

Immunodeficiency and other clinical immunology

Altered expression of IgG and complement receptors indicates a significant role of phagocytes in atopic dermatitis

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Background: Strict dietary precautions against allergic sensitization may benefit a group of predisposed children.

Objective. To develop new strategies for identifying these children, a better understanding of the processes that initiate sensitization and regulate and perpetuate the inflammatory response is needed.

Methods: We measured the expression of the receptors for the constant (Fc) region of IgG (FcγRI, FcγRII, and FcγRIII) and that for the complement fragments C3b and C3bi (CR1 and CR3) in neutrophils and monocytes from 39 children with atopic dermatitis, 17 disease control patients with acute infections, and 17 healthy control subjects. The capacity of phagocytes to produce reactive oxygen species was also determined. To find the best way of discriminating the patients with atopic dermatitis from control subjects, a stepwise logistic binary regression model was made.

Results: The stepwise logistic regression analysis was based on differences in individual receptor expression between the study groups. Because acute infections strongly affected receptor expression in both neutrophils and monocytes, to avoid diagnostic bias, children with acute infections were excluded from the analysis. The combination of the receptors CR1 in neutrophils and FcγRI and FcγRII in monocytes was the best indicator of atopic dermatitis. A significant correlation between the expression of CR1 in neutrophils and in monocytes, as well as reactive oxygen species production of phagocytes, and the severity of the eczema was detected.

Conclusions: These results suggest that a distinct receptor profile of phagocytic cells can be characterized in patients with atopic dermatitis, providing a new direction to the search for early identification of children predisposed to allergic sensitization. (*J Allergy Clin Immunol* 1997;99:707-13.)

Key words: Complement receptors, atopic dermatitis, Fc receptors, food allergy, hypersensitivity

Hereditary predisposition is the major denominator of atopic dermatitis, and hypersensitivity reactions contribute to the expression of the genetic predisposition. The

Abbreviations used

CR:	Receptor for the complement fragment
FcγR:	Receptor for the constant (Fc) region of IgG
FITC:	Fluorescein isothiocyanate
HBSS:	Hanks' balanced salt solution
ROC:	Receiver operating characteristics (curve)

relationship between environmental antigens and exacerbation of atopic dermatitis is particularly apparent in infancy so that dietary antigens predominate.^{1,2} Therefore allergic reactions to foods are common. Early identification of allergic sensitization is imperative to prevent sustained inflammation of the target organ. However, currently available tools to this end are of poor diagnostic accuracy.^{2,3} We hypothesized here that the inflammatory response induced by environmental antigens in atopic children may be manifested early in an overactive phagocytic process.

When appropriately triggered, phagocytes release in various inflammatory reactions toxic agents, such as reactive oxygen intermediates and lytic enzymes, into phagolysosomes and occasionally also to the outside of the cell with the potential for attack on adjacent normal tissue.⁴ The cells are efficiently triggered by the ligands of certain opsonin receptors on the phagocyte plasma membrane. These receptors include FcγRI, FcγRII, and FcγRIII for IgG and CR1 and CR3 for derivatives of C3, a component of the complement cascade. Cross-linking or immobilization of these receptors in the membrane by polyvalent immune complexes, rather than binding of ligand per se, is the triggering event. Human FcγRs bind IgG₁ and IgG₃ with high (FcγRI), low (FcγRII), and intermediate (FcγRIII) affinity. FcγRI is constitutively expressed on monocytes and macrophages and can be induced on neutrophils and eosinophils. FcγRII is constitutively expressed on all of these cell types. FcγRIII exists in two forms: FcγRIIIa is a transmembrane receptor present on monocytes and macrophages, whereas FcγRIIIb is linked to the outer leaflet of the plasma membrane of neutrophils and eosinophils by a glycosyl phosphatidylinositol anchor. Various phagocyte stimulants activate phospholipase C enzyme, which cleaves

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TABLE I. Clinical characteristics of healthy control subjects, disease control patients with acute infections, and children with atopic dermatitis

