IgE Hyperproduction Through Enhanced Tyrosine Phosphorylation of Janus Kinase 3 in NC/Nga Mice, a Model for Human Atopic Dermatitis

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IgE Hyperproduction Through Enhanced Tyrosine Phosphorylation of Janus Kinase 3 in NC/Nga Mice, a Model for Human Atopic Dermatitis

Masahiro Matsumoto,*‡ Chisei Ra,‡ Keiko Kawamoto,*‡ Hiroaki Sato,* Atsuko Itakura,* Junko Sawada,* Hiroko Ushio,* Hajime Suto,§ Kouichi Mitsuishi,§ Yoshiaki Hikasa,¶ and Hiroshi Matsuda*‡

IgE hyperproduction frequently observed in patients with atopic dermatitis (AD) may greatly contribute to the pathogenesis of AD, but its mechanisms are still unclear. NC/Nga mice raised in nonsterile circumstances spontaneously suffered from AD-like skin lesions with elevation of plasma IgE levels. We investigated mechanisms of the IgE hyperproduction in NC/Nga mice. Splenic T cells from SPF NC/Nga mice had a level of CD40 ligand (CD40L) expression comparable to that of BALB/c mice. Although there was no difference in the expression of CD40 on B cells between NC/Nga and BALB/c mice, B cells of NC/Nga mice produced much more IgE in the presence of soluble CD40L and IL-4. The stimulation with CD40L and/or IL-4 resulted in tyrosine phosphorylation of Janus kinase 3 (JAK3) in B cells, which was more strongly inducible in NC/Nga mice than in BALB/c mice. In B cells isolated from PBMC of AD patients with high serum IgE levels, JAK3 was constitutively phosphorylated at the tyrosine residue, and its phosphorylation was enhanced by the treatment with CD40L and/or IL-4 as was that in splenic B cells of NC/Nga mice with dermatitis and high IgE levels. Thus, it is suggested that constitutive and enhanced JAK3 phosphorylation in B cells highly sensitive to CD40L and IL-4 may be attributable to IgE hyperproduction in NC/Nga mice and patients with AD. The Journal of Immunology, 1999, 162: 1056–1063.

A topic dermatitis (AD) is a chronic and relapsing inflammatory skin disease with immunological disturbance, and its incidence is increasing in infants and children (1, 2). Patients with AD have been reported to have typical eczema with pruritus, most frequently accompanied by increased levels of total and/or specific IgE in the peripheral blood, and a personal or familial history of other atopic diseases (1, 2). In addition, immunological disturbance has been noticed in patients with AD, such as overexpression of IL-3, IL-4, IL-5, IL-10, granulocyte-macrophage CSF in the affected skin (3, 4). Defective IFN-γ production of T cells (5–7), IgE hyperproduction of B cells (8, 9), and defective IL-12-induced IFN-γ production may be caused by hyperproduction of IL-4 and IL-10 (10).

Since AD is frequently associated with elevated levels of serum IgE against many kinds of inhaled, ingested, and/or epidermally encroached allergens (2), a possible involvement of IgE Ab in the development of AD has been speculated. However, the mechanisms of IgE hyperproduction and the pathogenesis of AD have not been clarified.

NC/Nga mice were established as an inbred strain by Dr. K. Kondo in 1957 based on Japanese fancy mice (Nishiki-Nezumi) and has been reported to have some biological characteristics: liver and kidney esterase like a DBA/2 strain, high susceptibility to x-irradiation, and high susceptibility to anaphylactic shock from OVA (11, 12). Recently, we have demonstrated that inbred NC/Nga mice are available as an animal model for human AD (13). When NC/Nga mice were raised in air-uncontrolled conventional circumstances (conventional NC/Nga mice), all the mice spontaneously suffered from AD-like skin lesions with marked elevation in plasma levels of total IgE. In contrast, NC/Nga mice grown in specific pathogen-free circumstances (SPF NC/Nga mice) showed neither clinical signs nor IgE hyperproduction. The immunohistochemical examination of the skin lesion in conventional NC/Nga mice presented typical features of the affected skin observed in patients with AD, such as an increased number of mast cells and eosinophils with marked degranulation, infiltration of numerous CD4+ T cells and macrophages, and infiltration of a few CD8+ T cells. The activated mast cells and CD4+ T cells in the skin lesion expressed a positive reaction for IL-4 and IL-5, but little or no reaction for IFN-γ. We also demonstrated a close relationship between IgE levels and the onset or progression of these skin lesions in NC/Nga mice, and a contribution of circumstances to the pathogenesis of AD-like skin lesions in NC/Nga mice as well as in human patients with AD (1, 2). Therefore, it is important for clarifying the pathogenesis of AD to understand the mechanisms of IgE hyperproduction.

