INTERNATIONAL JOURNAL OF IMMUNOPATHOLOGY AND PHARMACOLOGY Vol. 20, no. 1, 59-67 (2007)

NUCLEAR FACTOR κB ACTIVITY IS INCREASED IN PERIPHERAL BLOOD MONONUCLEAR CELLS OF CHILDREN AFFECTED BY ATOPIC AND NON-ATOPIC ECZEMA

F. ANGELINI[1], G. DI MATTEO[1], S. BALESTRERO[1], E. BRUNETTI[2], G. MANCINO[2] P. ROSSI[1,3] and E. GALLI[2]

[1]Department of Pediatrics, Tor Vergata University, Rome; [2]San Pietro Research Center Fatebenefratelli Hospital, AFaR, Rome; [3]Division of Immunology and Infectious diseases, Bambino Gesù Children's Hospital, Rome, Italy

Received June 26, 2006 – Accepted November 28, 2006

Atopic and non-atopic eczema is an inflammatory cutaneous disease which is common in childhood and is associated with a dysregulation of the immune system. Many genes encoding immune receptors, cytokines, chemokines, chemokine receptors, and adhesion molecules involved in the development of the disease are under the control of transcription factors belonging to the nuclear factor (NF)-κB family. To investigate the role of NF-κB in the development of eczema, 20 children, affected by relapsing chronic eczema, were enrolled in this study. Eleven of the 20 children showed IgE immunoreactivity and had a positive prick test. The DNA binding activity of NF-κB in nuclear extracts of the patients' peripheral blood mononuclear cells (PBMC) was examined by electrophoretic mobility shift assay. We found that basal NF-κB-DNA binding activity in PBMC was significantly higher in the eczema patient group in comparison with the same parameter in the healthy age-matched control group. Moreover, we observed a significant correlation between NF-κB-DNA binding activity and patients' clinical score (SCORAD). Based on these observations we speculate that NF-κB can play an important role in the immunopathogenesis of eczema and therefore could be considered as a potential therapeutic target.

Eczema, atopic and non-atopic (1), is a chronic relapsing inflammatory skin syndrome characterized by typically distributed skin lesions (2-5). This condition is one of the most common skin disorders found in infants and children and its onset occurs in 45% of affected individuals during the first 6 months of life, in 60% during the first year, and in at least 85% before 5 years of age.

A combination of genetic, skin barrier, immunologic factors and triggering events, such as environmental, pharmacologic, psychological factors and infections, is considered responsible for the development and the severity of eczema. In particular, a large body of experimental data indicates that the most relevant factor in the pathogenesis of the disease is a dysregulation of the immune system.

It is well known that transcription of many genes overexpressed in eczema is controlled predominantly by the nuclear factor (NF)-κB. The NF-κB family is composed of five DNA-binding proteins: p50 and its precursor p105, p52 and its precursor p100,

Key words: atopic dermatitis, allergy, NF-κB

Mailing Address: Federica Angelini, MD, PhD.,
Department of Pediatrics,
Tor Vergata University,
Policlinico di Tor Vergata,
Via Montpellier 1, 00133 Rome, Italy
Tel: ++39 06 72596823 Fax: ++39 06 72596824, 06 20900530
e-mail:angelini@med.uniroma2.it

0394-6320 (2007)

cRel, Rel A (p65) and Rel B (6). NF-κB is retained in the cytoplasm in its inactive form by interaction with various inhibitors known as IκBs. Modification of IκB in response to a variety of pro-inflammatory signals, leads to the translocation of NF-κB subunits from the cytoplasm to the nucleus where they activate their target genes upon binding to their consensus sequences. The most common form of NF-κB is a heterodimer consisting of p50 and p65 subunits, which can form different complexes in response to specific stimuli, thus allowing for different DNA-binding specificities and trans activation potentials (7).

Whether NF-κB is directly involved in the pathogenesis of eczema has not yet been demonstrated, but several lines of evidence suggest that it may play a pivotal role in the skin inflammation which is characteristic of the disease. NF-κB is probably a crucial factor for the recruitment of activated lymphocytes to the site of skin inflammation since it controls the transcription of chemoattractants, such as eotaxin, IL-5, MCP-1, MIP-1, RANTES, and MCP-4, chemokine receptors, such as CCR3, and adhesion molecules such as ICAM-1, E-selectin, and VCAM-1 (8-9).

An important feature of eczema is that Th1 and Th2 cytokines are involved in the pathogenesis of cutaneous inflammation, and their specific expression varies in different phases of the disease. In acute eczema, infiltrating T cells are responsible for increased IL-4 and IL-13 expression, while chronic inflammation is characterized by the prominent production of IL-5, GM-CSF, IL-12 and IFN-γ. The transcription of all these cytokines is mediated by NF-κB (10-11). In particular, the over-expression of IL-12 contributes to the promotion of the switch to the Th1 cytokine profile typical of chronic eczema (12). Although NF-κB has been hitherto considered as mediator of acute inflammatory responses, it has been shown to be persistently activated also in chronic inflammatory conditions such as inflammatory arthritis (13), inflammatory bowel diseases (14), atherosclerosis (15), asthma (16-17), and, more recently, also in psoriasis (18-20) and eczema (21).

