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Increased number of immunoreactive nerve fibers in atopic dermatitis

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The presence of immunologic markers for neurofilaments, neuropeptides of sensory nerve fibers (Calcitonin gene-related peptide and substance P), for noradrenergic innervation (neuropeptide Y and Tyrosine hydroxylase), and Neuron-specific protein 9.5 was evaluated in frozen tissue sections from normal skin (n = 34) and from skin biopsies manifesting urticaria (n = 6), leukocytoclastic vasculitis (n = 4), systemic lupus erythematosus (n = 23), and atopic dermatitis (n = 40, of which 16 were from lesions induced by epicutaneous atopic allergen patch tests). In some normal skin specimens immunoreactive nerve fibers expressing Neuron-specific protein 9.5 were observed in the epidermis, dermis, and around blood vessels. For the other markers, immunolabeling was mainly observed in the dermis around blood vessels. Neurofilaments, which are scarce in normal skin epidermis, were present in higher density in the epidermis of affected skin in all disease conditions. Biopsies from urticaria and systemic lupus erythematosus showed a decrease in density of fibers immunolabeled for neuropeptides substance P and Calcitonin gene-related peptide and for Neuropeptide Y. In biopsies from skin with atopic dermatitis, an increased density of fibers was observed for all markers except Neuropeptide Y and Tyrosine hydroxylase. In this group, biopsies from positive atopic allergen patch tests showed an enhanced density of fibers labeled by antibody to Neuron-specific protein 9.5 and a lower density in labeling for Tyrosine hydroxylase. The data indicate a potential role of innervation and neuropeptides in dermatoses like atopic dermatitis. (J ALLERGY CLIN IMMUNOL 1992;90:613-22.)

Key words: Atopic dermatitis, dermatoses, inflammation, innervation, neuropeptides, skin

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The human skin is equipped with a rich supply of nerve elements serving receptor as well as effector functions. Environmental stimuli activate afferent sensory neurons that, by intervention of the central nervous system, give rise to mechanoreceptive (touch), thermal, and pain sensitization. Effector activities, for example, vasoconstriction/dilation and sweating, are mediated by impulses originating in the central nervous system and by the axon reflex of sensory nerves in the periphery. Varicosities along these nerves release neuropeptides that can act on specific receptors. These peptides are necessary for the initiation of inflammatory and reparative processes in injured skin.¹⁻⁶

Altered patterns of cutaneous innervation have been reported in many inflammatory dermatoses, including psoriasis, leprosy, and eczema.⁷⁻¹¹ Similar observations have been made for urticaria on evidence from tissue-fluid analysis and capsaicin-induced prevention of urticarial responses.^{12, 13} A symptom of these skin disorders is pruritus, where sensory C and possibly A Δ fibers are stimulated in the superficial dermis.^{6, 10} Peptidergic mediators have been implicated in skin inflammatory disorders.¹¹ Immunocytochemical and radioimmunoassay studies have shown elevated levels of substance P (SP), Calcitonin gene-related peptide (CGRP), Neuropeptide Y (NPY), Vasoactive intestinal peptide, and somatostatin in blister tissue and fluids.^{11, 13} These neuropeptides can manifest immunomodulatory activity,¹⁴ and they contribute to the cross talk between the autonomic and sensory nervous systems and the immune system in the skin.¹⁵

In a retrospective analysis we investigated the presence of neurofilaments, CGRP, SP, NPY, Tyrosine hydroxylase (TH), and Neuron-specific protein 9.5 (PGP9.5). This analysis was performed by analysis of immunohistochemistry on skin biopsies from patients with urticaria, vasculitis, systemic lupus erythematosus (SLE), and atopic dermatitis (AD).