	Healthy control subjects (n = 17)	Control patients with infection (n = 17)	Patients with atopic dermatitis (n = 39)
Mean gestational age (wk)	40	40	40
Range	39-42	39-41	38-41
Mean birth weight (gm)	3518	3560	3511
Range	3080-4100	2860-4210	2780-4420
Mean birth length (cm)	51	51	50
Range	49-54	49-54	48-53
Acute febrile infections			
Bacterial	0	12	0
Pyelonephritis (<i>Escherichia coli</i>)	0	4	0
Pneumonia	0	1	0
Otitis media	0	1	0
Septicemia (pneumococcus)	0	6	0
Viral	0	5	0
Bronchitis	0	3	0
Mononucleosis (Epstein-Barr)	0	1	0
Gastroenteritis (rotavirus)	0	1	0
Dietary restrictions			
Cow's milk	0	0	37
Egg	0	0	39
Other (cereals, vegetables, fruits)	0	0	29

the anchor and readily sheds the receptor from the membrane.⁴ CR1 binds dimeric C3b and, with a lower affinity, monomeric C3b. CR3 is a multifunctional receptor recognizing C3bi, fibrinogen, some other Arginine-Glycine-Aspartic acid sequence-containing proteins, and lipopolysaccharides. It also exhibits lectin-like properties, and it participates in adhesion events. The number of CR1 and CR3 on phagocytic leukocytes varies substantially depending on the activation state of the cells. Expression on the cell membrane can be upregulated by receptor translocation from intracellular pools in 10 to 15 minutes after activation of phagocytes with various stimuli. In addition to upregulation and downregulation of the expression, these receptor proteins undergo rapid activation and deactivation processes.⁵

The expression of these receptors was assessed in children with atopic dermatitis, in disease control patients with acute infection, and in healthy control subjects. Moreover, we measured the reactive oxygen species production in the peripheral blood phagocytes of the same subjects.

METHODS

Study subjects and design

Three groups of children (Table I), aged 1 to 55 months (mean age, 14 months), were studied with their parents' consent: (1) 39 patients with atopic dermatitis, (2) 17 patients with an acute infection, and (3) 17 healthy children. The study was approved by the Ethical Committee of Tampere University Hospital. The atopic dermatitis group comprised 39 children aged 1 to 55 months (mean age, 11 months) fulfilling the criteria of Hanifin⁶ for atopic eczema in children. They had been referred to a pediatric clinic on the basis of suspected food

allergy and were allocated to double-blind, placebo-controlled or open cow's milk challenge as described previously.² The inclusion criteria for the study were that the patient be free of symptoms and signs of acute infection and had not received any medication during the previous month. None of the patients was receiving systemic corticosteroid therapy.

Blood samples were collected for the assessment of FcγRI, FcγRII, and FcγRIII receptors; complement receptors CR1 and CR3; and reactive oxygen species production at presentation. The serum total IgE concentration (Phadebas IgE Prist; Pharmacia, Uppsala, Sweden) and cow's milk-specific IgE (RAST, Pharmacia) were determined. The severity of the skin condition was graded. Skin prick testing was performed with cow's milk and a long list of dietary antigens.

The second group included patients, aged 1 to 37 months (mean age, 16 months), who were admitted to the hospital for treatment of acute febrile infections. Blood samples were collected for the assessment of the receptors and reactive oxygen species production during the first 12 hours in the hospital. The third group comprised children, aged 1 to 38 months (mean age, 16 months), who were studied as outpatients after minor operations or previous investigations. All were found to be healthy. The criteria for enrollment were no acute infections for the previous month and no history of atopy or food allergy in the family.

Grading of atopic dermatitis

The severity of atopic dermatitis was scored according to the SCORAD method, established by the European Task Force on Atopic Dermatitis.⁷ The extent of the dermatitis was estimated by using the rule of nines. The intensity of the dermatitis was the sum of the scores for erythema, edema and/or papules, excoriation, lichenification, and dryness. The subjective manifestations, including pruritus and sleep loss, were assessed from parents' estimations. SCORAD was

TABLE II. FcγR and CR receptor expression of neutrophils in healthy control subjects, control patients with infection, and patients with atopic dermatitis

	Healthy control subjects (n = 17)	Control patients with infection (n = 17)	Patients with AD (n = 39)	p Value (Kruskal-Wallis test)
CR1				
Median	63	87*	87*	0.008
Lower-upper quartile	38-78	54-114	61-101	
CR3				
Median	680	772	780	0.12
Lower-upper quartile	300-844	580-982	486-1003	
FcγRI				
Median	21	42*	22†	0.006
Lower-upper quartile	15-37	23-74	15-26	
FcγRII				
Median	124	222*	144†	<0.0001
Lower-upper quartile	107-147	157-266	112-160	
FcγRIII				
Median	842	850	630	0.03
Lower-upper quartile	725-990	705-1098	524-874	

AD, Atopic dermatitis.

