



Ethnopharmacological communication

Effects of *Scutellariae radix* and *Aloe vera* gel extracts on immunoglobulin E and cytokine levels in atopic dermatitis NC/Nga miceJinkyung Kim^a, In seok Lee^a, Soojin Park^b, Ryowon Choue^{a,c,*}^a Department of Medical Nutrition, Graduate School of East-West Medical Science, Kyung Hee University, Yongin-si, Gyeonggi-do 446-701, Republic of Korea^b Department of Oriental Medical Food and Nutrition, Semyung University, 117 Semyung-ro, Jecheon, Chungbuk, Republic of Korea^c Research Institute of Clinical Nutrition, Kyung Hee University, Dongdaemoon-gu, Seoul 130-701, Republic of Korea

ARTICLE INFO

Article history:

Received 13 April 2010

Received in revised form 9 August 2010

Accepted 25 August 2010

Available online 15 September 2010

Keywords:

Atopic dermatitis

Aloe vera gel

Cytokines

IgE

Scutellariae radix

ABSTRACT

Aim of the study: The present study aimed to investigate the effects of *Scutellariae radix* (SR) and *Aloe vera* gel (AV), alone or in combination, on levels of immunoglobulin E (IgE) and inflammatory cytokines in spontaneous atopic dermatitis (AD)-like skin lesions.

Materials and methods: After spontaneous AD-like skin lesion was developed by adaptation to conventional conditions, mice were randomly assigned to control, SR (50 mg/kg, p.o.), AV (0.8 mg/kg, p.o.) and SR+AV (50 mg of SR and 0.8 mg of AV/kg, p.o.) groups, and were treated for 6 weeks.

Results: SR and SR+AV suppressed IL-5 levels compared with control, but had no effects on IgE levels ($P < 0.05$). AV increased IgE levels, but decreased both IL-5 and IL-10 compared with control ($P < 0.05$).

Conclusion: These results suggest that SR and AV modulate immunological responses in AD, mainly through influencing IL-5 or IL-10 levels.

© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease accompanied by severe itching, eczematous skin lesions and repeated cutaneous hyperactivity (Novak et al., 2003). Approximately 70–80% of AD patients present elevated blood immunoglobulin E (IgE) levels, with IgE antibodies against environmental and/or food allergens. The remaining 20–30% present low IgE levels, but develop allergic IgE-mediated sensitization later in life (Leung et al., 2004).

AD progresses through an acute pathway that produces a large number of the T-helper 2 (Th2) cytokines, IL-4, IL-5 and IL-13, and a chronic pathway in which AD results in the infiltration of inflammatory dendritic epidermal cells, macrophages and eosinophils, which produce IL-5, IL-12, and GM-CSF. Subsequently, local T-helper 1 (Th1) and interferon-gamma (IFN- γ) increase, and tissue remodeling occurs, with increased deposition of collagen and dermal thickening (Novak et al., 2003).

Scutellariae radix is name for crude drugs which is made by roots of *Scutellariae baicalensis* Georgi, Lamiaceae family. *Scutellariae radix* is officially listed in the Korea Pharmacopoeia and is

used traditionally against bacterial infections of the respiratory and gastrointestinal tract, and rheumatoid arthritis. It has been reported to anti-inflammatory and anti-oxidative activities and immune-modulating effects in various pathophysiological models (Lim, 2002; Park et al., 2004). *Aloe vera* (syn: *Aloe barbadensis* Miller) belongs to the Liliaceae family, and functions as a skin-conditioning or skin-soothing agent in cosmetic products. It ameliorates release of cytokine by bacterially induced human leukocytes (Cosmetic Ingredient Review Expert Panel, 2007).

To determine the immunomodulatory and clinical effects of *Scutellariae radix* (SR) and *Aloe vera* gel (AV) on AD animal model, we investigated the levels of IgE and various cytokines in NC/Nga mice fed extracts of the plants for 6 weeks.