MATERIAL AND METHODS

Biopsies

This retrospective study included 40 biopsies from patients with AD (21 males and 19 females; age range, 1.5 to 72 years; median age, 20 years). Twenty-four of these biopsies were from affected skin and showed the histologic characteristics of AD. Sixteen biopsies were taken from lesions after stripping the epidermis, and subsequent epicutaneous patch testing was performed with house dust, house dust mite, and grass pollen allergens according to methods previously described.¹⁶ Twenty-three biopsies were from patients with SLE (14 females, 9 males; age range, 0 to 72 years; median age, 33 years). Histopathologic study of this material showed changes characteristic of SLE, and

Abbreviations used

SP:	Substance P
CGRP:	Calcitonin gene-related peptide
NPY:	Neuropeptide Y
TH:	Tyrosine hydroxylase
PGP9.5:	Neuron-specific protein 9.5
SLE:	Systemic lupus erythematosus
AD:	Atopic dermatitis
PBS:	Phosphate-buffered saline

immunofluorescence demonstrated granular deposits of immunoglobulins and complement along the epidermal basement membrane. Four biopsies from patients with vasculitis (3 females, 1 male; age, 11 to 36 years; median, 30 years) were analyzed. Histopathologic study of that material revealed leukocytoclastic vasculitis; with immunofluorescence, immunoglobulin and complement deposits were observed in superficial blood vessel walls. Six biopsies were from patients with urticaria (4 females, 2 males; age range, 24 to 69 years; median, 46 years). With histopathologic study some characteristics of urticaria were manifest. Thirty-four skin biopsies showing no histologic abnormalities served as controls; 31 samples were biopsies and 3 samples were obtained at autopsy (20 males, 14 females; age range, 15 to 79 years; median, 37 years). Some of these specimens were from uninvolved skin of patients in the disease categories mentioned above. Except for autopsy tissue, almost all samples were 3 mm punch biopsies. Biopsies from patients were from the affected skin; those from controls were mainly taken from the extensor site of the upper arm. In all cases the histopathologic diagnosis was made on formalin-fixed tissue embedded in paraffin. The samples analyzed in this study were snap frozen and stored at -70°C before analysis.

Immunohistochemistry

Frozen sections of 10 μm thickness were taken up in series of three consecutive sections onto glass slides coated with gelatin. The slides were fixed in 100% acetone for 10 minutes at room temperature. The primary antisera are listed in Table I. An avidin-biotin immunoperoxidase method was used. First, sections were preabsorbed with 10% normal goat (or horse) serum in phosphate-buffered saline (PBS) for 15 minutes. Then the sections were incubated for 1 hour with primary antiserum at the predetermined optimal dilution (Table I). They were subsequently rinsed in PBS and incubated for 30 minutes with biotinylated goat anti-rabbit (1:250) (or horse anti-mouse [1:150]) serum (Vector Laboratories, Burlingame, Calif.). Antisera were diluted in PBS supplemented with 1% normal goat (or horse) serum. The sections were rinsed in PBS and finally incubated for 30 minutes with an avidin-biotin complex (Vector Laboratories) in PBS supplemented with 1% normal goat (or horse) serum. After rinsing, the immunoreaction was visualized by use of a mixture of 3'3'-diaminobenzidine tetrahydrochloride (0.35

TABLE I. Antibodies applied in this study

Antigen	Species	Origin*	Dilution	Reciprocal Remarks
Neurofilament 200kd	Mouse	Sigma†	15,000	Neuron-specific cytoskeletal protein
Neurofilament 160kd	Mouse	Sigma†	15,000	Neuron-specific cytoskeletal protein
Neurofilament 68kd	Mouse	Sigma†	700	Neuron-specific cytoskeletal protein
CGRP	Rabbit	Amersham‡	150	Neuropeptide, sensory nerves
SP	Rabbit	Amersham§	200	Neuropeptide, sensory nerves
NPY	Rabbit	Amersham	30	Neuropeptide, noradrenergic innervation
TH	Mouse	Ultraclone¶	100	Enzyme involved in noradrenalin synthesis
PGP9.5	Rabbit	Ultraclone#	2,000	Neural-neuroendocrine-specific carboxyl-terminal hydrolase

*Origin: Amersham, Bucks, UK; Sigma Chemical Co., St. Louis, Mo.; Ultraclone, Incstar, Stillwater, Minn.

†Specification by the supplier: Raised to neurofilaments purified from pig spinal cord; IgG1 subclass; no crossreactivity to other intermediate filament proteins; reacts to neurofilaments of 200kd, 160 kd, or 68 kd.

‡Specification by the supplier: raised to rat CGRP conjugated to bovine serum albumin; crossreactivity, none detected with other peptides.

