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REFERENCES

1. Tsai FY, Orkin SH. Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. *Blood* 1997;89:3636-43.
2. Tsai FY, Keller G, Kuo FC, Weiss M, Chen J, Rosenblatt M, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature* 1994;371:221-6.
3. Harigae H, Takahashi S, Suwabe N, Ohtsu H, Gu L, Yang Z, et al. Differential roles of GATA-1 and GATA-2 in growth and differentiation of mast cells. *Genes Cells* 1998;3:39-50.
4. Walsh JC, DeKoter RP, Lee HJ, Smith ED, Lancki DW, Gurish MF, et al. Cooperative and antagonistic interplay between PU.1 and GATA-2 in the specification of myeloid cell fates. *Immunity* 2002;17:665-76.
5. Babina M, Schulke Y, Kirchhof L, Gohl S, Franke R, Bohm S, et al. The transcription factor profile of human mast cells in comparison with monocytes and granulocytes. *Cell Mol Life Sci* 2005;62:214-26.
6. Ohmori S, Moriguchi T, Noguchi Y, Ikeda M, Kobayashi K, Tomaru N, et al. GATA2 is critical for the maintenance of cellular identity in differentiated mast cells derived from mouse bone marrow. *Blood* 2015;125:3306-15.
7. Inage E, Kasakura K, Yashiro T, Suzuki R, Baba Y, Nakano N, et al. Critical Roles for PU.1, GATA1, and GATA2 in the expression of human FcεRI on mast cells: PU.1 and GATA1 transactivate FCER1A, and GATA2 transactivates FCER1A and MS4A2. *J Immunol* 2014;192:3936-46.
8. Ishijima Y, Ohmori S, Uenishi A, Ohneda K. GATA transcription factors are involved in IgE-dependent mast cell degranulation by enhancing the expression of phospholipase C-gamma1. *Genes Cells* 2012;17:285-301.

9. Li Y, Liu B, Harmacek L, Long Z, Liang J, Lukin K, et al. The transcription factors GATA2 and microphthalmia-associated transcription factor regulate Hdc gene expression in mast cells and are required for IgE/mast cell-mediated anaphylaxis. *J Allergy Clin Immunol* 2018;142:1173-84.

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Murine neonatal skin mast cells are phenotypically immature and minimally sensitized with transplacentally transferred IgE



To the Editor:

IgE binds to mast cells (MCs) through the high-affinity IgE receptor (FcεRI) on their surfaces and mediates type I hypersensitivity by inducing degranulation on recognition of its cognate antigen. It is generally considered that IgE does not pass through the placenta, unlike IgG, which is transplacentally transferred from mothers to fetuses through the neonatal Fc receptors (FcRn).^{1,2} However, subtle amounts of IgE are detected in umbilical cord blood in human subjects,³ suggesting the possibility of transplacental transfer of IgE.

IgE in umbilical cord blood is detected mostly in the form of immune complexes (ICs) with anti-IgE IgG autoantibodies,⁴ and ICs can bind to FcRn; therefore we hypothesized that transplacental IgE transfer can occur in the form of ICs. In fact, an *in vitro* transwell assay using a kidney cell line transfected with human FcRn showed transcytosis of IgE in the form of ICs.⁴ However, there has been no study to show transplacental IgE transfer *in vivo*. Furthermore, it is completely unknown whether transplacentally transferred IgE can distribute to peripheral tissues, such as the skin, and exert pathogenic functions in neonates. Here we aimed to elucidate (1) whether maternal IgE is transplacentally transferred in the form of ICs *in vivo* and (2) whether the transferred IgE distributes to the peripheral tissues in neonates and induces degranulation of MCs, focusing on the skin.

First, to investigate whether maternal IgE passes through the placenta, we injected anti-2,4,6-trinitrophenyl (TNP) IgEa, an allotype of murine IgE that does not exist in C57BL/6 mice, into pregnant C57BL/6 mice intravenously in either the monomeric form or the IC form on pregnancy day 17. Twenty-four hours later, we analyzed serum levels of anti-TNP IgEa in fetuses using an ELISA (Fig 1, A). In the mothers' sera, 24 hours after administration, levels of IC IgEa were substantially lower than those of the monomeric IgEa (see Fig E1 in this article's Online Repository at www.jacionline.org), but in fetuses, subtle amounts of IgEa were detected only in fetuses whose mothers were injected with ICs (Fig 1, B). These results indicate that maternal IgE passes through the placenta mainly in the form of ICs.

