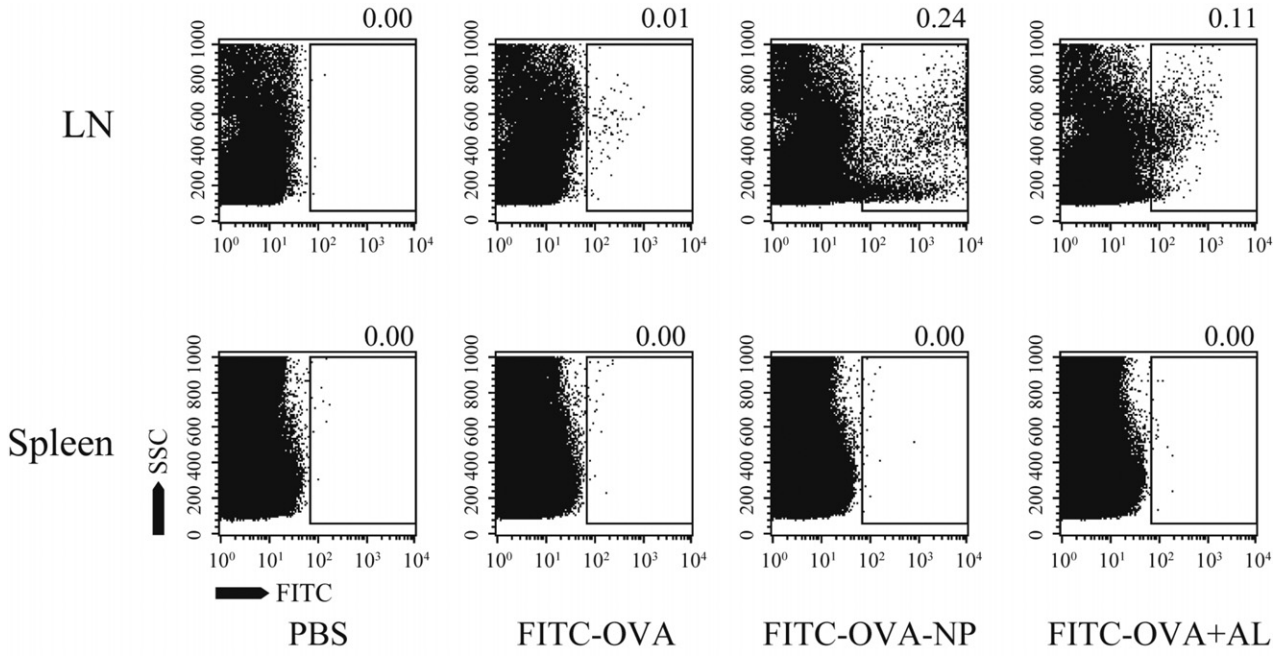

Fig. 2. Uptake of various forms of FITC-OVA by APCs in vivo. Mice (n=3) were injected subcutaneously with FITC-OVA, FITC-OVA-NPs, or FITC-OVA + aluminum (AL). After 3 days, cells from the regional LNs or spleen were collected and analyzed by flow cytometry. Mice injected with PBS were used as a control. Each number represents the percentage of $FITC^+$ cells. A representative result of three separate experiments is shown.