The observation that some pro-inflammatory cytokines involved in allergic response, such as TNF-α, are regulated by NF-κB and can themselves activate NF-κB expression, indicates that a feed-forward amplification loop exists that could form the basis for the persistence of chronic inflammatory processes in eczema (2, 12). Furthermore, interesting work performed on animal models of allergic asthma (22-24) and cutaneous inflammation (25-27) supports the idea that NF-κB plays a pivotal role in the induction and maintenance of the skin inflammation in eczema. To investigate the potential role of NF-κB in the pathogenesis of eczema we studied NF-κB-DNA binding activity in peripheral blood mononuclear cells (PBMC) of children affected by chronic atopic and non-atopic eczema.

MATERIALS AND METHODS

Patients

Twenty children (12 male and 8 female, aged between 6 and 144 months, mean 51.6, median 28.5), affected by relapsing chronic eczema for at least 6 months, were enrolled in this study. Eleven children out of 20 (55%) showed detectable IgE sensitization and positive prick test. Twelve out of 20 (60%) were suffering from mild-moderate eczema (SCORAD ranging between 10 and 25) and 8 out of 20 (40%) from severe eczema (SCORAD over 25). The characteristics of the patients are summarized in Table I. At the time of the enrolment no patient had been taking either local or oral antibiotics or corticosteroids or immunosuppressive drugs for at least 2 weeks.

Three patients (p1, p5 and p20), who were affected by severe eczema refractory to conventional specific therapies, underwent therapy with intravenous methylprednisolone bolus (20 mg/Kg/die) for 3 consecutive days (28), as inpatients. As control group, nine age-matched children were included in the study, 5 male and 4 female, aged between 2 and 168 months (mean 68.7, median 30), with neither family history nor clinical symptoms of allergy, and negative for skin prick test and serum IgE.

SCORAD

The severity of eczema was evaluated using the SCORAD Index (27). This index consists of several evaluation criteria (objective and subjective) of variable weight, giving a global quantitative score representing the intensity of disease at a given time. The objective criteria include the extent and intensity of the disease. The subjective signs assess the itching and sleep loss related to eczema.

Serum IgE quantitation

Serum samples were blind-coded prior to the quantitation of IgE. IgE specific for inhalant and food allergens were tested with Pharmacia RAST according

to the manufacturer's instructions. Titers were expressed as IU/ml.

Cells

Peripheral blood mononuclear cells (PBMC) were obtained from patients and healthy donors by gradient centrifugation over Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden). A minimum of $10x10^6$ cells was lysed immediately after separation. Reference PBMC obtained from adult healthy donors were cultured with or without PHA (5 μg/ml) (Sigma, St Louis, MO, U.S.A) for 1 hr before lysis.

Nuclear extracts

Nuclear extracts were prepared according to Andrews et al. (30). Briefly, cells were lysed in 400 μl of buffer A (10 mM HEPES-KOH, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitors for 15 min on ice. After centrifugation at 1,000 rpm for 1 min at 4°C, the nuclear pellet was lysed with 30-50 μl of buffer C (HEPES-KOH 20 mM, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM dithiothreitol (DTT), 0.2 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibitors for 20 min on ice. Following centrifugation at 12,000 rpm for 10 min, supernatants were harvested and protein concentration was determined (Bio-Rad protein assay). All reagents were obtained from Sigma (St Louis, MO, U.S.A).

Electrophoretic Mobility Shift Assay (EMSA)

The DNA binding activity of NF-κB was examined by EMSA using Lightshift chemioluminescent EMSA kit (Pierce Chemical Company, Rockford, USA). Briefly, for each binding reaction 30 fmol of biotin end-labeled double-stranded κB oligonucleotide (5'-AGTTGAGGGGACTTTCCCAGGC-3') (Invitrogen, Groningen, Netherlands) was incubated with 6 μg of nuclear extract obtained from patient or control PBMC in binding buffer in the presence of 1 μg poly(dI-dC) as nonspecific competitor. The reaction mixture was incubated for 15 min at RT and loaded on 5% not denaturing polyacrylamide (29:1 acrylamide: bis-acrylamide) gel in 0.5x tris-borate- EDTA (TBE) buffer. The gel was transferred to a Hybond-N+ nylon membrane (Amersham Biosciences, Uppsala, Sweden) at 380 mA for 30 min in 0.5x TBE buffer cooled to about 10°C. When the transfer was complete, the DNA was UV cross-linked and the detection of biotin-labeled κB DNA was performed according to the manufacture's procedure. The amount of NF-κB-DNA binding was quantified by densitometric analysis. Band intensity was normalized to unbound probe. NF-κB-DNA binding activity in PBMC derived from an adult healthy donor, stimulated with PHA (5 μg/ml) was considered 100% and used for normalization. Specificity of binding was determined by addition of 50 and 100 fold excess of unlabeled probe.