Next, we examined whether circulating IgE in the form of ICs can be distributed to the skin and bind to dermal MCs in neonates. First, we intraperitoneally administered anti-TNP IgEa in the form of ICs to postnatal day 0 (P0) mice. On the next day, we checked for the existence of IgE and anti-IgE IgG (goat IgG) on MCs in the skin by using flow cytometry. We detected surface binding of IgE and anti-IgE IgG (goat IgG) on MCs, but only approximately 7% of MCs were positive for IgE (and anti-IgE IgG; Fig 1, C). Consistent with this result,

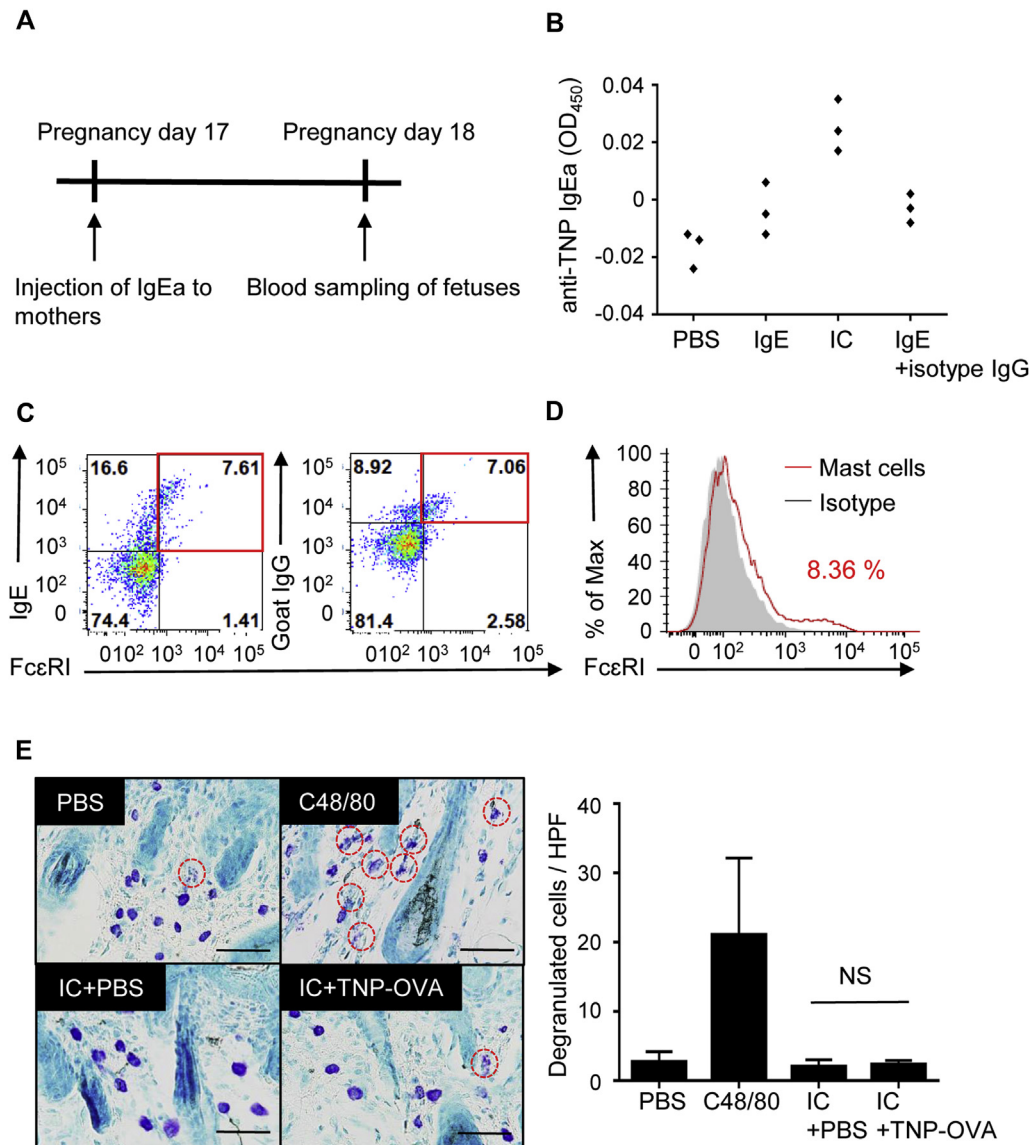


FIG 1. Maternal transfer of IgE and its ability to bind to MCs in neonatal skin. **A**, Experimental scheme. **B**, Serum levels of anti-TNP IgEa in fetuses ($n = 3$). **C**, Identification of IgE and goat anti-IgG on FcεRI on MCs using flow cytometric analysis. **D**, FcεRI expression on MCs in neonatal skin. **E**, Toluidine blue staining of fetal back skin treated with PBS, C48/80, ICs plus PBS, and ICs plus TNP-OVA. Degranulated MCs are indicated by red dotted circles. Scale bar = 50 μ m. Numbers of degranulated MCs per high-power field (HPF, $\times 40$ magnification; right panel; $n = 3$). P values are from the Student t test. NS, Not significant. Data are representative of 3 independent experiments.

