

Supplementary Materials: Transcutaneous Administration of Imiquimod Promotes T and B Cell Differentiation into Effector Cells or Plasma Cells

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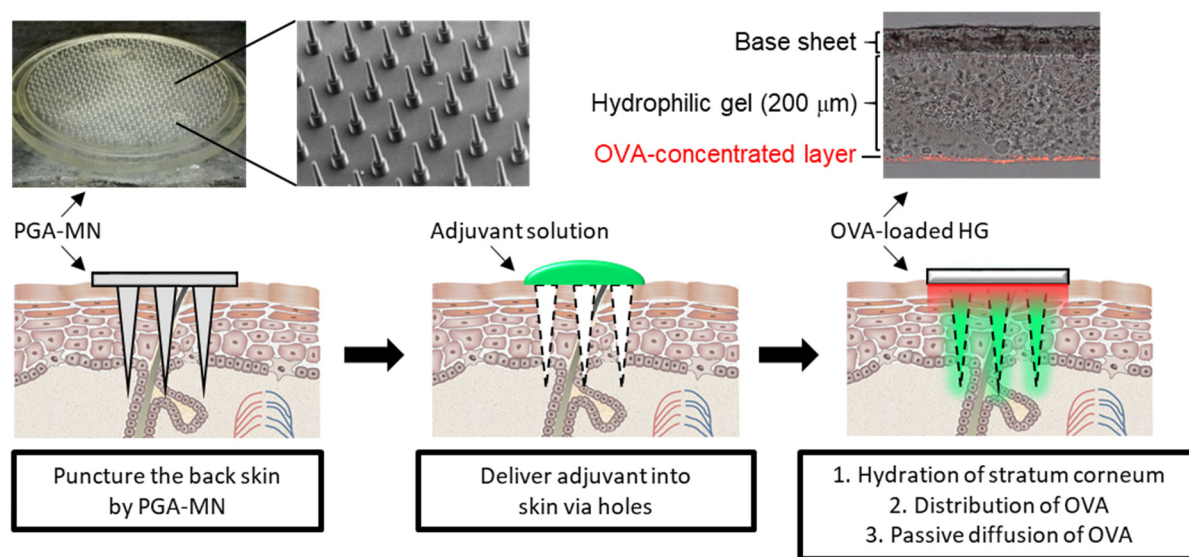
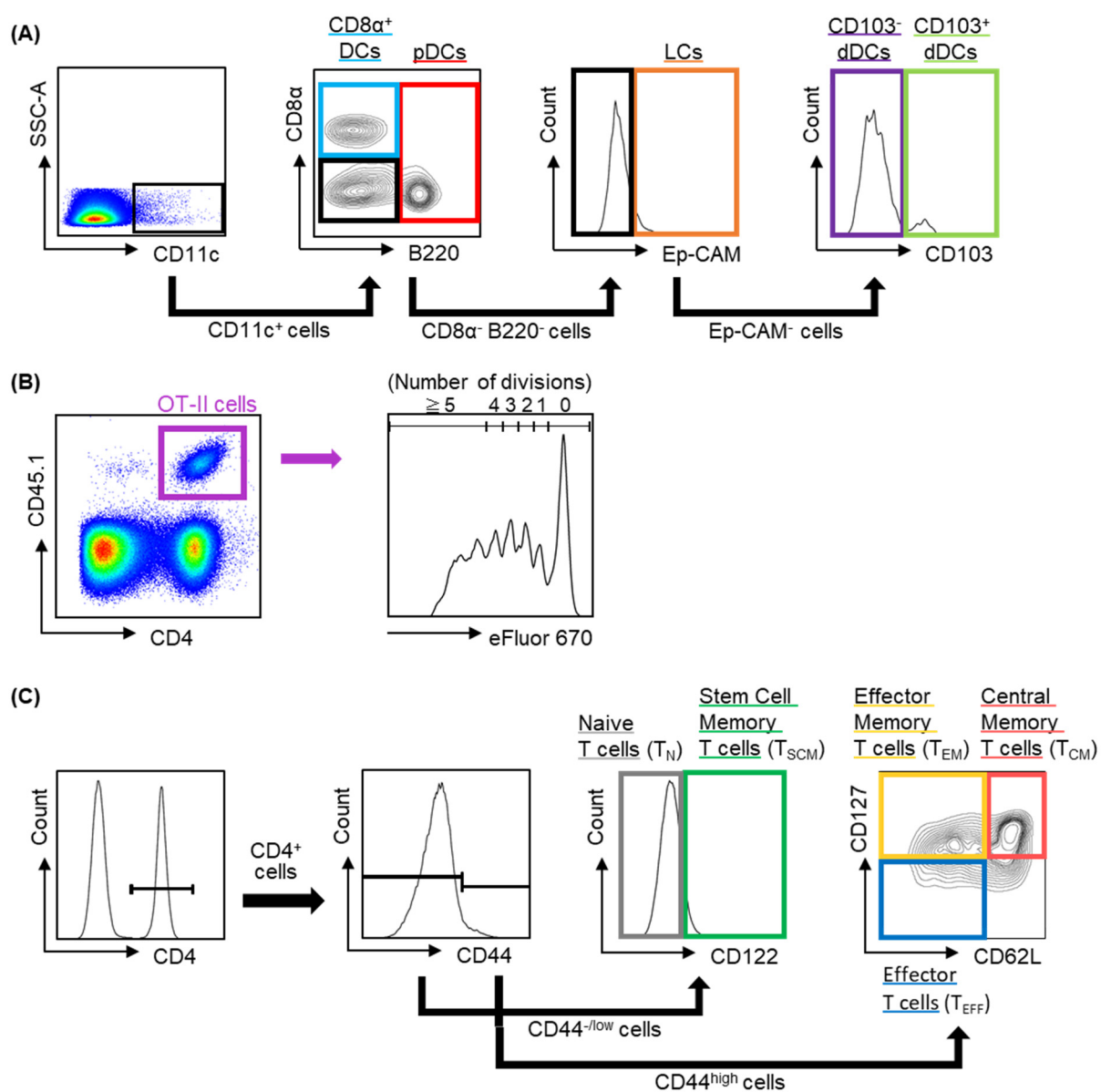