*Significant (Bonferroni-corrected) difference versus healthy control subjects.

†Significant (Bonferroni-corrected) difference versus control patients with infection.

obtained with the calculation: SCORAD = Extent ÷ 5 + 3.5 × intensity + subjective score.

Skin prick tests

Antihistamine medication was discontinued for periods of 3 days to 6 weeks before skin testing, depending on the drug's duration of action. Prick testing was done on the volar aspect of the forearm with a commercially available cow's milk allergen (ALK; Allergologisk Laboratorium, Horsholm, Denmark) and histamine dihydrochloride (10 mg/ml, ALK) as positive control as described previously.² Reactions were read after 15 minutes, and a response half of the histamine reaction size or more was recorded as positive on the condition that the mean diameter of the wheal was at least 3 mm and the negative control at the same time was 0 mm.

Receptor analysis

Monoclonal antibodies and flow cytometry were used to assess the expression of Fcγ receptors (FcγRI, FcγRII, FcγRIII) and complement receptors (CR1, CR3).

Reagents

Hanks' balanced salt solution (HBSS) without Ca²⁺ and Mg²⁺ ions (pH 7.4) was made without phenol red and supplemented with 0.1% gelatin. Fluorescein isothiocyanate (FITC)-labeled anti-FcγRI (CD64), anti-FcγRII (CD32), and anti-FcγRIII (CD16) were purchased from Medarex Inc. (Annandale, N.J.). FITC-conjugated anti-CR1 (CD35) was obtained from CLB Immune Reagents (Amsterdam, The Netherlands), and phycoerythrin-conjugated anti-CR3 (CD11b) was obtained from Becton Dickinson (Mountain View, Calif.). Isotype-matched monoclonal antibodies (mouse IgG-1 FITC-conjugate, mouse IgG-1 R-phycoerythrin conjugate, mouse IgG-2b R-phycoerythrin conjugate) were from Caltag Laboratories (San Francisco, Calif.).

Preparation of human leukocytes

Peripheral, ethylenediaminetetraacetic acid (EDTA)-anticoagulated (1.5 mg EDTA/1 ml blood) blood samples were collected from the study subjects. After 4 hours of storage at room temperature, blood erythrocytes were lysed with 0.83% ammonium chloride (1.5 ml blood, 8.5 ml ammonium chloride) at +20° C for 15 minutes. After lysing, leukocytes were centrifuged (400 g for 10 minutes at +4° C) and resuspended in 500 ml of ice-cold Ca²⁺ and Mg²⁺-free HBSS.

Measurements of receptor expression

Leukocytes (3 × 10⁵) were incubated with monoclonal antibodies in 12 × 75 mm polystyrene vials for 30 minutes at +4° C. Incubation volume was 90 μl. Control sample was incubated with isotype-matched monoclonal antibodies directed to an irrelevant antigen. After incubation, cells were washed with cold Ca²⁺ and Mg²⁺-free HBSS. Leukocytes were resuspended in 500 μl of cold Ca²⁺ and Mg²⁺-free HBSS, and they were ready for flow cytometry examination.

Flow cytometry

Flow cytometric analysis was performed with a FACScan (Becton Dickinson) flow cytometer with an argon ion laser. The laser excitation wavelength was 488 nm. Emitted light was collected through a 560 nm and 60 nm dichromic filter and through 530 nm and 585 nm bandpass filters. The fluorescence of 5000 cells was measured by using logarithmic amplification. A relative measure of receptor expression was obtained by determining the mean log fluorescence intensity after the two-color spectral compensation network adjustment.⁸ The percentage of positive cells was generally 98 to 100, unless otherwise indicated.

