

IL-1 β protein in human skin lymph does not discriminate allergic from irritant contact dermatitis

CH. U. BRAND, TH. HUNZIKER, N. YAWALKAR AND L. R. BRAATHEN
Dermatological Clinic, University of Berne, Switzerland

Recent data suggest a key rôle for IL-1 β in the induction phase of allergic contact dermatitis. In the present study, the protein levels of IL-1 β were measured in skin lymph derived from normal untreated skin as well as from irritant and allergic (induction and elicitation phase) contact dermatitis. IL-1 β increased in the course of both types of contact dermatitis, displaying the highest levels in irritant CD. Using a reverse transcriptase-polymerase chain reaction, low signal strength of IL-1 β mRNA was demonstrated in lymph cells derived from normal skin and allergic CD. In lymph cells collected 2 $\times$ daily during the induction phase of allergic contact dermatitis, no upregulation of the IL-1 β mRNA signal was found. Isolated CD1a⁺ lymph cells derived from normal skin as well as from the induction and elicitation phase of allergic contact dermatitis did not express IL-1 β mRNA. Our results demonstrate that in human skin lymph, the IL-1 β profiles do not discriminate between irritant and allergic contact dermatitis and that besides resident epidermal and dermal cells, circulating lymph cells may also contribute to IL-1 β protein production.

Key words: skin-derived lymph; contact dermatitis; cytokines; interleukin-1 β . © Munksgaard, 1996.

Accepted for publication 28 February 1996

Abbreviations

CD	Contact dermatitis
DNCB	2,4-dinitrochlorobenzene
DPCP	Diphenylcyclopropenone
IL	Interleukin
LC	Langerhans cells
LPS	Lipopolysaccharides
PBMC	Peripheral blood mononuclear cells
RT-PCR	Reverse transcriptase-polymerase chain reaction
SLS	Sodium lauryl sulphate

Interleukin-1 is a cytokine family produced in the skin by cells of the monocyte/macrophage lineage, lymphocytes, epithelial cells, endothelial cells and fibroblasts (1). 2 distinct DNA clones encoding for IL-1 have been isolated and referred to as IL-1 α and IL-1 β (2-4). Biologically-active IL-1 is present in human epidermis under normal conditions (5, 6). Upon injury or in inflammatory processes of the skin, IL-1 is released and participates in the regulation of local and systemic reactions. In immunological responses, IL-1 seems to be involved in the process of antigen presentation to T lymphocytes by Langerhans cells (LC) and other antigen presenting cells (7).

Contact dermatitis (CD) is classified into irritant and allergic, the former resulting from toxic chemicals/irritative agents and the latter representing an allergic hypersensitivity reaction. Although irritant CD is considered to be mediated by essentially non-immunological processes, and despite a considerable amount of clinical and experimental data, there are no established and fully reliable parameters to differentiate the 2 types of skin reactions.

Recently, Enk et al. (8) demonstrated in mice that the cytokine mRNA pattern of irritant CD differs from that in the induction phase of allergic CD, mainly by the selective upregulation of IL-1 β mRNA in the latter. In the present study, we compare the IL-1 β profiles in human skin lymph derived from irritant and the induction and the elicitation phase of allergic CD.

Materials and Methods

Experimental design

15 healthy volunteers, 3 female and 12 male, aged 25 to 32 years were investigated. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Berne; written informed consent was obtained from the volunteers. The technique used for cannulation of a lymph ves-

sel on the lower leg exclusively draining a defined skin area on the medial and upper part of the foot, has been described previously (9). Briefly, a superficial lymph vessel on the lower leg was isolated for 1–2 cm, and dissected free of fat and fibrous tissue. The tip of a specially prepared polyethylene tube was inserted in the distal direction. After closure of the wound, the other end of the polyethylene tube was immersed in 1 ml 0.9% NaCl with 20 IU heparin in a 10-ml sterile plastic vial. The vial was taped to the calf and the leg was covered with an elastic bandage. Lymph was collected at 8 a.m. and 5 p.m. each day for a period of 9 days. The volume of the lymph was measured and total cells were counted. The cells were then separated by centrifugation at 300 g for 5 min and the lymph fluid immediately snap frozen and stored at -70°C .

Control experiments: 2 volunteers were designated as negative controls, i.e., after cannulation, the drained skin area remained untreated during the 14-day experiment.