Table 1. *Clinical features, IgE levels, prick test and NF-κB activity of the AEDS patients.*

Pts.	Sex	Age (mos)	Familiarity	Onset (mos)	AEDS (mos)	SCORAD	Other diagnosis	Total IgE (IU/ml)	Specific IgE (IU/ml) Dust mite	Casein	Prick test	NF-κB activity (% PHA)
P1	F	23	yes	3	20	68	none	1.477	>100	23.7	dust mite +++, cow's milk +++	87
P2	M	20	yes	12	7	17	none	8.2	neg	neg	neg	89
P3	M	41	yes	2	39	20	none	5.3	neg	neg	dust mite +	80
P4	F	10	neg	4	6	45	none	97	neg	neg	neg	128
P5	F	123	yes	5	118	25	asthma+rhinitis	7658	>100	neg	dust mite +++, lolium++	78
P6	M	94	neg	4	90	10	asthma	238	5.2	neg	dust mite +	45
P7	M	25	yes	4	21	15	none	15.4	neg	neg	neg	59
P8	F	24	yes	4	20	10	none	340	neg	4.8	cow's milk +++	28
P9	M	13	neg	7	3	12	none	13,2	neg	neg	neg	29
P10	F	28	yes	1	27	28	none	3	neg	0.75	neg	62
P11	F	54	yes	24	30	10	asthma	98.60	1.61	neg	dust mite+++, lolium+++	43
P12	M	104	neg	12	92	12	asthma	187	neg	neg	dust mite +++	61
P13	M	29	yes	7	22	31	none	36	neg	3.41	cow's milk +++	60
P14	M	101	yes	3	98	62	none	3.181	>100	4.89	dust mite++, cow's milk +++	79
P15	F	144	yes	6	138	10	asthma+rhinitis	285	3.50	neg	dust mite++	48
P16	F	9	neg	3	5	28	none	3.5	neg	neg	neg	99
P17	M	15	yes	1	14	58	none	140	neg	0.7	cow's milk ++	302
P18	M	32	neg	5	27	16	rhinitis	17.4	neg	neg	neg	106
P19	M	6	yes	1	4	8	none	67	neg	0.4	cow's milk ++	50
P20	M	136	yes	24	112	40	none	10	neg	neg	neg	86

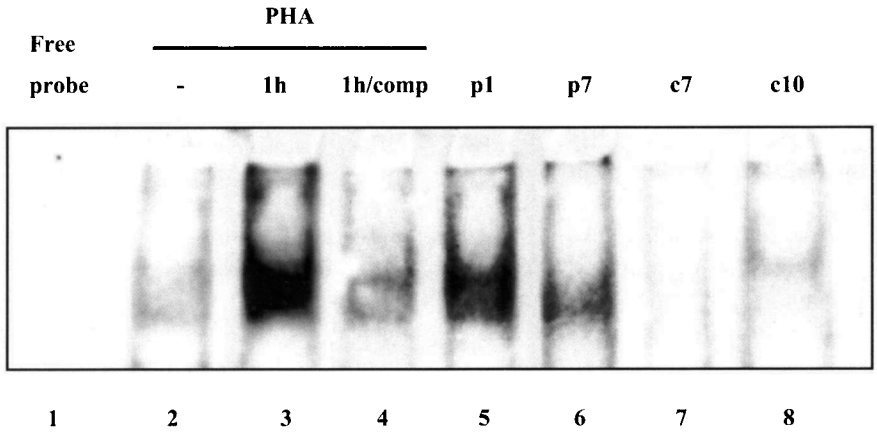

Fig. 1. *Representative EMSA experiment. NF-kB DNA binding activity of nuclear extracts derived from patients (p1, p7) and age-matched controls (c7 and c10) PBMC. Healthy donor PBMC unstimulated (lane 2) or stimulated with PHA for 1 h (lane 3) are also shown. Competition experiment by using 50X excess of unlabeled probe is also shown in lane 4.*