Measurement of reactive oxygen species production

Reagents. Gelatin, Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), and zymosan (Zymosan A from *Saccharomyces*

TABLE III. FcγR and CR receptor expression of monocytes in healthy control subjects, control patients with infection, and patients with atopic dermatitis

	Healthy control subjects (n = 17)	Control patients with infection (n = 17)	Patients with AD (n = 39)	p Value (Kruskal-Wallis test)
CR1				
Median	71	92	85	0.12
Lower-upper quartile	60-91	68-104	77-101	
CR3				
Median	737	1003	813	0.19
Lower-upper quartile	300-1038	709-1272	501-1028	
FcγRI				
Median	86	133*	72†	0.0006
Lower-upper quartile	68-138	103-194	57-113	
FcγRII				
Median	139	203*	147†	0.0005
Lower-upper quartile	97-152	165-226	126-185	
FcγRIII				
Median	114	197	113†	0.04
Lower-upper quartile	89-148	120-253	93-164	

AD, Atopic dermatitis.

*Significant (Bonferroni-corrected) difference versus healthy control subjects.

†Significant (Bonferroni-corrected) difference versus control patients with infection.

cerevisiae) were purchased from Sigma Chemical Company (St. Louis, Mo.). Stock solution of 10 mmol/L luminol was prepared in 0.2 mol/L sodium borate buffer (pH 9.0) and stored at -20° C. Zymosan (20 mg/ml) was prepared by boiling for 20 minutes and washing twice with HBSS. For opsonization, the suspension was incubated in 65% pooled serum from healthy volunteers for 60 minutes at +37° C and washed twice in HBSS. Unopsonized and opsonized zymosan were resuspended (20 mg/ml) in HBSS. Zymosan suspensions were stored at -70° C.

Measurement of chemiluminescence. Zymosan-induced luminol-dependent chemiluminescence was measured with an automated luminometer set-up, allowing the simultaneous and continuous measurement of 25 samples (LKB Wallac 1251 luminometer; LKB Wallac, Turku, Finland) connected with a microcomputer. The chemiluminescence emission was measured at +37° C for 60 minutes with diluted whole blood.⁹ Capacity of phagocytes to emit chemiluminescence was expressed in millivolts. Reaction mixtures contained a total volume of 0.5 ml of HBSS supplemented with 0.1% gelatin and including 0.4 mmol/L luminol and 1 mg of zymosan. Both opsonized and unopsonized zymosan were used. Whole blood was diluted 1:500 in 0.1% gelatin-HBSS. The reaction was initiated with addition of 50 μl of diluted blood (final dilution, 1:5000).

Statistics

The data are given as medians with lower and upper quartiles. The Kruskal-Wallis test was used for comparing differences between groups. In cases in which the results of Kruskal-Wallis test were significant ($p < 0.05$), pairwise comparisons were carried out to identify which group differences accounted for the overall p value. The Mann-Whitney U test was used in the pairwise comparisons, and Bonferroni's correction was used to adjust for multiplicity. Spearman's rank correlation coefficient was used to determine the degree and significance of association between receptor expression levels and the severity of atopic eczema (SCORAD scores) and between reactive oxygen species production and SCORAD scores. Level of

significance was less than 0.05. A stepwise logistic binary regression analysis was done to identify the best combination of receptors for detecting atopic sensitization. The probability of atopic sensitization estimated by the logistic model was then used as a new diagnostic variable. A child with the estimated probability higher than the specified cutoff points was classified as atopic. When compared with the true disease status, the sensitivity and specificity of the new diagnostic test variable was calculated by using different cutoff points. Sensitivity and specificity were calculated according to the formulas:

Sensitivity (%) = Number of test positive subjects among atopic subjects ÷ Total number of atopic subjects × 100

Specificity (%) = Number of test negative among healthy control subjects ÷ Total number of healthy control subjects × 100

To evaluate the effect of moving the cutoff point, the receiver operating characteristics (ROC) curve was used. In the ROC curve each point represents one of the cutoff points of the estimated probabilities 0.30, 0.50, 0.70, and 0.90.

