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Immunohistochemical Study of Some Chemokines in Atopic Epidermis: Extrinsic versus intrinsic types.

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Abstract

Background:

The atopic reaction is mediated through 3 main types of cells; T-cells, APCs and keratinocytes. The role of these cells is orchestrated by complex interplay of adhesion molecules, cytokines and other inflammatory mediators.

Aim of the study:

was to semiquantitatively assess expression of some keratinocyte-derived chemokines in the epidermis of atopic skin (acute to subacute lesions) and to explore any differences in the degree and pattern of epidermal expression between intrinsic and extrinsic subsets of AD.

Material and methods :

Twenty seven cases of atopic dermatitis (16 of the extrinsic and 11 of the intrinsic type) plus 14 control subjects were evaluated immunohistochemically for the epidermal expression pattern and intensity of some chemokines namely TSLP, GM-CSF, TARC, CTACK and RANTES and for LC density in the epidermis.

Results:

Enhanced expression of TSLP in the lesional epidermis could be seen in ADi group and significantly more in ADe. For GM-CSF, expression was significantly enhanced in the lesional epidermis of ADe only but not in ADi. Lesional skin of both ADi and ADe showed high TARC expression levels but significantly more in ADe than ADi. Expression of both CTACK and RANTES were faint in atopic epidermis and did not differ from that of normal skin. Decrease in the LC count was seen in the lesional skin of both groups but was significant only in the ADe group. The non-lesional atopic epidermis did not show significantly different expression patterns from normal epidermis except for TARC which was significantly enhanced in ADe but not in ADi.

Conclusion:

The enhanced expression of TSLP, GM-CSF and TARC by atopic epidermis may play a role in the evolution of atopic lesions in general and in the characteristic T_{h2} polarization of the inflammatory reaction in particular. The difference in the expression pattern between both subgroups my play a role in determining the exact lineage of the atopic patient (i.e. intrinsic versus extrinsic). TSLP and GM-CSF are likely to augment LC population number and activity in the skin thus increasing the tendency of the skin to react to external allergens which is characteristic of ADe. On the other hand, higher levels of TSLP and TARC in ADe compared to ADi may be responsible for stronger T_{h2} polarization of the inflammatory reaction. This may explain some reported differences between ADe and ADi at the tissue or serum levels.

Introduction and Aim of the Work:

Atopic dermatitis is a chronically relapsing eczematous skin disease resulting from complex interactions between genetic and environmental factors.[1] A large number of immunological and non-immunological abnormalities have been reported in AD patients whether in the skin or serum.[2,3]

While the primary defect seems to be genetically determined, many triggering factors have been reported including stress, external irritants, scratching and microbial agents. [4]

In spite of controversies as regards the exact pathophysiology of eczematous lesion (and even the exact type of immune reaction), three main types of cells have been confirmed to play the major role in the evolution of characteristic pathology of AD namely T-lymphocytes, antigen presenting cells (APCs) and keratinocytes.[5]

The role played by these effector cells is orchestrated by a growing list of cytokines (or chemokines), adhesion molecules and other inflammatory mediators that control trafficking and action of the inflammatory cells and subsequently determine the nature, extent and duration of the inflammatory reaction.[6]

In the last few years, more light has been shed on the role of

keratinocytes in AD where a lot of constitutive and induced abnormalities have been demonstrated at the immunological (both innate and adaptive) and non- immunological levels (e.g. as regards barrier function and microbial colonization). Immunologically, the expression by epidermal cells of a large number of cytokines has been shown to be dysregulated in atopic skin in general or in lesional areas only. The actions of many of these cytokines could be of significance for the atopic reaction (e.g. for expression of adhesion molecules, recruitment, maturation, survival &/or activation of inflammatory cells especially APCs, determination of the polarity of the reaction and perpetuation of the inflammation).[5]

At the same time, the distinction between two subtypes of AD has been recently suggested which are the extrinsic type (ADe) and intrinsic type (ADi). The former type is mainly characterized by elevated serum IgE levels and polyvalent IgE sensitization against inhalant and/or food allergens in skin test or serum while in the intrinsic type, there is no specific IgE sensitization and total serum IgE is not elevated (i.e. <100 kU/L). Other distinctive features of intrinsic AD include later age of onset, mild female predominance, only mild to moderate eosinophilia, lower serum levels of IL-4 and of CD23+ B cells, lower density of tissue eosinophils, lower tissue expression of IL-1 ß , IL-5 and IL-13 as well as Fc RI/Fc RII expression ratio on the CD1a+ epidermal DCs of less than 0.5 in lesional skin. ADi is estimated to represent only 16-45% of all AD cases.[7,8] Although additional differences between these two types are continuously reported whether at the tissue or serum level,[8,9,10] many other aspects of the immune reaction have not yet been explored in a comparative way between both subtypes.

The lack of external allergy and of elevated serum IgE levels in the intrinsic group may point to some difference(s) in the evolution pathway between these two subtypes. The aim of the study was to explore other possible differences between ADi and ADe. The study has focused on the immunological role of keratinocytes and in-particular the expression of some keratinocyte-derived chemokines that could be relevant to the atopic reaction.

Material and Methods

Twenty seven patients with typical atopic dermatitis (AD) and who attended the Dermatology Outpatient Clinic in Bou-Shahri Medical Centre – Kuwait, were randomly selected to participate in the study. Fourteen age-matched healthy individuals with no personal or family history of atopy were included as control . Informed consent was obtained from all patients (or their parents) and from control individuals.

The diagnosis of atopic dermatitis was based on the criteria described by Hanifin and Rajka.[11]

All the patients had not been treated with any systemic or topical drugs for at least 1 month before the beginning of the study.

To investigate whether the patients were ADi or ADe and apart from suggestive clinical data, blood samples from all subjects were tested for total serum IgE titre and eosinophil count.

Total serum IgE was measured using an ultrasensitive enzyme-linked immunoassay, IM x total IgE assay (Abbott Laboratories®, Abbott Park, IL, USA), based on microparticle enzyme immunoassay. The results were expressed in IU/mL –1 (1 IU/·mL = 2.4 µg of IgE/L). The minimum sensitivity of the assay was 0.048 IU/mL.

In addition, all AD patients were also checked in-vitro for ingestant- and inhalant-specific serum IgE levels using the multiple antigen simultaneous testing chemiluminescent assay (MAST-CLA) system (MAST Immunosystems, Mountain View, Calif., USA).

MAST-CLA In vitro testing : A three step technique was applied as described by Brown et al. (1985)[12] , in the form of overnight incubation of the serum with multiple allergens fixed on strands of cellulose, 4 hour incubation with enzyme labeled antibody and 30-minute chemiluminescent reaction, which produces a visible image (immunograph) on high-speed Polaroid instant film. The densities of the bands produced on the film are quantified with a microprocessor-controlled infrared transmittance densitometer. Scoring was performed according to the serum level of specific IgE as shown in **table (1)**. A CLA class greater than or equal to 2+, corresponding to an IgE concentration equal to or more than 71 IU/mL (or KU/L), was considered significant.