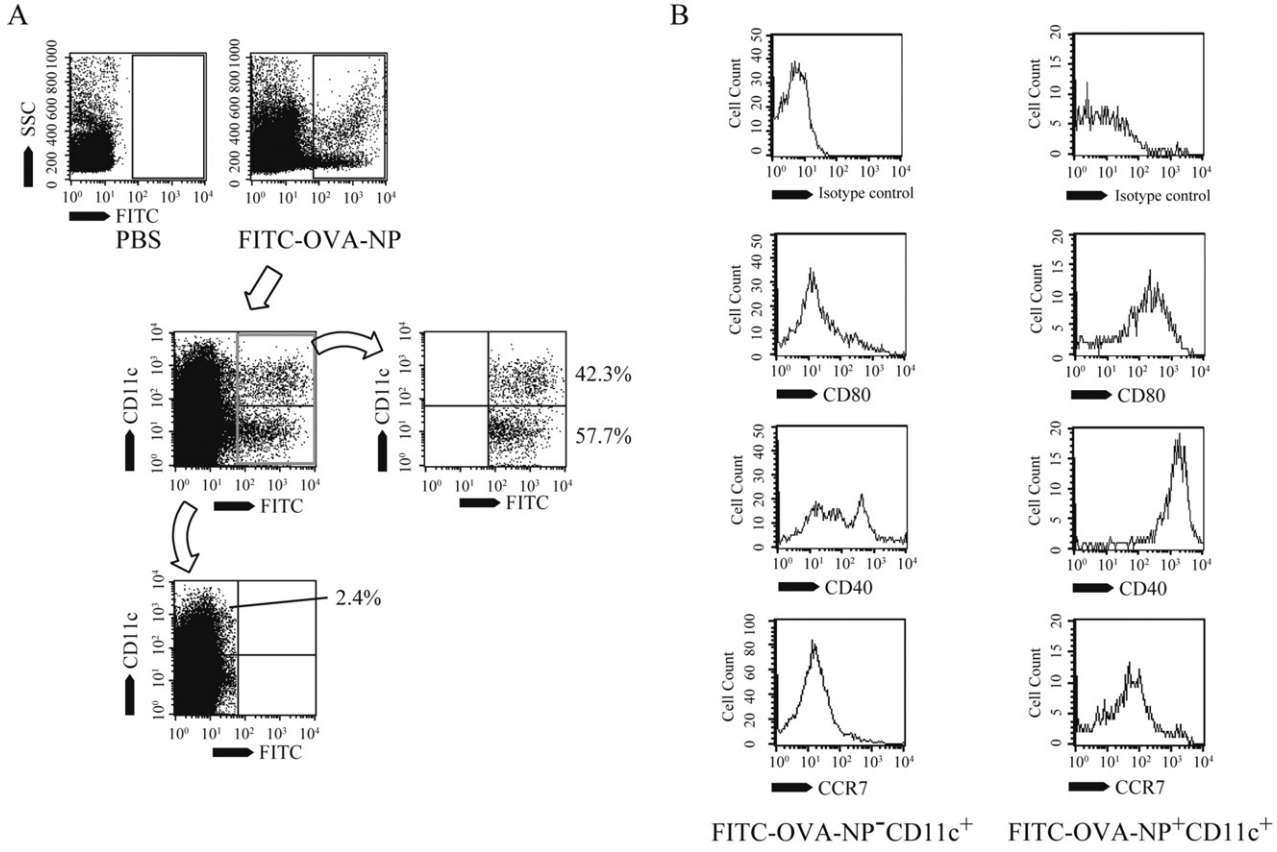

Fig. 3. Maturation of DCs by antigen-carrying γ-PGA NPs in vivo. (A) Mice (n=3) were subcutaneously injected with FITC-OVA-NPs. After 3 days, cells from the regional LNs were isolated, stained with the anti-CD11c mAb, and analyzed by flow cytometry. Mice injected with PBS were used as a control. Each number represents the percentage of cells in the respective gates. (B) FITC-OVA-NP^-CD11c^+ and FITC-OVA-NP^+CD11c^+ cells were further analyzed by the expression of CD80, CD40, CD86, and CCR7.