cutaneous MCs in neonates exhibited low expression of FcεRI (Fig 1, D). Furthermore, we histologically evaluated degranulation of MCs in the neonatal skin after antigen challenge. Anti-TNP IgEa in the form of ICs was administered intraperitoneally to neonatal (P0) mice, and the next day, TNP-ovalbumin (OVA) was intradermally injected into the skin. However, MC degranulation was minimally induced, even when monomeric IgE was administered instead of ICs (Fig 1, E, and see Fig E2 in this article's Online Repository at www.jacionline.org). On the other hand, significant degranulation was observed in neonatal skin injected with Compound 48/80 (C48/80), an MC activator, suggesting that the degranulation ability of MCs was not impaired in neonatal

skin. To test whether anti-TNP IgEa in both the monomeric and the IC forms has the ability to induce degranulation, we performed similar experiments using adult skin and confirmed significant degranulation of MCs with increased vascular permeability (see Fig E3 in this article's Online Repository at www.jacionline.org). These results suggest that, unlike adult skin, MCs in neonatal skin are minimally sensitized with IgE, possibly because of their low expression levels of FcεRI. Expression levels of FcεRI on MCs in the skin increased as age progressed: less than 10% of MCs expressed FcεRI up to 2 weeks of age; however, FcεRI expression gradually increased, and approximately 80% of MCs expressed FcεRI at 12 weeks of age (Fig 2, A).