IgE level (IU/ml)	Class (& score)
> 150	Very strong (4)
151 – 250	Strong (3)
71 – 150	Moderate (2)
31 - 70	Weak (1)
0 - 30	Undetectable (0)

Table (1): Scoring of the MAST-CLA test results

Histopathologic and immunohistochemical evaluation:

From each patient a 5 mm-punch biopsy was obtained from both lesional and non-lesional skin, preserved immediately in 10% formalin and subsequently embedded in paraffin blocks. Four micron sections were prepared for subsequent hematoxylin & eosin (H&E) as well as for immunohistochemical staining. One biopsy of normal skin (at matching sites)

has been also taken from each control subject.

Histopathologic examination of H&E stained sections was carried out under light microscopy to confirm the diagnosis and to record histopathologic features. The density of the dermal mononuclear infiltrate was given a score of 0 to 6 and the average score of four sections per biopsy was calculated.

(N.B. Only lesions that were judged clinically and confirmed histopathologically to be in the acute or subacute stage were subjected to immunohistochemical evaluation.).

Immunohistochemical Technique

i. Four-micron thick tissue sections cut from the representative paraffin-embedded tissue blocks, overlaid on APES (Sigma, St. Louis, USA) coated slides, were deparaffinized (2 changes of Xylene X 5 minutes each, 1 change of acetone X 1 min) followed by rehydration in decreasing ethanol concentrations (95% ethanol X 3 mins., 70% ethanol X 3 mins., distilled water X 1 min).

ii. For staining with all the antibodies, the tissue sections were then subjected to antigen unmasking by heating the sections immersed in 10 mmol. citrate buffer pH 6.0 (2.1 gm of anhydrous citric acid crystals dissolved in 1L of distilled water and pH adjusted to 6.0, if necessary) inside a 600 watt microwave oven in full power for 35 minutes, allowed to cool to room temperature and then washed briefly with 0.05 M Tris-Hcl buffer pH 7.4.

ii. Endogenous peroxidase activity was then quenched by immersing the sections in methanolic H 2 O 2 (1 part 3% H 2 O 2 plus 4 parts absolute methanol) for 30 minutes. After brief rinsing, the sections were placed in 0.05 M Tris-Hcl buffer pH 7.4 for 10 minutes.

iii. Excess buffer was tapped off followed by a careful wipe around the specimen. Sections were then overlaid with adequate amount of primary antibody diluted optimally using 0.05 M Tris-Hcl buffer pH 7.4 containing 1% bovine serum albumin (Sigma, St. Louis, USA) followed by incubation at 4 0 C overnight.

iv. The slides were then washed with three changes (5 mins each) of 0.05 M Tris-Hcl buffer pH 7.4 followed by incubation for 30 minutes at room temperature after application of biotinylated secondary (link) antibody in phosphate buffered saline containing carrier protein and 15 mmol. sodium azide (LSAB Plus Kit, DAKO, Denmark).

v. After three washings (5 mins each) in Tris-Hcl buffer, peroxidase conjugated streptavidin was applied to cover the specimens and incubated at room temperature for 30 minutes.

vi. Slides were rinsed with 3 changes of Tris-Hcl buffer for 5 mins each. Sections were then covered with substrate chromogen solution prepared

freshly by dissolving 1 mg of 3,3′ – diaminobenzidine tetrahydrochloride (Sigma, St. Louis, USA) in 1 ml of 0.05 M Tris-Hcl buffer pH 7.4 containing 1 µl of hydrogen peroxide. The slides were incubated at room temperature for 5 to 10 minutes under microscopic control till the optimal development of brown colored peroxidase reaction product.

vii. After rinsing in distilled water, the sections were lightly counterstained with Harris' hematoxylin, followed by mounting with cover slips with DPX as mounting medium.

viii. Precaution was taken so that drying of tissue sections strictly did not occur at any time during the entire procedure of immunostaining. All incubations were done inside humid chambers.

ix. Controls: During each batch of staining, positive and negative controls appropriate for the particular antibody were incorporated.

Table (2) shows the details of antibodies utilized in the study.

1ry Ab				2ry Ab		
1ry Ab	Manufacturer	source	Working dilution	Manufacturer	source	Working dilution
Anti-TSLP	R&D**	sheep	2-15 µg/ml	Abcam*	Rabbit	0.7361111
Anti-TARC	R&D	goat	5-15 µg/ml	Abcam	Rabbit	0.7361111
Anti-CTACK	R&D	goat	15 µg/ml	Abcam	Rabbit	0.7361111
Anti-Rantes	R&D	goat	0.5-5 µg/ml	Abcam	Rabbit	0.7361111
Anti-GM-CSF	Abcam	mouse	5 µg/ml	Abcam	Rabbit	0.7361111
Anti-Langerin#	R&D	goat	2-15 µg/ml	Abcam	Rabbit	0.7361111

*ABCAM - Cambridge – UK
**R&D Systems – Minneapolis – MN - USA
As a marker of Langerhans cells. 13

Table (2): Primary and secondary antibodies used in the study.

Scoring method:

The scoring method for chemokines was modified from that described by Sinicrope et al. [14] Four sections per specimen have been examined for each marker evaluated in the study using a light microscope equipped with SIS Image Analysis Computer System. The mean percentage of positive tumor

cells was determined in at least five fields per section (at 400-fold magnification) and assigned one of the following 8 categories: 0 = <5%; 1 = 5–12.5%; 2 = 12.5–25%; 3 = 25–37.5%; 4 = 37.5-50%, 5 = 50-67.5%, 6 = 67.5-75%, 7 = 75-87.5%, 8 = >87.5%. (N.B. the score refers to the percent of positively stained cells). The intensity of immunostaining was scored as follows: 1+, weak; 2+, moderate; and 3+, intense. Because the lesions showed heterogeneous staining, the dominant pattern was used for scoring. The scores indicating percentage of positive cells and staining intensity were multiplied to produce a weighted score for each field. The average weighted score for the 20 fields examined for each specimen (5 fields/section X 4 sections) is then calculated. Cases with weighted scores <1 were defined as negative, and cases were otherwise defined as positive.

For Langerin reactivity, only the cell with a nucleus and clear immunoreactivity was judged to be a positive cell. Positive cell count only was recorded.

Statistical analysis.