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3 Abbreviations used in this paper: AD, atopic dermatitis; SPF, specific pathogen-free; CD40L, CD40 ligand; JAK3, Janus kinase 3; PE, phycoerythrin; MFI, mean fluorescent intensity; SH-PTP1C, phosphotyrosine phosphatase containing Src homology 2 domain.

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For IgE synthesis by B cells in vivo, at least two stimuli are necessary; IL-4 (14) and a cognate signal induced by the interaction of CD40 on B cells and CD40 ligand (CD40L) on activated T cells (15, 16). Defects in CD40L expression and mutations of a CD40L molecule induce X-linked severe immunodeficiency with IgM hyperproduction in humans (17–21), and the blockade of CD40/CD40L interaction with injection of anti-CD40L mAb inhibits the Ig class switch in mice (22), demonstrating that transient expression of CD40L on T cells may be indispensable for the Ig class switch to IgE synthesis in vivo. In in vitro culture systems, IL-4 (23) can induce IgE production by B cells in the presence of CD40L (24, 25), soluble CD40L (15, 26), anti-CD40 mAb (8, 27), anti-IgM Ab (15), LPS (15, 28–31), or hydrocortisone (32, 33). Namely, IL-4 is an essential factor for IgE synthesis both in vivo and in vitro. IL-4 elicits its biological effects through the specific receptor complex: the α-chain that was cloned as a high affinity receptor (34, 35) and the γ-chain that was shared with receptors for IL-2, IL-4, IL-7, IL-9, and IL-15 (36–42). Janus kinase 3 (JAK3), a member of Janus kinase family of nonreceptor protein tyrosine kinases, is involved in IL-4 signaling (43). JAK3 associates with a member of Janus kinase family of nonreceptor protein tyrosine kinases, is involved in IL-4 signaling (43). JAK3 associates with γ-chain (42, 43) and is phosphorylated in the tyrosine residue upon binding of IL-4 to the IL-4R complex on B cells (42–45). Ligation of CD40L to CD40 on B cells leads to tyrosine phosphorylation of JAK3 as well (46).

In the present study we have demonstrated that B cells isolated from NC/Nga mice possess a higher potency for IgE synthesis, probably due to a higher sensitivity to CD40L and IL-4, compared with those of BALB/c mice that are a high responder strain in IgE production (30). The higher sensitivity of B cells of NC/Nga mice to CD40L and IL-4 was suggested to be due to enhanced activation of JAK3. Moreover, B cells of conventional NC/Nga mice with AD-like skin lesions showed constitutive phosphorylation of JAK3 comparable to those of AD patients with IgE hyperproduction.

Materials and Methods

Animals

SPF NC/Nga mice (H-2d) were maintained in a filter-laminar flow enclosure in a biosecure room and were provided with autoclaved food and water ad libitum. Several mating pairs of SPF NC/Nga mice were moved to an air-uncontrolled conventional room. Skin lesions very similar to those in human AD spontaneously appeared from the age of 8 wk in all the progeny. SPF BALB/c mice were purchased from Clea Japan (Tokyo, Japan). Male mice were used at 4–12 wk of age.

Cytokines and Ab

Recombinant murine and human IL-4 were purchased from Genzyme (Cambridge, MA) and Pharmingen (San Diego, CA), respectively. Murine soluble CD40L was provided by Dr. C. R. Maliszewski (Immunex, Seattle, WA) (15). Phycoerythrin (PE)-conjugated rat anti-mouse CD4 mAb, FITC-conjugated rat anti-mouse CD45R/B220 mAb, FITC-conjugated rat anti-mouse CD90 (Thy1.2) mAb, hamster anti-mouse CD40L mAb, and mouse anti-human CD40 mAb were obtained from Pharmingen. Rat anti-mouse CD40 mAb and goat anti-rat IgG (H+L) F(ab’1)_2 conjugated with magnetic beads were provided by Serotec (Oxford, U.K.) and Miltenyi Biotec (Sunnyvale, CA), respectively. FITC-conjugated goat anti-hamster IgG and PE-conjugated goat anti-rat IgG were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA). Rabbit Ab against mouse JAK3 and human JAK3 were purchased from Upstate Biotechnology (Lake Placid, NY). Mouse anti-phosphotyrosine mAb and peroxidase-conjugated goat anti-rabbit IgG were provided by Sigma (St. Louis, MO) and BioMakor (Rehovot, Israel), respectively.