Figure S1. Poke and patch method.

The PGA-MN patch was applied to the epilated back skin of each mouse. Then, the adjuvant solution was dropped into the holes formed using PGA-MNs, and OVA-loaded HG was applied to the holes.



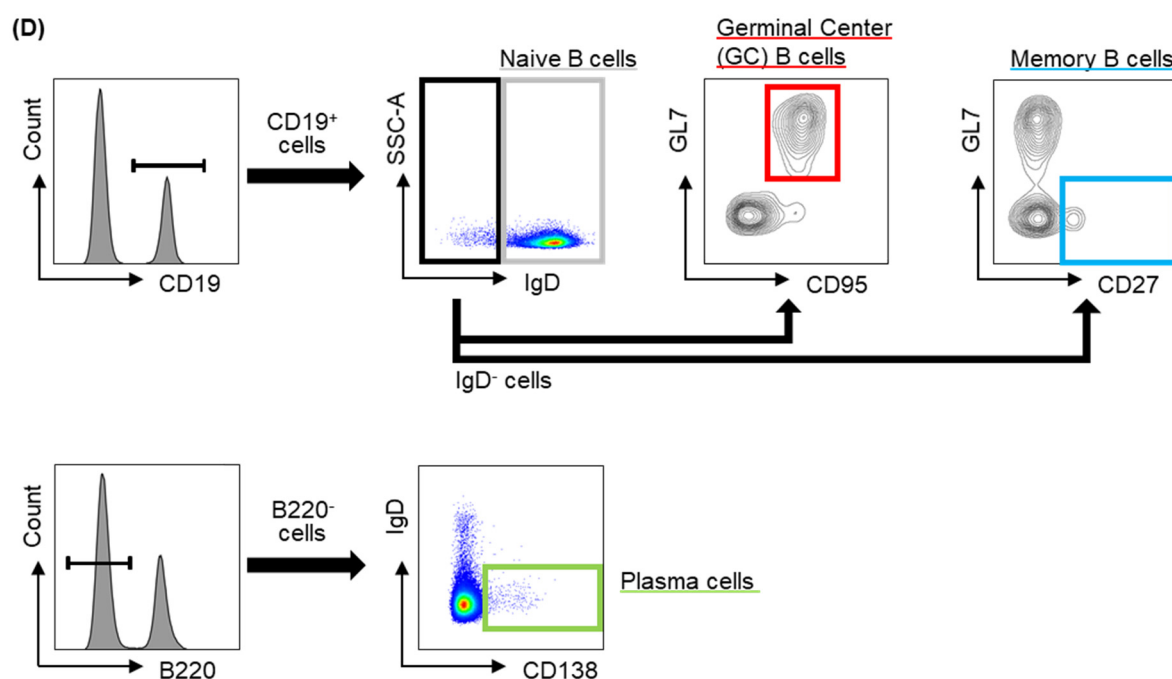


Figure S2. Gating and analyzing strategy using flow cytometry.

(A) CD11c⁺ cells were divided into five subsets: CD8α⁺ DCs (CD8α⁺, B220⁻), pDCs (B220⁺), LCs (CD8α⁻, B220⁻, Ep-CAM⁺), CD103⁻ dDCs (CD8α⁻, B220⁻, Ep-CAM⁻, CD103⁻), and CD103⁺ dDCs (CD8α⁻, B220⁻, Ep-CAM⁻, CD103⁺). (B) C57BL/6 mice (CD45.2⁺) were administered eFluor 670-labeled OT-II cells (CD4⁺, CD45.1⁺). On the next day, the mice were immunized with OVA. Three days later, the proliferation of the transferred OT-II cells (CD4⁺, CD45.1⁺) in the dLNs was analyzed by FCM. The number of divisions was determined based on the fluorescence intensity of eFluor 670, and the number of OT-II cells is shown for each number of divisions. (C) CD4⁺ T cells were divided into five subsets: T_N cells (CD44^{low}, CD122⁻), T_{SCM} cells (CD44^{low}, CD122⁺), T_{CM} cells (CD44^{high}, CD127⁺, CD62L^{high}), T_{EM} cells (CD44^{high}, CD127⁺, CD62L^{low}), and T_{EFF} cells (CD44^{high}, CD127⁻, CD62L^{low}). (D) B cells were divided into four subsets: naive B cells (CD19⁺, IgD⁺), GC B cells (CD19⁺, IgD⁻, GL7⁺, CD95⁺), memory B cells (CD19⁺, IgD⁻, GL7⁻, CD27⁺), and plasma cells (B220⁻, IgD⁻, CD138⁺).