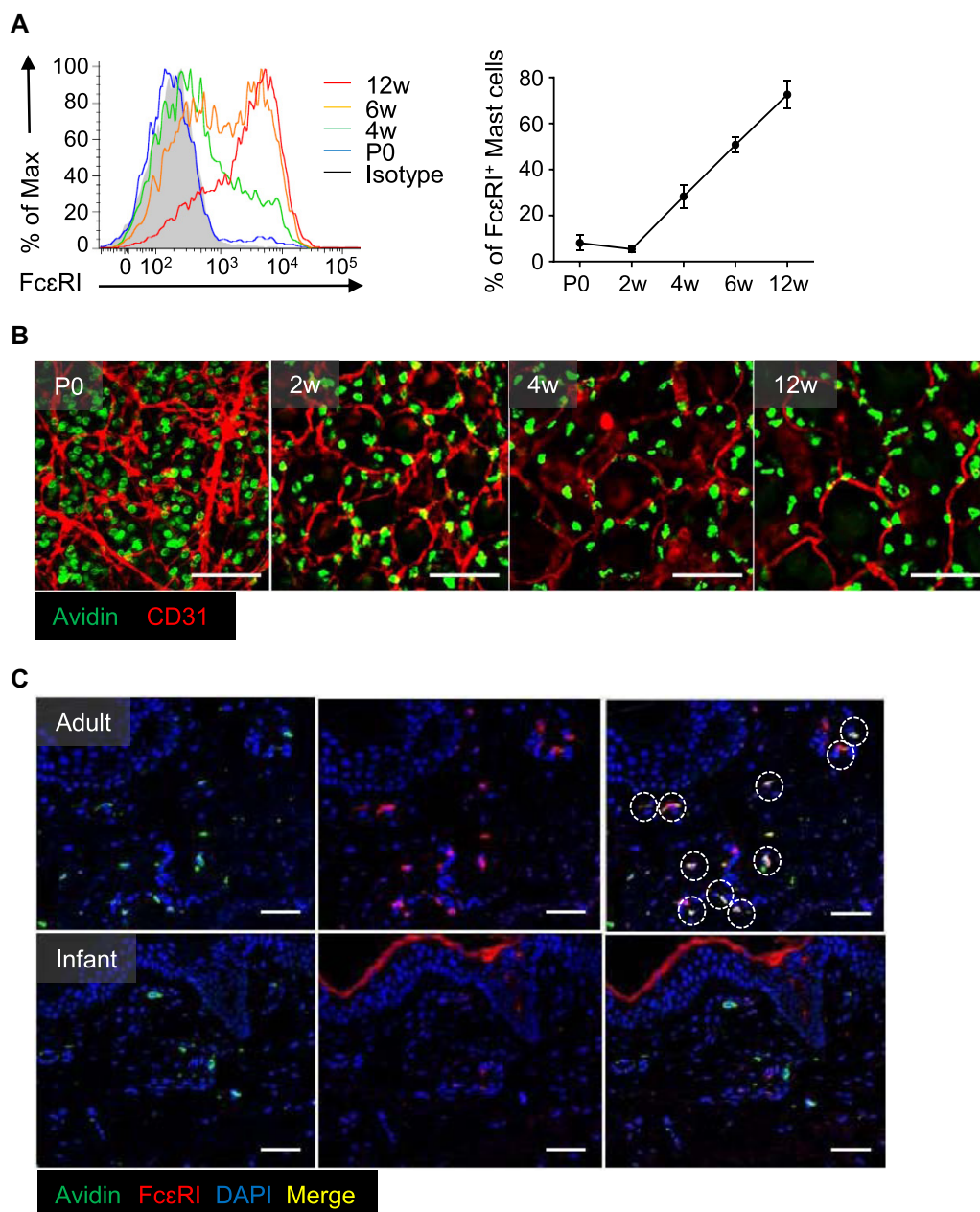


FIG 2. Expression of FcεRI in neonatal skin. **A**, FcεRI expression on MCs in mice of different ages: P0, 2 weeks old (2w), 4 weeks old (4w), 6 weeks old (6w), and 12 weeks old (12w; n = 4). **B**, Immunostaining of avidin (green) and CD31 (red) in ear skin of mice of different ages. Scale bar = 50 μm. **C**, Immunostaining of avidin (green), FcεRI (red), and 4'-6-diamidino-2-phenylindole dihydrochloride (DAPI; blue) in human adult and infant (1-month-old) skin. Double-positive cells are indicated by white dotted circles. Scale bar = 50 μm.

We also investigated the positional relationship between MCs and blood vessels by immunostaining the neonatal skin because MCs are known to capture circulating IgE in the blood by elongating their dendrites.⁵ MCs in neonatal skin generally showed rounded shapes and did not appear to attach to the blood vessels. However, as age progressed, the number of MCs with a spindle shape increased, and they were located near or along the blood vessels (Fig 2, B). Collectively, these data suggest that dermal MCs in neonatal mice seem to be immature in terms of the expression of FcεRI, morphology, and disposition.

Finally, to examine human relevance, we checked FcεRI expression on MCs in the skin of human infants using immunohistochemistry. Although more than 80% of dermal MCs in adults and approximately 75% in 5-month-old infants were positive for FcεRI, less than 10% of MCs in the skin of infants less than 3 months old (3 cases) were positive for FcεRI (Fig 2, C, and see Fig E4 in this article's Online Repository at www.jacionline.org). These results suggest the possibility that MCs in the skin of human infants also have low expression levels of FcεRI.

Here we provide *in vivo* evidence that maternal IgE was transplacentally transferred in the form of ICs in mice. Although the precise mechanism remains unclear, we speculate that FcRn on the placenta mediate the transfer. We also demonstrate that MCs exhibited a low level of FcεRI expression and rounded morphology and were less prone to sensitization with IgE in the neonatal skin. Findings of a low level of FcεRI expression are consistent with a recent study showing the ontogeny of MCs in various organs.⁶ Expression levels of FcεRI increased age dependently, suggesting that MCs gradually matured in the skin after birth in both mice and human subjects. Complete maturation can be induced *in situ* under the influence of several factors, including the skin microbiome.^{7,8} MCs in an immature state might be beneficial for neonates to prevent unintended IgE-induced degranulation.