§Specification by the supplier: raised to synthetic SP conjugated to bovine serum albumin; crossreactivity, none detected with amphibian bombesin.

||Specification by the supplier: raised to unconjugated synthetic porcine NPY; crossreactivity, no significant cross-reactivity with PYY.

¶Specification by the supplier: raised to TH from rat PC12 cells; IgG1 subclass; recognizes an epitope in the midportion of the TH molecule where extensive species homology exists; no crossreactivity to dihydropterine reductase, dopamine- β -hydroxylase, phenylethanolamine-N-methyltransferase, phenylalanine hydroxylase, and tryptophan hydroxylase (western blotting).

#Specification by the supplier: raised to human PGP9.5.

gm/L), nickel-ammoniumsulfate (6-hydrate, 25 gm/L) and H_2O_2 (0.1%) for 3 to 5 minutes. Sections were counterstained with Nuclear Fast Red (Sigma Chemical Co., St. Louis, Mo.), dehydrated, and embedded in synthetic mounting medium DPX (Aldrich Chemical Co., Milwaukee, Wis.). The immunolabeling product for individual antibodies is designated as "immunoreactive." Control material was incubated with an irrelevant antiserum, including a rabbit antimouse antiserum (Dakopatts; DAKO Immunoglobulins, Glostrup, Denmark) or a monoclonal antibody to human IgD (Dakopatts) of the same subclass (IgG1) as the monoclonal antibodies applied. The control procedure also included the replacement of primary antisera with PBS. No staining was observed, with the exception of eosinophilic and neutrophilic polymorphonuclear granulocytes, when present.

Semiquantitative scoring

The density of immunoreactive fiberlike structures in three consecutive sections was assessed by two independent observers. In cases of disagreement, mean scores were taken. The scores were expressed as absent (0), incidental (0.5), few (1), moderate (2), many (3), and abundant (4). Scores were given in relation to each of the following skin components: epidermis, superficial dermis, and superficial blood vessels. Also the occurrence in hair follicles or in sebaceous and eccrine sweat glands, if present, was noted but not scored. Statistical analysis was performed for groups defined according to the histopathologic diagnoses men-

tioned herein, by means of the nonparametric Kruskal-Wallis one-way analysis of variance (ANOVA) and the Mann-Whitney U test. The software applied was SPSS.PC version 3.0. For individual groups of biopsies, the difference in score with regard to normal skin was calculated. In addition, for the AD group the difference between affected skin and skin after patch testing was analyzed. Differences were considered significant at values of $p < 0.05$.

RESULTS

The semiquantitative scores of individual markers in epidermis, dermis, and around blood vessels, for individual disease categories and controls, are shown in Table II. Scores with the most significant differences after aggregation are graphically represented in Fig. 1. In the following section we describe the immunohistology and statistical results for individual markers.

Neurofilaments

The distribution and density of immunoreactive fibers observed was similar for labeling by antibodies to neurofilament 200, neurofilament 160, and neurofilament 68. The aggregated data are presented in Fig. 1, *a* in combined form for the epidermis. In normal skin, some immunoreactive fibers were pres-

TABLE II A. Semiquantitative score of immunoreactive fibers in various skin compartments

Category	No.	Neurofilament 200			Neurofilament 160			Neurofilament 68		
		Epidermis	Dermis	Vessels	Epidermis	Dermis	Vessels	Epidermis	Dermis	Vessels
Normal control	34	0 0 - 0.5	1 0 - 3	1 0 - 2	0 0 - 1	0 0 - 1	1 0.5 - 2	0 0 - 1	2 0 - 3	1 1 - 3
Vasculitis	4	1 0 - 2	1 1 - 2	0 0 - 2	1 0 - 1	1 0 - 2	1 0 - 1	0 0 - 2	1 0 - 2	0 0 - 2
SLE	23	0.5 0 - 2	1 0 - 3	1 0 - 2	0 0 - 0.5	1 0.5 - 3	1 1 - 2	0.5 0 - 0.5	2 0.5 - 3	1 1 - 3
Urticaria	6	1 0 - 2	1 1 - 3	1 1 - 2	0 0 - 1	1 0 - 3	2 0.5 - 3	1 0 - 2	2 1 - 2	2 1 - 3
Atopic dermatitis affected skin	24	0.5 0 - 1	2 1 - 4	2 0 - 3	0 0 - 1	1 0.5 - 3	1 0 - 3	1 0 - 2	2 0.5 - 3	2 0 - 3
Atopic dermatitis patch test	16	1 0 - 2	2 0.5 - 3	1 0.5 - 2	1 0 - 2	1 1 - 3	2 1 - 2	1 0 - 2	2 1 - 3	2 1 - 3