Irritant CD: In 6 volunteers, 10% sodium lauryl sulphate (SLS) in aqueous solution was applied 48 h after cannulation to the drained skin area on the medial and upper part of the foot. The skin was then occluded with a plastic membrane. The application was repeated after another 24 h. The occlusive dressing remained for 36 h.

Allergic CD: Diphenylcyclopropanone (DPCP) and 2,4-dinitrochlorobenzene (DNCB) were used as sensitizing agents. They were diluted in acetone to obtain the concentrations appropriate for induction (2%) and elicitation (1%–10%) of contact hypersensitivity.

Induction of CD: 5 volunteers were painted with DNCB 2% on the drained skin area of the foot on day 2 after the cannulation.

Elicitation of CD: In the 1st volunteer, sensitization was induced by painting DPCP 2% once on a 6 cm $\times$ 6 cm area of the left forearm some weeks prior to the cannulation. The degree of sensitization was tested 3 weeks later by patch testing with DPCP serial dilutions on the upper back. On days 2 and 3 after the cannulation, an elicitation reaction was then induced in the drained skin area by painting with the minimal dermatitis-eliciting concentration, determined by patch testing. In the 2nd volunteer, DPCP 2% was painted on the drained skin area on day 4 for induction, and on days 11, 12 and 13 challenge doses of 0.05%, 0.1% and 0.5% DPCP, respectively, were applied to the same skin area for elicitation of CD.

The skin reactions were evaluated 2 $\times$ daily and quantified by visual scoring as: 0, no response; 0.5, slight, patchy erythema; 1, uniform erythema; 2, erythema and edema; 3, erythema, edema and papules, vesicles or blisters.

II. LC isolation

For purification of LC, lymph cells obtained at various time points from normal untreated skin as well as from the induction and elicitation phase of allergic CD were reacted with FITC-conjugated mouse anti-CD1a monoclonal antibody (1:10, 60 min, 4°C ; Serotec, Oxford UK), washed and then incubated with 4×10^7 sheep anti-mouse IgG antibody-coated Dynabeads[®] M-450 (Dynal, Oslo, Norway). Highly purified LC were obtained by at least 4 repeated washings each followed by renewed immunomagnetic separation.

III. Cytokine analysis

ELISA: The IL-1 β concentrations of the different lymph samples were determined at the same time using commercially available ELISA kits (Quantikine ELISA kits, R&D systems/Biermann Diagnostica, Bad Nauheim, Germany) in all lymph samples. According to the manufacturers specification, the detection limit of the assay is 0.3 pg/ml, and there is no measurable cross reactivity to other cytokines.

To allow a comparison of the values of the probands, the values measured in each volunteer at day 2, i.e., before the intervention, were considered to be the base line values (=1). Thereupon all values of each volunteer were calculated as a % of this 2nd-day value, and expressed as IL-1 β level index.

Reverse transcriptase-polymerase chain reaction analysis. Total RNA from the whole lymph cell population (10^6 lymph cells) and from purified LC ($1-9 \times 10^4$ cells) derived from the induction phase of allergic CD (3 volunteers, postoperative days 1–9), the elicitation phase of allergic CD (1 volunteer, day 10) and normal skin (2 volunteers, at the 4th, 9th and 13th postoperative days) as well as from LPS-stimulated PBMCs, was extracted by guanidinium thiocyanate/phenol-chloroform procedure (10). After extraction, the amount of total RNA was quantified by spectrophotometry. Using equal amounts of RNA, single-strand cDNA was synthesized by reverse transcription using a cDNA Synthesis Kit (Boehringer Mannheim, Mannheim, Germany). PCR was performed with equal amounts of cDNA to amplify IL-1 β and β -actin (as an internal standard) specific messages. The primer sets for IL-1 β and β -actin were obtained from Stratagene (La Jolla, CA). The predicted sizes of the PCR products are 332 bp for IL-1 β and 661 bp for β -actin. The reaction consisted of 35 cycles for IL-1 β and 30 cycles for β -actin of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 2 min. All samples were subjected to denaturation at 94°C for 5 min before the 1st cycle and to final extension at 72°C for 10 min.