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REFERENCES

1. Avrech OM, Samra Z, Lazarovich Z, Caspi E, Jacobovich A, Sompolsky D. Efficacy of the placental barrier for immunoglobulins: correlations between maternal, paternal and fetal immunoglobulin levels. *Int Arch Allergy Immunol* 1994;103:160-5.
2. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. *Nat Rev Immunol* 2007;7:715-25.
3. Michel FB, Bousquet J, Greillier P, Robinet-Levy M, Coulomb Y. Comparison of cord blood immunoglobulin E concentrations and maternal allergy for the prediction of atopic diseases in infancy. *J Allergy Clin Immunol* 1980;65:422-30.
4. Bundhoo A, Pavoglio S, Rafti E, Dhongade A, Blumberg RS, Matson AP. Evidence that FcRn mediates the transplacental passage of maternal IgE in the form of IgG anti-IgE/IgE immune complexes. *Clin Exp Allergy* 2015;45:1085-98.
5. Cheng LE, Hartmann K, Roers A, Krummel MF, Locksley RM. Perivascular mast cells dynamically probe cutaneous blood vessels to capture immunoglobulin E. *Immunity* 2013;38:166-75.
6. Li Z, Liu S, Xu J, Zhang X, Han D, Liu J, et al. Adult connective tissue-resident mast cells originate from late erythro-myeloid progenitors. *Immunity* 2018;49:640-653, e5.
7. Wang Z, Mascarenhas N, Eckmann L, Miyamoto Y, Sun X, Kawakami T, et al. Skin microbiome promotes mast cell maturation by triggering stem cell factor production in keratinocytes. *J Allergy Clin Immunol* 2017;139:1205-1216, e6.
8. Gentek R, Ghigo C, Hoeffel G, Bulle MJ, Msallam R, Gautier G, et al. Hemogenic endothelial fate mapping reveals dual developmental origin of mast cells. *Immunity* 2018;48:1160-1171, e5.

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<https://doi.org/10.1016/j.jaci.2019.05.011>

METHODS

Mice

All experiments were performed using P0 to 12-week-old C57BL/6 background mice (Shimizu, Kyoto, Japan). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Kyoto University Graduate School of Medicine.

Animal treatment

An anti-TNP mouse monoclonal IgE-producing hybridoma cell line (IGELa2) was obtained from the American Type Culture Collection (Manassas, Va).

Female mice on pregnancy day 17 were intravenously administered either PBS, 200 μ g of anti-TNP IgEa, 200 μ g of anti-TNP IgEa in the form of ICs with anti-IgE IgG (R35-72; BD Biosciences, San Jose, Calif), or 200 μ g of anti-TNP IgEa reacted with isotype IgG (eBRG1; Invitrogen, Carlsbad, Calif) to test the transplacental transfer of maternal IgE. ICs were formed, as described previously; anti-TNP IgEa and anti-IgE IgG or isotype IgG with the same molecular weight were incubated for 1 hour at room temperature (RT) before injection.^{E1}

Adult female mice were intradermally injected with either PBS or 3 μ g of anti-TNP IgEa with or without 2.4 μ g of goat anti-mouse IgE polyclonal IgG to assess type I hypersensitivity in adult ear skin (Abcam, Cambridge, United Kingdom). Anti-TNP IgEa and goat anti-mouse IgE polyclonal IgG (Abcam) were incubated for 1 hour at RT before injection to form ICs. The next day, 200 μ L of 0.5% Evans blue (Sigma-Aldrich, St Louis, Mo) was intravenously injected, and 1 hour later, 10 μ g of TNP (14)-OVA (BioResearch Technologies, Hoddesdon, United Kingdom) was administered intradermally. Ear thickness was measured 30 minutes after injection, and ear skin was sampled. Evans blue leakage in ear skin was assessed by means of absorbance at 620 nm with a microplate reader (FlexStation 3; Molecular Devices, San Jose, Calif).

P0 mice were intraperitoneally administered either PBS or 10 μ g of anti-TNP IgEa in the form of ICs with 8 μ g of goat anti-mouse IgE polyclonal IgG to assess degranulation of MCs in neonatal skin (Abcam). The next day, 10 μ g of TNP (14)-OVA (BioResearch Technologies) was applied intradermally. For the positive control, 1 μ g of C48/80 (Sigma-Aldrich) was intradermally injected to induce MC degranulation. Fetal back skin was sampled 30 minutes after injection.

ELISA for detection of serum mouse anti-TNP IgEa antibody

Nunc-Immuno Plates (Sigma-Aldrich) were coated with TNP (14)-OVA at 2 μ g/mL overnight at 4°C. After blocking the plates against nonspecific binding with 1% BSA in 50 mmol/L Tris and 0.14 mol/L NaCl for 1 hour, serum was applied to each well overnight at 4°C. After washing with PBS, biotin-conjugated rat anti-mouse IgEa (UH297; BioLegend, San Diego, Calif) was applied, followed by streptavidin-horseradish peroxidase (BD Biosciences). Color development was induced with the 3,3',5,5'-tetramethylbenzidine microwell peroxidase substrate system, and absorbance at 450 nm was measured with a microplate reader (FlexStation 3; Molecular Devices).