Immunization

Mice were injected s.c. with 0.2 ml of PBS containing 1 μg of OVA grade V (Sigma) conjugated with 4 mg of aluminum hydroxide (Boehringer Ingelheim, Heidelberg, Germany), and 2 wk later received an i.p. boost of 2.5 μg of OVA conjugated with 10 mg of aluminum hydroxide. Blood was collected from the retro-orbital plexus with glass capillary tubes and heparinized immediately. Plasma samples were stored at −20°C until quantitative analyses for total IgE.

Cell isolation

Thyl1.2 T cells, CD4+ T cells, and B220+ B cells were positively collected from spleens of NC/Nga and BALB/c mice by magnetic cell sorting according to the manufacturer’s instructions. Briefly, single cell suspensions were prepared from spleens in RPMI 1640 (Life Technologies, Grand Island, NY) containing 10% FCS (HyClone, Logan, UT), according to the method described previously (47). After treatment with ammonium chloride to deplete erythrocytes, spleen cells were resuspended in RPMI 1640 with 10% FCS at a concentration of 10^6 cells/ml. For isolation of CD90+ cells and B220+ cells, the cells were incubated with the specific mAb conjugated with FITC at a concentration of 25 μg/ml in PBS containing 0.5% BSA (Sigma), 5 mM EDTA, and 0.01% sodium azide (isolation buffer) for 15 min at 4°C. For CD4+ T cell isolation, before treatment with anti-CD4 mAb a T cell-rich fraction was collected from spleen cells using nylon wool columns, and then was incubated with PE-conjugated rat anti-mouse CD4 mAb at a concentration of 25 μg/ml for 15 min at 4°C. The cells treated with these primary mAb were resuspended in isolation buffer at a concentration of 10^7 cells/ml, mixed with a 1/5 dilution of goat anti-rat IgG (H+L) F(ab’1)_2 conjugated with magnetic beads, and incubated for 15 min at 4°C. Labeled cells were loaded on separation columns with a magnet, and then the column was washed with ice-cold isolation buffer. The column was taken away from the magnet, and the cells carrying the relevant surface Ag were recovered by washing the column with ice-cold isolation buffer. Specimens of individual sorted cell populations were analyzed by an EPICS flow cytometer (Coulter, Hialeah, FL); the purity of the cells was >90%. There were no significant differences in numbers of Thyl1.2 T cells, CD4+ T cells, CD8+ T cells, or B220+ B cells in spleens between BALB/c and NC/Nga mice.

Flow cytometric analysis

To examine the expression of CD40L on CD4+ T cells and CD40 on the B220+ cells, double immunofluorescence staining was performed. CD4+ T cells were stained with PMA (10 ng/ml; Sigma) and ionomycin (0.25 μM; Sigma) for 4, 6, 8, and 24 h (48, 49) in RPMI 1640 supplemented with 10% FCS, 10^−3 M 2-ME, 50 U/ml penicillin, and 50 μg/ml streptomycin at a concentration of 10^7 cells/ml in the 24-well culture plate (Nunc, Roskilde, Denmark). Freshly prepared or stimulated CD4+ T cells were harvested and incubated with hamster anti-mouse CD40L mAb (1 μg/ml) and PE-conjugated rat anti-mouse CD4 mAb (1 μg/ml) at a concentration of 10^7 cells/ml in 100 μl of PBS containing 2% FCS and 0.1% sodium azide for 1 h at 4°C. Cell preparations were then stained with FITC-conjugated goat anti-hamster IgG for 30 min at 4°C. To determine CD40 expression on the B220+ B cells, Thyl1.2- cells were stimulated with IL-4 (200 U/ml) for 24, 48, and 72 h at a concentration of 10^7 cells/ml in the 24-well culture plate. freshly prepared or stimulated B220+ B cells were treated with rat anti-mouse CD40 mAb (50 μg/ml) for 1 h at 4°C, and then were stained with PE-conjugated goat anti-rat IgG for 30 min at 4°C at a concentration of 10^7 cells/ml. After washing three times, the cells were re incubated with FITC-conjugated rat anti-mouse B220 mAb for 1 h at 4°C. The cell preparations were analyzed by a flow cytometer shortly after the double staining.