RESULTS

Clinical characteristics of the patients with atopic dermatitis

There was a family history of atopic disorders in 33 (85%) of the patients with atopic dermatitis. The mean age at onset of dermatitis was 2 months (range, 1 to 9 months); and the duration of breast-feeding, exclusively and totally, was 3 months (range, 0 to 6 months) and 6 months (range, 1 to 14 months), respectively.

The median (lower-upper quartile) serum total IgE concentration in these patients was 35 kU/L (range, 6 to 161 kU/L). Cow's milk-specific IgE antibodies (RAST, 0.4 kU/L) were detected in 46%; and results of skin prick

tests with cow's milk were positive in 36% of the patients with atopic dermatitis.

The diagnosis of cow's milk allergy was given to 37 (94%) of the patients. These patients continued to receive a cow's milk-free diet with tolerated substitute formulas (extensively hydrolyzed or amino acid formula) after the challenge. On the basis of the clinical history and results of skin testing and RAST or challenge, the diet additionally excluded egg, cereals (e.g., wheat, barley, rye, and oat), and various fruits and vegetables in most of the cases (Table I).

The severity of the eczema, as estimated by the SCORAD score, was 31 (19 to 44), median (lower-upper quartile). The extent of the eczema was 19% (4 to 20), the intensity score was 4 (2 to 7), and the score for subjective symptoms was 11 (7 to 15).

Receptor analysis

A stepwise logistic binary regression model was used to find a combination of variables that best describes the probability of atopy. This analysis was based on differences in individual receptor expression between healthy control subjects, control patients with infection, and patients with atopic dermatitis in both neutrophils (Table II) and monocytes (Table III). Because acute infections strongly influence receptor expression in both neutrophils and monocytes, to avoid diagnostic bias, children with acute infections were excluded from the analysis. Consequently, the results of logistic regression can be interpreted and applied only when simultaneous acute infection is ruled out.

Stepwise binary logistic regression analysis of receptor variables showed significant and independent predictive effects for CR1 in neutrophils and FcγRI and FcγRII in monocytes.

The regression equation took the form of:

Probability (atopy) =

$$1 \div (1 + e^{(-4.40 + 0.0415 \cdot \text{CR1} - 0.0252 \cdot \text{Fc}\gamma\text{RI} + 0.0334 \cdot \text{Fc}\gamma\text{RII})})$$

where CR1 is in neutrophils, and FcγRI and FcγRII are in monocytes. The model yields continuous results between 0 and 1, indicating the probability of atopy. The probability of atopy estimated by the logistic model was then used as a new diagnostic variable. With the model above, each child's probability was then calculated, and the child was classified as atopic or nonatopic. If the estimated probability was higher than the specific cutoff point, it was an indication of atopy. The indices of sensitivity and specificity depend on the selection of the cutoff point between 0 and 1, as shown in the ROC curve in Fig. 1. The sensitivity and specificity of the logistic model were higher than those determined by the univariate techniques. If the cutoff point and probability of 0.70 is used, the model has a sensitivity of 0.80 (80%) and a specificity of 0.82 (82%).

Reactive oxygen species production

The reactive oxygen species production was different between the groups: $p = 0.01$ for unopsonized zymosan

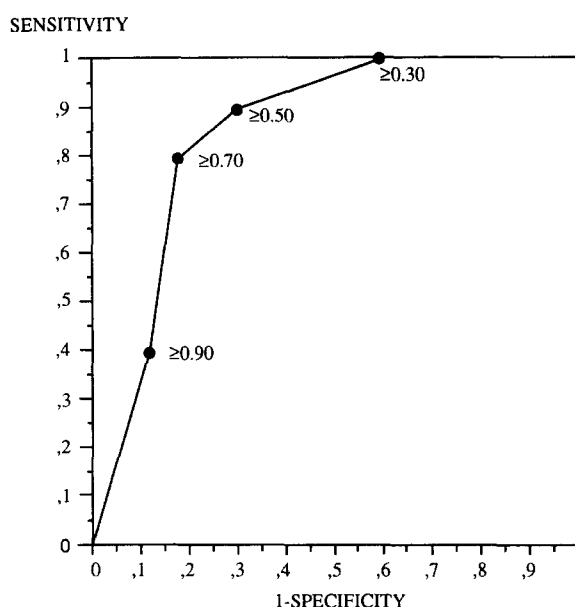


FIG. 1. ROC curve for logistic regression model as a diagnostic test for atopic sensitization. Cutoff points are predicted probabilities of atopic sensitization.