Comparative analysis between two groups was done through unpaired T-test using graphpad software downloaded from the website: http://www.graphpad.com/quickcalcs/ttest1.cfm [15]

Correlation between different parameters was done through graphpad software downloaded from the website: http://calculators.stat.ucla.edu/correlation.php [16]

Results

The study included 27 patients suffering from atopic dermatitis (acute to subacute stage) in addition to 14 healthy subjects of matched age and sex as control.

According to the results of laboratory and skin tests, the patients have been divided into 2 subcategories; extrinsic group (16 patients) and intrinsic group (11 patients)

Clinical data of the material of the study and of the biopsied lesions are shown in **table (3)**

	CONTROL	TOTAL PATIENTS	EXTRINSIC GROUP	INTRINSIC GROUP
NUMBER	**14**	**27**	**16**	**11**
AGE (YEARS)	10 – 42 (28.8 +/- 12.2)	7 – 43 (25.7 +/- 11.9)	7 – 38 (23.9 +/- 9.2)	10 – 43 (29.0 +/- 10.2)
SEX (M/F)	6/8	11/16	6/10	5/6
DURATION OF ILLNESS (MONTH)	-------	3 MONTHS – 22 YEARS	3 MONTHS – 22 YEARS	6 MONTHS – 15 YEARS
ASSOCIATED ATOPIC DISORDERS	-------	11 cases	**11 cases**:** asthma -7 allergic sinusitis -7	0
SITE OF BIOPSY	Arm (5) Thigh (5) Back (3) Axilla (1)	Arm (11) Thigh (10) Back (3) Axilla (3)	Arm (7) Thigh (6) Back (2) Axilla (1)	Arm (4) Thigh (4) Back (1) Axilla (2)
DURATION OF BIOPSIED LESIONS (MONTHS)	----------	1.4 (+/- 1.3)	1.7 (+/- 0.9)	1.1 (+/-1.1)

**Three cases had multiple extracutaneous allergies.

Table (3): Clinical data of the study material

Results of laboratory tests are shown in **tables (4&5).**

	CONTROL	TOTAL PATIENTS	EXTRINSIC GROUP	INTRINSIC GROUP
	Range (mean +/- SD)	Range (mean +/- SD)	Range (mean +/- SD)	Range (mean +/- SD)
EOSINOPHILS (percentage in blood)	1 –6% 3.14 +/- 1.7	1 – 17% 9.1 +/- 4.6	7 – 17 % 12.1 +/- 2.9	1 – 11 % 5.6 +/- 2.3
TOTAL SERUM IgE (IU/ml)	8 – 66 (IU/ml) (32.9 +/- 19.5)	12 – 1560 (IU/ml) (495.8+/- 95.4)	120 – 1560 (IU/ml) (807.2 +/- 407.8)	12 – 95 (IU/ml) (34.6 +/-23.5)

Table (4): Results of laboratory investigations

Allergen	Number of +ve cases*	% of +ve cases
Mites	4	14.80%
Pollen grain	3	11.10%
Animal dander	2	7.40%
Moulds	2	7.40%
Cow's milk	3	11.10%
Egg yolk	2	7.40%
Wheat	2	7.40%
crab	2	7.40%
Others	4	14.80%

*Some cases had multiple allergies.

Table (5): Results of MAST-CLA test

All biopsied lesions were judged clinically and confirmed histopathologically to be in the acute to subacute stage. The density of dermal mononuclear infiltrate were not significantly different between the two subsets of patients. **(table 6).**

	Mean	S/D
Total patient group	3.1	1.9
ADe	3.3	1.6
ADi	2.9	1.8

Table (6): Average score for the density of dermal inflammatory infiltrate.

Immunohistochemical examination revealed significantly increased immunoreactivity for TSLP in lesional epidermis of atopic patients compared to control ($p<0.001$) The expression was also significantly higher in ADe lesional epidermis compared to ADi lesional epidermis ($p<0.05$) Non-lesional atopic epidermis showed only occasional staining for TSLP which was, however, not significantly different in either subgroup from normal epidermis ($p>0.05$).

The staining was frequently seen throughout the lesional epidermis, or only confined to the lower or upper parts of epidermis.

For TARC, Significantly increased immunoreactivity could be seen in atopic epidermis compared to normal epidermis which only occasionally showed very faint focal staining ($p<0.001$) Non-lesional atopic epidermis showed also significantly enhanced staining for TARC compared to normal skin ($p<0.001$) but less than that of lesional skin. When evaluated separately, ADe lesional epidermis showed higher reactivity than that of ADi skin ($p<0.001$) and both were significantly higher than normal skin ($p<0.001$). Non-lesional epidermis showed also staining for TARC in ADe group that was significantly higher than ADi ($p<0.001$) and only staining in the ADe group was significantly different from control epidermis ($p<0.001$).

For GM-CSF, significantly increased expression could be seen in lesional epidermis of ADe only compared to control ($p<0.001$). Reactivity was significantly higher in ADe than ADi. ($p<0.001$) Non-lesional epidermis showed only faint staining that was not significantly different in either subgroup from control.

Immunoreactivity for RANTES and CTACK in both AD subgroups was not prominent and only occasional and focal. Overall score for each of them was not significantly different from that of control whether at the level of lesional or non-lesional epidermis ($p>0.05$).

The average langerin-positive cell density in the epidermis was lower in AD patients than in control epidermis but when the two subgroups were evaluated separately the difference was significant only in lesional ADe epidermis ($p<0.05$).

Strongly positive correlation could be only seen between TSLP and TARC immunoreactivity of lesional skin ($\pi =0.836$). No other significant correlations could be found between any two parameters evaluated in the study.

Results of immunohistochemical examination are shown in **tables (7 to 11)** and figs **(1 to 3)** .

CONTROL GROUP (N=14)	
Chemokine	Weighted Score (mean +/- SD)
TSLP	0
GM-CSF	0.6 (+/- 0.85)
CTACK	1.1 (+/-1.3)
RANTES	0.35 (+/-0.4)
TARC	0

Table(7): Results of semiquantitative assessment of immunohistochemical reactivity of epidermis for chemokines. (control group)

	ATOPIC DERMATITIS TOTAL PATIENT GROUP (n = 27)			
	Non-lesional		lesional	
	Score	*p-value*	Score	*p-value*
TSLP	1.1 (+/- 3)	>0.05	8.8 (+/- 5.3)	<0.001*
GM-CSF	0.4 (+/- 0.8)	>0.05	2.7 (+/- 3.2)	<0.001*
CTACK	0.3 (+/- 0.5)	>0.05	0.63 (+/- 0.8)	>0.05
RANTES	0.4 (+/- 0.7)	>0.05	0.7 (+/- 0.8)	>0.05
TARC	2.2 (+/-2.8)	<0.001*	8.2 (+/- 5)	<0.001*

*=Statistically significant

Table(8): Results of semiquantitative assessment of immunohistochemical reactivity of epidermis for chemokines. (Total patient group)