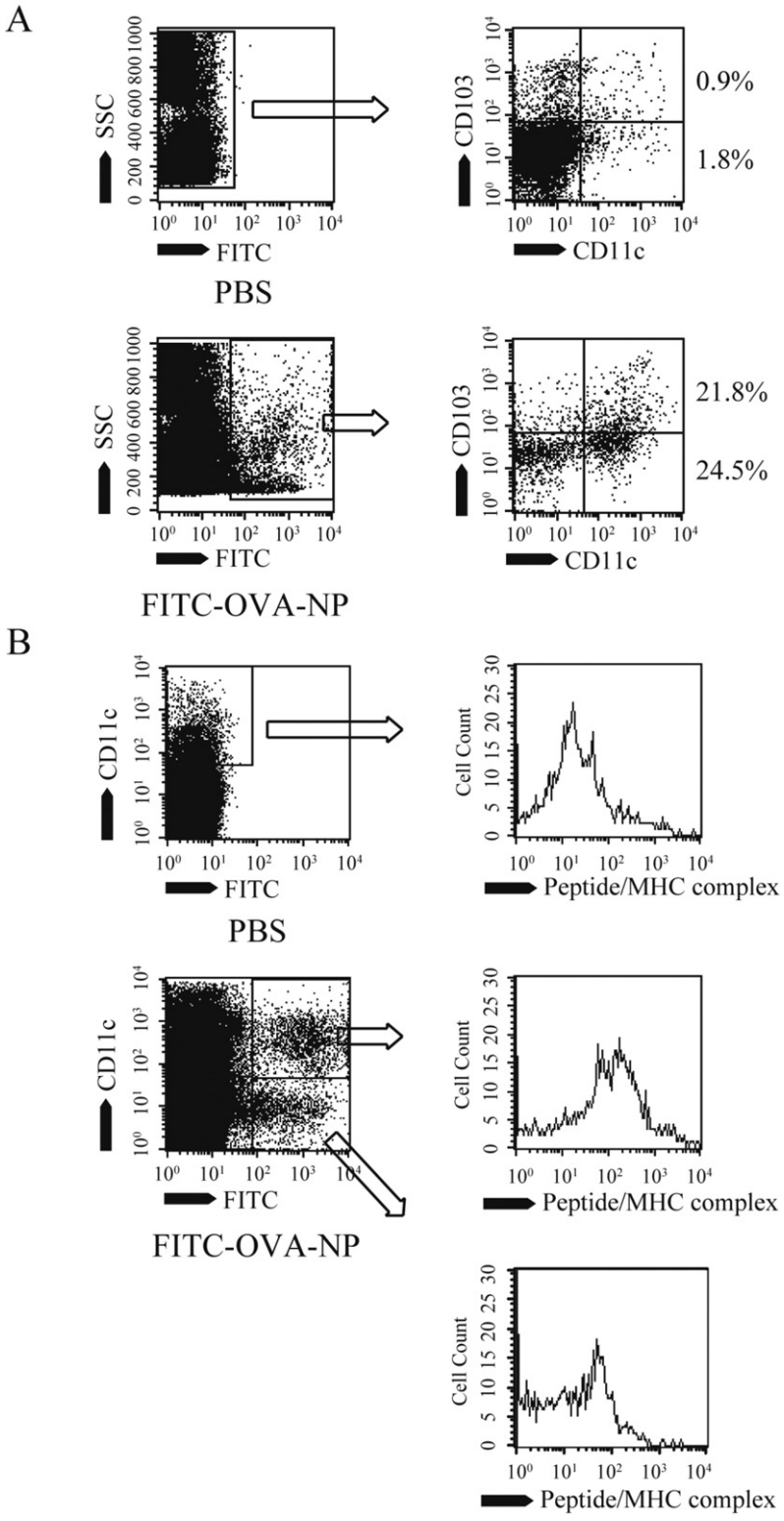

Fig. 4. Presentation of NP-associated antigens by skin-derived DCs in vivo. (A) Mice (n=3) were subcutaneously injected with FITC-OVA-NPs. After 3 days, cells from the regional LNs were isolated, stained with the anti-CD11c and anti-CD103 mAbs, and analyzed by flow cytometry. Mice injected with PBS were used as a control. Each number represents the percentage of cells in the respective gates. (B) FITC-OVA-NPs were injected, as described above. Cells from the LNs were isolated, stained with the anti-CD11c and anti-H-2K^b/SIINFEKL complex mAbs, and analyzed by flow cytometry. Mice injected with PBS were used as a control.

NPs by APCs was investigated in vivo. The regional LNs and spleen were collected from mice on day 3 after subcutaneous injection of PBS, FITC-OVA, FITC-OVA-NPs, or FITC-OVA + aluminum. The NP-associated form of FITC-OVA was taken up by APCs much more efficiently than FITC-OVA alone or FITC-OVA + aluminum (Fig. 2). Again, FITC$^+$ cells were not observed in the spleen.