and $p = 0.07$ for opsonized zymosan. The distinction was due to increased reactive oxygen species production in the patients with acute infections: unopsonized zymosan (median, 6; lower-upper quartile, 4-8) and opsonized zymosan (median, 21; lower-upper quartile, 18-44) compared with the healthy control subjects (median, 4; lower-upper quartile, 3-5) and (median, 15; lower-upper quartile, 13-22), respectively. In patients with atopic dermatitis the levels for unopsonized zymosan (median, 4; lower-upper quartile, 3-6) were indistinguishable from those of healthy control subjects, and for opsonized zymosan (median, 21; lower-upper quartile, 15-32), were comparable to those of control patients with infection.

Receptor expression and reactive oxygen production as compared with the severity of the atopic eczema

A direct correlation between the SCORAD score and complement receptor expression (CR1) in neutrophils ($\rho = 0.40$, $p = 0.01$) and monocytes ($\rho = 0.39$, $p = 0.01$) was detected. This was due to a significant correlation between the frequency of CR1 in neutrophils and in monocytes and the intensity of the eczema ($\rho = 0.43$, $p = 0.008$ and $\rho = 0.41$, $p = 0.01$, respectively). In parallel, a significant correlation was found between the SCORAD score and the intensity of the eczema and between the SCORAD score and the amount of reactive oxygen species production (unopsonized zymosan: $\rho = 0.38$, $p = 0.02$ and $\rho = 0.39$, $p = 0.01$, respectively; opsonized zymosan: $\rho = 0.46$, $p = 0.005$ and $\rho = 0.46$, $p = 0.005$, respectively).

DISCUSSION

Atopy is immunologically characterized by increased systemic production of allergen-specific IgE. IgE responses are under the control of cytokines produced by competing signals from T helper 1- and T helper 2-like cells. Apart from inducing IgE synthesis, IL-4 is obligatory for the development of the T helper 2 phenotype, which leads to enhanced IgE production, eosinophilia, and atopic disease. At initial antigen exposure in early infancy, transient hypersensitivity is frequently induced, which is reversed to antigen-specific hyporesponsiveness at subsequent encounter.¹⁰ A critical balance is generated and maintained between protective mucosal immunity, including a vigorous immune response to pathogenic antigens, and specific systemic hyporesponsiveness to ubiquitous antigens. An important question is: "Why do atopic patients fail to terminate an inflammatory response initiated by newly encountered environmental antigens such as food?"¹¹

Phagocytosis is responsible for the early activation of the inflammatory response before antibody production. Neutrophils constitute the first step in the reaction, followed by activated monocytes. Specific surface receptors of phagocytes enable identification of soluble opsonins bound to the target. Acute infections are envisaged by a sharp rise of the receptors' frequency and rapid activation of their function.¹² Judging from our observation of a significant rise in complement CR1 receptor expression in patients with atopic dermatitis compared with healthy control subjects, it is obvious that the balance of signals from phagocytes in atopic sensitization bears a close resemblance to that from inflammatory cells. However, the results of this study show that the expression of complement and IgG receptors on phagocytes from atopic patients is distinctive from those of control patients with infection.

Could the distinction in the receptor expression between control patients with infection and patients with atopic dermatitis reflect differences between acute and chronic inflammation, or is the profile of the receptor expression disease-specific? Previous data on clinical conditions associated with acute inflammation such as acute bacterial infection,¹² thermal injury,¹³ surgery,¹⁴ and intense interval exercise¹⁵ demonstrate that the frequency of each receptor tends to be elevated. In contrast, in clinical conditions associated with chronic inflammation, the pattern of phagocytosis receptor expression appears to be disease-specific, showing increases or decreases in only few of the receptor intensities. In rheumatoid arthritis the expression of CR1, CR3, and FcγRI on monocytes has been shown to be increased,^{16,17} whereas that of CR3 and FcγRIII on neutrophils has been unaltered¹⁸ or raised.¹⁹ In systemic lupus erythematosus the frequency of CR1 has been shown to be decreased,²⁰⁻²² and that of CR3, increased on neutrophils²³; whereas the expression of FcγRI on monocytes has been found to be increased, and that of FcγRII²⁴ and FcγRIII, decreased.^{25,26} In atopic sensitization, as determined in this study by a logistic regres-

sion analysis, the distinctive pattern of receptor expression was identified as elevated CR1 on neutrophils and reduced FcγRI/FcγRII ratio on monocytes.