Table 1. The individual IL-1 β protein levels (pg/ml) and the IL-1 β level index in human skin lymph derived from normal skin, irritant CD as well as from the induction and elicitation phase of allergic CD

post-operative day	mean daily IL-1 β levels (pg/ml) [IL-1 β level index ^{a)}]														
	normal untreated skin					induction of allergic contact dermatitis					elicitation of allergic CD				
	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer	volunteer
1	0.0 [0.0]	0.0 [0.0]	3.0 [0.4]	0.9 [0.5]	0.0 [0.0]	0.0 [0.0]	0.0 [0.0]	1.3	0.0	0.7 [0.2]	0.0 [0.0]	8.7 [0.5]	1.6 [0.3]	0.0 [0.0]	0.0 [0.0]
2	1.6 [1.0]	3.6 [1.0]	6.9 [1.0]	1.9 [1.0]	0.4 [1.0]	1.0 [1.0]	1.0 [1.0]	1.3	0.0	3.1 [1.0]	2.8 [1.0]	18.8 [1.0]	5.5 [1.0]	2.5 [1.0]	1.4 [1.0]
3	1.8 [1.2]	1.3 [0.4]	4.4 [0.6]	2.4 [1.3]	0.1 [0.2]	2.2 [2.1]	2.2 [2.1]	1.2	4.1	5.0 [1.6]	2.3 [0.8]	23.5 [1.3]	4.4 [0.8]	3.0 [1.2]	6.4 [0.5]
4	1.5 [1.0]	1.8 [0.5]	4.6 [0.7]	1.0 [0.5]	0.0 [0.0]	2.2 [2.1]	2.2 [2.1]	1.0	0.0	6.2 [2.0]	3.5 [1.2]	9.9 [1.8]	7.1 [2.9]	5.4 [0.4]	5.4 [0.4]
5	2.7 [1.7]	0.3 [0.1]	4.3 [0.6]	2.6 [1.4]	2.3 [6.0]	6.0 [5.8]	6.0 [5.8]	1.2	0.0	3.4 [1.1]	0.9 [0.3]	10.5 [1.9]	2.1 [0.9]	50.1 [3.6]	50.1 [3.6]
6	2.6 [1.7]	1.0 [0.3]			0.8 [2.0]	3.4	2.2 [2.1]	1.2	0.0	2.8 [0.0]	1.3 [0.5]	15.8 [2.9]	1.8 [0.7]	6.4 [0.5]	6.4 [0.5]
7	1.9 [1.2]	0.6 [0.2]	2.7 [0.4]		0.0 [0.0]	3.0	1.3 [1.3]	1.7	3.3	1.0 [0.3]	2.7 [0.9]	10.8 [2.0]	3.0 [1.2]	4.9 [0.3]	4.9 [0.3]
8	2.4 [1.5]	0.9 [0.3]	3.9 [0.6]		0.1 [0.3]	3.7 [3.6]	3.7 [3.6]	0.5				18.1 [1.0]	43.5 [7.9]	3.1 [1.3]	7.8 [0.6]
9	2.2 [1.4]				0.2 [0.4]	2.2		1.7	0						

^{a)} see materials and methods.

after the last cycle. The PCR cocktail contained 1.25 U Taq polymerase (Boehringer Mannheim), 0.1 mM dNTPs (Perkin-Elmer, Foster City, CA), 10-fold concentrated PCR buffer (100 mM Tris-HCl, pH 8.3; 500 mM KCl; 15 mM MgCl₂; 0.01% w/v gelatine; autoclaved), 1 μ M of primers and deionized water in a total volume of 50 μ l. For each experiment, internal controls lacking template were performed and verified to be negative. In addition, PCR using total RNA as template to check for DNA contamination, revealed no amplification. PCR products were finally analyzed by electrophoresis on 3% agarose gels, visualized by ethidium bromide staining, and the size of the amplified products was compared to molecular weight markers run in parallel.

Results

I. Skin reaction

Irritant CD:

SLS induced erythema appeared after 24 h. The inflammation was still increasing at the time of the removal of the plastic occlusion, i.e., after 36 h, and continued for another day, presenting as a mild to moderate erythema $\pm$ slight edema. In all volunteers, the reaction disappeared within 36 to 60 h.

Allergic CD:

Induction of CD: 2 out of 5 volunteers exhibited an erythematous and edematous skin reaction without papules 1 day after painting with DNCB 2%, which was strictly limited to the application site and lasted for 3 days. Clinically, this reaction was classified as toxic. 3 volunteers showed no skin reaction.

Elicitation of CD: 1 day after the 2nd painting with DPCP, the 1st volunteer developed an elicitation reaction with erythema and papules spreading slightly beyond the application site. The peak reaction was on day 5, while on day 10, there were no clinical signs of the skin reaction left. Concomitantly, there was a flare-up reaction at the sensitization site on the left forearm. In the 2nd volunteer, painting with DPCP 0.05% on day 12, i.e., 5 days after resolution of the induction reaction, induced a hardly discernible erythema 24 h later. 2 further applications of DPCP 0.1 and 0.5% on days 13 and 14, respectively, resulted in a strong eczematous reaction with large blisters spreading far beyond the application site.