	ATOPIC DERMATITIS (INTRINSIC SUBGROUP) (n = 11)			
	Non-lesional		lesional	
	Score	*p-value*	Score	*p-value*
TSLP	0.1 (+/- 0.3)	>0.05	5.2 (+/- 3.0)	<0.001*
GM-CSF	0.3 (+/- 0.5)	>0.05	1.3 (+/- 2.1)	>0.05
CTACK	0.3 (+/- 0.4)	>0.05	0.7 (+/- 0.8)	>0.05
RANTES	0.4 (+/- 0.7)	>0.05	0.6 (+/- 0.8)	>0.05
TARC	0.4 (+/- 0.6)	>0.05	3.6 (+/- 2.0)	<0.001*

*=Statistically significant

Table(9): Results of semiquantitative assessment of immunohistochemical reactivity of epidermis for chemokines. (Intrinsic subgroup)

	ATOPIC DERMATITIS (EXTRINSIC SUBGROUP) (n= 16)			
	Non-lesional		lesional	
	Score	*p-value*	Score	*p-value*
TSLP	0.75 (+/- 3.8)	>0.05	11.3 (+/- 4.8)	<0.001*
GM-CSF	0.5 (+/- 1.0)	>0.05	3.75 (+/- 3.6)	<0.01*
CTACK	0.3 (+/- 0.6)	>0.05	0.6 (+/- 0.8)	>0.05
RANTES	0.4 (+/- 0.7)	>0.05	0.75 (+/- 0.8)	>0.05
TARC	3.5 (+/- 2.7)	<0.001*	11.4 (+/- 3.8)	<0.001*

*=Statistically significant

Table(10): Results of semiquantitative assessment of immunohistochemical reactivity of epidermis for chemokines. (Extrinsic subgroup)

	Control	TOTAL PATIENTS		INTRINSIC AD		EXTRINSIC AD	
		Non-lesional	lesional	Non-lesional	lesional	Non-lesional	lesional
Score Mean (+/-SD)	53.2 (+/-14.3)	47.7 (+/- 17.9)	4.2 (+/-16.3)	45.9 (+/-15.2)	50.3 (+/-16.6)	48.5 (+/-10.9)	39.3 (+/-20.8)
p-value		**> 0.05**	**< 0.05**	**> 0.05**	**> 0.05**	**> 0.05**	**<0.05***

*=Statistically significant

N.B. Figures shown refer to the average score of Langerin +ve cells per mm^2 of epidermis.

Table (11): Results of Langerin +ve cell scoring.

Positive correlation could be seen between the density of dermal inflammatory infiltrate and TSLP, TARC and to a less extent GM-CSF in ADe but not ADi group. No correlation could be seen between the density of infiltrate and other parameters evaluated in the study except for some negative correlation with langerin +ve cell count mainly in ADe group. **(Table 12)**.

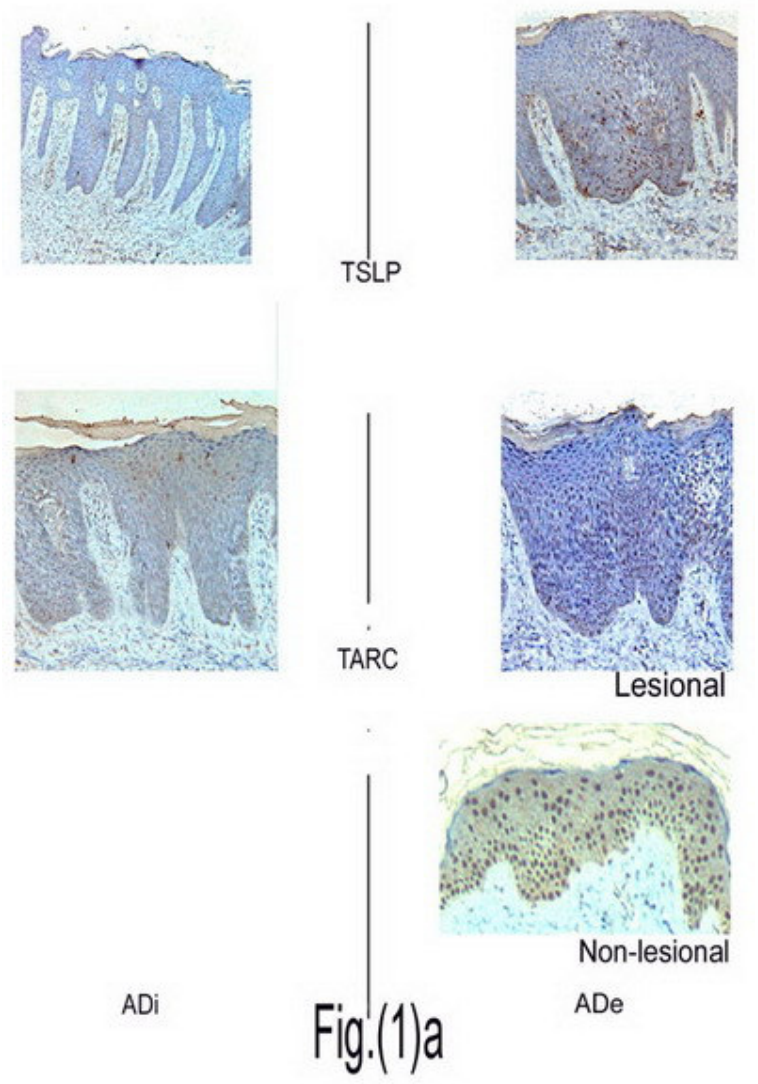


Fig.(1)a: -Immunohistochemical reactivity for TSLP: Strong epidermal staining of lesional skin of ADe and to a less extent ADi. -Strong staining for TARC in the lesional and non lesional skin of ADe and to a less extent in the lesional skin only of ADi