3.3. Maturation of DCs by antigen-carrying γ-PGA NPs in vivo

To analyze the uptake of antigen-carrying γ-PGA NPs by APCs in vivo, the regional LNs were collected from mice on day 3 after subcutaneous injection of PBS or FITC-OVA-NPs. Approximately 42% of the FITC-NP$^+$ cells were CD11c$^+$ (Fig. 3A). In contrast, only 2.4% of FITC-NP$^-$ cells were CD11c$^+$. Since the delivery of antigens into DCs followed by their maturation is a critical step for initiating the adaptive immunity, the LN cells were examined for the expression of CD80, CD40, CD86, and CCR7. As shown in Fig. 3B, the expression was highly upregulated in FITC-NP$^+$CD11c$^+$ cells, as compared with FITC-NP$^-$CD11c$^+$ cells.

3.4. Presentation of NP-associated antigens by skin-derived DCs in vivo

It has recently been shown that skin-derived CD103$^+$ DCs present antigens to CD8$^+$ T cells through the cross-presentation pathway in vivo [20,21]. Therefore, FITC-OVA-NP$^+$ cells were examined whether they were CD103$^+$ DCs. The regional LNs were collected from mice on day 3 after subcutaneous injection of PBS (control) or FITC-OVA-NPs and stained with the anti-CD11c and anti-CD103 mAbs. Among FITC-OVA-NP$^+$ cells, 21.8% of the cells were CD103$^+$CD11c$^+$ (Fig. 4A) in NP-injected mice. Only 0.9% of the FITC-OVA-NP$^+$ cells were CD103$^+$CD11c$^+$ in PBS-injected mice, which was considered to be the background. To determine whether the antigen was processed and presented by DCs, FITC-OVA-NP$^+$CD11c$^+$ DCs were stained with the mAb recognizing the antigen peptide/MHC class I complex. The expression of the complex was much higher in FITC-OVA-NP$^+$CD11c$^+$ cells than in FITC-OVA-NP$^+$CD11c$^-$ cells (Fig. 4B), suggesting that the antigen peptides of γ-PGA NPs can be presented by the MHC class I molecule of DCs (cross-presentation).

3.5. Activation of antigen-specific CD8$^+$ T cells by antigen-carrying γ-PGA NPs in vitro

DCs were treated with antigen-carrying γ-PGA NPs and examined for their ability to induce antigen-specific CD8$^+$ T cell responses in vitro. BMDCs were incubated with PBS, OVA, OVA-NPs, or OVA + LPS and cocultured with OVA-specific CD8$^+$ T cells. As shown in Fig. 5, although BMDCs treated with OVA-NPs or OVA + LPS were able to induce the proliferation of OVA-specific CD8$^+$ T cells in a dose-dependent fashion, BMDCs treated with OVA-NPs were a more potent inducer of OVA-specific CD8$^+$ T cell proliferation than those treated with OVA + LPS. In contrast, BMDCs treated with PBS or OVA alone hardly induced the proliferation of OVA-specific CD8$^+$ T cells.

3.6. Activation of antigen-specific CD8$^+$ T cells by antigen-carrying γ-PGA NPs in vivo

To confirm the activation of antigen-specific CD8$^+$ T cells by antigen-carrying γ-PGA NPs in vivo, mice were subcutaneously immunized once with PBS, OVA, OVA-NPs, or OVA + aluminum. The number of OVA-specific CD8$^+$ T cells was determined by the tetramer-staining assay on day 15 after immunization. The mice immunized with OVA-NPs displayed significant increase of OVA-specific CD8$^+$ T cells, (Fig. 6A). However, such CD8$^+$ T cells were hardly detected in mice immunized with PBS, OVA, or OVA + aluminum. To examine the kinetics of OVA-specific CD8$^+$ T cells, the cells were measured at various time points after immunization. OVA-specific CD8$^+$ T cells could be identified on day 7 and did reach the peak level on day 10 (Fig. 6B). These results indicate that the immunization with antigen-carrying γ-PGA NPs strongly activate antigen-specific CD8$^+$ T cells not only in vitro but also in vivo.

