Application of moisturizer to neonates prevents development of atopic dermatitis

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Supported in part by Health and Labour Sciences Research Grants for Research on Allergic Diseases and Immunology from the Ministry of Health, Labour and Welfare of Japan (H22-Mori-27600007 to H.S. and H23-Nanchito-Ippan-001-8040 to M.A. and H.S. as principal investigators), funding from the Japan Environment and Children’s Study (JECS; to Y.O. and H.S.), and grants from the National Center for Child Health and Development (20S-1 to Y.O. and 23S-3 to H.S.).

Disclosure of potential conflict of interest: K. Horimukai has received research support from the Ministry of Health, Labour and Welfare (the grant no. of MHLW is hencforth H22-Mori-27600007 and H23-Nanchito-Ippan-001, unless otherwise specified); is employed by the National Center for Child Health and Development and the Jikei University Katsushika Medical Center; and has received payment for lectures from Maruhou and GlaxoSmithKline K.K. K. Morita has received research support from the Ministry of Health, Labour and Welfare and Shiseido and is employed by the National Center for Child Health and Development. M. Narita is employed by the National Center for Child Health and Development and has received payment for lectures from GlaxoSmithKline K.K. M. Kondo, Y. Shigematsu, K. Moritomu, and T. Takimoto have received research support from the Ministry of Health, Labour and Welfare and is employed by the National Center for Child Health and Development. H. Kitazawa is employed by the National Center for Child Health and Development and Miyagi Children’s Hospital. M. Nozaki, Y. Yoshida, H. Maruhou, and K. Morita have received research support from the Ministry of Health, Labour and Welfare. H. Niizuki has received research support from the Ministry of Health, Labour and Welfare; is employed by the National Center for Child Health and Development; has received payment for lectures from GlaxoSmithKline; and has received travel support from Kyowa Kirin. H. Sago has received research support from the Ministry of Health, Labour and Welfare and the Japan Society for Promotion of Science; is employed by the National Center for Child Health and Development; and has received payment for lectures from GE Healthcare, Gene Tech, Johnson & Johnson, Kissel Pharmaceutical, Eisai, Bayer Health Care, and ASKA Pharmaceutical. E. Inoue has received research support from the Ministry of Health, Labour and Welfare; has consultanit arrangements with Tokyo Women’s Medical University and Stagen; is employed by the National Center for Child Health and Development; and has received payment for lectures from Takeda Pharmaceutical, Chugai Pharmaceutica, and the Japan Clinical Cancer Research Organization. N. Kamemura has received research support from the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science and Technology and is employed by the University of Tokushima. H. Kido has received research support from the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science and Technology; is employed by the University of Tokushima; and has received payment for lectures from Taisho Toyo Pharmaceutical and Teijin. J. Hisatsune has received research support from the Ministry of Health, Labour and Welfare and is employed by Hiroshima University. M. Sugai has received research support from the Ministry of Health, Labour and Welfare; is employed by Hiroshima University; has received research for lectures from Saiseikai Kure Hospital and Hiroshima CDC; and has received payment for education presentations and travel support from Kochi University, Tokushima University, Nagasaki University, and Ehime University. H. Murota has received research support from the Ministry of Education, Culture, Sports, Science and Technology. T. Sasaki has received research support from the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science and Technology and is employed by KOSE Endowed Program for Skincare and Allergy Preventive Medicine. M. Amagai has received research support from the Ministry of Health, Labour and Welfare, MSD K.K., and Maruhou and has consultant arrangements with Daichi Sankyo, Novartis Pharma K.K., and GlaxoSmithKline K.K. A. Matsuda has received research support from the Ministry of Health, Labour and Welfare, is employed by the National Center for Child Health and Development; has received payment for lectures from Japan Multiplex bio-Analyses Consortium, Benesse, Japan Blood Products Organization, and Affymetrix Japan; and has received payment for educational presentations from Tokyo University of Science. K. Matsumoto has received research support from the Ministry of Health, Labour and Welfare and the National Institute for Biomedical Innovation (NIBio ID10-43); is employed by National Research Institute for Child Health and Development; has received payment for lectures from Merck Sharp and Dohme K.K., Ono Pharmaceutical, GlaxoSmithKline K.K., Kyorin Pharmaceutical, Otsuka Pharmaceutical K.K., Mitsubishi Tanabe Pharma, AstraZeneca K.K., Siemens Healthcare, Abbott Japan, and Sunovion Danippon Pharma; has received payment for manuscript preparation from Maruhou; and has received payment for educational presentations from Gifu Pharmaceutical University. H. Saito has received research support and travel support from the Ministry of Health, Labour and Welfare; is employed by the National Center for Child Health and Development; has received research support from the Japan Society for the Promotion of Science (21390303 & 23390202); has received payment for lectures from Teijin Pharma, Shiseido, Merck Sharp and Dohme K.K., Taiho Pharmaceutical, Nippon Boehringer-Ingelheim, Ono Pharmaceutical, GlaxoSmithKline K.K., Pfizer Japan, Novartis Pharma K.K., Kyowa Hakko Kirin, Kyorin Pharmaceutical, and Daiichi Sankyo; has received payment for manuscript preparation from Taiho Pharmaceutical; has received payment for educational presentations from Shimane University and Tohio University; and has received travel support from the Shimane University Japanese Society of Allergology and the Japanese Society of Pediatric Allergy & Clinical Immunology and Pfizer Japan. Y. Ohya has received research support and travel support from the Ministry of Health, Labour and Welfare; is employed by the National Center for Child Health and Development; has received payment for research support from the Ministry of Health, Labour and Welfare, the National Center for Child Health & Development (23S-3), the Environmental Restoration & Conservation Agency, Shiseido, Maruhou, and the National Institute for Environmental Studies; has received payment for lectures from Merck Sharp and Dohme K.K., GlaxoSmithKline K.K., Malho, Teijin Pharma, Shiseido, Abbott Japan, Sanofi K.K., Siemens AG, Kyowa Hakko Kirin, Ltd., and Nikkei Radio broadcasting; has received payment for manuscript preparation from the University of Tokyo Press, Tokyo Igakusha, and the Asahi Shimbun; has received payment for educational presentations from Japan Allergy Foundation, Japan Pharmacists Education Center, NHK Educational, and the Korean Pediatric Society; and has received travel support from the Japanese Society of Child Health, Japanese Society of Pediatric Dermatology, Tokyo Metropolitan Government, National Institute for Environmental Studies, Ministry of the Environment, and the Cabinet Office. I. Katayama declares no relevant conflicts of interest.