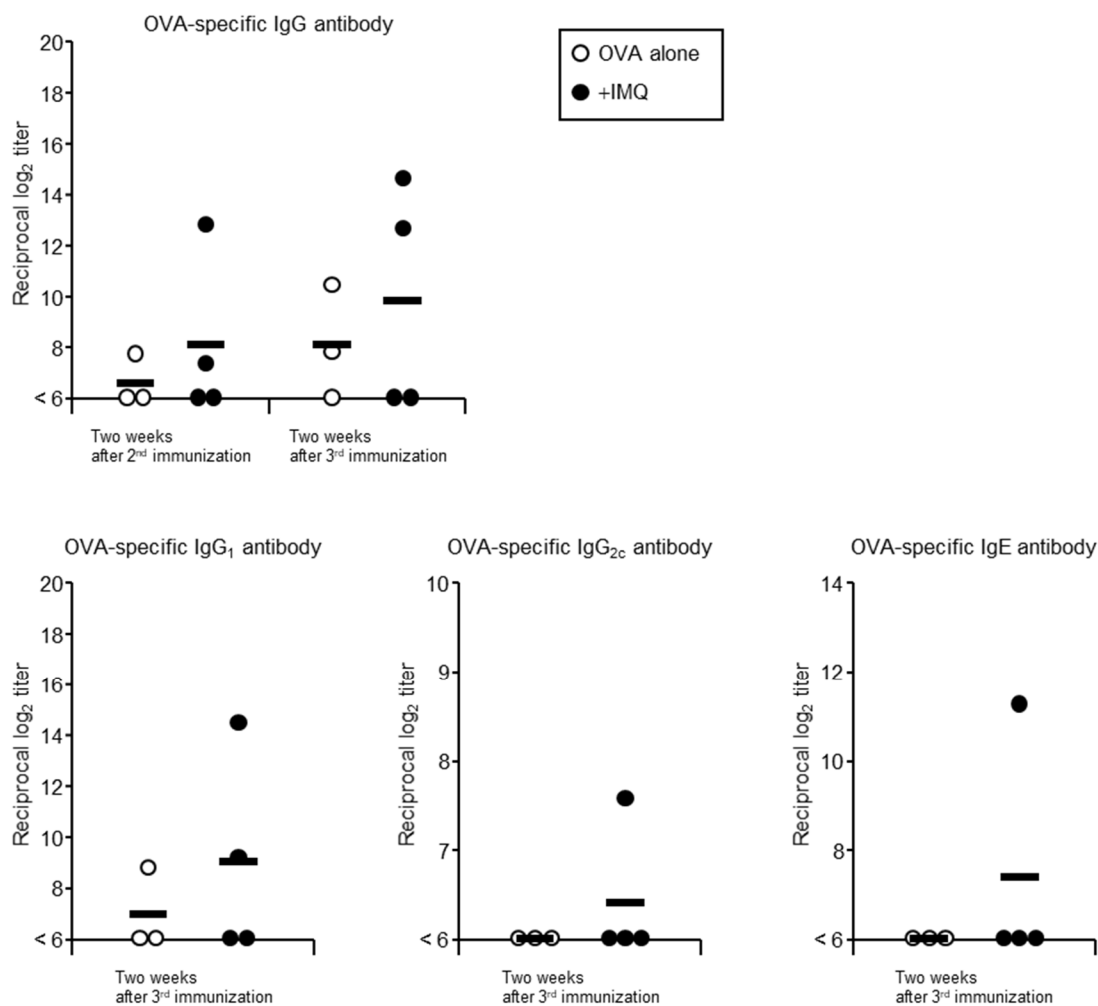


Figure S3. OVA-specific antibody production using HG loaded with OVA and IMQ.

HG loaded with OVA (50 µg) alone or with OVA and IMQ (50 µg) were applied to C57BL/6 mice three times every 2 weeks. Sera collected every 2 weeks were assayed to determine OVA-specific total IgG titers, IgG subclass titers, and IgE titers by ELISA analysis.