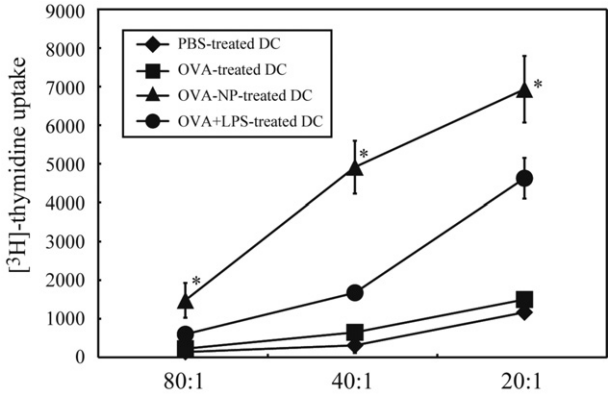

Fig. 5. Activation of antigen-specific CD8$^+$ T cells by antigen-carrying γ-PGA NPs in vitro. BMDCs were cultured with PBS, OVA, OVA-NPs, or OVA + LPS. After 24h, cells were collected and cocultured with autogenic OVA-specific CD8$^+$ T cells for 4 days. Proliferative response was measured by ^{3}H-thymidine uptake. Data are expressed as means ± SD. Statistical analysis was carried out between the OVA-NP-treated DC group and other groups (*p <0.05).

4. Discussion

Although particulates are widely used as antigen carriers, their adjuvant mechanisms in vivo are poorly understood. In this study, we provide the evidence that APCs migrate from the injection site to the regional LNs after the uptake of γ-PGA NPs in vivo. The uptake and presentation of antigens by APCs are essential for the induction of antigen-specific immune responses [22]. In a steady state, APCs circulate through peripheral tissues, where they capture antigens and migrate to the secondary lymphoid organs [23]. After injection with γ-PGA NPs (both FITC-NPs and FITC-OVA-NPs), APCs migrated to the regional LNs from the skin in vivo (Figs. 1 and 2). In addition, NP-associated antigens (FITC-OVA-NPs) could enhance the antigen uptake by APCs than antigens alone in vivo (Fig. 2). Although γ-PGA NPs appeared to be taken up by both CD11c$^+$ cells (DCs) and CD11c$^-$ cells (presumably, macrophages and B cells), a considerable number (approximately 40%) of CD11c$^+$ cells was found to capture γ-PGA NPs (Fig. 3A). Thus, γ-PGA NPs have great potential as an effective antigen carrier that mainly targets DCs in vivo.

Antigen uptake by DCs and subsequent their maturation is a key step for initiating and regulating the adaptive immunity [7,10,20–23]. The interaction between antigen components and DCs appears to trigger off the maturation of DCs and enhance their antigen-presenting capacity, costimulatory molecule expression, and cytokine production. Our previous studies showed that γ-PGA NPs were efficiently taken up by DCs and did induce their maturation through the TLR4 and MyD88 signaling pathway in vitro [12,15,24,25]. However, the evidence of DC maturation after uptake of γ-PGA NPs and migration from the skin to the regional LNs in vivo has not been demonstrated yet. In this study, the uptake of γ-PGA NPs by DCs resulted in their enhanced expression of costimulatory molecules in vivo. In addition, a high level of CCR7 expression was observed in DCs capturing γ-PGA NPs in vivo (Figs. 2B and 4B). CCR7 plays important roles in DC migration to the LNs and the induction of maturation signals [23].

Mature DCs move into the regional LNs and present the processed antigens with the MHC class I molecule through the antigen cross-presentation pathway [7,10,21]. During this process, cytosolic proteins are hydrolyzed by the proteasome complex to yield peptides that are translocated into the endoplasmic reticulum through