2. Materials and methods

2.1. Materials

Water extract powder of SR was obtained from Sun Ten Pharmaceutical Co. (Taipei, Taiwan). Baicalin, baicalein and wogonin which were active substances of SR were 95.89 ± 0.95 mg (9.6%), 43.03 ± 0.55 mg (4.3%), 8.02 ± 0.10 mg (0.8%) per g, respectively, as a result of HPLC. AV was obtained from Namyang Co. (Chungbuk, Korea), which was ATIValue® 200:1 powder concentrated under vacuum and produced by Aloecop Inc. (USA). This material was approved by KFDA and quality is controlled as a material for health functional food which contained above 10% polysaccharide.

* Corresponding author at: Department of Medical Nutrition, Graduate School of East-West Medical Science, Kyung Hee University, Seocheon-dong, Giheung-gu, Yongin-si, Gyeonggi-do, 446-701, Republic of Korea. Tel.: +82 31 201 3779; fax: +82 31 201 3819.

E-mail address: rwcho@khu.ac.kr (R. Choue).

Table 1
Composition of AIN-93G diet.

Ingredient	g/kg diet
Cornstarch	397.486
Casein (>85% protein)	200.000
Dextrinized cornstarch (90–94% tetrasaccharides)	132.000
Sucrose	100.000
Soybean oil (no additives)	70.000
Fiber	50.000
Mineral mix (AIN-93G-MX) ^a	35.000
Vitamin mix (AIN-93-VX) ^b	10.000
L-Cystine	3.000
Choline bitartrate (41.1% choline)	2.500
tert-Butylhydroquinone (TBHQ), mg	14.0

^a Mineral mix composition (AIN-93G-MX): calcium carbonate anhydrous (40.04% Ca) 357.00 g/kg, potassium phosphate monobasic (22.76% P, 28.73% K) 196.00 g/kg, potassium citrate, tri-potassium monobasic (33.16% K) 70.78 g/kg, sodium chloride (39.34% Na, 60.66% Cl) 74.00 g/kg, potassium sulfate (44.87% K, 18.39% S) 46.60 g/kg, magnesium oxide (60.32% Mg) 24.00 g/kg, ferric citrate (16.5% Fe) 6.06 g/kg, zinc carbonate (52.14% Zn) 1.65 g/kg, sodium meta-silicate-9H₂O (9.88% Si) 1.45 g/kg, manganous carbonate (47.79% Mn) 0.63 g/kg, cupric carbonate (57.47% Cu) 0.30 g/kg, chromium potassium sulfate-12H₂O (10.42% Cr) 0.275 g/kg, boric acid (17.5% B) 0.0815 g/kg, sodium fluoride (45.24% F) 0.0635 g/kg, nickel carbonate (45% Ni) 0.0318 g/kg, lithium chloride (16.38% Li) 0.0174 g/kg, sodium selenate anhydrous (41.79% Se) 0.01025 g/kg, potassium iodate (59.3% I) 0.01 g/kg, ammonium paramolybdate-4H₂O (54.34% Mo) 0.00795 g/kg, ammonium vanadate (43.55% V) 0.0066 g/kg, powdered sucrose 221.026 g/kg.

^b Vitamin mix composition (AIN-93-VX): nicotinic acid 3.00 g/kg, calcium pantothenate 1.60 g/kg, pyridoxine-HCl 0.70 g/kg, thiamin-HCl 0.60 g/kg, riboflavin 0.60 g/kg, folic acid 0.20 g/kg, biotin 0.02 g/kg, vitamin B12 (cyanocobalamin) (0.1% in mannitol) 2.50 g/kg, vitamin E (all-*rac*- α -tocopheryl acetate; 500 IU/g) 15.00 g/kg, vitamin A (all-*trans*-retinyl-palmitate; 500,000 IU/g) 0.80 g/kg, Vitamin D₃ (cholecalciferol) (400,000 IU/g) 0.25 g/kg, Vitamin K1 (phyloquinone) 0.075 g/kg, powdered sucrose 974.655 g/kg.

2.2. Animals and diets

Five-week-old male NC/Nga mice were provided by SLC (Shizuoka, Japan). All experimental protocols were approved by the animal care and use review committee of Kyung Hee University (IACUC, protocol number: KHUASP-09-010). To induce spontaneous AD-like skin lesions, the animals were maintained under conventional laboratory conditions without air filtration as described previously (Aioi et al., 2001) at ambient temperature (24 ± 2 °C), humidity (55 ± 5%) and light (12-h light/dark cycle). They were allowed to access a non-purified pellet diet and tap water *ad libitum*. AD-like skin lesions were observed on the ears, eyes, nose, neck and legs as well as loss of hair in NC/Nga mice over 8 weeks of environmental stimuli. The mice were then fed an AIN-93G diet (Hanlive, Seoul, Korea) and water *ad libitum*, supplemented with plant extracts for 6 weeks. The composition of AIN-93G diet is shown in Table 1.