TABLE II B. Semiquantitative score of immunoreactive fibers in various skin components

Category	No.	CGRP			SP			NPY		
		Epidermis	Dermis	Vessels	Epidermis	Dermis	Vessels	Epidermis	Dermis	Vessels
Normal control	34	0 0 - 1	0 0 - 2	0 0 - 2	0 0 - 0.5	0.5 0 - 1	0.5 0 - 1	0 0 - 1	0.5 0 - 2	1 0 - 2
Vasculitis	4	0 0 - 0	0 0 - 0	0 0 - 1	0 0 - 0	0 0 - 1	0 0 - 0.5	0 0 - 0	0 0 - 2	0.5 0 - 1
SLE	23	0 0 - 0.5	0 0 - 2	0 0 - 1	0 0 - 0	0 0 - 0.5	0.5 0 - 0.5	0 0 - 0	0 0 - 1	0 0 - 1
Urticaria	6	0 0 - 0	0 0 - 2	0 0 - 0	0 0 - 0	0 0 - 0	0 0 - 0.5	0 0 - 0	0 0 - 0	0 0 - 1
Atopic dermatitis Affected skin	24	0 0 - 0	1 0 - 1	1 0 - 1	0 0 - 0	1 0 - 1	0.5 0.5 - 1	0 0 - 1	0 0 - 2	0 0 - 2
Atopic dermatitis Patch test	16	0 0 - 0	1 0.5 - 1	1 0 - 1	0 0 - 0	1 0.5 - 2	1 0.5 - 2	0 0 - 0.5	1 0 - 2	0 0 - 1

Data presented are median values and ranges determined by semiquantitative scoring.

ent in the superficial dermis and around blood vessels. Only rarely was labeling in the epidermis observed (Fig. 2, *a*). The presence of immunoreactive fibers in vasculitis cases was similar to that seen in normal skin. A significantly higher density of immunoreactive neurofilament fibers was observed in the epidermis of

lesional skin of patients with SLE, urticaria, and AD (Fig. 1, *a*, illustrated in Fig. 2, *b* and *c*). In the AD group, biopsies from affected skin did not differ from those taken from skin after patch tests. In the superficial dermis and around blood vessels, SLE biopsies manifested a higher density than normal skin

PGP9.5		
Epidermis	Dermis	Vessels
0	1	1
0 - 2	0 - 4	1 - 4
0	0.5	0
0 - 0	0 - 1	0 - 0
0	1	0
0 - 0.5	0 - 3	0 - 1
0	1	0.5
0 - 0.5	0 - 3	0 - 3
0	1	1
0 - 1	0 - 2	0 - 2
0	2	1
0 - 1	0 - 3	0 - 3

TH		
Epidermis	Dermis	Vessels
0	0	0.5
0 - 1	0 - 3	0 - 2
0	0	0
0 - 0	0 - 0	0 - 2
0	0	0
0 - 1	0 - 2	0 - 2
0	0.5	0.5
0 - 0	0 - 1	0 - 2
0	1	2
0 - 0	0 - 2	0 - 3
0	0	0
0 - 0	0 - 2	0 - 2

($p = 0.004$) (Fig. 2, c). But SLE lesional skin with a marked mononuclear cell infiltrate contained very few immunoreactive-neurofilament fibers (Fig. 2, d).

Neuropeptides in sensory nerves (CGRP [Fig. 1, b] and SP)

In normal skin the distribution and relative density of immunoreactive CGRP and immunoreactive SP fi-

bers were similar. They were very rarely seen in the epidermis, whereas they were more common around blood vessels and in association with trespassing nerve bundles. Similar observations were made in vasculitis biopsies. A lower mean density of immunoreactive CGRP was found in biopsies from patients with SLE, and a significantly higher density was observed in biopsies from patients with AD (Fig. 1, b, illustrated in Fig. 3). In skin with urticaria, a low mean density was observed, but in some cases clear labeling was seen in the upper dermis around blood vessels (Fig. 4).