Cell preparation and flow cytometry of murine skin samples

Back skin of neonates or dorsal halves of ear skin of mice were collected and floated for 30 minutes at 37°C on 5 mg/mL Dispase II (Wako, Osaka, Japan) dissolved in RPMI 1640 (Invitrogen) containing 10% heat-inactivated

FCS. After separating the epidermis from the dermis, the dermis was minced with scissors and incubated for 1 hour at 37°C in cRPMI containing 0.33 mg/mL Liberase TL (Roche, Basel, Switzerland) and 0.05% DNase I (Sigma-Aldrich) dissolved in RPMI 1640 (Invitrogen) containing 10% FCS. Cell suspensions were filtered with a 40- μ m cell strainer mesh. Cells were stained with the following antibodies: anti-mouse antibody to CD45 (30-F11; BD Biosciences), CD11b (M1/70; BD Biosciences), CD200R3 (Ba13; BioLegend), c-Kit (2B8; BD Biosciences), Fc ϵ RI (MAR-1; eBioscience, San Diego, Calif), IgE (R35-72; BD Biosciences), and goat IgG (Invitrogen). Fixable viability dye (eBioscience) was used to gate out dead cells. Flow cytometry was performed with an LSRFortessa (BD Biosciences) and analyzed with FlowJo (TreeStar, San Carlos, Calif). MCs were detected as the CD45⁺CD11b⁻CD200R3⁺c-Kit⁺ fraction.

Histologic examination of murine ear skin

Skin tissues were fixed with 10% formalin in PBS and embedded in paraffin. Sections with a thickness of 5 μ m were prepared, stained with toluidine blue, and examined by using light microscopy.

Whole-mount staining of murine ear skin

Dorsal halves of ear skin were collected and floated for 30 minutes at 37°C on 35.56 mg/mL ammonium thiocyanate (Wako) dissolved in PBS to separate the epidermis and dermis. Then the dermis was fixed with 4% paraformaldehyde for 1 hour at RT, washed with PBS, and stained overnight at 4°C with avidin-fluorescein isothiocyanate (BioLegend) and anti-CD31 antibody (MEC13.3; BD Biosciences). Samples were mounted with antifade mounting medium (Invitrogen). Fluorescent images were analyzed with a Nikon confocal microscope (Nikon A1RMP; Nikon, Tokyo, Japan).

Immunostaining of human skin samples

Adult skin samples were obtained from healthy skin. Infant skin samples were obtained from patients who were given a diagnosis of Mongolian spots or giant nevi. This study was approved by the Medical Ethics Committee of the Kyoto University Graduate School of Medicine and was conducted according to the principles of the Declaration of Helsinki.

Paraffin-embedded skin samples were deparaffinized and subjected to heat-mediated antigen retrieval. Double-immunofluorescent staining was performed for avidin and Fc ϵ RI. Antibodies used were avidin-fluorescein isothiocyanate (BioLegend) and anti-human Fc ϵ RI (9E1; Abcam). Fluorescent images were analyzed with a Nikon confocal microscope (Nikon A1RMP).

Statistical analysis

Data are presented as the means $\pm$ SEMs, and each data point is representative of at least 3 independent experiments. Statistical analyses were performed with GraphPad Prism software (GraphPad Software, La Jolla, Calif) by using either a parametric Student *t* test or 1-way ANOVA. All *P* values of less than .05 were considered to indicate statistical significance and indicated with an asterisk.

REFERENCE

- E1. Paveglia S, Puddington L, Rafti E, Matson AP. FcRn-mediated intestinal absorption of IgE anti-IgE immune complexes in mice. *Clin Exp Allergy* 2012;42:1791-800.

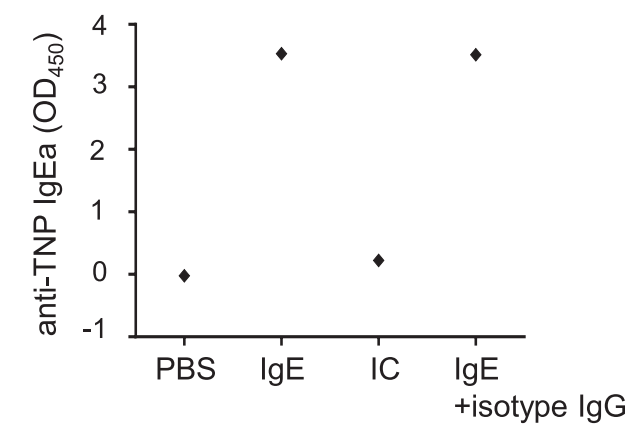


FIG E1. Serum levels of anti-TNP IgEa in mothers (n = 1). Data are representative of 3 independent experiments.