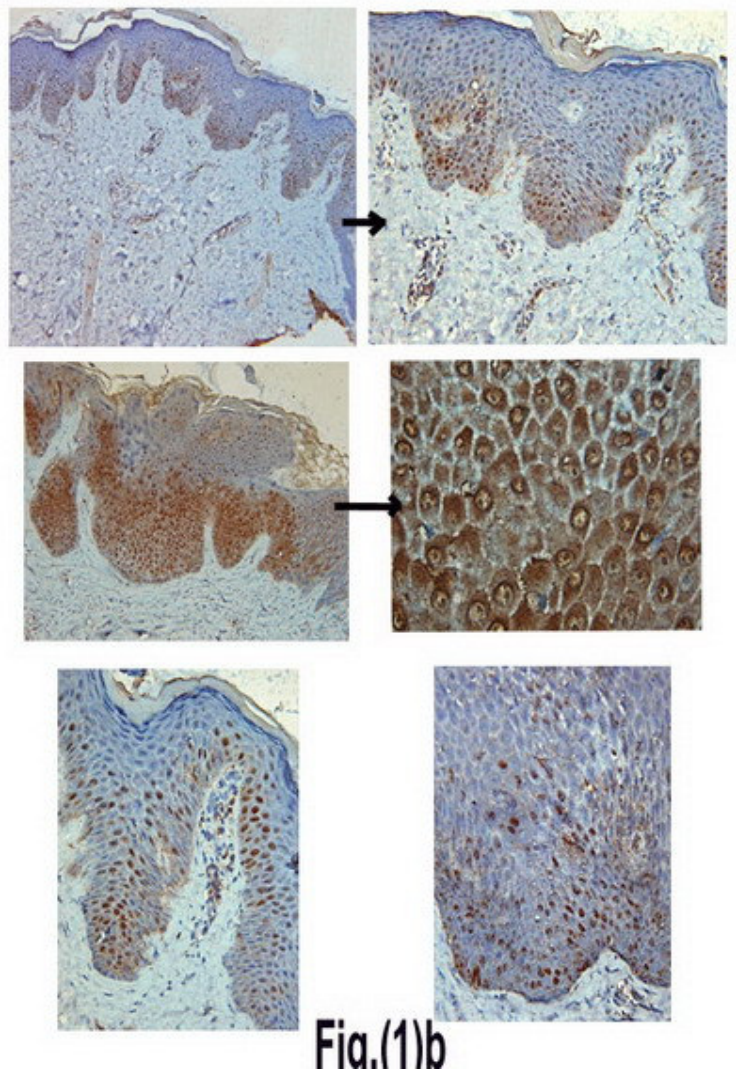


Fig.(1)b: Different patterns and intensities of staining for TSLP
in ADe lesional skin: Nuclear vs. cytoplasmic, diffuse vs., patchy.

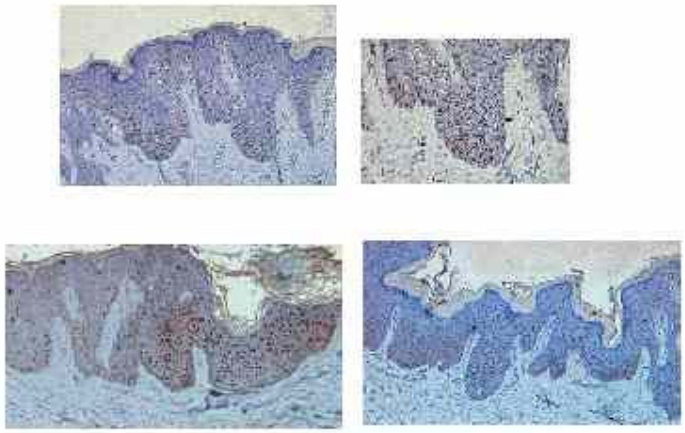


Fig.(1)c: Different patterns and intensities of staining for TARC
in ADe lesional skin.

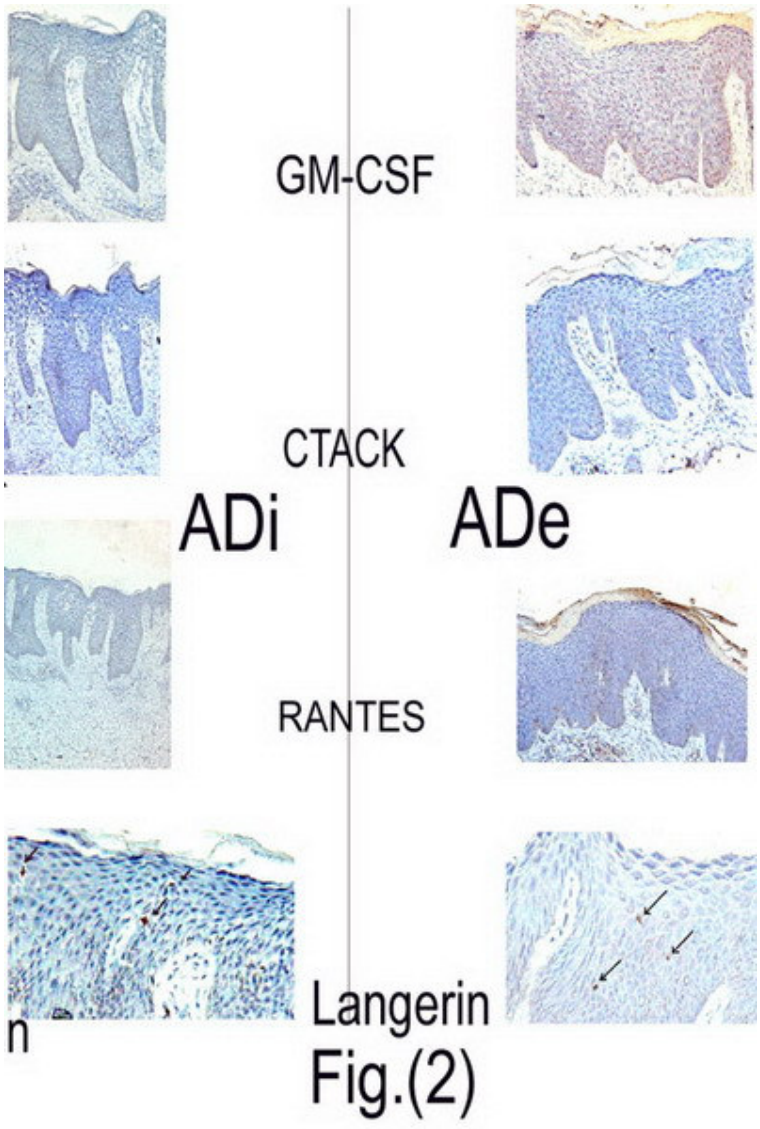


Fig.(2):
-Immunohistochemical reactivity for GM-CSF: Moderate epidermal staining of lesional skin of ADe and weak staining of ADi lesional skin
-Immunohistochemical reactivity for CTACK and RANTES: Only weak epidermal staining of lesional skin of ADe and ADi
- Immunohistochemical staining for Langerin +ve cells: Arrows refer to positively labeled cells in lesional skin of ADe and ADi.