Received for publication June 17, 2014; revised July 23, 2014; accepted for publication July 23, 2014.

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Background: Recent studies have suggested that epidermal barrier dysfunction contributes to the development of atopic dermatitis (AD) and other allergic diseases.

Objective: We performed a prospective, randomized controlled trial to investigate whether protecting the skin barrier with a moisturizer during the neonatal period prevents development of AD and allergic sensitization.

Methods: An emulsion-type moisturizer was applied daily during the first 32 weeks of life to 59 of 118 neonates at high risk for AD (based on having a parent or sibling with AD) who were enrolled in this study. The onset of AD (eczematous symptoms lasting >4 weeks) and eczema (lasting >2 weeks) was assessed by a dermatologist specialist on the basis of the modified Hanifin and Rajka criteria. The primary outcome was the cumulative incidence of AD plus eczema (AD/eczema) at week 32 of life. A secondary outcome, allergic sensitization, was evaluated based on serum levels of allergen-specific IgE determined by using a high-sensitivity allergen microarray of diamond-like carbon–coated chips.

Results: Approximately 32% fewer neonates who received the moisturizer had AD/eczema by week 32 than control subjects (P = .012, log-rank test). We did not show a statistically significant effect of emollient on allergic sensitization based on the level of IgE antibody against egg white at 0.34 kU/L CAP-FEIA equivalents. However, the sensitization rate was significantly higher in infants who had AD/eczema than those who did not (odds ratio, 2.86; 95% CI, 1.22-6.73).

Conclusion: Daily application of moisturizer during the first 32 weeks of life reduces the risk of AD/eczema in infants. Allergic sensitization during this time period is associated with the presence of eczematous skin but not with moisturizer use. (J Allergy Clin Immunol 2014;134:824-30.)

Key words: Atopic dermatitis, atopy, allergic sensitization, food allergy, IgE, randomized controlled trial

The prevalence of atopic dermatitis (AD) among children continues to increase, reaching 20% in some parts of the world; almost half of all children experience eczema within the first 2 years of life. AD reduces quality of life, such as by disturbing sleep, and should be considered a significant global burden of disease.

Skin barrier dysfunction contributes to the development of AD, and dry skin often causes inflammation of eczematous skin. Filaggrin, a key component of the epidermal differentiation complex, is required for barrier function. Disruption of the gene encoding filaggrin (FLG) is associated with development of AD, as well as ichthyosis. Children with mutations in FLG have increased transepidermal water loss, even before AD develops.

The skin stratum corneum of infants is intact shortly after birth, but the water-sustaining barrier function of skin becomes adult like only after the first year of life. Therefore it has been proposed that intensive emollient use in early life could prevent AD, especially in infants at high risk for AD (based on having a parent or sibling with AD). This hypothesis was investigated in a pilot study, and a large-scale randomized controlled trial (RCT) is underway (Barrier Enhancement for Eczema Prevention trial; http://www.beepstudy.org/).

We initiated an RCT in 2010 to test the effects of an emulsion-type moisturizer (2e [Douhet] emulsion; Shiseido, Tokyo, Japan) in neonates at high risk for AD.

Several cohort studies have provided evidence that infants with eczema tend to have other allergic diseases, such as asthma, rhinitis, and food allergy. Moreover, topical application of peanut oil to neonatal skin increased the infant’s risk of peanut allergy, indicating epicutaneous sensitization to allergens.

Loss-of-function mutations in FLG are associated with a wide range of allergic diseases and sensitization to airborne and food antigens, even though filaggrin expression is limited to the skin and oral mucosa and has not been detected in the respiratory or intestinal mucosa.

Primary prevention of allergic disease has been studied for many years. However, studies of avoidance of food allergens, aeroallergens, or both have generally produced disappointing results. In this study we investigate whether daily application of moisturizer to neonates at high risk for AD prevents allergic sensitization, as well as development of AD. In addition to the outcomes of this RCT, we report that the presence of skin lesions (including AD) is a risk factor for allergic sensitization.