2.3. Experimental groups

A total of 48 AD-induced mice were randomly allocated into four experimental groups of twelve animals each. Control group animals received 0.2 ml of distilled water as vehicle, SR group animals received SR extract (50 mg/kg, p.o.) (Lim, 2004), AV group animals received AV extract (0.8 mg/kg, p.o.) (Langmead et al., 2004), and the SRAV group animals received both SR extract (50 mg/kg, p.o.) and AV extract (0.8 mg/kg, p.o.) for 6 weeks. Before oral supplementation, blood was drawn from the rear of jawbone by Goldenrod™ animal lancet (MEDIpoin, Inc., New York, USA). At the end of the experimental period, mice were fasted overnight, blood was collected from the retro-orbital venous plexus under mild anesthesia, and animals were euthanized. Forty-seven mice were analyzed because one SRAV group mouse was lost during blood collection.

2.4. Measurement of blood parameters

Plasma IgE levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions (Bethyl, TX, USA). Cytokine (IL-2, IFN- γ , TNF- α , IL-4, IL-5, and IL-10) levels were measured using a MILLIPLEX® mouse cytokine panel kit, following the manufacturer's instructions (Millipore, USA).

2.5. Statistical analysis

Results are presented as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison tests or Student's *t*-test ($P < 0.05$).

3. Results

3.1. Effects of *Scutellariae radix* and *Aloe vera* gel on plasma IgE levels

There were no significant intergroup differences in the spontaneous AD model mice in baseline plasma IgE levels (Table 2). In contrast, the final plasma levels of IgE in the AV group were significantly higher than those in SR group ($P < 0.05$). Mice supplemented with SR extract appeared to have the lowest plasma IgE levels among the four experimental groups. Plasma IgE levels of the SRAV group were between the SR group and the AV group with no significant differences compared to the control group.

3.2. Effects of *Scutellariae radix* and *Aloe vera* gel on cytokine release

Neither SR nor AV significantly affected serum levels of Th1 cytokines including IL-2, IFN- γ and TNF- α in NC/Nga mice (Fig. 1a). Among the Th2 cytokines, including IL-4, IL-5 and IL-10, serum levels of IL-5 were lower significantly in the supplemented groups compared to the control group ($P < 0.05$ for each, Fig. 1b). Interestingly, the IL-10 concentration was lower significantly in the AV group only compared to the control group ($P < 0.05$).

4. Discussion

This study explored the systemic efficacy of *Scutellariae radix* and *Aloe vera* gel, alone or in combination, against atopic dermatitis in NC/Nga mice. We found that human AD-like skin lesions, itching, erythema, scale, dehairing and hemorrhage, developed in the NC/Nga mice during 8 weeks of conventional laboratory conditions without air filtration. Among 51 mice, 3 showed no lesions; 24 showed mild inflammation with some scales, small areas of bleeding and little dehairing; and 24 spontaneously showed severe inflammation with severe hemorrhage and dehairing.

Human AD patients are characterized by increased levels of blood IgE (Jin et al., 2009). Most studies have demonstrated a significant correlation between disease severity and blood IgE levels (Jin et al., 2009; Chen et al., 2005), although one recent study reported no association (Leung et al., 2004). NC/Nga mice are known to display mutations on chromosome 9, which is linked to increase IgE production, as well as increased Th2 response (Matsumoto et al., 1999; Yagi et al., 2002). Subsequent cross-linking of IgE with allergens triggers the release of histamine and other inflammatory mediators. Therefore, considerable attention has been given to the regulation of IgE antibody synthesis.

In this study we found an increase in IgE in the mice, similar to Matsuda (Matsuda et al., 1997). SR extract suppressed IgE release (−16.4%) compared to initial IgE levels, although the dif-

Table 2Effect of *Scutellariae radix* and *Aloe vera* gel on the IgE production in NC/Nga mice.

