Pustular Irritant Dermatitis due to Croton Oil

Evaluation of the Role Played by Leukocytes and Complement

WAKIO TORINUKI and HACHIRO TAGAMI

Department of Dermatology, Tohoku University School of Medicine, Sendai 980, Japan

Torinuki W, Tagami H. Pustular irritant dermatitis due to croton oil: evaluation of the role played by leukocytes and complement. Acta Derm Venereol (Stockh) 1988; 68: 257–260.

To elucidate the pathomechanisms of irritant pustular dermatitis and to evaluate the role of leukocytes in pustulation induced by croton oil, we compared the skin responses in leukopenic- and decomplemented guinea pigs with those in control saline-injected animals, to 1% croton oil application. Both decomplemented and control animals responded similarly to croton oil, showing erythema and pustulation at 24 h after topical application; microscopically numerous mononuclear and polymorphonuclear cells infiltrated the skin. Meanwhile, the clinical and histopathological response of leukopenic animals to croton oil was significantly depressed. Time-course study of inflammatory infiltrate in the dermis of controls revealed that mononuclear cells preceded the infiltration of polymorphonuclears. Although leukocytes constitute an important component in croton oil dermatitis, our results suggest that unclarified chemical mediators, other than complement-derived chemotactic factors, play a crucial role as a primary chemoattractant in the production of pustulation at the croton oil-applied site. (Received October 22, 1987.)

W. Torinuki, Department of Dermatology, Ichihara Hospital of Teikyo University, Ichihara-shi, Chiba-ken, Japan.

Toxic (irritant) contact dermatitis occurs more commonly than allergic contact dermatitis in many situations. There are some types of irritant dermatitis in which sterile pustule formation occurs. Well known examples are those produced by patch test with the salts of heavy metals (1) and by irritation with petroleum hydrocarbons (2). Pustular irritant dermatitis is also produced by application of croton oil (3, 4). Epidermal necrosis and separation from the dermis accompanied by cellular infiltration in the dermis are said to be characteristic in the experimental croton oil dermatitis in guinea pigs. The dermal infiltrate consists of both mononuclear and polymorphonuclear cells (PMNs). Therefore, leukocytes seem to be an important factor in the toxic reaction elicited by croton oil. Since no sensitization is involved in the toxic reaction, the pathomechanism of the inflammation must depend upon direct damage of the cutaneous tissue, perhaps via a release of pro-inflammatory chemical mediators.

Among various endogenous chemical mediators, complement-derived fragments, e.g., C5a, have been reported to have biological activities, particularly PMN chemotactic properties (5).

In this study, we have evaluated the role of leukocytes in the toxic pustular contact reaction to croton oil, by comparing the response in guinea pigs decomplemented by treatment with cobra venom factor (CVF) and animals rendered leukopenic with cyclophosphamide monohydrate (CY), with that in saline-treated animals. A further aim of this study was to assess whether C5a might play a crucial role in pustulation due to croton oil application.

MATERIALS AND METHODS

Animals

Female albino guinea pigs of the Hartley strain, weighing 300–400 g, were used throughout the experiments. The dorsal area was depilated 3 days before topical application of croton oil. Decomplementation was performed in guinea pigs by i.p. injection of 300 U/kg of CVF (Cordis Labs., USA) 16 h before topical application (6). Leukopenic animals were obtained by i.p. injection of 300 mg/kg of

CY (Aldrich Chemical, USA) 4 days before topical application (7). Our pilot studies demonstrated that such treatments resulted in a decrease in the total complement hemolytic activity of about 97% and in the total leukocyte count of about 93%, respectively. These depressed values persisted at least until 5 days after CVF and until 7 days after CY, respectively. Animals that were injected with saline served as controls.

Topical application of croton oil

One percent croton oil in acetone (10 μl/cm²/animal) was topically applied to 16 cm² area on the back of decomplemented-, leukopenic- and control animals, respectively. Clinical skin changes such as erythema and pustule were evaluated by two independent observers 24 h after topical application.

Method of testing for leukocytes in dermis

At 2, 6, 12 and 24 h after topical application of 1% croton oil, 4-mm punch biopsy samples were taken from 3 or 4 control animals, and were fixed in formalin for routine hematoxylin and eosin preparations. In the case of decomplemented and leukopenic animals, biopsy specimens were taken at 24 h after topical application. A previous described counting procedure was employed (8, 9). Twenty fields just below the dermo-epidermal junction were counted (1000×, oil-immersion) and results were presented as the average number of cells per high-power field. Granulocytes were differentiated on morphological grounds into eosinophils and PMNs. Mononuclear cells (small and medium-sized lymphocytes, monocytes) were counted as a single group. In every animal the dermal cellular infiltrate in nontreated skin was counted, and their numbers were subsequently subtracted from the counts of the test areas to obtain a 'test minus normal count' for every animal and cell type. Statistical analysis was accomplished by Student's *t*-test.