Noradrenergic innervation (NPY [Fig. 1, c] and TH [Fig. 1, d])

In normal skin, immunoreactive NPY and immunoreactive TH fibers were observed around blood vessels and to a lesser extent around hair follicles and sebaceous glands. Except for a few cases, no intra-epidermal staining was seen. Skin biopsies from patients with vasculitis showed similar results. The density of immunoreactive NPY fibers was lower in skin of patients with SLE and urticaria and similar to control specimens in skin of patients with AD (Fig. 2, c). A different picture was observed for TH. Here, the mean density of immunoreactive TH fibers was similar in skin of patients with SLE and urticaria to that found in normal skin, and a higher mean density was observed in skin of patients with AD (Fig. 2, d, illustrated in Fig. 5). Also, when compared with skin after patch testing, the higher mean density in affected skin was statistically significant ($p = 0.005$). Immunoreactive TH fibers were most dense around sweat glands, hair follicles, and blood vessels. Often leukocytes with a mast-cell appearance were labeled.

Other neural markers (PGP9.5 [Fig. 1, e])

Immunoreactivity for PGP9.5 in normal healthy skin was present in the epidermis, dermis, and around vessels. The highest mean density was observed in skin of patients with AD after patch test provocation, being statistically significant when compared with normal skin ($p = 0.003$) and affected skin of patients with AD ($p = 0.03$). In skin of patients with vasculitis, SLE, and urticaria, the mean density was similar to that found in normal skin.

DISCUSSION

The distribution and relative density of fibers that we found to be immunoreactive for neuropeptides and neurofilaments in normal healthy skin correspond to findings from the literature.¹⁻⁶ The highest density of immunoreactive fibers occurs in the dermis around blood vessels (Table II). This was observed for all