	Control	SR	AV	SRAV
Initial (ng/ml)	130.6 ± 69.51	137.6 ± 45.42	134.8 ± 23.36	137.6 ± 16.23
Final (ng/ml)	136.2 ± 46.40 ^{ab}	115.1 ± 15.89 ^b	167.6 ± 41.18 ^a	138.4 ± 38.76 ^{ab}

Data are means (g) ± S.D. of each group.

^{a–c}Values without the same superscript letter are significantly different at $P < 0.05$.

ference was not statistically significant. We cannot compare this result to other studies because of an absence of known research on effects of SR on AD. Lim (2004) documented that wogonin from SR extracts inhibited IgE production in Con-A-induced MLN lymphocytes or DS-induced colitis. In our study, the IgE levels in the AV group were higher than that of the SR, and the IgE levels of the SRAV group were between the SR and AV groups, therefore it can be speculated that SR extract supplements reversed the effects of AV on IgE release.

T-helper cells can be divided into subgroups with distinct regulatory and influencing functions based on their cytokine profiles. Th1 lymphocytes express IFN- γ , IL-2 and TNF- α , while Th2 lymphocytes produce IL-4, IL-5 and IL-10. This cytokine release is important for the initiation, regulation, and perpetuation of inflammation in AD. Acute AD skin lesions exhibit Th2-dominant inflammation. Specifically, the Th2 cytokines IL-4 and IL-5 and the Th1 cytokine IFN- γ play important roles in the inflammation and hypertrophy of the skin in AD.

In this study, SR administration selectively affected IL-5 release, and AV strongly affected both the release of IL-5 and IL-10. Other

cytokines released from Th1 and Th2 cells were not significantly affected by the administration of plant extracts. IL-10 plays an important role in the Th2 response to antigen and in the development of skin eosinophilia in the AD murine model (Laouini et al., 2003). IL-10 is released by keratinocytes after mechanical injury might promote the Th2 response to EC sensitization by polarizing skin dendritic cells to support Th2 differentiation (Jin et al., 2009).

The AV-derived material has fungicidal, antimicrobial and antiviral activities, and has been shown to be effective in wound healing and infectious skin disease treatment in animals (Choi and Chung, 2003). Recently, the inner gel component of AV was shown to suppress bacterially induced pro-inflammatory cytokines, TNF- α and IL-1b in human leukocytes (Habeb et al., 2007). One case report presented a study in which individuals with acute eczema and dermatitis received AV topically (Vazquez et al., 1996), but oral application of AV for AD has yet not been reported.

We hypothesized that oral administration of AV extract might improve significantly the symptoms of AD, and that dual administration of AV and SR might enhance the response. However, the