Because the frequency of a receptor does not directly reflect its activity, we measured the capacity of the phagocytes to produce reactive oxygen intermediates by stimulating the cells with various ligands. The results indicate that the capacity to generate and release functionally active products is increased in the phagocytes of patients with atopic dermatitis compared with control subjects. Interestingly, in phagocytes of patients with acute infections, the greater capacity to produce functionally active products was observed when both unopsonized and opsonized zymosan were used as stimulants, whereas in those of atopic children this capacity was observed only with opsonized zymosan, substantiating differences in receptor expression between these two patient groups. Free radicals and lytic enzymes can interfere with the integrity of mucosal defense barriers and consequently may be of pathogenic significance in local inflammation. In the gut, acute inflammatory response induces dysfunction in the intestine's mucosal barrier and disturbs the physiologic protein transfer,^{27,28} which may provide the means to circumvent natural immunosuppressive mechanisms and lead to exaggeration of immune response required for atopic sensitization.²⁹ In like manner, environmental stimuli may induce production of specific proinflammatory cytokines by keratinocytes with ensuing upregulation of adhesion molecules and recruitment of immunocompetent cells.³⁰ It was therefore interesting to note that the severity of the atopic eczema, particularly its intensity, was related to the activity of phagocytosing cells.

In the industrialized countries, atopic diseases are in the process of augmentation in prevalence and severity. Early recognition of atopic sensitization with manipulation of the environmental factors responsible for sensitization thus appears to be increasingly attractive. We propose that examining the activity of the phagocytic process in children at risk may be of use in early identification of atopic inflammation. Investigations are in progress to determine whether such a test could be suitable for general screening, especially in the newborn period.

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REFERENCES

1. Sampson HA, McCaskill CC. Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. *J Pediatr* 1985;107:669-75.
2. Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. *J Allergy Clin Immunol* 1996;97:9-15.
3. Zeiger RS. Atopy in infancy and early childhood: natural history and role of skin testing. *J Allergy Clin Immunol* 1985;75:633-9.
4. Unkeless JC, Boros P, Fein M. Structure, signaling and function of FcγR. In: Gallin JT, Goldstein IM, Snyderman R, editors. *Inflammation: basic principles and clinical correlates*. New York: Raven Press Ltd, 1992:497-510.
5. Wright S. Receptors for complement and the biology of phagocytosis.