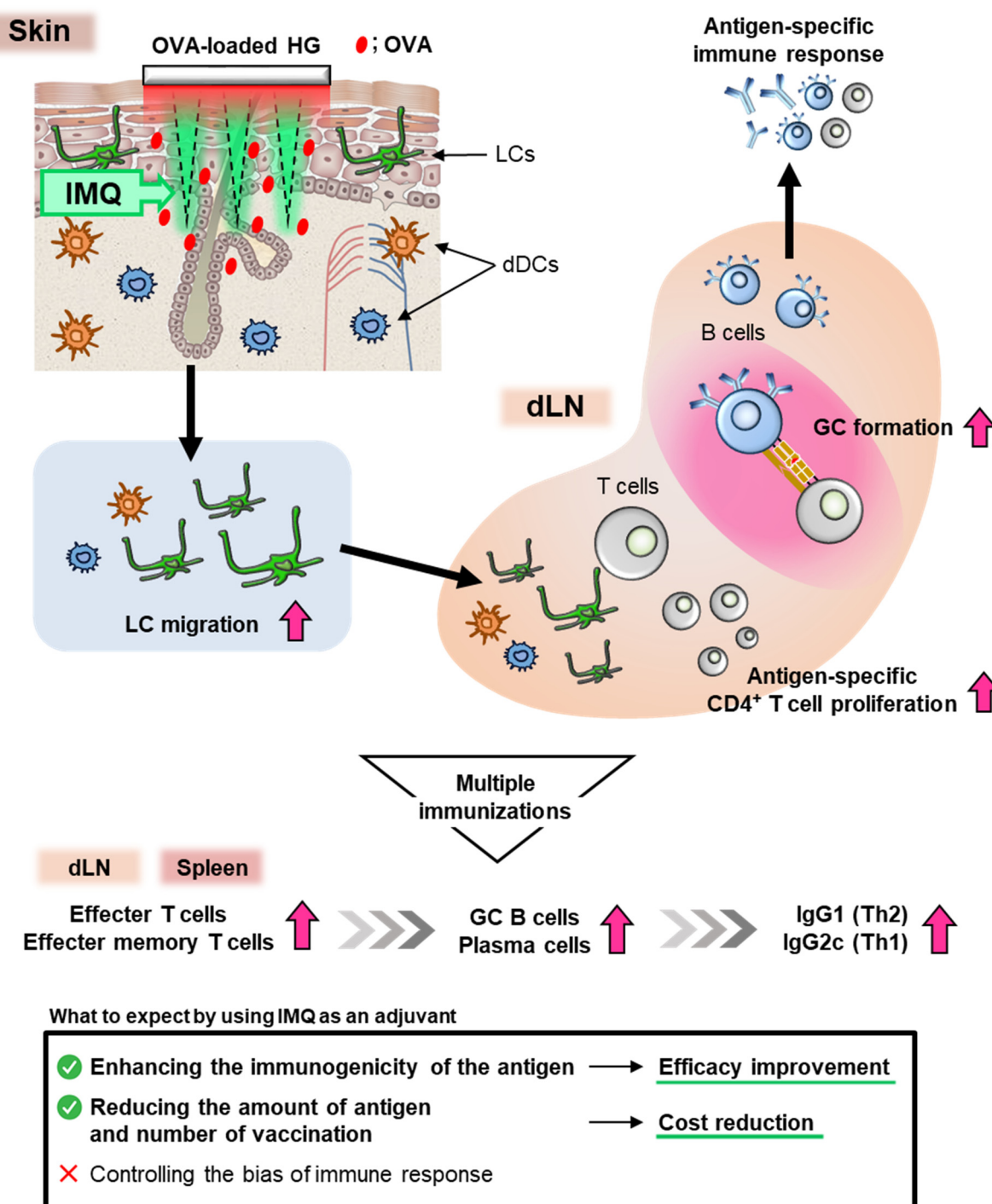


Figure S4. Mechanism of the adjuvant activity of IMQ after transcutaneous vaccination.

Transcutaneously administered IMQ enhances antigen-specific primary immune responses by promoting LC migration, antigen-specific CD4⁺ T cell proliferation, and GC formation. Furthermore, when performing multiple immunizations, IMQ systemically increased the abundance of TEM cells to enhance TEFF cell responses, promote GC responses, and trigger differentiation into plasma cells. However, IMQ induces IgG1 antibodies (Th2 type IgG subclass) and IgG2c antibodies (Th1 type IgG subclass) in parallel. Accordingly, by using IMQ as an adjuvant in combination with transcutaneous vaccination, we can expect efficacy improvement and cost reduction, but not versatility expansion.