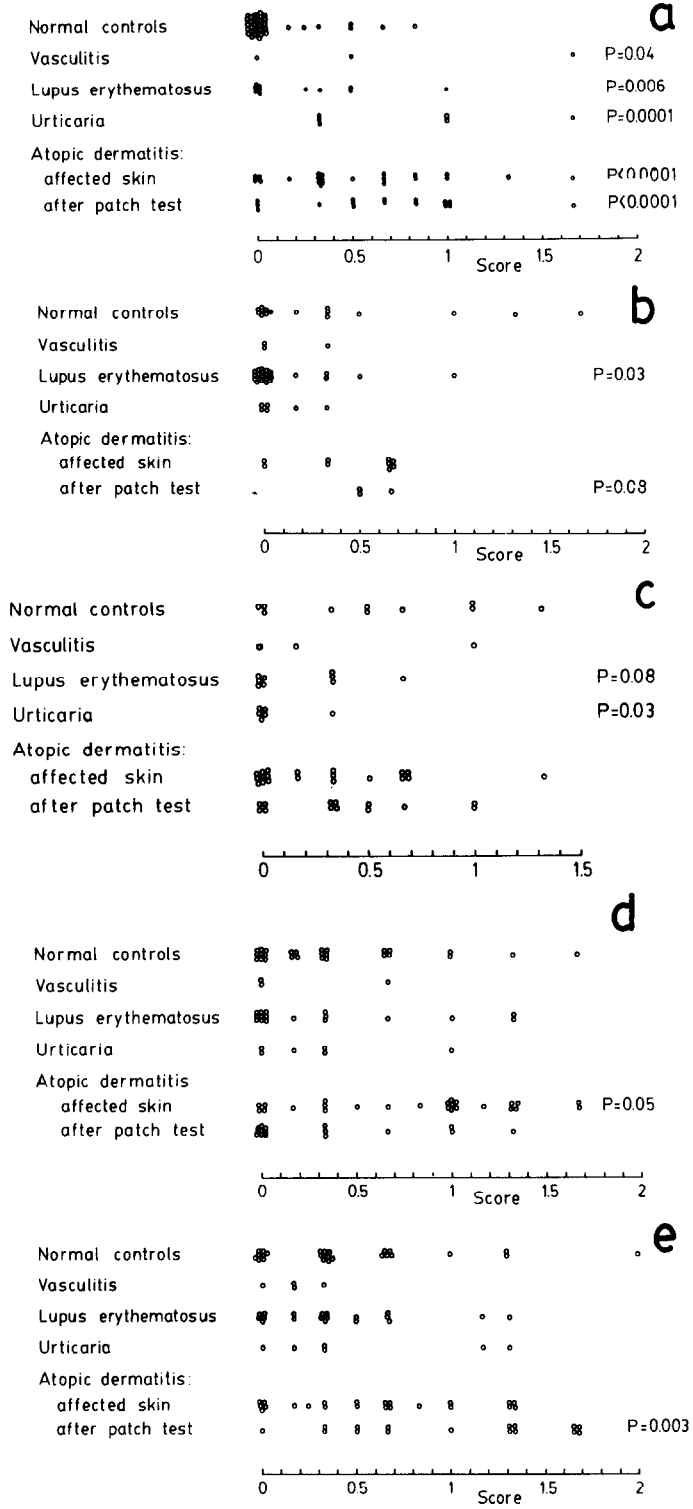


FIG. 1. Scores of individual neural markers in skin compartments. Figures present data of normal control biopsies of skin without histopathologic abnormalities and biopsies of skin with leukocytoclastic vasculitis, SLE, urticaria, and AD. Data subdivided into biopsies from affected skin and biopsies from lesions after epicutaneous patch tests with atopic allergens. Data shown: **a**, neurofilaments in the epidermis; **b**, mean of CGRP in epidermis, dermis, and around blood vessels; **c**, mean of NPY in epidermis, dermis, and around blood vessels; **d**, mean of TH in epidermis, dermis, and around blood vessels; **e**, mean of PGP9.5 in epidermis, dermis, and around blood vessels. Statistically significant differences from normal skin (Mann-Whitney U test) are indicated.