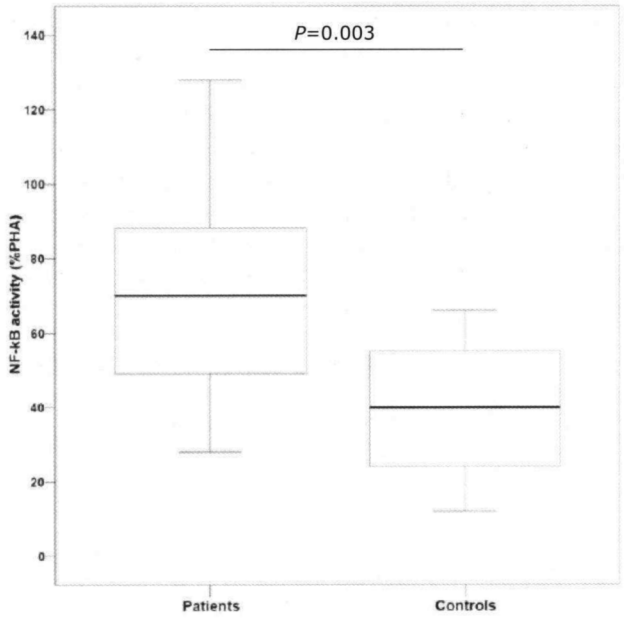

Fig. 2. *Comparison between median NF-κB-DNA binding activity in 20 children with eczema and 9 healthy children. NF-κB-DNA binding activity was assessed in PBMCs by EMSA, as described in Materials and Methods and expressed as percent of NF-κB-DNA binding activity induced in PBMC from an healthy donor after 1 h stimulation with PHA (5 μg/ml).*

Statistics

Data are expressed as mean ± SEM or median (upper and lower quartile) as appropriate. Interval scale variables were tested for normality by the Shapiro-Wilk method. The sample size was originally planned by the use of the effect size method. Thus, for a desired effect size of 1.2, 80% statistical power, alpha error 0.05, and a case / control ratio of 0.5, 18 cases and 9 controls had to be enrolled. The nQuery Advisor v.1.0 power analysis software was employed.

The differences of NF-κB-DNA binding between normal subjects and eczema patients and the prevalence of increased NF-κB-DNA binding in IgE-mediated and non IgE-mediated eczema groups were assessed with

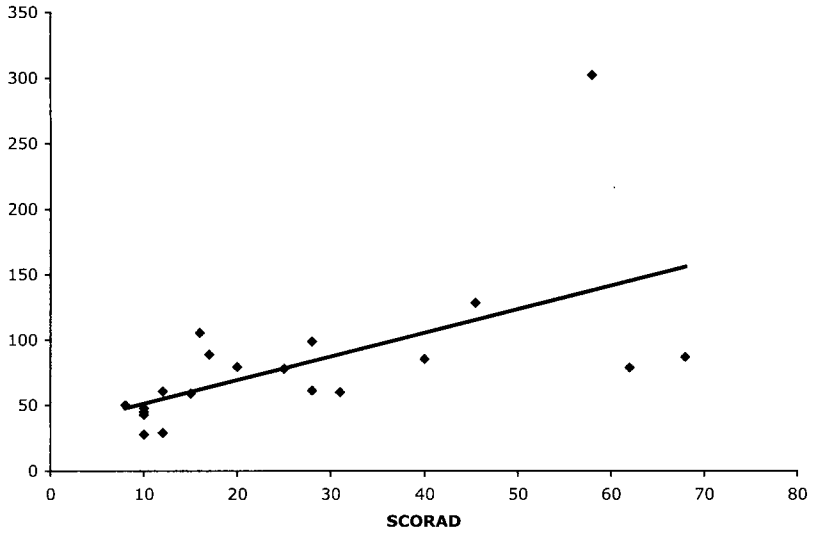

Fig. 3. *Correlation between NF-κB activity and SCORAD in 20 children with eczema. NF-κB binding activity was expressed as percent of NF-κB-DNA binding activity induced in PBMC from an healthy donor after 1 h stimulation with PHA (5 μg/ml). Each dot represents the mean of at least two experiments (P=0.008).*