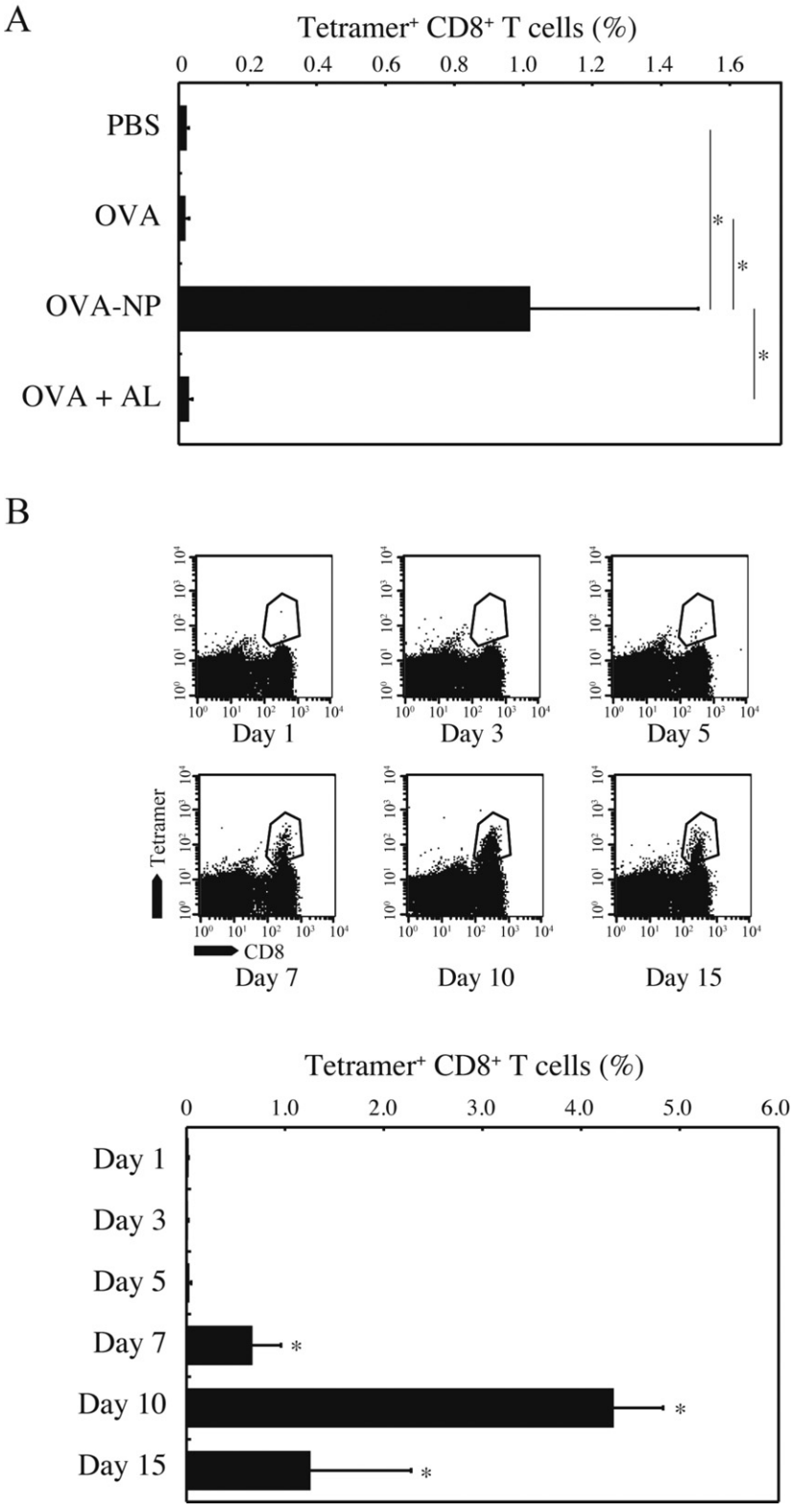

Fig. 6. Activation of antigen-specific $CD8^+$ T cells by antigen-carrying γ-PGA NPs in vivo. (A) Mice were subcutaneously injected once with PBS, OVA, OVA-NPs, or OVA + AL (aluminum). Spleen cells were isolated on day 15 after immunization. The cells were stained with the anti-CD8 mAb and H-$2K^b$/$OVA_{257–264}$ tetramer. Data are expressed as the percentages of pentamer-binding $CD8^+$ T cells among the gated $CD8^+$ T cells, as determined by flow cytometry. The experiments were performed for three mice per each group, and data represent means ± SD in each group. Statistical analysis was carried out among the 4 groups (*$p < 0.05$). (B) Mice were subcutaneously injected once with OVA-NPs. Spleen cells were isolated at the indicated time points. OVA-specific $CD8^+$ T cells were analyzed, as described above. The experiments were performed for three mice per each group, and data represent means ± SD in each group. Statistical analysis was carried out between the day 1 group and other groups (*$p < 0.05$).