Cell culture

IgE synthesis of B cells was analyzed by an in vitro experimental system with T cell-B cell collaboration (25). CD4+ T cells activated with PMA and ionomycin (see above) were fixed in 1% paraformaldehyde for 10 min at 4°C. A total of 10^6 B220+ B cells isolated from spleens of SPF NC/Nga and BALB/c mice were cultured in 200 μl of RPMI 1640 supplemented with 10% FCS, 10^−4 M 2-ME, 50 U/ml penicillin, and 50 μg/ml streptomycin together with 2 × 10^5 fixed CD4+ T cells in the presence of 200 U/ml murine IL-4 in the 96-well flat-bottom culture plate (Nunc) for 9 days at 37°C in a humidified atmosphere flushed with 5% CO2 in air. In experiments to determine a potential for IgE production and the sensitivity to IL-4, splenic B cells were incubated with various doses of IL-4 in the presence of soluble CD40L or LPS (from Salmonella typhimurium; Sigma). The culture supernatants were collected and stored at −20°C until quantitative analyses for total IgE. To assay cytokine productivity of T cells, 2 × 10^6 Thy1.2+ T cells were incubated in 1 ml of RPMI 1640 with 5 μg/ml Con A, FCS, 2-ME, and antibiotics in the 24-well culture plate at 37°C in a humidified atmosphere flushed with 5% CO2. Three days later the culture supernatants were collected and stored at −20°C until use.
Isolation of human B cells

Human B cells were isolated from PBMC from four patients with AD and four normal healthy volunteers after obtaining their informed consents. All the patients had elevated levels of serum total IgE (7,330–12,680 U/ml), and the healthy donors had no history of allergic diseases. PBMC were separated from heparinized venous blood by density gradient centrifugation using Lymphoprep (Nycomed, Oslo, Norway). CD19+ B cells were isolated from PBMC using Dynabeads (Dynal, Oslo, Norway) according to the manufacturer’s instructions. To remove the magnetic beads from B cells, the cells with the beads were incubated with DETACHABeads (Dynal) for 10 min at room temperature. More than 98% cells were CD20 positive as assessed by flow cytometric analysis.

Measurement of total IgE and IgG1

IgE levels were measured by a sandwich ELISA using two kinds of rat anti-mouse IgE mAb (6HD5 and HMK12) according to a previously described method (50). 6HD5 mAb recognizes different epitopes of Fc fragments of IgE that are unrecognized by HMK12 mAb. Briefly, immunoplates (Maxi-sorp, Nunc) were coated with 50 μl of 6HD5 mAb (5 μg/ml) and blocked with PBS supplemented with 1% BSA. Collected samples or standard mouse IgE (SPE7, Seikagaku Kogyo, Tokyo, Japan) were added to the wells and incubated for 1 h at room temperature. After washing with PBS containing 0.05% Tween-20, 50 μl of biotinylated HMK12 mAb (1 μg/ml) was added, and the plates were incubated for 1 h, then 50 μl peroxidase-conjugated avidin (1/2000; Dakopatts, Glostrup, Denmark) was added. After incubation for 1 h, the reaction products were visualized with 0.4 mg/ml orthophenylenediamine and 0.012% H2O2. IgG1 levels were measured by a sandwich ELISA using two kinds of rat anti-mouse IgG1 mAb (PharMingen) according to the process stated above. The absorbance at 490 nm wavelength was measured by an ImmunoMini (NJ-2300, Nippori Intermed, Tokyo, Japan). The sensitivity of this assay was 2 ng/ml.

Cytokine assay

Murine IL-4 levels were measured by an ELISA kit provided by BioSource (Camarillo, CA) according to the manufacturer’s instructions.