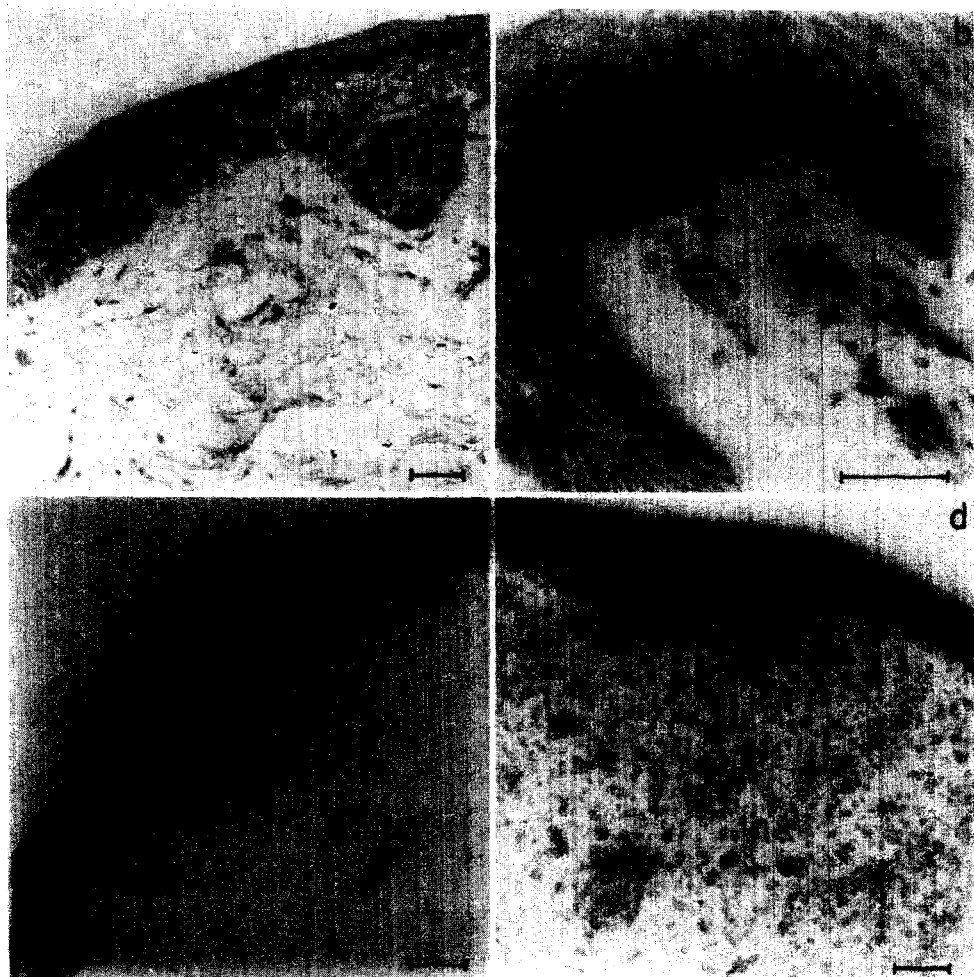


FIG. 2. Neurofilament immunolabeling. **a**, Normal healthy skin, without neurofilament 200 labeling product (score 0). **b**, Skin in patient with AD shows intraepidermal and subepidermal sensory nerves indicated by *arrows* (score 3). **c**, Skin in patient with SLE, shows many sensory nerves in and underneath the epidermis indicated by *arrows* (score 3). **d**, SLE lesional skin, with apparent absence of sensory nerve fibers (score 0). *Bar*, 50 μ m.

markers investigated: neurofilaments, neuropeptides SP and CGRP associated with sensory nerves, NPY and TH as markers for noradrenergic innervation, and PGP9.5. For SP, the findings in the literature vary from negative to positive for fibers in the superficial dermis^{5, 17}; our data confirm findings in the latter report. The immunoreactivity at other skin sites varied according to the antigen investigated. For instance, normal epidermis was negative for markers of noradrenergic innervation, and it was only rarely positive for PGP9.5, neurofilaments, and neuropeptides CGRP and SP. Similar observations have been reported by others.^{1, 2, 4, 6} For visualization of cutaneous innervation, the choice of anti-PGP9.5 antibody or antibodies to neurofilaments is recommended.^{3, 4}

The dermatoses investigated manifested a number of alterations in the immunoreactivity for nerve mark-

ers. This observation should be interpreted in light of the restrictions imposed by the study design. Since this investigation was retrospective, we were unable to perform a follow-up analysis on material from the same patient. Skin in patients with SLE, urticaria, and AD showed a statistically significant increase in the density of immunoreactive NF fibers in the epidermis (Fig. 1, *a*). Peptides associated with sensory nerves were decreased in skin of patients with SLE and urticaria (Fig. 1, *b*), and the scores for immunoreactive noradrenergic nerves (Fig. 1, *c*) were lower. On the other hand, skin of patients with AD showed a significant increase for all markers, except TH and NPY. For SP, a similar increase has been reported by others.¹⁷ For NPY, Pincelli et al.¹⁷ have shown staining of dendritic epidermal cells in skin of patients with AD. We were unable to reproduce these findings in



FIG. 3. Substance P immunolabeling in skin of a patient with AD. Immunoreactive fibers are associated with superficial dermal vessels (score 2), one of which is indicated by an arrow. Leukocytes also show labeling (arrowhead). Bar, 25 μ m.



FIG. 4. CGRP immunolabeling in urticarial skin. Immunoreactive fibers (one indicated by an arrow) are present in the superficial dermis and around dilated blood vessels (score 2). Bar, 25 μ m.

the present study. This discrepancy may be ascribed to differences in study material, technique, and antibody applied. We conclude that the skin of patients with AD shows an increased density of immunoreactive nerve fibers. This does not occur in skin of patients with urticaria and SLE, except for immunoreactive neurofilament fibers in the epidermis.