METHODS

Trial design and participants

We performed an investigator-blinded, randomized, controlled, parallel-group study at the National Center for Child Health and Development (NCCHD) in Tokyo, Japan, from November 2010 through November 2013 (Fig 1). The NCCHD is the only national hospital for mothers and children in Japan, performing more than 1600 deliveries per year. After receiving approval from the institutional review board (IRB) of the NCCHD in August 2010, we invited expectant mothers with family histories of AD who visited the prenatal clinic of the NCCHD to participate in this trial. A high familial risk of AD was defined as a history of physician-diagnosed AD for at least 1 of the unborn baby’s parents or siblings. Informed consent was obtained from the parents before delivery. After birth, the study doctors and a dermatology specialist confirmed the eligibility of each neonate on the basis of the inclusion criteria (eg, absence of treatment with corticosteroids) and exclusion criteria (eg, abnormal skin disorders, such as ichthyosis), which had been registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; UMIN000004544). The enrolled neonates were then randomly assigned to the intervention (n = 59) or control (n = 59) group (Fig 2).

The intervention group began receiving daily application of an emulsion-type emollient (2e [Douhet] emulsion) from the first week of life; petroleum jelly was prescribed to each infant in both groups on request by the IRB. Emollient was applied each day for 32 weeks. All infants were examined by the same blinded dermatologist from the NCCHD at scheduled visits and at weeks 4, 12, 24, and 32 of life. At each visit, the dermatologist examined the skin condition of the infant and recorded a diagnosis of AD, eczema, skin rash without pruritus, or healthy skin without any lesions. The worldwide and most validated criteria for diagnosis did not specify a time frame for AD development, describing a chronic or relapsing course, and therefore it was not possible to diagnose an infant’s AD immediately after his or her pruritic skin lesion emerged.
by temporal observation) was analyzed by using the log-rank test. The
problems (Fig 1).
made a diagnosis of skin rash. When given a diagnosis of AD/eczema, infants
rash or eczematous skin did not show any sign of pruritus, the dermatologist
Because AD and infantile eczema, as defined above, were essentially synon-
tions that lasted for at least 4 weeks,' ' and infantile eczema was defined as the
criteria for an incident case of AD according to our definition of infantile
eczema and AD. In our trial AD was defined as ‘itchy eczema at typical loca-
case, setting the time for AD development to at least 2 weeks. The same
measures, at UMIN-CTR (UMIN000004544). We proposed that protection of
skin barrier with a moisturizer beginning in the neonatal period would be a
safe and effective strategy for prevention of AD and allergic sensitization. The
primary outcome measure was the cumulative rate of incidence of AD,
eczema, or both by temporal observation. The diagnostic criteria for infantile
eczema, AD, or both (AD/eczema) were developed based on a modification of
the United Kingdom Working Party’s criteria and were applied by a
dermatology specialist, as described above. Briefly, those criteria were a
pruritic skin condition of at least 2 weeks’ duration, visible flexural dermatitis
(and/or on the cheeks and extensor surfaces), a history of dry skin, and a family
history in a first-degree relative of the enrolled neonate.
Secondary outcome measures were the presence of allergen-specific IgE,
transepidermal water loss (to measure stratum corneum hydration and pH at
birth [baseline] and at weeks 4, 12, 24 and 32 of life; Vapo Meter, SW-4002;
Delfin Technologies, Kuopio, Finland), stratum corneum hydration (Moisture
Meter, SC-5; Delfin Technologies), stratum corneum pH (epidermal; Skin-pH-
Meter, PH905; Courage & Khazaka Electronic GmbH, Kohn, Germany), and
skin colonization by Staphyloccocus aureus (measured at the cheek).
Onset of allergic diseases, such as food allergy (registered on November 10,
2010), and onset of asthma were added as outcome measures on April 12,
2011, in response to a recommendation by the evaluation committee of the
Ministry of Health, Labour and Welfare. Skin barrier functions were assessed
by using the previously described methods.21
Outcomes
We registered this trial design, including the hypothesis and outcome
measures, at UMIN-CTR (UMIN000004544). We proposed that protection of
the skin barrier with a moisturizer beginning in the neonatal period would be a
safe and effective strategy for prevention of AD and allergic sensitization. The
primary outcome measure was the cumulative rate of incidence of AD,
eczema, or both by temporal observation. The diagnostic criteria for infantile
eczema, AD, or both (AD/eczema) were developed based on a modification of

FIG 1. Study design.

Simpson et al20 have modified the Hanifin-Rajka criteria for an incident case,
setting the time for AD development to at least 2 weeks. The same
authors proposed setting the time frame as at least 4 weeks.20 We incorporated
these criteria for an incident case of AD according to our definition of infantile
eczema and AD. In our trial AD was defined as “itchy eczema at typical loca-
tions that lasted for at least 4 weeks,” and infantile eczema was defined as the
same eczema that lasted at least 2 weeks. Then these criteria were registered.
statistical analyses
Analyses of the primary and secondary outcomes were conducted
according to the intent-to-treat principle and based on the full analysis set,
which included all randomized subjects. For an analysis of allergic
sensitization, subjects without serum specific IgE (detected by using the
diamond-like carbon [DLC] chip with high-density allergen immobilization
and high sensitivity22 at week 32; n = 2 for the intervention group and n = 5
for the control group) were excluded.
The primary outcome (cumulative rate of incidence of AD, eczema, or both
by temporal observation) was analyzed by using the log-rank test. The
significance level was set at .05. The Kaplan-Meier method was used to estimate
the cumulative incidence of AD/eczema for each group, and the Cox
regression model was applied to estimate the hazard ratio between groups. The
Mann-Whitney U test and χ² test with the Yates correction were used with
continuous and categorical variables, respectively, to analyze secondary
outcomes. Demographic and baseline data are presented as means, SDs, and
proportions, as appropriate.
Once the data were collected from all subjects, we conducted several post
hoc analyses. To evaluate the association between sensitization to foods and
AD, we constructed a contingency table that dichotomized serum levels of
antigen-specific IgE (based on results from the DLC assay) measured at
week 32 at several cutoff values. The odds ratio (OR) and 95% CI were
used to evaluate the degree of association. Statistical analyses were conducted
with SPSS 17.0 software for Windows (SPSS, Chicago, Ill) and R software