Western blot analysis of tyrosine-phosphorylated JAK3

Tyrosine-phosphorylated JAK3 was detected as described previously (43). Briefly, 2 × 107 B220+ B cells isolated from NC/Nga and BALB/c mice were incubated with 100 ng/ml soluble CD40L and/or 200 U/ml murine anti-CD40 mAb and/or 200 U/ml human IL-4 for 15 min at 37°C. The cells were then subjected to 7.5% SDS-PAGE, and the resolved samples were transferred electrophoretically to Immobilon-P membranes (Millipore, Bedford, MA). The membranes were immunoblotted with rabbit anti-JAK3 Ab (2), we determined total IgE levels in the peripheral blood of conventional NC/Nga mice. Plasma levels of total IgE in conventional NC/Nga mice began to be increased from the age of 8 wk with aging (Fig. 2), which had a significant correlation with the onset and progression of dermatitis (13). In contrast, SPF NC/Nga and BALB/c mice showed neither elevation of IgE levels (Fig. 2)

Statistical analysis

Two-tailed Student’s t test was performed for statistical analysis of the data, and p < 0.05 was taken as the level of significance.

Results

Development of AD-like skin lesions and IgE hyperproduction in conventional NC/Nga mice

NC/Nga mice that were raised in nonsterile air-uncontrolled conventional circumstances, started to scratch their faces, necks, ears, and dorsal skins from the age of 8 wk, resulting in dermatitis that consisted of eczema, erythema, hemorrhage, superficial erosion, deep excoriation, scaling, and dryness (Fig. 1). Grades of the dermatitis became more severe with aging and reached a plateau at the age of 17 wk (13). This kind of skin lesion was also observed in human patients with AD (1, 2); histopathologic findings of the skin lesions were very similar to those for human AD (13). Since >80% of patients with AD showed elevated levels of serum IgE Ab (2), we determined total IgE levels in the peripheral blood of conventional NC/Nga mice. Plasma levels of total IgE in conventional NC/Nga mice began to be increased from the age of 8 wk with aging (Fig. 2), which had a significant correlation with the onset and progression of dermatitis (13). In contrast, SPF NC/Nga and BALB/c mice showed neither elevation of IgE levels (Fig. 2).
and IgG levels (data not shown) nor any clinical sign of dermatitis (data not shown). These findings and our previous studies suggested that IgE produced in NC/Nga mice might be involved in both the onset and the development of AD skin lesions.

Plasma levels of total IgE after immunization with OVA and aluminum hydroxide

To determine IgE production in vivo, mice were immunized with OVA conjugated with aluminum hydroxide twice. The immunization with OVA and aluminum hydroxide led to increased levels of total IgE in each strain of mouse, but the levels in SPF NC/Nga mice were threefold higher than those in BALB/c mice 2 wk after the first immunization (Table I). Two weeks after the second immunization, levels of total IgE in SPF NC/Nga mice were markedly increased compared with those in BALB/c mice.

IgE synthesis by B cells cocultured with activated CD4\(^+\) T cells

We attempted to demonstrate high productivity of B cells for IgE in an in vitro experimental system with cognate interaction between T and B cells. Splenic B220\(^+\) B cells of SPF NC/Nga and BALB/c mice were incubated with 200 U/ml IL-4 and paraformaldehyde-fixed CD4\(^+\) T cells expressing CD40L on the cell surface that had been activated with PMA and ionomycin. As shown in Table II, both B cells of NC/Nga and BALB/c mice incubated with the activated CD4\(^+\) T cells could synthesize IgE in the presence of 200 U/ml IL-4. IgE levels in culture supernatants of B cells of NC/Nga mice were higher than those in BALB/c mice in the presence of CD4\(^+\) T cells from each mouse strain. In contrast, no significant IgE production by B cells was detected in the presence of the activated CD4\(^+\) T cells alone, IL-4 alone, or nonactivated CD4\(^+\) T cells with IL-4 (data not shown). Thus, splenic B cells from SPF NC/Nga mice could produce increased IgE regardless of the source of activated T cells.