a transporter associated with antigen presentation. In the endoplasmic reticulum, the peptides bind to the MHC class I molecule and are transported to the cell surface for recognition by $CD8^+$ T cells [26]. In fact, it has been shown that γ-PGA NPs release carrying antigens to the cytoplasm of DCs [27]. DCs migrating from the skin could present OVA-derived peptides by the MHC class I molecule after subcutaneous injection of FITC-OVA-NPs (Fig. 4B). Furthermore, BMDCs treated with OVA-NPs stimulated OVA-specific $CD8^+$ T cells in vitro (Fig. 5). Skin-derived $CD103^+$ DCs are considered to be essential for the cross-presentation of antigens to $CD8^+$ T cells [20,21]. Therefore, the uptake of antigens by $CD103^+$ DCs is important for cognate T cells to differentiate into antigen-specific $CD8^+$ T cells. $CD103^+$ DCs capturing γ-PGA NPs were found in the regional LNs after subcutaneous injection with γ-PGA NPs (Fig. 4A). Taken together, antigen-carrying NPs are taken up by $CD103^+$ DCs and present NP-associated antigens to $CD8^+$ T cells in vivo.

Activation of the cytolytic immune response that clears infected host cells is a key area of research for improving vaccines [1]. The adaptive cytolytic response is primarily mediated by $CD8^+$ T cells [28,29]. $CD8^+$ T cells can fulfill direct destruction of infected cells via recognition of pathogen-derived antigens presented in the context of MHC class I. The generation of antigen-specific $CD8^+$ T cells is most likely mediated by cross-presenting DCs [30,31]. Our previous studies showed that antigen-specific $CD8^+$ T cells induced by antigen-carrying γ-PGA NPs differentiated to the memory phenotype and remained stable for a long period of time [13,15]. Although one of the important aspects in vaccine development is rapid induction of antigen-specific $CD8^+$ T cells, the kinetics of antigen-specific $CD8^+$ T cells generated by immunization with γ-PGA NPs remains to be elucidated. In this study, a significant level of OVA-specific $CD8^+$ T cells was detected on day 7 after single immunization with OVA-NPs. These findings suggest that antigen-carrying NPs can induce rapidly the antigen-specific effector and long-lived memory $CD8^+$ T cells in vivo.

Most vaccines developed so far are live-attenuated, inactivated, and subunit ones [15]. Live-attenuated vaccines have an advantage of inducing both humoral and cellular immune responses. However, live-attenuated vaccines can still infect and replicate in the hosts, thereby their safety is difficult to control. Inactivated and subunit vaccines induce only humoral immunity and require multiple doses. Especially, subunit vaccines are poorly immunogenic and require a booster vaccination every few years. Therefore, a number of DNA vaccines capable of inducing humoral and cellular immune responses are currently under investigation [32]. In addition, a lot of attempts have been made to develop effective vaccine adjuvants [2,33]. In this regard, γ-PGA NPs are capable of inducing both humoral and cellular immune responses [12–19]. In addition, various proteins, peptides, and chemicals can be encapsulated into the particles and/or immobilized onto their surfaces [14]. The injection of antigens in combination with γ-PGA NPs has been shown to enhance not only antigen uptake but also DC maturation in vivo. Thus, it is possible that combinations of other immunization techniques such as DNA vaccines with γ-PGA NPs are more effective for inducing antigen-specific humoral and cellular immune responses.

5. Conclusion

The present results clearly show that DCs capturing γ-PGA NPs migrate into the regional LNs from their injection site. In addition, antigen-carrying γ-PGA NPs promote the maturation of DCs and induce cross-presentation of antigens by the MHC class I molecule of DCs. Furthermore, single immunization with antigen-carrying γ-PGA NPs induces rapid activation of antigen-specific $CD8^+$ T cells. Therefore, γ-PGA NPs can effectively induce the adaptive immune responses through the DC-mediated immune system in vivo and may have great potential as an antigen-delivery system and vaccine adjuvant.

Acknowledgements

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