II. LC isolation

The % and the counts of LC obtained in the different lymph samples ranged from 1 to 9% and from $1-9 \times 10^4$ cells, respectively.



Figure 1. IL-1 β mRNA expression in lymph cells derived from normal skin as well as from LPS-stimulated PBMCs. Total mRNA was extracted and reverse transcribed. A PCR using primers specific for IL-1 β and β -actin was performed and 10 μ l of each PCR sample was electrophoresed on a 3% agarose gel and visualized with ethidium bromide. Top IL-1 β , bottom β -actin: lane 1 represents lymph cells derived from normal skin, lane 2 represents PBMCs stimulated with LPS, lane 3 represents negative control, lane 4 represents control without RT.

LC were isolated and highly purified by positive selection with mouse anti-CD1a monoclonal antibodies and sheep anti-mouse antibody-coated Dynabeads. In the specimen with purified LC, we detected only FITC-CD1a-positive, large, dendritic, cells with peripheral Dynabeads and no FITC-negative cells, suggesting a 100% pure LC population.

III. Cytokine analysis

ELISA: In some volunteers, values of the 2nd post-operative day were increased, most probably due to

the surgical procedure of the lymph vessel cannulation. A clear day-night difference was evident. The cytokine levels in samples collected during the day were usually higher than in those collected during the night. Significant inter-individual variations were found among the volunteers. To allow a better comparison of the values of the different probands, the values are also expressed as IL-1 β level index (Table 1).

Compared to the individual baseline values, the IL-1 β levels increased in volunteers of all experimental groups, i.e., in all 6 volunteers of irritant and in 5 out of 7 volunteers of allergic CD, but also in 1 volunteer from the control group. In irritant CD, in 4 out of 6 volunteers, peak IL-1 β lymph levels were detected at days 3/4, while in allergic CD, in 5 out of 7 volunteers, peak IL-1 β lymph levels were not seen before day 5. Whereas in allergic CD the highest values increased up to 6 pg/ml, in irritant CD, values up to 50 pg/ml were detected. However, when looking at the IL-1 β level index, we did not find clear-cut differences between irritant and allergic CD; similar to the values obtained in lymph derived from normal untreated skin, 3 volunteers of each group showed an index ≤ 2 , whereas in the others, a mean index of about 5 was reached.

Reverse transcriptase-polymerase chain reaction analysis: Transcripts for IL-1 β mRNA were reproducibly detected in the whole lymph cell population (Fig. 1, lane 1) from normal untreated skin as well as from allergic CD. The size of the product coincided with the predicted bp of 332. We were not able to detect IL-1 β mRNA in the isolated CD1a $^{+}$ lymph cells nor to find an upregulation of the IL-1 β mRNA signal strength in the course of the induction phase of allergic CD. Strong specific signals for IL-1 β were also detected in LPS stimulated PBMCs, which were chosen as a positive control (lane 2), whereas no signals were found in the negative control (water blanks; lane 3) and the control without RT (lane 4). The PCR amplification of β -actin mRNA, which remained unchanged, is shown as a positive control for equivalent loading and integrity of the RNA used in the analysis.

Discussion

We have established a technique for long-term collection of human skin-derived lymph (9). By inducing specific skin lesions, this system allows one to get insight into local pathomechanisms as well as signal transmission in selected skin disorders. The signals detected in the lymph reflect, e.g., immunological processes in the skin, but probably also determine the reaction pattern in the lymph node.

Using this model, we compared the IL-1 β protein profiles in human skin lymph in the course of irritant and allergic CD, since IL-1 β has recently been proposed as key cytokine for allergic skin reactions (8).

The significant interindividual variations in the values of the 15 volunteers may be due to the varying topography of the superficial lymph vessels cannulated, resulting in differences concerning the exact localisation and size of the drained skin area. Furthermore, the inter-individual variations in intensity and time course of the skin reactions may also contribute to the differences in IL-1 β lymph levels. To allow a comparison among the values of the individual volunteers, an IL-1 β index was created. In the course of the experiments, 50% of the volunteers of both, irritant and allergic CD, showed IL-1 indices exceeding 2. Thus, IL-1 β protein in human skin-derived lymph does not discriminate allergic from irritant CD. Since even in lymph derived from one control experiment, a small increase in the IL-1 β index was documented, the lymph levels of IL-1 β protein may be influenced not only by allergic and irritant reactions, but also by mechanical injuries such as, e.g., operative trauma.