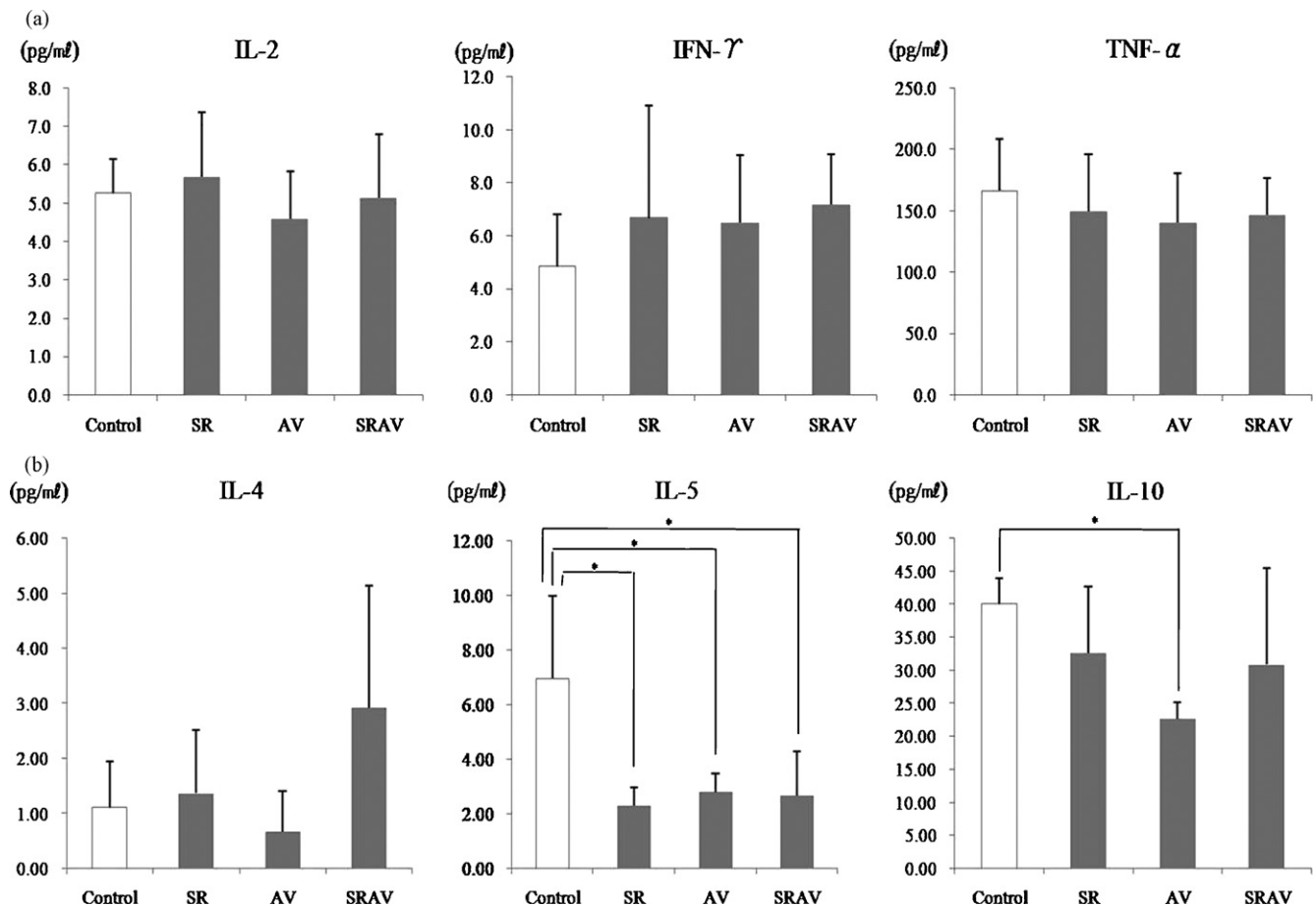


Fig. 1. Effect of *Scutellariae radix* and *Aloe vera* gel on the levels of serum cytokine in NC/Nga mice. (a) Th1 cytokines: data are means (g) ± S.D. of each group ($n = 6$). All groups are not significantly different compared with control group at $P < 0.05$. (b) Th2 cytokines: data are means ± S.D. of each group ($n = 6$). $P < 0.05$, significantly different from the control group.

combined application of SR and AV did not show synergistic effects on the release of various cytokines in the AD animal model.

In conclusion, supplements SR and AV extracts might act independently as potent immunologic modulators in a spontaneous AD animal model, mainly through inhibition of IL-5 and IL-10. The underlying mechanisms should be explored to determine if SR and AV extracts are promising nutraceutical ingredients for improving clinical and immune responses in AD patients.

Acknowledgments

This work was supported by a grant from the Kyung Hee University (20040052). The authors gratefully acknowledge Namyang, for generously providing the *Aloe vera* gel extract.