RESULTS
Characteristics of neonates
We invited 183 expectant mothers from families at high risk for AD to participate in the study; 118 neonates were enrolled and randomly assigned to 2 groups of 59 infants each (Fig 2). Two infants assigned to the control group were found to have accidently received and used the emollient after opening the blinded data; 1 withdrew consent, and another completed the study without skin lesions. All 118 neonates were included as the intent-to-treat population (Table 1) and the 2 infants who mistakenly received the intervention were classified into the control group. During the trial, 8 families withdrew informed consent (2 infants in the intervention group and 6 infants in the control group). The dermatologist withdrew an infant in the intervention group from the study because she or he had a hemangioma. After the second scheduled examination, we found that the incidence of AD was significantly lower in the intervention group than in the control group and reported this observation to the IRB of the NCCHD. The trial was discontinued at the recommendation of the NCCHD’s IRB on November 30, 2013; by this time, 10 neonates had left the study (6 in the intervention group and 4 in the control group).
TABLE I. Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Intervention group (n = 59)</th>
<th>Control group (n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant girl, no. (%)</td>
<td>26/59 (44.1)</td>
<td>24/59 (40.7)</td>
</tr>
<tr>
<td>Birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ages of mothers at delivery (y)</td>
<td>35.8 ± 4.80</td>
<td>35.0 ± 4.85</td>
</tr>
<tr>
<td>Cesarean section, no. (%)</td>
<td>16/59 (27.1)</td>
<td>13/59 (22.0)</td>
</tr>
<tr>
<td>Mean gestational age (wk)</td>
<td>39.1 ± 0.97</td>
<td>39.0 ± 1.07</td>
</tr>
<tr>
<td>Mean birth weight (g)</td>
<td>3074 ± 363</td>
<td>3034 ± 366</td>
</tr>
<tr>
<td>Breast-feeding at 1 mo (%)</td>
<td>29/58 (50.0)</td>
<td>28/58 (48.3)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food allergy (%)</td>
<td>24/59 (40.7)</td>
<td>21/59 (36.8)</td>
</tr>
<tr>
<td>Bronchial asthma (%)</td>
<td>24/59 (40.7)</td>
<td>21/59 (36.8)</td>
</tr>
<tr>
<td>Allergic rhinitis (%)</td>
<td>46/59 (78.0)</td>
<td>48/59 (84.2)</td>
</tr>
<tr>
<td>Mean no. of siblings</td>
<td>0.34 ± 0.58</td>
<td>0.38 ± 0.62</td>
</tr>
<tr>
<td>Environmental exposures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking in the family, no. (%)</td>
<td>10/59 (16.9)</td>
<td>7/57 (12.3)</td>
</tr>
<tr>
<td>Any pet, no. (%)</td>
<td>12/58 (21.4)</td>
<td>13/57 (23.2)</td>
</tr>
<tr>
<td>Dog, no. (%)</td>
<td>8/58 (13.8)</td>
<td>6/57 (10.5)</td>
</tr>
<tr>
<td>Cat, no. (%)</td>
<td>2/58 (3.4)</td>
<td>4/57 (7.0)</td>
</tr>
<tr>
<td>Skin barrier function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEWL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean lower leg</td>
<td>8.31 ± 2.67</td>
<td>8.40 ± 2.92</td>
</tr>
<tr>
<td>Mean forehead</td>
<td>8.29 ± 4.77</td>
<td>7.62 ± 3.15</td>
</tr>
<tr>
<td>Stratum corneum hydration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean lower leg</td>
<td>13.7 ± 5.93</td>
<td>13.5 ± 5.94</td>
</tr>
<tr>
<td>Mean forehead</td>
<td>20.6 ± 10.7</td>
<td>19.2 ± 11.6</td>
</tr>
<tr>
<td>Mean pH</td>
<td>5.65 ± 0.59</td>
<td>5.61 ± 0.39</td>
</tr>
</tbody>
</table>

TEWL, Transepidermal water loss.

Among 118 infants evaluated, 47 had AD/eczema (19/59 in the intervention group and 28/59 in the control group), 13 had skin rash without pruritus (6 in the intervention group and 7 in the control group), and 31 did not have any skin lesions (20 in the intervention group and 11 in the control group). There were 5 infants (2 in the intervention group and 3 in the control group) who used moisturizers for skin disorders other than AD/eczema. The dermatology specialist stopped giving the emollient to 3 infants whose skin lesions seemed to be the result of urticaria or contact dermatitis caused by emulsion-type emollients (related adverse events). After several days, however, the doctor judged that these skin lesions were not adverse events because they disappeared rapidly and similar lesions were not seen when the same emollients were used again. These 3 infants did not have AD/eczema or skin rash when they were followed for 32 weeks. Among 8 families who withdrew consent, 2 families in the intervention group said that it was difficult for them to visit the NCCHD. There were no infants from families that withdrew consent who had skin lesions. In summary, adverse events caused by this emulsion-type emollient were not observed during this RCT.