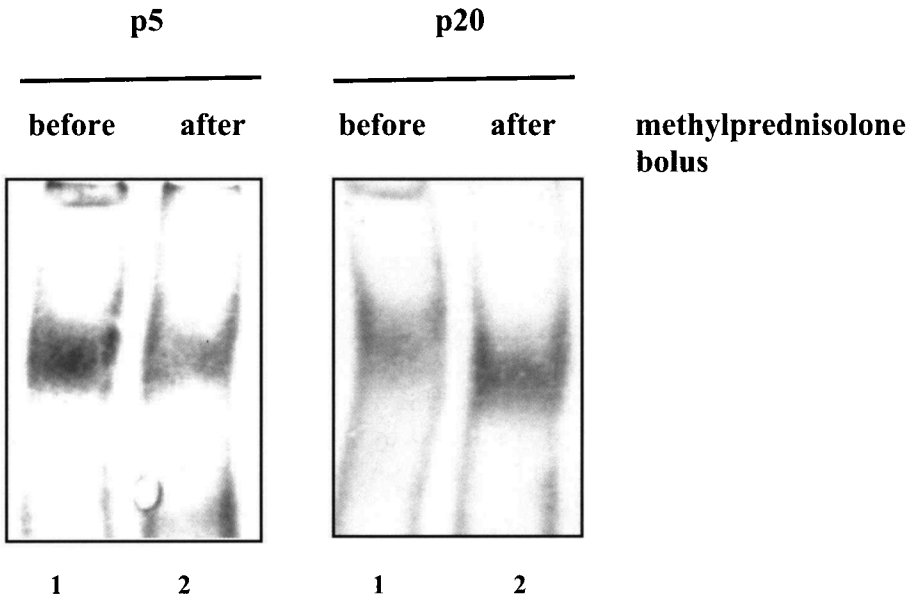

Fig. 4. *NF-κB-DNA binding activity of nuclear extracts obtained from patients 5 (p5) and patient 20 (p20) before (lanes 1) and after (lanes 2) treatment with intravenous methyprednisolone bolus (20 mg/Kg/die) for 3 consecutive days. Representative EMSA experiment (n=3).*

the independent 2-samples Student's *t* test or the Mann-Whitney's *U* non-parametric test when indicated. The relationship between NF-κB-DNA binding activity and severity of the disease (SCORAD), age of the patients, duration of the disease and age at onset was assessed by Pearson's correlation test. A P value <0.05 was considered statistically significant.

RESULTS

In the present study we tested basal NF-κB-DNA binding activity in PBMC derived from 20 children affected by eczema. The clinical characteristics, IgE serum levels, prick test and NF-κB DNA binding activity of the patients are reported in Table I. For

each patient the percentage value reported in Table I represents the average NF-κB-DNA binding activity of a minimum of 2 experiments.

In Fig. 1 a representative EMSA experiment is shown, which included nuclear extract prepared from unstimulated PBMC of eczema patients and healthy children. Excess cold double strand NF-κB oligonucleotide out-competed NF-κB for DNA binding (lane 4), confirming the specific nature of the binding. The signals for NF-κB-DNA binding corresponding to the patients (lane 5 and 6) were more intense compared to the signals corresponding to controls (lanes 7 and 8), in which NF-κB-DNA binding was almost undetectable. Nuclear extracts from PBMC derived from an adult healthy donor, left unstimulated (lane 2) and stimulated with PHA for 1 h (lane 3), were used for normalization. NF-κB-DNA binding activity detected in nuclear extracts was significantly higher in PBMC from patients than in PBMC from 9 healthy age-matched controls: 70% (28-302) *vs.* 40% (12-66), *U* 28.8; *P*=0.003 (Fig. 2).

We then correlated basal NF-κB-DNA binding activity with the SCORAD of eczema patients. Our data showed a positive correlation between NF-κB-DNA binding activity and severity of dermatitis (Pearson's correlation coefficient 0.59; *P*=0.008) (Fig. 3). In our cohort no correlation was observed between NF-κB-DNA binding activity and age of the patients, duration of disease and age at the onset.

Additionally, we found a higher average NF-κB-DNA binding activity in non-IgE-mediated eczema children than in IgE-mediated eczema children (82% *vs.* 49%), if we exclude 1 child (p17) affected by severe allergy to cow's milk, in which NF-κB-DNA binding activity was 302% (Table I).

Two out of 3 patients who underwent treatment with intravenous methylprednisolone bolus, showed a prompt improvement of the skin inflammation (p1-SCORAD: before=68 and after=26 and p5-SCORAD: before=25 and after=8), while the third patient failed to respond to the treatment (p20-SCORAD: before=40 and after=48).

For p5 and p20 we had the opportunity to study NF-κB-DNA binding activity both before and 24 hrs after treatment with methylprednisolone bolus. In patient p5 we observed a substantial reduction of the NF-κB-DNA binding after treatment (before=59.1% and after=35.2%), associated with a marked improvement of the clinical picture which was still persisting at 6 months follow up.

On the other hand, patient p20, who did not clinically respond to methylprednisolone bolus, showed no reduction of NF-κB-DNA binding (before=71% and after=85%). The lower relative position of the NF-κB complex after therapy suggests a different dimer composition and needs further investigation (Fig. 4).

DISCUSSION

The crucial role of NF-κB in the transcription of many genes involved in allergic inflammation is well documented and several observations in animal models of asthma (22-24) and in asthmatic patients have been reported (17, 31). Nevertheless, no study has been devoted to investigate NF-κB-DNA binding activity in children affected by eczema, and only one report has been published on adult patients on this issue (21).

Here we report, for the first time, a statistically significant increase of NF-κB-DNA binding basal activity in nuclear extracts from PBMC of 20 children affected by chronic eczema, suggesting, for this transcription factor, a role in the pathogenesis of this disease.