- sis. In: Gallin JT, Goldstein IM, Snyderman R, editors. *Inflammation: basic principles and clinical correlates*. New York: Raven Press Ltd, 1992:477-95.
6. Hanifin JM. Epidemiology of atopic dermatitis. *Monogr Allergy* 1987;21:116-31.
7. European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: The SCORAD Index. *Dermatology* 1993;186:23-31.
8. Owens MA, Loken MR. *Flow cytometry principles for clinical laboratory practice*. New York: Wiley-Liss, 1995.
9. Lilius EM, Mäki AL, Proskin J, Rajamäki A. Leucocytes as immunosensors: whole blood chemiluminescence (CL) in human leukemias. In: Schölmerich J, Andreessen R, Kapp A, Ernst M, Woods W, editors. *Bioluminescence and chemiluminescence: new perspectives*. Chichester: Wiley & Sons, 1987:53-6.
10. Weiner HL, Friedman A, Miller A, Khoury SJ, Al-Sabbagh A, Santos L, et al. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu Rev Immunol* 1994;12:809-37.
11. Holt PG. Immunoprophylaxis of atopy: light at the end of the tunnel. *Immunol Today* 1994;15:484-9.
12. Simms HH, Frank MM, Quinn TC, Holland S, Gaither TA. Studies on phagocytosis in patients with acute bacterial infections. *J Clin Invest* 1989;83:252-60.
13. Vindenes H, Bjerknes R. Activation of polymorphonuclear neutrophilic granulocytes following burn injury: alteration of Fc-receptor and complement receptor expression and of opsonophagocytosis. *J Trauma* 1994;36:161-7.
14. Tellado JM, Christou NV. Activation state of polymorphonuclear leukocytes in surgical patients: characterization of surface receptor expression. *Surgery* 1993;113:624-30.
15. Gray AB, Telford RD, Collins M, Baker MS, Weidemann MJ. Granulocyte activation induced by intense interval running. *J Leukoc Biol* 1993;53:591-7.
16. Gadd SJ, Felzmann T, Majdic O, Maurer D, Petera P, Chen WJ, et al. Phenotypic analysis of functionally associated molecules on peripheral blood and synovial fluid monocytes from arthritis patients. *Rheumatol Int* 1992;12:153-7.
17. McCarthy D, Taylor MJ, Bernhagen J, Perry JD, Hamblin AS. Leucocyte integrin and CRI expression on peripheral blood leukocytes of patients with rheumatoid arthritis. *Ann Rheum Dis* 1992;51:307-2.
18. McCarthy DA, Bernhagen J, Taylor MJ, Hamblin AS, James I, Thompson PW, et al. Morphological evidence that activated polymorphs circulate in the peripheral blood of patients with rheumatoid arthritis. *Ann Rheum Dis* 1992;51:13-8.
19. Mishima K, Ichikawa Y, Shimizu H, Yoshida M, Arimori S. Cell surface antigens expressed on polymorphonuclear cells in the peripheral blood from patients with inflammatory rheumatic diseases: two-color flow-cytometric analysis. *Tokai J Exp Clin Med* 1992;17:41-51.
20. Wilson JG, Ratnoff WD, Schur PH, Fearon DT. Decreased expression of the C3b/C4b receptor (CRI) and the C3d receptor (CR2) on B lymphocytes and of CRI on neutrophils of patients with systemic lupus erythematosus. *Arthritis Rheum* 1986;29:739-47.
21. Fyfe A, Holme ER, Zoma A, Whaley K. C3b receptor (CRI) expression on the polymorphonuclear leukocytes from patients with systemic lupus erythematosus. *Clin Exp Immunol* 1987;67:300-8.
22. Mir A, Porteu F, Levy M, Lesavre P, Halbwachs-Mecarelli L. C3b receptor (CRI) on phagocytic cells from SLE patients: analysis of the defect and familiar study. *Clin Exp Immunol* 1988;73:461-6.
23. Buyon JP, Shadick N, Berkman R, Hopkins P, Dalton J, Weissmann G, et al. Surface expression of Gp 165/95, the complement receptor CR3, as a marker of disease activity in systemic lupus erythematosus. *Clin Immunol Immunopathol* 1988;46:141-9.
24. Szucs G, Kawai M, Suranyi P, Kiss E, Csipo I, Szegedi G. Correlations of monocyte phagocytic receptor expressions with serum immune complex level in systemic lupus erythematosus. *Scand J Immunol* 1994;40:481-4.
25. Clark MR, Liu L, Clarkson SB, Ory PA, Goldstein IM. An abnormality of the gene that encodes neutrophil Fc receptor III in a patient with systemic lupus erythematosus. *J Clin Invest* 1990;86:341-6.
26. Enenkel B, Jung D, Frey J. Molecular basis of IgG Fc receptor III defect in a patient with systemic lupus erythematosus. *Eur J Immunol* 1991;21:659-63.
27. Sanderson IR, Walker WA. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterology* 1993;104:622-39.
28. Majamaa H, Isolauro E. Evaluation of gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol* 1996;97:985-90.
29. Fargeas MJ, Theodorou V, More J, Wal JM, Fioramonti J, Bueno L. Boosted systemic immune and local responsiveness after intestinal inflammation in orally sensitized guinea pigs. *Gastroenterology* 1995;109:53-62.
30. Barker JNWN, Mitra RS, Griffiths CEM, Dixit VM, Nickoloff BJ. Keratinocytes as initiators of inflammation. *Lancet* 1991;337:211-4.