Expression of CD40L on CD4\(^+\) T cells

We examined CD40L expression on CD4\(^+\) T cells isolated from spleens of SPF NC/Nga and BALB/c mice by flow cytometric analysis using anti-CD40L mAb. Although CD40L was not detected on freshly isolated CD4\(^+\) T cells of either SPF NC/Nga or BALB/c mice, 8-h incubation with PMA and ionomycin induced CD40L expression at a maximal level, expressing very similar profiles of the fluorescence intensity observed on CD4\(^+\) T cells from both NC/Nga and BALB/c mice (Fig. 3A). After 8 h CD40

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<tr>
<th>Groups</th>
<th>Mice</th>
<th>IgE Concentrations (ng/ml)</th>
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<tbody>
<tr>
<td>I</td>
<td>BALB/c</td>
<td>60 ± 23^*</td>
</tr>
<tr>
<td>II</td>
<td>BALB/c</td>
<td>54 ± 5^*</td>
</tr>
<tr>
<td>III</td>
<td>NC/Nga</td>
<td>177 ± 24</td>
</tr>
<tr>
<td>IV</td>
<td>NC/Nga</td>
<td>258 ± 21</td>
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\(^*\) p < 0.05, when compared with group I.

\(^†\) p < 0.01, when compared with group II.
Expression of CD40 on B cells

We next examined CD40 expression on splenic B220⁺ B cells of SPF NC/Nga and BALB/c mice by flow cytometric analysis using rat anti-mouse CD40 mAb. The fluorescent intensity for CD40 on freshly isolated B cells from NC/Nga mice was comparative to that on cells from BALB/c mice (Fig. 3B). Since CD40 expression on PBMC is increased by stimulation with IL-4 (8), we investigated an expression profile of CD40 on B cells following stimulation with IL-4. When B220⁺ B cells isolated from spleens of SPF NC/Nga and BALB/c mice were stimulated with 200 U/ml IL-4 for 24 h, the mean fluorescent intensities (MFI) for CD40 on B cells from NC/Nga and BALB/c mice were 11.4 and 11.0, respectively. Additional stimulation for 24 h induced increased MFI for CD40, but there was no significant difference between the mouse strains (Fig. 3B). Thus, there was no difference in CD40 expression on splenic B cells between NC/Nga and BALB/c mice even when the cells were stimulated with IL-4.

Sensitivity of B cells to CD40L for IgE synthesis

To determine responsiveness of B cells to CD40L, splenic B220⁺ B cells were incubated with various doses of soluble CD40L (1–1000 ng/ml) and a fixed dose of IL-4 (200 U/ml) for 9 days. When 100 ng/ml CD40L was added, significant levels of IgE were detected in cultures of B cells from both groups of mice, but IgE levels in cultures of B cells from NC/Nga mice were higher than those in cultures of B cells from BALB/c mice (Fig. 4). Costimulation with 1000 ng/ml CD40L and 200 U/ml IL-4 to B cells of NC/Nga mice led to a marked increase in levels of IgE, whereas there was no significant difference in a total number of viable B cells during the incubation for 9 days between NC/Nga and BALB/c mice. Thus, significant differences in the sensitivities of splenic B cells to CD40L were observed between NC/Nga and BALB/c mice.

Sensitivity of B cells to IL-4 for IgE synthesis

To determine the sensitivities of B cells to IL-4 for IgE synthesis, B220⁺ B cells isolated from spleens of SPF NC/Nga and BALB/c mice were incubated with various doses of IL-4 (25–200 U/ml) and the fixed dose of soluble CD40L (100 ng/ml) for 9 days. As shown in Fig. 5, IL-4 induced IgE production by splenic B cells of NC/Nga and BALB/c mice in a dose-dependent manner. The minimal doses of IL-4 required for IgE synthesis by B cells of NC/Nga and BALB/c mice were 50 and 100 U/ml, respectively; B cells from NC/Nga mice produced higher levels of IgE at individual doses of IL-4 than those of BALB/c mice (Fig. 5). Since IL-4 is also able to induce IgE synthesis by B cells with LPS (14, 22–26), in the presence of 10 μg/ml LPS, B220⁺ B cells isolated from spleens of SPF NC/Nga and BALB/c mice were incubated with various doses of IL-4 (6.25–200 U/ml). IL-4 induced IgE production by B cells of NC/Nga and BALB/c mice in a dose-dependent manner (Fig. 6). IgE levels in the culture supernatants of B cells of NC/Nga mice were higher than those of BALB/c mice at each dose of IL-4, and the minimal effective doses of IL-4 for IgE synthesis by B cells of NC/Nga mice and BALB/c mice were 6.25 and 25 U/ml, respectively (Fig. 6). Thus, splenic B cells from NC/Nga mice were more sensitive to IL-4 for IgE synthesis than those from BALB/c mice.
BALB/c mice, resulting in the elevated in vitro production of IgE by B cells of NC/Nga mice.