It has been shown that Rel B deficient mice develop skin lesions similar to atopic dermatitis, probably due to impaired IκBα stability (25) and that IκB deficiency in mice results in widespread dermatitis accompanied with sustained NF-κB response (26). In addition, it was recently demonstrated that mice prone to developing atopic dermatitis display Th2 hyper-responsiveness associated with increased NF-kB-DNA binding activity, and the consequent skin inflammation is reduced by specific NF-κB inhibition (27). Our observations are consistent with these finding, demonstrating that a significant increase of basal NF-κB-DNA binding activity occurs in PBMC of children affected by chronic eczema and that there is a positive correlation between NF-κB-DNA binding activity and severity of the disease.

These results, taken together, suggest that NF-κB is a key mediator of the skin inflammation in atopic eczema, although, given the redundancy of the NF-κB pathway, it would be relevant, in our patients, to

characterize the dimer composition (32).

Interestingly, we observed that children affected by non-IgE-mediated eczema showed an increased NF-κB activity compared to those affected by IgE-mediated eczema. This observation needs more investigation but potentially suggests a lack of positive correlation between NF-κB transcriptional activity and IgE levels.

It is worth recalling that NF-κB is an important regulator of keratinocyte proliferation and differentiation (33), extending the notion that NF-κB plays a key role in both homeostasis of the epidermis and development of skin appendages. Indeed several studies performed on experimental animal models (34) and on patients affected by primary immune deficiencies with skin abnormalities (35-36), suggest that a defect of components of the NF-κB pathway, such as IKKα IKKγ(NEMO) or IκBα, might play an important role in skin pathology, also by altering the constitutive structure and biology of the skin (37).

Eczema patients have an increased risk of cutaneous infections by *S. aureus*, *Herpes simplex*, *molluscum contagiosum* and fungi, such as *Malassezia furfur* (*Pityrosporum ovale)* and *Aspergillus fumigatius* (38-42). Since these microorganisms can trigger the Toll-like receptor (TLR) pathways, therefore inducing an increase of NF-κB activity, it was important to discriminate, in our study, between allergic inflammation and inflammation induced by pathogens frequently present in the skin lesions of patients affected by eczema. Indeed, a careful clinical examination of our patients was performed to exclude patients showing clinical signs of staphylococcal, fungal and/or herpetic skin lesions, therefore avoiding a bias in the interpretation of the experimental data.

The characterization of the composition of NF-κB dimers bound to the DNA in the PBMC from eczema patients, and the investigation of other molecules upstream NF-κB would be interesting issues; unfortunately the scarcity of the material obtained from our young patients did not allow us to perform these experiments.

Further support for the hypothesis that NF-κB may be important in dermatitis comes from the notion that glucocorticosteroids, the most effective treatment for eczema, are potent inhibitors of these transcription factors (43-44). Eczema patients might be refractory to conventional therapies and methylprednisolone bolus has been proposed for their management, although corticosteroid resistance has been occasionally observed (28). The number of patients treated with this novel protocol is still limited, but it is noteworthy that, in our patients, the success of methylprednisolone bolus therapy correlated with systemic NF-κB-DNA binding activity,

In conclusion, our study may contribute to understanding the basic mechanism of eczema and also aid in designing novel therapeutic strategies targeting NF-κB. A variety of new agents aimed at modulating NF-κB activity is in various stages of investigation (45-46), and the identification and targeting of NF-κB subunits critical for disease development could prevent the potential side effects of the inhibition of NF-κB pathway.

ACKNOWLEDGEMENTS

We wish to thank Giovanni L. Pappagallo for the statistical analysis and Giovanni Monteleone for technical advice.

REFERENCES

1. **Johansson S.G., T. Bieber, R. Dahl, et al.** 2004. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization October 2003. *J. Allergy Clin. Immunol. 113:832.*
2. **Akdis C.A. and M. Akdis.** 2003. Immunological differences between intrinsic and extrinsic types of atopic dermatitis. *Clin. Exp. Allergy 33:1618.*
3. **Galli E., R. Ciccone, P. Rossi, A. Casati, E. Brunetti and G. Mancino.** 2003. AD: molecular mechanism clinical aspects and new therapeutical approaches. *Curr. Mol. Med. 3:127.*
4. **Williams H.C.** 2005. Clinical practice. Atopic dermatitis. *N. Engl. J. Med. 352:2314.*
5. **Boguniewicz M., Y.M. Donald and D.Y. Leung.** 2006. Atopic Dermatitis. *J. Allergy Clin. Immunol. 113:651.*
6. **Ghosh S., M.J. May and E.B. Kopp.** 1998. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Ann. Rev. Immunol. 16:225.*