Because the IRB recommended permitting application of petroleum jelly when the parents thought it necessary, we calculated the amount of these 2 types of moisturizers used by each group based on their diaries. The mean daily amount of emulsion-type moisturizer used by the intervention group was 7.86 ± 4.34 g (0 g for the control group, excluding the 2 infants placed in the wrong group). The mean daily amount of petroleum jelly applied to the control group was 0.101 ± 0.286 g (mean frequency of use, 0.235 d/wk). Petroleum jelly (20 g per bottle) was prescribed to all neonates born at the NCCHD, but we had no information about how much was used by the intervention group. Nevertheless, only a few of the parents occasionally used a small, almost ignorable, amount of the jelly on their infants.

Primary and secondary outcomes

During their first 32 weeks of life, 19 infants in the intervention group had AD/eczema compared with 28 infants in the control group. Calculation of cumulative incidence values for AD/eczema by using the Kaplan-Meier method showed that the intervention group maintained intact skin for a significantly longer period than the control group (P = .012, log-rank test; Fig 3). Cox regression analysis showed the risk of AD/eczema to be significantly lower in the intervention group (hazard ratio, 0.48; 95% CI, 0.27-0.86).

In analyses of secondary outcomes (levels of allergen-specific IgE), we evaluated the serum levels of anti–egg white and anti-ovomucoid IgE in infants at 32 weeks,22 as described in the Methods section of this article’s Online Repository. IgE antibody data were converted to CAP-FEIA data after confirming the correlation between the data sets (see Fig E1 in this article’s Online Repository). However, we were not able to demonstrate a statistically significant effect of emollient on the rate of allergic sensitization based on level of IgE antibody against egg white (0.34 kU/L CAP-FEIA equivalents); the proportions of infants who were sensitized by allergen were similar in the intervention and control groups (Table II18 and see Fig E2 in this article’s Online Repository).

The intervention group had significantly higher levels of stratum corneum hydration in the lower leg at weeks 12 and 24 compared with those seen in the control group (see Fig E3 in this article’s Online Repository). In both groups 6.1% of infants (7/115 cases measured) had positive test results for S aureus in cheek samples at birth, and 22.4% had positive test results (19/85 cases measured) at week 12. There was no significant difference between percentages of infants with positive test results for S aureus in the intervention (26.0%)
TABLE II. Allergic sensitization at week 32

<table>
<thead>
<tr>
<th>Level of specific IgE</th>
<th>Intervention group (n = 48)</th>
<th>Control group (n = 44)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg white (kU/L)*</td>
<td>0.35</td>
<td>42% (20/48)</td>
<td>45% (20/44)</td>
</tr>
<tr>
<td></td>
<td>≥0.70</td>
<td>38% (18/48)</td>
<td>45% (20/44)</td>
</tr>
<tr>
<td>Ovomucoid (kU/L)*</td>
<td>≥0.35</td>
<td>19% (9/48)</td>
<td>6.8% (3/44)</td>
</tr>
<tr>
<td></td>
<td>≥0.70</td>
<td>13% (6/48)</td>
<td>4.5% (2/44)</td>
</tr>
</tbody>
</table>

*We converted the levels of specific IgE (binding unit of IgE [BUe/mL]) measured with a DLC chip into CAP-FEIA equivalents (kU A/L) based on a previously described method. 22 Cutoff values for allergic sensitization were set at 0.35 or greater or 0.7 or greater.
†The χ² test was used to calculate the difference between the 2 study groups.

TABLE III. Numbers of Infants with AD/eczema and allergic sensitization at week 32

<table>
<thead>
<tr>
<th>Level of specific IgE</th>
<th>With AD/eczema (n = 43)</th>
<th>Without AD/eczema (n = 49)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg white (kU/L)*</td>
<td>≥0.35</td>
<td>56% (24/43)</td>
<td>33% (16/49)</td>
</tr>
<tr>
<td></td>
<td>≥0.70</td>
<td>56% (24/43)</td>
<td>29% (14/49)</td>
</tr>
<tr>
<td>Ovomucoid (kU/L)*</td>
<td>≥0.35</td>
<td>19% (8/43)</td>
<td>8.2% (4/49)</td>
</tr>
<tr>
<td></td>
<td>≥0.70</td>
<td>12% (5/43)</td>
<td>6.1% (3/43)</td>
</tr>
</tbody>
</table>

*We converted the levels of specific IgE (binding unit of IgE [BUe/mL]) measured with a DLC chip into CAP-FEIA equivalents (kU A/L) based on a previously described method. 22 Cutoff values for allergic sensitization were set at 0.35 or greater or 0.7 or greater.
†The χ² test was used to calculate the difference between the 2 study groups.