**IgG1 synthesis by B cells cocultured with CD40L and IL-4**

CD40L and IL-4 are sufficient to induce murine B cells to switch Ig isotypes to IgG1 as well (15). Therefore, in the presence of 100 ng/ml CD40L and 200 U/ml IL-4, B cells were cultured for 9 days, and IgG1 levels in the culture supernatants were measured. Significantly high levels of IgG1 as well as IgE were detected in supernatants from splenic B cells of NC/Nga mice compared with those from cells of BALB/c mice (Table III).

**Tyrosine phosphorylation of JAK3 in B cells**

JAK3, a member of Janus kinase family of nonreceptor protein tyrosine kinase, associates with CD40 (46) and with the γ-chain that constitutes the IL-4R complex (40, 42) and mediates CD40L and IL-4 signalings through its phosphorylation in lymphoid cells (40, 43, 44, 46). Therefore, we attempted to determine phosphorylation of JAK3 in murine splenic B cells and peripheral blood B cells from patients with AD and in those from healthy nonatopic donors. No tyrosine phosphorylation of JAK3 was detectable in unstimulated B cells of spleens in SPF NC/Nga mice and BALB/c mice (Fig. 7, A and C). On the other hand, splenic B cells isolated from conventional NC/Nga mice with marked elevation of plasma IgE levels elicited constitutive tyrosine phosphorylation of JAK3. CD40L (100 ng/ml) or IL-4 (200 U/ml) increased its phosphorylation, and the additional effect was detected in B cells costimulated with CD40L and IL-4 (Fig. 7, A and C).

Addition of anti-CD40 mAb and/or 200 U/ml IL-4 led to tyrosine phosphorylation of JAK3 in B cells isolated from healthy subjects, whereas little or no positive reaction was observed in the absence of the reagents (Fig. 7, B and D). In contrast, in B cells from AD patients with elevated levels of serum IgE, JAK3 was constitutively phosphorylated at the tyrosine residue, and phosphorylation was enhanced by treatment with anti-CD40 mAb and/or IL-4 (Fig. 7, B and D), very similar to a pattern of the phosphorylation observed in splenic B cells from conventional NC/Nga mice.

**IL-4 synthesis by Thy1.2+ T cells**

IL-4 is an essential cytokine for IgE synthesis in mice. Therefore, we investigated the productivity of IL-4 by Thy1.2+ T cells. Thy1.2+ T cells isolated from spleens of SPF NC/Nga and BALB/c mice were incubated with 5 μg/ml Con A for 72 h, and IL-4 concentrations in the culture supernatants were measured by an ELISA. The stimulation with an optimal dose of Con A led to significant IL-4 production of Thy1.2+ T cells in NC/Nga and BALB/c mice, but the level of IL-4 in the former was about one-third that in the latter (Table IV).

**Discussion**

NC/Nga mice raised in conventional circumstances spontaneously suffered from AD-like skin lesions with IgE hyperproduction, and there was a close relationship between plasma total IgE levels and unstimulated B cells of spleens in SPF NC/Nga mice and BALB/c mice (Fig. 7, A and C). On the other hand, splenic B cells isolated from conventional NC/Nga mice with marked elevation of plasma IgE levels elicited constitutive tyrosine phosphorylation of JAK3. CD40L (100 ng/ml) or IL-4 (200 U/ml) increased its phosphorylation, and the additional effect was detected in B cells costimulated with CD40L and IL-4 (Fig. 7, A and C).
Table IV. IL-4 production by Thyl.2+ T cells

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<thead>
<tr>
<th>Mice</th>
<th>IL-4 concentration (pg/ml)</th>
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<tr>
<td>BALB/c</td>
<td>45.1 ± 7.7</td>
</tr>
<tr>
<td>NC/Nga</td>
<td>14.7 ± 2.3*</td>
</tr>
</tbody>
</table>

* Thyl.2+ T cells isolated from spleens of SPF NC/Nga and BALB/c mice were incubated with Con A (5 μg/ml) at a concentration of 2 × 10^6 cells/ml for 72 h. IL-4 levels in the collected medium were measured by an ELISA. Each value is expressed as the mean ± SE of four separate experiments in duplicate.

References