7. **Li Q. and I.M. Verma.** 2002. NF-kappaB regulation in the immune system. *Nat. Rev. Immunol. 2:725.*
8. **Chen C.C., C.L. Rosenbloom, D.C. Anderson and A.M. Manning.** 1995. Selective inhibition of E-selectin, vascular cell adhesion molecule-1, and intercellular adhesion molecule-1 expression by inhibitors of IkappaB-alpha phosphorylation. *J. Immunol. 155:3538.*
9. **Santamaria Babi L.F., M.T. Perez Soler, C. Hauser and K. Blaser.** 1995. Skin-homing T cells in human cutaneous allergic inflammation. *Immunol. Res. 14: 317.*
10. **Grewe M., C.A. Bruijnzeel-Koomen, E. Schopf, T. Thepen, A.G. Langeveld-Wildschut, T. Ruzicka and J. Krutmann.** 1998. A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol. Today 19:359.*
11. **Shimada Y., K. Takehara and S. Sato.** 2004. Both Th2 and Th1 chemokines are in sera from patients with atopic dermatitis. *J. Dermatol. Sci. 34:201.*
12. **Girolomoni G., S. Sebastiani, C. Albanesi and A. Cavani.** 2001. T-cell subpopulations in the development of atopic and contact allergy. *Curr. Opin. Immunol. 13:733.*
13. **Campbell I.K., S. Gerondakis, K. O'Donnell and I.P. Wicks.** 2000. Distinct roles for the NF-kappaB1 (p50) and c-Rel transcription factors in inflammatory arthritis. *J. Clin. Invest. 105:1799.*
14. **Schreiber S., S. Nikolaus and J. Hampe.** 1998. Activation of nuclear factor kappa B inflammatory bowel disease. *Gut 42:477.*
15. **Collins T. and M.I. Cybulsky.** 2001. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? *J. Clin. Invest. 107:255.*
16. **Bureau F., G. Bonizzi, N. Kirschvink, S. Delhalle, D. Desmecht, M.P. Merville, V. Bours and P. Lekeux.** 2000. Correlation between nuclear factor-kappaB activity in bronchial brushing samples and lung dysfunction in an animal model of asthma. *Am. J. Respir. Crit. Care. Med. 161:1314.*
17. **Gagliardo R., P. Chanez, M. Mathieu, A. Bruno, G. Costanzo, C. Gougat, I. Vachier, J. Bousquet, G. Bonsignore and A.M. Vignola.** 2003. Persistent activation of nuclear factor-kappaB signaling pathway in severe uncontrolled asthma. *Am. J. Respir. Crit. Care. Med. 168:1190.*
18. **Butt C., S. Sun, L. Peddle, C. Greenwood, S. Hamilton, D. Gladman and P. Rahman.** 2005. Association of nuclear factor-kB in psoriatic arthritis. *J. Rheumatol. 32:9.*
19. **Johansen C., E. Flindt, K. Kragballe, J. Henningsen, M. Westergaard, K. Kristiansen and L. Iversen.** 2005. Inverse regulation of the nuclear factor-kB binding to the p53 and interleukin-8 kB response elements in lesional psoriatic skin. *J. Invest. Dermatol. 124:1284.*
20. **Lizzul P.F., A. Aphale, R. Malavivya, Y. Sun, S. Masud, V. Dombrovskiy and A.B. Gottlieb.** 2005. Differential expression of phosphorylated NF-kB/RelA in normal and psoriatic epidermis and downregulation of NF-kB in response to treatment with etanercept. *J. Invest. Dermatol. 124:1275.*
21. **Dieckhoff K., P. Graf, B. Beinhauer, C. Schwaerzler, J.M. Carballido, C. Neumann, K. Zachmann and T. Jung.** 2005. Deficient translocation of c-Rel is associated with impaired Th1 cytokine production in T cells from atopic dermatitis patients. *Exp. Dermatol. 14:17.*
22. **Yang L., L. Cohn, D.H. Zhang, R. Homer, A. Ray and P. Ray.** 1998. Essential role of nuclear factor kappaB in the induction of eosinophilia in allergic airway inflammation. *J. Exp. Med. 188:1739.*
23. **Donovan C.E., D.A. Mark, H.Z. He, H.C. Liou, L. Kobzik, Y. Wang, G.T. De Sanctis, D.L. Perkins and P.W. Finn.** 1999. NF-kappa B/Rel transcription factors:c-Rel promotes airway hyperresponsiveness and allergic pulmonary inflammation. *J. Immunol. 163:6827.*
24. **Poynter M.E., C.G. Irvin and Y.M. Janssen-Heininger.** 2002. Rapid activation of nuclear factor-kappaB in airway epithelium in a murine model of allergic airway inflammation. *Am. J. Pathol. 160:1325.*
25. **Barton D., H. Hogen Esch and F. Weih.** 2000. Mice lacking the transcription factor RelB develop T cell-dependent skin lesions similar to human atopic dermatitis. *Eur. J. Immunol. 30:2323.*
26. **Klement J.F., N.R. Rice, B.D. Car, S.J. Abbondanzo, G.D. Powers, P.H. Bhatt, C.H. Chen, C.A. Rosen and C.L. Stewart.** 1996. IkappaBalpha deficiency results in a sustained NF-kappaB response and severe widespread dermatitis in mice. *Mol. Cell. Biol. 16:2341.*