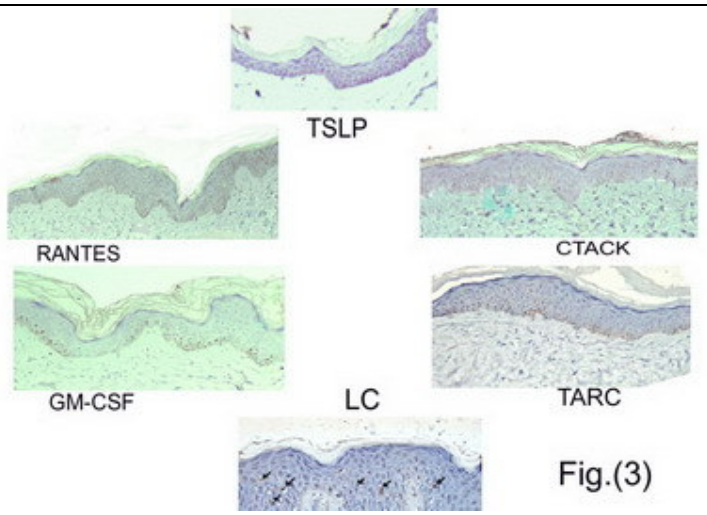
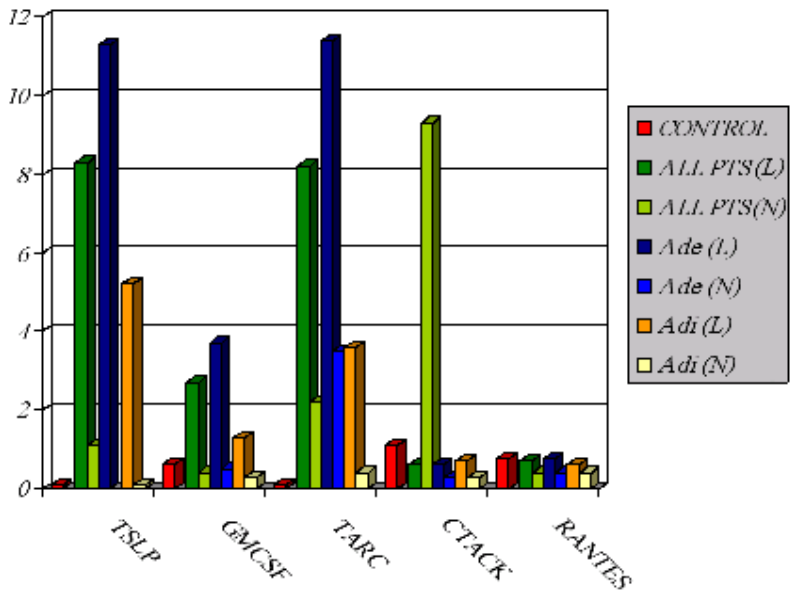


Fig.(3): Immunohistochemical reactivity of normal control epidermis. Negative staining for TSLP and TARC and only very weak staining for GM-CSF, CTACK and RANTES.



Diagrammatic representation of immunohistochemical scores of different chemokines in patients and control.
(L: Lesional N: Non-lesional)

Fig.(4): Comparative epidermal levels of different chemokines in patients and control.

Discussion

Immunologically, the atopic reaction is usually described as an IgE-mediated delayed hypersensitivity reaction. Pathologically, atopic dermatitis is characterized by prominent T-cell infiltration with polarization towards $T_{(h2)}$ in early phase and combined $T_{(h1)}/T_{(h2)}$ in late stages.[17]

However, allergen-specific T-cells have been shown to represent only a minority of infiltrating T-cells in lesional skin. Therefore, other factors which lead to the activation of T-cells at the site of inflammation are probably involved in the pathogenesis of atopic dermatitis.[18,19,amp;20]

Recently, Keratinocytes have been suggested as one of the major players in the atopic reaction and their role in the evolution of skin lesions is being more clearly unraveled in the past few years. [5]

In the present study, a statistically significant increase in the reactivity for TSLP, GM-CSF and TARC could be detected in atopic lesional epidermis in relation to that of normal skin. As regards non-lesional atopic epidermis, only the reactivity for TARC was significantly increased compared to the normal control skin.

When evaluated separately, significant differences between extrinsic and intrinsic cases could be also found in the present study as regards the epidermal expression of TSLP, GMCSF and TARC. While the staining of only lesional skin for TSLP and GMCSF of ADe was significantly higher than that of ADi lesions, the expression of TARC in both lesional and non-lesional skin was significantly higher in ADe epidermis than that of ADi skin.

Although no firm conclusions can be made as regards the significance of these findings in atopic skin and of the demonstrated differences between ADi and ADe skin in particular, some postulations can be made.

Previous studies could show that AD skin in both types exhibits a tissue profile that is pro-inflammatory and at the same time polarized towards T(h2) (in the acute phase) and towards mixed T(h1)/T(h2) in the chronic phase. [17]

However, ADe cases are distinguished by the additional feature of reactivity to one or more external allergens and by serum levels of IgE, IL-4 and CD45+Bcells as well as tissue levels of IL-5 and IL-13 that are higher than those of ADi cases. Whether these differences were the underlying cause of enhanced reactivity to external allergens in ADe patients or just the result of (successful) immune sensitization to these allergens can not be ascertained.[7]

On the light of these earlier findings, the results of the present study could be interpreted. TSLP is well known to have prominent effects on APCs. Thymic stromal lymphopoietin (TSLP) is an interleukin (IL)-7–like cytokine which activates dendritic cells (DCs) and induces them to prime naïve CD4+ T cells to produce moderate to high levels of $T(h2)$ cytokines (IL-4, IL-5, and IL-13), and downregulate IL-10 and interferon (IFN)-gamma expression, thereby promoting a proallergic phenotype. TSLP also induces the secretion of

T(h 2)-attracting chemokines (macrophage-derived chemokine [MDC—CCL22] and thymus and activation-regulated chemokine [TARC—CCL17]). SO, the release of keratinocyte-derived TSLP might play a key role in the enhanced reactivity to allergens (through activation of APCs) and also in the T(h 2) polarization observed in acute lesions and might augment this effect by recruiting more T(h 2) cells. [5,21]

Similarly, GMCSF has many actions relevant to allergic inflammation by aiding in the activation/maturation, recruitment, and survival of monocytes, APCs, and eosinophils, increasing the number of Langerhans cells and enhancing the antigen-presenting capacity of Langerhans cells and other dendritic cells. [22] In this way, the overexpression of this mediator by ADe keratinocytes may promote the ability of the skin to respond to external antigens and may thus explain the extrinsic differentiation of this subset of atopic cases.

However, the inability to demonstrate significantly increased population of LCs in the epidermal compartment in AD skin of both subsets is somewhat unexpected. One explanation is that suggested by Esche et. al. (2004)[5] who proposed enhanced migration of the mature, activated APCs to the regional lymph nodes -in response to TSLP stimulation- as a possible cause of relative paucity of APCs associated with the enhanced expression of TSLP observed in atopic skin. Bieber et. al. (1989)[23] reported higher density of IgE-bearing dendritic cells in the epidermis of AD patients compared to normal skin. Although the present study could not confirm this finding, the possibility is there of a selective increase in this particular subset of APCs that is not necessarily accompanied by a significant increase in the overall count of APCs.