A number of explanations for the observed changes in immunoreactive nerve fibers may be offered. First, the changes are caused by the disease process. This may underlie the lower density of immunoreactive profiles in the dermis of patients with SLE and urticaria; that is, the infiltrates may damage the nerve structures. For neurofilament immunolabeling, biopsies of skin of patients with SLE showed a higher expression in the dermis than controls, but this difference was not apparent when a mononuclear cell infiltrate was pronounced (Figs. 2, *c* and *d*). Second, the changes in nerve fibers may play a role in the pathogenesis of the inflammatory response. The enhanced expression of neurofilaments in the epidermis suggests such an explanation because nerves identified

by neurofilament immunolabeling (Fig. 1, *a*) can serve as mediators of inflammation.¹⁸ In patients with SLE and urticaria we did not find an increase in immunoreactivity for neuropeptides of sensory nerves, for example, SP and CGRP. For patients with AD a different picture emerges. The higher immunoreactivity for most of the markers investigated suggests that nerve components play a role in the disease process. Neuropeptides, including SP and CGRP,^{19, 20} are able to produce inflammatory reactions on intradermal injection in normal skin. Also the weal and flare response after trauma is ascribed to sensory neuropeptides.²¹ One mechanism triggering this response may operate via T lymphocytes, which are susceptible to stimulation by these peptides and subsequently initiate an inflammatory response.^{22, 23} Patients with AD have been reported to be anergic or hyperreactive on such a challenge.^{21, 22} This apparent discrepancy may be related to the ill-defined balance between the increased vulnerability to neuropeptide action on the one hand and exhaustion of the responding cellular components on the other. Alternatively, allergen challenge may

influence the neuropeptide status. In this study, biopsies taken from patients with AD after atopic allergen challenge showed a higher density for PGP9.5 and a lower density for TH than biopsies from affected skin. That difference forms a first indication of changes in nerve components after allergen challenge. This point warrants further study in serial biopsies from the same patients before a definite conclusion can be made.

In selecting the material for this retrospective study, our choice focused on inflammatory skin lesions. We conclude that lesions in patients with AD show detectable alterations in immunoreactive nerve profiles. This is also the case for neurofilament labeling in patients with urticaria, leukocytoclastic vasculitis, and SLE. In view of the present immunohistochemical data, future studies should focus on the role of sensory nerves and neuropeptides associated with sensory innervation in the etiology and pathogenesis of these dermatoses.

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FIG. 5. TH immunolabeling in skin of a patient with AD. Immunoreactive TH fibers, indicated by an arrow, are associated with a superficial dermal blood vessel and perivascular infiltrate (score 3). Bar, 25 μ m.

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Fish hypersensitivity. II: Clinical relevance of altered fish allergenicity caused by various preparation methods

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In double-blind, placebo-controlled, oral food challenges with fish, a 12-fold higher false-negative rate was found compared with other food antigens. In an effort to elucidate this discrepancy, cooked lyophilized fish extracts (used in double-blind, placebo-controlled, oral food challenges) were compared with cooked, nonlyophilized fish extracts (used in open challenges) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, immunoblot, and ELISA-inhibition assays. Altered fish allergenicity as a result of food processing was examined with canned tuna and salmon. Forty-five children and young adults with food allergies, including 18 patients with IgE-mediated hypersensitivity to fish, were challenged with canned tuna. All 45 challenges with canned tuna were negative. Two of these patients are allergic to salmon and also have negative reactions to challenges with canned salmon. In vitro investigation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of tuna and salmon extracts revealed a striking loss of definable protein fractions in the canned fish extract when compared with raw and cooked fish extracts, and immunoblot analyses demonstrated minimal IgE-specific binding to the canned fish extracts. In addition, decreased allergenicity of the canned tuna and salmon was demonstrated by ELISA-inhibition assay and by negative oral challenges with canned salmon in two patients allergic to salmon. Collectively, these findings suggest that some of the major allergens responsible for IgE-mediated food allergy to fish are more labile than previously recognized. (J ALLERGY CLIN IMMUNOL 1992;90:622-9.)

Key words: Fish allergy, food hypersensitivity, double-blind, placebo-controlled oral food challenges, lyophilization, ELISA—inhibition assay, immunoblotting

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Allergenic food proteins are characteristically heat and acid stable, largely resistant to proteolysis and digestion, and their primary amino acid structures are bound by IgE.¹ Indeed, these inherent physicochemical characteristics of allergenic food proteins may play a major role in their allergenicity. Furthermore, despite the wide variety of foods consumed, only a small number are responsible for more than 90% of the food-allergic reactions in both pediatric and adult populations.²

Unfortunately for the population with food allergies, most allergenic food proteins require extensive alteration to decrease their allergenicity.³⁻⁶ This is an issue that has gained notoriety in the infant formula