27. **Tenda Y., M. Yamashita, M.Y. Kimura, A. Hasegawa, C. Shimizu, M. Kitajima, A. Onodera, A. Suzuki, N. Seki and T. Nakayama.** 2006. Hyperresponsive TH2 cells with enhanced nuclear factor-kappa B activation induce atopic dermatitis-like skin lesions in Nishiki-nezumi Cinnamon/ Nagoya mice. *J. Allergy Clin. Immunol. 118:725.*
28. **Galli E., L. Chini, V. Moschese, F. Paone, A. Menichelli, G. Fraioli and P. Rossi.** 1994. Methylprednisolone bolus: a novel therapy for severe atopic dermatitis. *Acta Paediatr. 83:315.*
29. **European Task Force on Atopic Dermatitis.** 1993. Severity scoring of atopic dermatitis: the SCORAD index. *Dermatology 18:23.*
30. **Andrews N.C. and D.V. Faller.** 1991. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res. 19:2499.*
31. **Hart L.A., V.L. Krishnan, I.M. Adcock, P.J. Barnes and K.F. Chung.** 1998. Activation and localization of transcription factor, nuclear factor-kappaB, in asthma. *Am. J. Respir. Crit. Care Med. 158:1585.*
32. **Marienfeld R., M.J. May, I. Berberich, E. Serfling, S. Ghosh and M. Neumann.** 2003. Rel-B forms transcriptionally inactive complexes with RelA/p65. *J. Biol. Chem. 278:19852.*
33. **Takao J., T. Yudate, A. Das, S. Shikano, M. Bonkobara, K. Ariizumi and P.D. Cruz.** 2003. Expression of NF-kappaB in epidermis and the relationship between NF-kappaB activation and inhibition of keratinocyte growth. *Br. J. Dermatol. 148:680.*
34. **Hu Y., V. Baud, T. Oga, K.I. Kim, K. Yoshida and M. Karin.** 2001. IKK-alpha controls formation of the epidermis independently of NF-kappaB. *Nature 410:710.*
35. **Zonana J., M.E. Elder, L.C. Schneider, S.J. Orlow, C. Moss, M. Golabi, S.K. Shapira, P.A. Farndon, D.W. Wara, S.A. Emmal and B.M. Ferguson.** 2000. A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). *Am J. Hum. Genet. 67:1555.*
36. **Courtois G., A. Smahi, J. Reichenbach, et al.** 2003. A hypermorphic IkappaBalpha mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. *J. Clin. Invest. 112:1108.*
37. **Bell S., K. Degitz, M. Quirling, N. Jilg, S. Page and K. Brand.** 2003. Involvement of NF-kappaB signalling in skin physiology and disease. *Cell Signal 15:1.*
38. **Breuer K., A. Kapp and T. Werfel.** 2001. Bacterial infections and atopic dermatitis. *Allergy 56:1034.*
39. **Faergemann J.** 2002. Atopic dermatitis and fungi. *Clin. Microbiol. Rev. 15:545.*
40. **Mambula S.S., K. Sau, P. Henneke, D.T. Golenbock and S.M Levitz.** 2002. Toll-like receptor (TLR) signaling in response to Aspergillus fumigatus. *J. Biol. Chem. 277:39320.*
41. **Meier A., C.J. Kirschning, T. Nikolaus, H. Wagner, J. Heesemann and F. Ebel.** 2003. Toll-like receptor (TLR) 2 and TLR4 are essential for Aspergillus-induced activation of murine macrophages. *Cell. Microbiol. 5:561.*
42. **Wollenberg A., Wetzel S., Burgdorf W.H. and J. Haas.** 2003. Viral infections in atopic dermatitis: pathogenic aspects and clinical management. *J. Allergy Clin. Immunol. 112:667.*
43. **Auphan N., J.A. Di Donato, C. Rosette, A. Helmberg and M. Karin.** 1995. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science 270:286.*
44. **De Bosscher K., W. Vanden Berghe, L. Vermeulen, S. Plaisance, E. Boone and G. Haegeman.** 2000. Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. *Proc. Natl. Acad. Sci. USA 97:3919.*
45. **Henderson W.R. Jr., E.Y. Chi, J.L. Teo, C. Nguyen and M. Kahn.** 2002. A small molecule inhibitor of redox-regulated NF-kappa B and activator protein-1 transcription blocks allergic airway inflammation in a mouse asthma model. *J. Immunol. 169:5294.*
46. **Nakamura H., M. Aoki, K. Tamai, M. Oishi, T. Ogihara, Y. Kaneda and R. Morishita.** 2002. Prevention and regression of atopic dermatitis by ointment containing NF-κB decoy oligodeoxynucleotides in NC/Nga atopic mouse model. *Gene Ther. 9:1221.*