The demonstrated increase in the staining for TARC could be of significance for selective recruitment of T(h 2) cells which express CCR4 for which TARC is the specific ligand. Subsequent release of IL-4, 5, and 13 by the attracted T(h 2) cells may act to recruit and activate more effector cells e.g. eosinophils and basophils with subsequent perpetuation of the proallergic inflammatory reaction. [24] The observed strong correlation between expression of TARC and that of TSLP is expected keeping in mind that TSLP is a major stimulus for TARC secretion by keratinocytes.[5,21]

The overexpression (whether spontaneous or reactive) by AD keratinocytes of these chemokines –as shown in the present study- may be an inherent feature of atopic patients in general. However, there is relatively higher production of TARC by ADe keratinocytes in both lesional and non lesional skin in contrast to high (but less than that of ADe) production by lesional skin only in ADi skin. T(h 2) polarization can be thus mediated through TSLP (upgraded in ADe significantly more than ADi skin) and by TARC (also significantly increased in both types of AD but more in ADe skin). So, T(h 2) polarization is likely to take place in a significantly exaggerated (or more committed) way in ADe compared to ADi; a fact that may explain the previously reported differences between the two AD subtypes as regards levels of eosinophils, B-cells, IgE, IL-4, IL-5 and IL-13 in the

serum &/or skin.[7]

Positive correlation between the density of dermal inflammatory infiltrate and epidermal expression of TSLP, TARC and GM-CSF again highlights the significance of these chemokines in the pathogenesis of atopic reaction. However, when evaluated separately, only the ADe showed significant correlation while correlation was poor in the ADi subgroup. This observation besides the inability to demonstrate any significant difference in the density of inflammatory reaction between the two subtypes may suggest that the difference between the two groups can be a qualitative rather than a quantitative one and may even involve different evolutionary pathways.

The inability to demonstrate significant difference between immunohistochemical reactivity of normal and atopic skin for RANTES or CTACK contradicts with the findings of previous studies which reported enhanced expression of the former by keratinocytes of lesional atopic skin. [25] Other studies have also reported expression of the latter chemokine (CTACK) in the basal layer of normal control and non-lesional atopic skin with significantly increased expression (both basal and suprabasal) in lesional atopic skin. [26]

The discrepancy can be attributed to the selection criteria adopted in the present study which was confined to the early (acute or subacute) stages when the T-cell subset is expected to be predominantly of the h 2 polarity while RANTES is likely to attract both T(h 1) and T(h 2) cells and is therefore more likely to play a role in the later stages or in more chronic forms of atopic lesions. [5,25]

Similarly, CTACK exerts a non-polarized pro-inflammatory effect by attracting CLA+ T-cells[5,26] and may thus act at a later stage to self perpetuate the inflammatory reaction. In fact, many of the previous studies have mainly focused on chronic lesions which may explain the difference from our work.

Conclusion

Findings of the present study in the form of enhanced epidermal expression of TSLP, GM-CSF and TARC may be significant for the determination of atopic reactivity in general and the proallergic nature in particular (whether intrinsic or extrinsic). The question remains as to whether the observed differences between these two AD subtypes are primary (constitutive) or induced abnormalities that occur late in the evolution of atopic lesions.

Moreover, certain difficulties are traditionally encountered in the interpretation of any findings related to AD research. An important limitation is the poorly defined nature of immune reaction in AD which does not exactly conform to one of the well known classic types of immune reactions (Type I, Type IV or mixed). There is also lack of a comprehensive scenario or an orderly sequence of events that can depict the evolution of eczema in atopic

skin. In addition, there is the complex interplay of multiple etiological and triggering factors in determining the atopic state and in the initiation &/or perpetuation of the skin reaction e.g. genetic (intrinsic), environmental (external) and other factors. Another difficulty is the enormous number of abnormalities reported in AD whether at the serum or the tissue level and the great deal of interaction between most of these abnormal features in-vivo. This is largely responsible for the inability to differentiate between primary and secondary defects as well as between early and late (or downstream) events. In fact, different subsets of patients seem to have different abnormalities that all lead to the same clinical manifestation. It is also becoming more and more clear that different cells and mediators play roles at different times and stages of the disease.[2,3,6,7,17]

Moreover, the distinction between the two subsets of AD may not be as sharp as was suggested initially. In fact, some authors[27] have suggested the possibility of transition of the intrinsic to the extrinsic form and even ultimately to some sort of an autoimmune reaction. If true, any existing differences between these two forms may just represent a secondary event in the evolution of the disease not a primary feature of the skin or of the immune system. In this case, the assignment of a particular patient to any of these two subsets may not be an inevitable but rather an accidental one and may be just related to the stage at the time of evaluation or to the (suitable) exposure to external allergens resulting in successful sensitization.

References

1. Nurnberg, W. (2005): Inpatient rehabilitation of chronic dermatoses illustrated by atopic dermatitis. Hautarzt.56(7): 644-8.

2. Meagher, L. J., Wines, N.W. and Cooper, A. J. (2002): Atopic dermatitis: Review of immunopathogenesis and advances in immunosuppressive therapy. Australasian Journal of Dermatology 43(4): 247

3. Wuthrich B. (1989): Atopic neurodermatitis Wien Med Wochenschr. 139(6-7): 156-65.

4. Yassky, E. G. (2004): Exogenous Factors in Atopic Dermatitis (3): 228-236

5. Esche, C., de Benedetto, A. and Beck, L.A. (2004): Keratinocytes in atopic dermatitis: inflammatory signals. Curr Allergy Asthma Rep. 4(4):276-84.

6. Leung, D.Y.M., Boguniewicz, M., Howell, M. D., Nomura, I. and A. Hamid, Q. (2004): New insights into atopic dermatitis. J. Clin. Invest. (113): 651-657

7. Schmid-Grendelmeier P.; Simon D.; Simon H-U; Akdis C.A.; Wüthrich B. (2001): Epidemiology, clinical features, and immunology of the "intrinsic" (non-IgE-mediated) type of atopic dermatitis (constitutional dermatitis). Allergy. (56)9: 841-849

8. Stary, G., Bangert, Ch., Stingl, G. and Kopp, T. (2005): Dendritic cells in atopic dermatitis: expression of FcepsilonRI on two distinct inflammation-associated subsets. Int Arch Allergy Immunol. (138)4: 278-90

9. Jeong, C.W., Ahn, K.S., Rho, N.K., Park, Y.D., Lee, D.Y., Lee, J.H., Lee, E.S., Yang, J.M.. Stary, G., Bangert, C., Stingl, G., Kopp, T. (2003): Differential in vivo cytokine mRNA expression in lesional skin of intrinsic vs. extrinsic atopic dermatitis patients using semiquantitative RT-PCR. Clin. Exp. Allergy. 33(12):1717-24.