In mice, Enk et al. (8) have demonstrated an upregulation of IL-1 β mRNA in epidermal cells in the very early phase of the induction of allergic CD. Thereby, the IL-1 β mRNA signal was shown to come almost entirely from epidermal LC. In humans, however, keratinocytes also act as a source of this cytokine (1). Thus, some important species differences have to be kept in mind. In our experiments, we detected IL-1 β mRNA signals in the total lymph cell population derived from both, normal skin as well as allergic CD, indicating that besides resident epidermal and dermal cells, circulating lymph cells may also contribute to the IL-1 β production. In lymph cells sampled 2 $\times$ daily during the whole course of induction of allergic CD, however, we were not able to detect an upregulation of IL-1 β mRNA. To obtain enough lymph for cell and cytokine analysis, sampling periods of 8 to 15 h are unavoidable. Thereby, very early differences in the IL-1 β mRNA expression might be masked (8). In the migrating CD1a⁺ lymph cells derived from normal skin as well as from the induction and elicitation phase of allergic CD, no IL-1 β mRNA signals could be detected. Thus, the source(s) of the IL-1 β mRNA in human skin lymph cells is still not defined.

In conclusion, in skin lymph, IL-1 β does not seem to represent an "indicator cytokine" to distinguish allergens from irritants. Similar to data obtained from skin biopsies (11), we find no differences between allergic and irritant CD. Our lymph cannulation data do not exclude that an early and specific

upregulation of IL-1 β mRNA in the induction phase of allergic CD might also occur in humans. Since this upregulation takes place within a very short period after application of the allergen, it is not detectable with our system, and it may even be restricted to the microenvironment of the epidermis.

Acknowledgements

We thank J. Mirkowitch and N. Weier for excellent technical help. These investigations were supported by a grant from the Swiss National Fund (32-38813.93).

References

1. Cork M J, Duff G W. Interleukin 1. In: Luger T A, Schwarz T (eds): *Epidermal growth factors and cytokines*. New York: Marcel Dekker, 1993: 293-431.
2. Auron P E, Webb A A, Rosenwasser L C, Mucci S F, Rich A, Wolff S M, Dinarello C A. Nucleotide sequence of human monocyte interleukin 1 precursor cDNA. *Proc Natl Acad Sci USA* 1984; 81: 7907-7911.
3. March C L, Mosley B, Larsen A, Cerretti D P, Braedt G, Price V, Gillis S, Henly C S, Kronheim S R, Grabstein K, Conlon P J, Hopp T P, Cosman D. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* 1985; 315: 641-647.
4. Lomedico P T, Gubler U, Hellmann C P, Dukovich M, Giri J C, Pan Y E, Collier K, Semionow R, Chua A O, Mizel S B. Cloning and expression of murine interleukin-1 cDNA in *Escherichia coli*. *Nature* 1984; 312: 458-462.
5. Gahring L C, Buckley A, Daynes R A. Presence of epidermal derived thymocyte activating factor IL-1 in normal human stratum corneum. *J Clin Invest* 1985; 76: 1585-1591.
6. Hauser C, Saurat J H, Schmitt A, Jannin F, Dayer J M. Interleukin 1 is present in normal human epidermis. *J Immunol* 1986; 136: 3317-3323.
7. Kurt-Jones E A, Beller D I, Mizel S B, Unanue E R. Identification of membrane associated interleukin 1 in macrophages. *Proc Natl Acad Sci USA* 1985; 82: 1204-1208.
8. Enk A H, Katz S I. Early molecular events in the induction phase of contact sensitivity. *Proc Natl Acad Sci USA* 1992; 89: 1398-1402.
9. Brand C U, Hunziker T, Braathen L R. Isolation of human skin-derived lymph. *Arch Dermatol Res* 1992; 284: 123-126.
10. Chomczynski P, Sacchi S. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extract. *Anal Biochem* 1987; 162: 156-160.
11. Hoefakker S, Caubio M, Van 't Erve E H M, Roggeveen M J, Boersma W J A, Van Joost T H, Notten W R F, Claassen E. In vivo cytokine profiles in allergic and irritant contact dermatitis. *Contact Dermatitis* 1995; 33: 258-266.

Address:

Ch. U. Brand
Dermatological Clinic
Inselspital
University of Berne
3010 Berne
Switzerland

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.