References

- Aioi, A., Tonogaito, H., Suto, H., Hamada, K., Ra, C., Ogawa, H., Maibach, H., Matsuda, H., 2001. Impairment of skin barrier function in NC/Nga Tnd mice as a possible model for atopic dermatitis. *British Journal of Dermatology* 144, 12–18.
- Chen, L., Lin, S.X., Overbergh, L., Mathieu, C., Chan, L.S., 2005. The disease progression in the keratin14 IL-4-transgenic mouse model of atopic dermatitis parallels the up-regulation of B cell activation molecules, proliferation and surface and serum IgE. *Clinical & Experimental Immunology* 142, 21–30.
- Choi, S., Chung, M., 2003. A review on the relationship between *Aloe vera* components and their biologic effects. *Seminars in Integrative Medicine* 1, 53–62.
- Cosmetic Ingredient Review Expert Panel, 2007. Final report on the safety assessment of *Aloe andongensis* extract, *Aloe andongensis* leaf juice, *Aloe arborescens* leaf extract, *Aloe arborescens* leaf juice, *Aloe arborescens* leaf protoplasts, *Aloe barbadensis* flower extract, *Aloe barbadensis* leaf, *Aloe barbadensis* leaf extract, *Aloe barbadensis* leaf juice, *Aloe barbadensis* leaf polysaccharides, *Aloe barbadensis* leaf water, *Aloe ferox* leaf extract, *Aloe ferox* leaf juice, and *Aloe ferox* leaf juice extract. *International Journal of Toxicology* 26, 1–50.
- Habeeb, F., Stables, G., Bradbury, F., Nong, S., Cameron, P., Plevin, R., Ferro, V.A., 2007. The inner gel component of *Aloe vera* suppresses bacterial-induced pro-inflammatory cytokines from human immune cells. *Methods* 42, 388–393.
- Jin, H., He, R., Oyoshi, M., Geha, R.S., 2009. Animal models of atopic dermatitis. *Journal of Investigative Dermatology* 29, 31–40.
- Langmead, L., Feakins, R.M., Goldthorpe, S., Holt, H., Tsironi, E., De Silva, A., Jewell, D.P., Rampton, D.S., 2004. Randomized, double-blind, placebo-controlled trial of oral *Aloe vera* gel for active ulcerative colitis. *Alimentary Pharmacology & Therapeutics* 19, 739–747.
- Laouini, D., Alenius, H., Bryce, P., Oettgen, H., Tsitsikov, E., Geha, R.S., 2003. IL-10 is critical for Th2 responses in a murine model of allergic dermatitis. *The Journal of Clinical Investigation* 112, 1058–1066.
- Leung, D.Y., Boguniewicz, M., Howell, M.D., Nomura, I., Hamid, Q.A., 2004. New insights into atopic dermatitis. *The Journal of Clinical Investigation* 113, 651–657.
- Lim, B.O., 2002. Effect of ganhuangenin obtained from *Scutellaria radix* on the chemical mediator production of peritoneal exudate cells and immunoglobulin E level of mesenteric lymph node lymphocytes in Sprague–Dawley rats. *Phytotherapy Research* 16, 166–170.
- Lim, B.O., 2004. Efficacy of wogonin in the production of immunoglobulins and cytokines by mesenteric lymph node lymphocytes in mouse colitis induced with dextran sulfate sodium. *Bioscience, Biotechnology, and Biochemistry* 68, 2505–2511.
- Matsuda, H., Watanabe, N., Geba, G.P., Sperl, J., Tsudzuki, M., Hiroi, J., Matsumoto, M., Ushio, H., Saito, S., Askenase, P.W., Ra, C., 1997. Development of atopic dermatitis-like skin lesion with IgE hyperproduction NC/Nga mice. *International Immunology* 9, 3461–3488.
- Matsumoto, M., Ra, C., Kawamoto, K., Sato, H., Itakura, A., Sawada, J., Ushio, H., Suto, H., Mitsuishi, K., Hikasa, Y., Matsuda, H., 1999. IgE hyperproduction through enhanced tyrosine phosphorylation of Janus kinase 3 in NC/Nga mice, a model for human atopic dermatitis. *The Journal of Immunology* 162, 1056–1063.
- Novak, N., Bieber, T., Leung, D.Y., 2003. Immune mechanisms leading to atopic dermatitis. *Journal of Allergy and Clinical Immunology* 112, S128–S134.
- Park, S., Hahm, K.B., Oh, T.Y., Jin, J.H., Choue, R., 2004. Preventive effect of the flavonoid, wogonin, against ethanol-induced gastric mucosal damage in rats. *Digestive Diseases and Sciences* 49, 384–394.
- Vazquez, B., Avila, G., Segura, D., Escalante, B., 1996. Antiinflammatory activity of extracts from *Aloe vera* gel. *Journal of Ethnopharmacology* 55, 69–75.
- Yagi, R., Nagai, H., Iigo, Y., Akimoto, T., Arai, T., Kubo, M., 2002. Development of atopic dermatitis-like skin lesions in STAT6-deficient NC/Nga mice. *The Journal of Immunology* 168, 2020–2027.