TABLE IV. Allergic sensitization based on cutoff levels of IgE specific for egg white at week 32

<table>
<thead>
<tr>
<th>Cutoff values for specific IgE for egg white</th>
<th>Skin lesion (+) vs others, OR (95% CI)</th>
<th>AD/eczema (+) vs others, OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLC chip [(BUe/mL)*]</td>
<td>CAP-FEIA (kU/L)*</td>
<td></td>
</tr>
<tr>
<td>843.4 6.09 4.00 (1.44-11.1) 3.39 (1.35-8.49)</td>
<td>182.2 0.34 3.24 (1.32-7.96) 2.61 (1.12-6.08)</td>
<td></td>
</tr>
<tr>
<td>607.0 3.93 3.84 (1.44-10.2) 2.94 (1.22-7.12)</td>
<td>167.8 0.31 2.90 (1.21-6.93) 2.63 (1.13-6.12)</td>
<td></td>
</tr>
<tr>
<td>540.3 2.18 3.76 (1.46-9.67) 2.62 (1.11-6.20)</td>
<td>151.6 0.28 2.57 (1.08-6.08) 2.41 (1.04-5.59)</td>
<td></td>
</tr>
<tr>
<td>474.8 2.18 3.76 (1.46-9.67) 2.62 (1.11-6.20)</td>
<td>142.5 1.21 4.04 (1.57-10.4) 2.88 (1.21-6.81)</td>
<td></td>
</tr>
<tr>
<td>412.5 1.11 4.00 (1.57-10.4) 2.88 (1.21-6.81)</td>
<td>137.2 0.32 2.84 (1.17-6.85) 2.38 (1.02-5.52)</td>
<td></td>
</tr>
<tr>
<td>361.7 0.66 3.73 (1.49-9.36) 2.86 (1.22-6.73)</td>
<td>130.6 0.32 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
<tr>
<td>364.4 0.67 4.35 (1.69-11.2) 3.16 (1.33-7.49)</td>
<td>127.3 0.32 2.08 (1.17-6.85) 2.61 (1.12-6.08)</td>
<td></td>
</tr>
<tr>
<td>407.2 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>120.6 0.32 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
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<tr>
<td>434.2 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>117.8 0.32 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
<tr>
<td>500.6 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>115.1 0.28 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
<tr>
<td>540.3 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>112.8 0.28 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
<tr>
<td>574.2 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>109.6 0.28 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
<tr>
<td>607.0 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>106.8 0.28 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
<tr>
<td>640.8 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>104.0 0.28 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
<tr>
<td>674.8 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>101.2 0.28 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
<tr>
<td>704.8 0.32 3.05 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td>98.4 0.28 2.39 (1.36-7.97) 2.88 (1.23-6.72)</td>
<td></td>
</tr>
</tbody>
</table>

We have shown in this and previous studies that measurements of IgE by using a DLC chip correlate with those determined by using CAP-FEIA (see Fig E1). To prove the accuracy of CAP-FEIA equivalents measured by using a DLC chip in allergic sensitization, we calculated ORs for allergic sensitization using 25 different cutoff levels, ranging from 0.1 to 8.0 kU A/L (Table IV). We found that ORs for allergic sensitization were greater for infants with AD/eczema than those without AD/eczema and for infants with compared with those without skin lesions when cutoff values were set at 25 different levels.

We detected loss-of-function mutations in FLG in 6 of the 57 DNA samples from infants. We were not able to demonstrate whether development of AD/eczema correlates with the presence of mutations, probably because of the small sample size (data not shown).

DISCUSSION

In a prospective RCT we investigated whether protection of the skin barrier with an emollient during the first 32 weeks of life prevents AD/eczema development in infants. A previous uncontrolled pilot study investigated whether a moisturizer can prevent AD,26 but to our knowledge, this is the first RCT to investigate this question.

This trial was performed at only the NCCHD, mainly because of its logistic support. We tested the effects of an emulsion-type moisturizer (2e [Douhet] emulsion) because it is widely used, including for infants, and its composition has been disclosed. Studies to investigate the effects of other moisturizers on other populations are needed to support our findings.
One limitation of our study involves the diagnosis of AD. Worldwide and most validated criteria for the diagnosis of AD did not define the time frame of signs and symptoms, resulting in its inability in diagnosis for early onset of AD in infancy. For this trial, we made the diagnosis of AD/eczema based on modified criteria proposed by Simpson et al. We confirmed that levels of anti–egg white IgE compared with infants without AD/eczema. Further-more, we found infants with skin lesions to have a more than 3-fold greater risk for allergic sensitization than infants without skin lesions based on 20 of 25 different cutoff points (range, 0.1-8.0 kU/L CAP-FEIA equivalents). Collectively, these findings indicate that the presence of eczematous skin, rather than a lack of emollient use, induces or promotes sensitization to allergens, such as egg white, during the first 8 months of life. The mechanisms of this process are unclear. Levels of tight junction proteins (eg, claudin-1) between epidermal cells are significantly decreased in patients with AD compared with those seen in nonatopic subjects. Also, Langerhans cells were reported to elongate their dendrites, penetrate keratinocyte tight junctions, and take up antigens when the Langerhans cells were activated by means of tape stripping. These results could provide information on how eczematous skin promotes allergen sensitization.

Future directions

Findings from our RCT support our hypothesis that daily application of a moisturizer would prevent development of AD/eczema during the first 32 weeks of life. Contrary to our hypothesis, however, allergen sensitization, as assessed on the basis of acquisition of anti–egg white IgE, was not affected by application of the emollient. Our post hoc analysis revealed that the incidence of allergic sensitization was significantly increased among infants with skin lesions, including those caused by AD/eczema, compared with that seen in infants without these lesions. However, studies of a larger number of subjects might find that moisturizer use reduces allergic sensitization by preventing development of AD/eczema. In this post hoc analysis skin rash that did not fulfill the present criteria for AD/eczema, such as a lack of pruritus, was proposed to contribute to allergen sensitization. Allergic sensitization sometimes precedes and predicts the development of eczema, and we have described the presence of low-affinity IgE against food antigens in blood and cord blood samples from newborns. Therefore further studies should examine whether sensitization might occur through the placenta or neonatal gastrointestinal tract. It will be interesting to examine the temporal sequence of allergic sensitization, especially of epicutaneous sensitization to food antigens, by separately measuring levels of low-affinity and ordinary IgEs against food antigens.