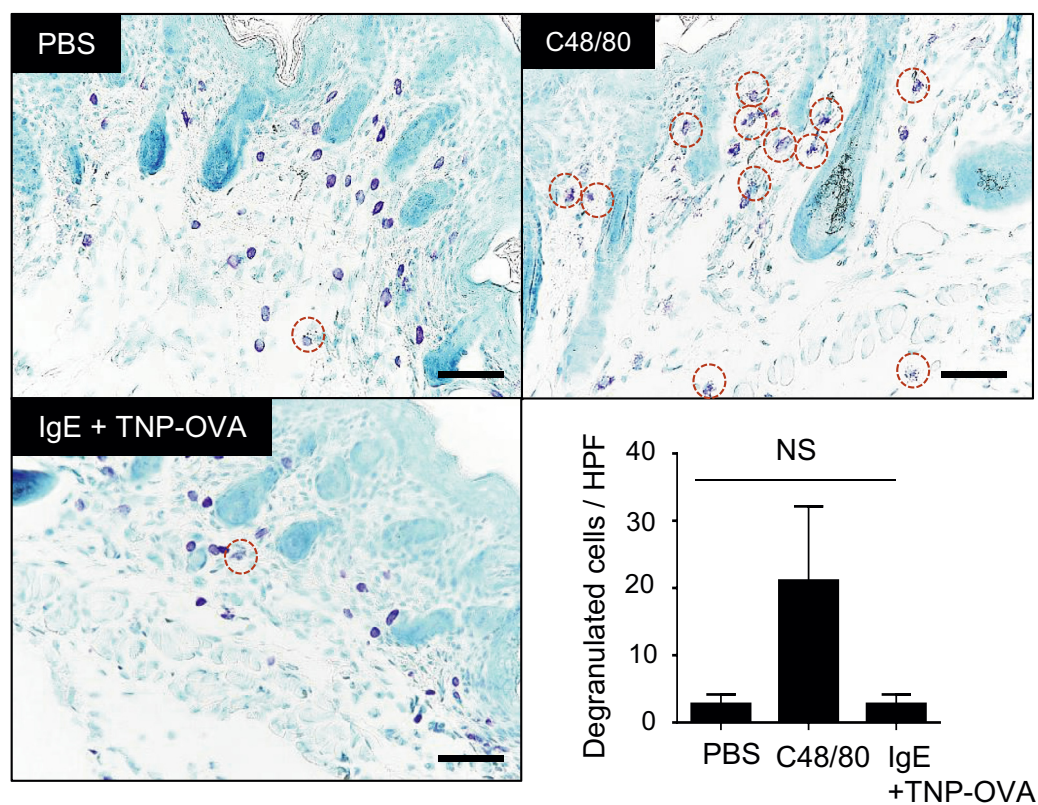


FIG E2. Toluidine blue staining of fetal back skin after antigen challenge when sensitized with monomeric IgE. Degranulated MCs are shown with *red dotted circles*. Scale bar = 50 μ m. Numbers of degranulated MCs per high-power field (HPF; $\times 40$ magnification; *right panel*; $n = 3$). NS, Not significant.

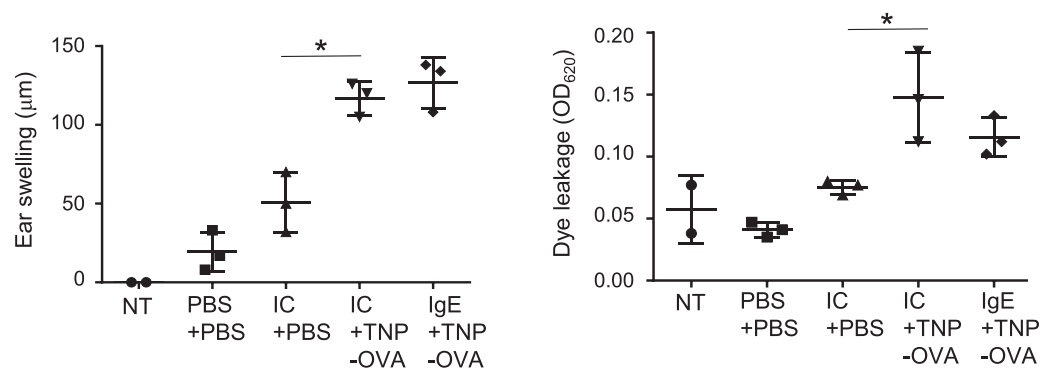


FIG E3. Ear swelling (*left panel*) and dye leakage (*right panel*) of adult ears after antigen challenge when sensitized with ICs or monomeric IgE (n = 3). P values are from the Student t test. *P < .05.