10. Oppel T, Schuller E, Gunther S, Moderer M, Haberstok J, Bieber T, Wollenberg A. (2000): Phenotyping of epidermal dendritic cells allows the differentiation between extrinsic and intrinsic forms of atopic dermatitis. Br J Dermatol. (143)6:1193-8.

11. Hanifin, J.M. and Rajka, G. (1980): Diagnostic features of atopic dermatitis. Acta Derm. Venereol. (Stockh) 92(suppl):.44-7

12. Brown, C.R., Higgins, K.W., Frazer, K., Schoelz, L.K., Dyminski, J.W., Marinkovich, V.A., Miller, S.P. and Burd, J.F. (1985): Simultaneous determination of total IgE and allergen-specific IgE in serum by the MAST chemiluminescent assay system. Clinical Chemistry. (31): 1500 - 1505

13. Mizumoto, N. and Takashima, A. (2004): CD1a and langerin: acting as more than Langerhans cell markers. J. Clin. Invest. (113): 658-660

14. Sinicrope, F.A,, Ruan, S.B., Cleary, K.R., Stephens, L.C., Lee, J.J. and Levin, B. (1995): bcl-2 and p53 oncoprotein expression during colorectal tumorigenesis. Cancer Res. (55): 237-241

15. http://www.graphpad.com/quickcalcs/ttest1.cfm

16. http://calculators.stat.ucla.edu/correlation.php

17. Thepen, T., Langeveld-Wildschut, E.G., Bihari, I.C. (1996): Biphasic response against aeroallergen in atopic dermatitis showing a switch from an initial TH2 response to a TH1 response in situ: an immunocytochemical study. J Allergy Clin Immunol (97)3: 828-37

18. Sager, N., Feldmann, A., Schilling, G., Kreitsch, P. and Neumann, C. (1992): House dust mite-specific T-cells in the skin of subjects with atopic dermatitis: Frequency and lymphokine profile in the allergen patch test. J. Allergy Clin. Immunol. (89): 801-10.

19. van Reijsen, F.C., Bruijnzeel-Koomen, C.A., Kalthoff, F.S., Maggi, E., Romagnani, S. and Westland, J.K. (1992): Skin derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. J. Allergy Clin. Immunol. (90): 184-93.

20. Werfel, T, Morita, A., Grewe, M., Renz, H., Wahn, U. and Krutmann, J. (1996): Allergen-specificity of skin-infiltrating T-cells is not restricted to a type 2 cytokine pattern in chronic skin lesions of atopic dermatitis. J. Invest. Dermatol. (107): 871-6.

21. Soumelis, V., Reche, P.A. and Kanzler, H. (2002): Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. Nat. Immunol. (3): 673-680. ?

22. Pastore, S., Fanales-Belasio, E. and Albanesi, C. (1997): Granulocyte macrophage colony-stimulating factor is overproduced by keratinocytes in atopic dermatitis: implications for Sustained dendritic cell activation in the skin. J. Clin. Invest. 1997 (99): 3009-3017

23. Bieber, T., Dannenberg, B., Prinz, J.C., Rieber, E.P., Stolz, W., Braun Falco, O. and Ring, J. (1989): Occurrence of IgE-bearing epidermal Langerhans cells in atopic eczema: a study of the time course of the lesions and with regard to the IgE serum level. J Invest. Dermatol. (93):215-9

24. Uchida,T., Suto, H., Ra, Ch., Ogawa, H., Kobata, T.and Okumura, K. (2002): Preferential expression of Th2-type chemokine and its receptor in atopic dermatitis. International Immunology. (14)12: 1431-1438

25. Giustizieri, M.L., Mascia, F. and Frezzolini, A. (2001): Keratinocytes from patients with atopic dermatitis and psoriasis show a distinct chemokine production profile in response to T cell-derived cytokines. J. Allergy Clin. Immunol. (107): 871-877

26. Homey, B.; Alenius, H.; Muller, A.; Soto, H.; Bowman, E. P.; Yuan, W.; McEvoy, L.; Lauerma, A. I.; Assmann, T.; Bunemann, E.; Lehto, M.; Wolff, H.; Yen, D.; Marxhausen, H.; To, W.; Sedgwick, J.; Ruzicka, T.; Lehmann, P. and Zlotnik, A. (2002.): CCL27-CCR10 interactions regulate T cell-mediated skin inflammation. Nature Med. (8): 157-165,

27. Bieber, T. and Novak, N. (2005): New concepts of atopic dermatitis: From non-IgE-mediated via IgE-mediated to autoimmune inflammation. Allergy Clin. Immunol. Int: (J. World Allergy Org.) (17)1: 26-29

الملخص العربي

دراسة كيميائية نسيجية مناعية لبعض الكيموكينات (الحرائك الخلوية الكيماوية الجاذبة)

فى طبقة البشرة فى حالات الاكزيما التأتبية:

مقارنة بين النوعين الداخلى والخارجى

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فى هذه الدراسة تم قياس مستوى التفاعل المناعى للبشرة بالنسبة لكل من **TSLP/GMCSF/TARC/RANTES/CTACK** و أيضا عدد خلايا لانجرهانز فى طبقة البشرة فى **16** حالة اكزيما تأتبية من النوع الخارجى و **11** حالة اكزيما تأتبية من النوع الداخلى بالاضافة الى **14** من الأشخاص الأصحاء كعينة ضابطة وذلك عن طريق الفحص الكيميائى النسيجى المناعى..

ولقد أظهرت الدراسة زيادة ذات مغزى فى مستويات **TSLP/GMCSF/TARC** في أماكن الاكزيما بين المرضى بوجه عام اكثر منها فى الجلد العادى. وعند مقارنة نوعى الاكزيما (الداخلى والخارجى) كان الارتفاع فى النوع الخارجى أكثر منه فى النوع الداخلى و بالنسبة للأماكن غيرالمصابة لدى المرضى لم يكن هناك فرق واضح عن الجلد العادى فيما عدا ارتفاع ذى مغزى فى مستوى **TARC** بين مرضى النوع الخارجى فقط. كما ظهرنقص ذومغزى احصائى فى كثافة خلايا لانجرهانز فى طبقة البشرة لدى مرضى الاكزيما من النوع الخارجى.

وبناء على هذه النتائج يمكن افتراض دور لكيموكينات البشرة فى نشوءالاكزيما التأتبية بوجه عام بينما يمكن للفروق الملاحظة بين نوعى الاكزيما أن تفسر القابلية المميزة للنوع الخارجى للتفاعل ضد المحسسات الخارجية كما يمكن ربطها ببعض الفروق التى تم اكتشافها فى دراسات سابقة بين النوعين سواء على مستوى الدم أومستوى الجلد.