We thank Professor Emiko Noguchi for providing information regarding the primer design for FLG mutations. We also thank Ms Kazuko Hayase and Ms Akiko Maruta of the NCCHD for their excellent assistance.

**Primary prevention of allergic sensitization**

Several cohort studies revealed that early-onset eczema increases the risk for allergic diseases, such as asthma, allergic rhinitis, and food allergy. The presence of AD was the main skin-related risk factor for food allergen sensitization in young infants. We confirmed that levels of anti–egg white and anti-ovomucoid IgEs measured by using a DLC chip correlate with those from CAP-FEIA. IgE-mediated egg allergy is one of the most common forms of food allergy; IgE against egg white is often used as a marker of atopy in infants. In our study we were not able to show the significant effect of emollient on the prevention of allergic sensitization based on the level of IgE antibody against egg white; similar proportions of infants were sensitized in the intervention and control groups. However, we showed that a higher proportion of infants with AD/eczema had allergic sensitization based on serum concentrations of anti–egg white IgE compared with infants without AD/eczema. Furthermore, we found infants with skin lesions to have a more than 3-fold greater risk for allergic sensitization than infants without skin lesions based on 20 of 25 different cutoff points (range, 0.1-8.0 kU/L CAP-FEIA equivalents). Collectively, these findings indicate that the presence of eczematous skin, rather than a lack of emollient use, induces or promotes sensitization to allergens, such as egg white, during the first 8 months of life.

The mechanisms of this process are unclear. Levels of tight junction proteins (eg, claudin-1) between epidermal cells are significantly decreased in patients with AD compared with those seen in nonatopic subjects. Also, Langerhans cells were reported to elongate their dendrites, penetrate keratinocyte tight junctions, and take up antigens when the Langerhans cells were activated by means of tape stripping. These results could provide information on how eczematous skin promotes allergen sensitization.

**Clinical implications:** Daily application of emollient reduces the risk of AD/eczema by 32 weeks. We might be able to reduce the prevalence of allergic sensitization by preventing the development of AD/eczema.

**REFERENCES**


METHODS
Interventions, randomization, and blinding

The research pediatricians (K.H. and K.M.) at the Division of Allergy of the NCCHD enrolled participants who met our criteria. Randomization of neonates into 2 groups was performed by means of random permuted blocks of size 4 at the Clinical Research Center of the NCCHD. The effect of intervention was evaluated as the cumulative incidence of AD/eczema, as registered at the UMIN-CTR. Dermatologists in the Division of Dermatology of the NCCHD examined the infants at scheduled visits in an investigator-blinded manner. The list of randomization was kept at the Clinical Research Center of the NCCHD until the end of the study to maintain the blinded state of the investigators.

The emollient used was an emulsion-type moisturizer, 2e emulsion, which was purchased from Shiseido. It was selected because it is commercially available and in widespread use in Japan, including for patients with AD and infants, and its composition has been disclosed. It contains glycerin, silyl, butylene glycol, behenyl alcohol, betyl alcohol, hydrogenated polydecene, dimethicone, squalane, pentaerythrityl tetraethoxysilane, Simmondsia chinesis (JOJOBA) seed oil. PEG-60 glyceril stearate, PEG-5 glyceryl isostearate, carbomer, potassium hydroxide, sodium metaphosphate, phenoxyethanol, tlcpherol, and water (see also http://2e.shiseido.co.jp/products/emulsion.html) but not preservatives or mineral oils. The moisturizer was applied at least once daily to the whole body surface of infants in the intervention group. The participating families in both groups were routinely given a 20-g bottle of petroleum jelly at birth. As recommended by the IRB, we permitted all the families to use the petroleum jelly when they believed it necessary. They recorded the amounts of emulsion-type moisturizer and petroleum used each day. The families also kept a daily diary regarding their infants’ skin condition (rash, erythema, itch, or scratch) and the areas to which the moisturizers were applied. We instructed the parents/caregivers to use commercially available soap with mild cleansing potency for their baby’s bathing. Parents were instructed to bath their babies at least once a day. These instructions were just based on local customs. Blood samples (200 μL) were collected from each infant at weeks 1 (birth), 12, and 32. Swab samples to determine skin colonization were collected at weeks 1, 4, 12, and 32. Physical condition and skin barrier functions, such as the stratum corneum water concentration, were also evaluated at weeks 1, 4, 12, and 32.