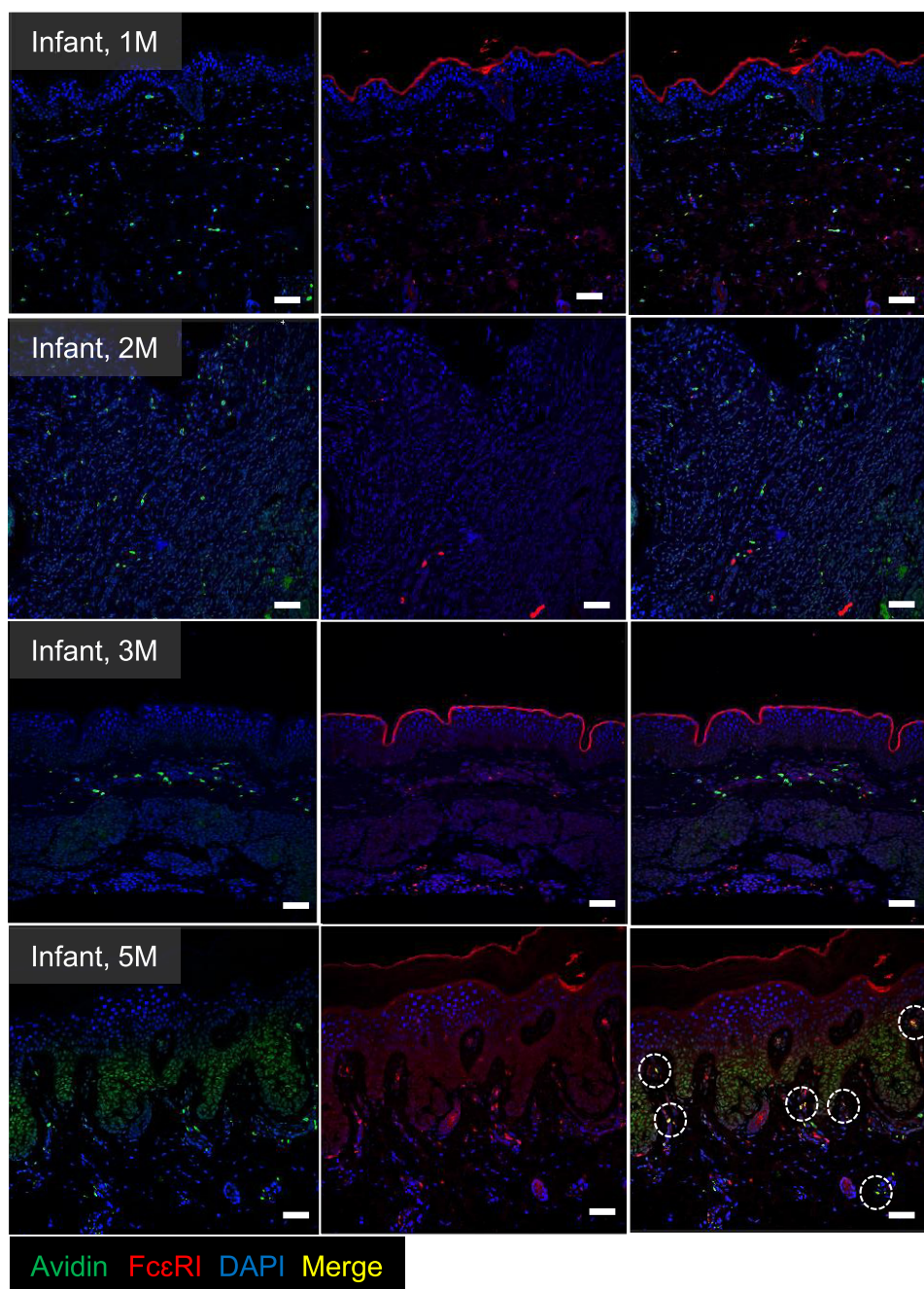


FIG E4. Immunostaining of avidin (green), FcεRI (red), and 4'-6-diamidino-2-phenylindole dihydrochloride (DAPI; blue) in 1-month-old (1M), 2-month old (2M), 3-month old (3M), and 5-month old (5M) human infant skin. Double-positive cells are shown with white dotted circles. Scale bar = 50 μm. The ratio of FcεRI/avidin double-positive cells among avidin single-positive cells (bottom panel).