Sample size

The sample size was calculated based on the preliminary results of our unpublished cohort study at the NCCHD. In that study infants at 6 to 8 months of age had a 47% cumulative prevalence of eczema, which was based on a modification of the questionnaire described in the International Study of Asthma and Allergies in Childhood report. Our experience shows that the rate of eczema assessed by using the modified International Study of Asthma and Allergies in Childhood questionnaire is always considerably higher than the rate measured with a DLC chip method to measure allergen-specific IgE antibodies because it requires less than 2 to 5 μL of blood, although we sometimes measured the same allergen-specific IgE antibodies using the ImmunoCAP solid-phase IgE assay (CAP-FEIA; Thermo Scientific, Uppsala, Sweden) when the blood sample volume was sufficient. The DLC chip, but not CAP-FEIA, can detect low-affinity IgE antibodies that are present in fetuses and neonates. IgE antibody levels measured with a DLC chip correlate well with those determined by using CAP-FEIA when adult samples are used, and we confirmed this correlation by using our own neonatal samples when we had a sufficient blood volume to test. We successfully measured 3 allergen-specific IgE antibodies (to egg white, ovo-mucoid, and milk) using both a DLC chip and CAP-FEIA methods (Fig E1). For anti-milk antibody, correlation between the values obtained by using the 2 methods was not sufficiently high, suggesting the presence of low-affinity IgE antibodies. As a consequence, levels of anti-egg white and anti-ovomucoid IgE antibodies measured with a DLC chip correlated significantly with those determined by using CAP-FEIA and were used in further analyses. We were not able to validate the correlation between IgE antibodies detected with the DLC chip and those detected with CAP-FEIA in our samples at 1 and 12 weeks.

FLG mutation analysis

The representative FLG mutation sites found in the Japanese population were detected by using the primer sets described below. The p.R501*, p.S2889*, and p.S3296* mutations were screened by using TaqMan analysis (Life Technologies, Thermo Fisher Scientific, Waltham, Mass), as described previously. The following mutations were screened for by using TaqMan analysis with newly developed primers and probes. The c.3321delA mutation was screened with 2 primers (5’-TGATAGTGGAGGACATTCAAGGA-3’ and 5’-TTCATGAGTGCTACCTGGTAGAT-3’) and 2 probes (5’-VIC-ACCTCCCCCTGACAG-MGB-3’ and 5’-FAM-ACCTCCCCCTGACAG-MGB-3’). The p.Q1701* mutation was screened with 2 primers (5’-AGCA GACAGGCTCCACAGACT-3’ and 5’-CTGTTGCTGCTACTCTTCTCAGG-3’) and 2 probes (5’-VIC-CAGACAGGCTCCACAGACT-3’ and 5’-FAM-ACCTCCCCCTGACAG-MGB-3’). The p.S2554* mutation was screened with 2 primers (5’-GCAAGACAGCAGAGAAGACC-3’ and 5’-CTGATGGCTGGTGTTCTGCTG-3’) and 2 probes (5’-VIC-CAGACAGGCTCCACAGACT-3’ and 5’-FAM-ACCTCCCCCTGACAG-MGB-3’). The p.K4022* mutation was screened with 2 primers (5’-CCCTTGGTAAGAAGATCATC-MGB-3’ and 5’-FAM-CGTGGTGGTAAAGATCATC-MGB-3’) and 2 probes (5’-CTGGTGGTGGTAAAGATCATC-MGB-3’ and 5’-FAM-CGTGGTGGTAAAGATCATC-MGB-3’).

Bacterial culture of Staphylococcus aureus

Bacteria on the swabs obtained from the cheeks of infants were inoculated onto No. 110 Staphylococcus species–selective agar plates (Nissui Pharmaceutical, Tokyo, Japan) and cultured. Each bacterial colony was examined regarding the expression of femA and femB genes to confirm the presence of S. aureus.
REFERENCES


FIG E1. Correlation of allergen-specific IgE values determined by using a DLC chip system and CAP-FEIA. The values of anti-egg white (A), anti-ovomucoid (B), and anti-milk (C) IgE antibodies derived from 72, 48, and 29 infants, respectively, could be determined by using both the CAP-FEIA and DLC chip methods, and the correlations between these values obtained from the same samples were tested by means of linear regression analysis. BUe, Binding unit of IgE.
FIG E2. Allergic sensitization at weeks 12 and 32: comparison between the intervention and control groups. The serum levels of egg white–specific IgE (binding unit of IgE [BUe]/mL) in infants at weeks 12 and 32 were measured with a DLC chip and converted into CAP-FEIA equivalents (kUA/L) by using a previously described method. Note that high correlation between these 2 data sets with the present samples was confirmed only at week 32. The values obtained from AD/eczema-positive infants are shown in warm colors, and those from AD/eczema-negative infants are shown in cold colors.
FIG E3. Stratum corneum hydration (SCH) change in the lower leg (A) and forehead (B) in each group. SCH values (relative impedance) on the outside of the lower leg (Fig E3, A) and forehead (Fig E3, B) were shown at baseline (week 0) and at 4, 12, 24, and 32 weeks of age. Symbols (circles and triangles) and bars stand for means and SDs. SCH values were significantly higher for the lower leg in the intervention group at 12 weeks of age compared with those in the control group (P < .05, ANOVA).
FIG E4. Allergic sensitization at weeks 12 and 32. Serum levels of egg white–specific IgE (binding unit of IgE [BUe]/mL) in infants at weeks 12 and 32 were measured with a DLC chip and converted into CAP-FEIA equivalents (kUA/L). 

A. The AD/eczema-positive group had a higher proportion of infants sensitized with egg white at 0.35 kUA/L CAP-FEIA equivalents at week 32 compared with the other group ($P = 0.043$).

B. The skin lesion–positive group had a higher proportion of infants sensitized with egg white at 0.70 kUA/L CAP-FEIA equivalent at week 12 ($P = 0.0059$) and week 32 ($P = 0.0017$) compared with the other group.