RESULTS

Clinical and microscopic assessment at 24 h

One percent croton oil produced weak erythema within 2 h at test areas. The visible reaction increased and was strongest at 24 h. Therefore, the clinical reactions were assessed at 24 h after application. Seven of 9 controls and 5 of 8 decomplemented animals developed erythema and pustulation. However, all the 9 leukopenic animals showed only erythema without any pustulation. These results are shown in Table I.

Fig. 1 shows the quantitation of the inflammatory infiltrate in leukopenic-, decomplemented- and control animals at 24 h after application. Both decomplemented- and control animals showed a significant increase in the mononuclear and PMN count in the upper dermis as compared with the leukopenic animals ($p<0.001$). Mononuclears predominated in all tested animals. There was no significant difference in cell number between decomplemented- and control animals.

Leukocytes in the skin until 12 h after topical application

We also counted the leukocytes in the skin of control animals at various times after application (Fig. 2). Quantification of the inflammatory infiltrate in the upper dermis revealed that mononuclear and PMNs increased gradually after application. However, the

Table I. *Macroscopic changes on control, decomplemented and leukopenic guinea pigs at 24 h after topical application of 1% croton oil*

	Guinea pigs[a]		
Grade	Control	Decomplemented	Leukopenic
I (erythema)	2/9 (22%)	3/8 (37%)	9/9 (100%)
II (erythema + pustule)	7/9 (78%)	5/8 (63%)	0/9 (0%)

[a] Number of animals that reached to each grade/number of animals tested.

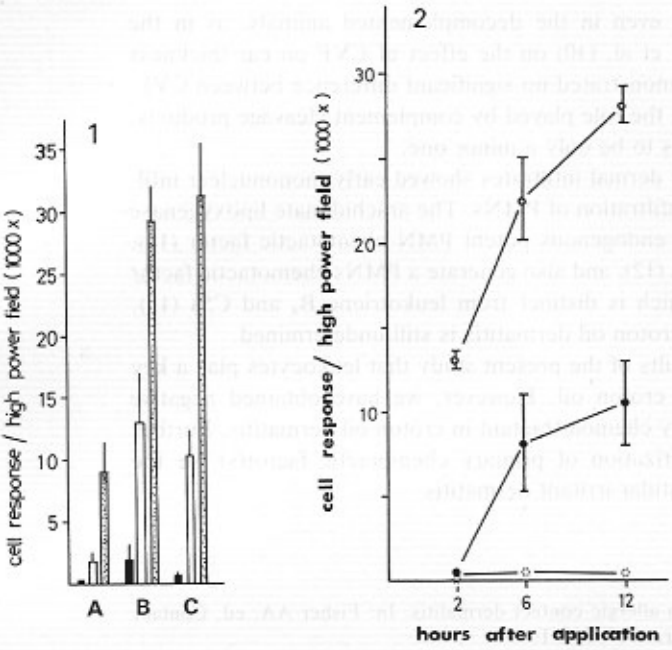

Fig. 1. Quantitation of inflammatory infiltration in the upper dermis in 4 leukopenic (A), 4 decomplemented (B) and 4 control (C) guinea pigs, 24 h after topical application of 1% croton oil. Columns show means of the cell responses (test count minus normal) for eosinophil (■), polymorphonuclear (□) and mononuclear (▦) cells. Vertical range bars show standard deviation.

Fig. 2. Quantitation of dermal leukocyte infiltration in 3 control animals at various times after topical application of 1% croton oil (mean ± SD). Cell response is given as the number of cells per high-power field in tested skin minus the corresponding number of cells in normal skin. ○—○, mononuclear cells; ●—●, polymorphonuclear cells; ☆—☆, eosinophils.

infiltration of the former preceded the latter. Mononuclears invaded the dermis soon after application, whereas PMNs began to appear at 6 h after application. No significant change in the eosinophil count was noted.

DISCUSSION

Croton oil, a toxic irritant agent, produces inflammatory responses characterized by erythema and pustulation in the skin. The histopathological findings in such skin showed accumulation of large numbers of PMNs as well as lymphocytes (3). Recently, Anderson & Groth (4) and Anderson (9) have shown that the dermal cellular infiltrate decreased in a dose-dependent fashion after CY administration, and the macroscopic appearance of the test reactions decreased generally. Our study also confirmed the suppressive effect of leukopenia on the pustular changes and on the cellular infiltrate in the inflamed skin, suggesting that leukocytes play a major role in the skin inflammation to croton oil. In the present study, we found that leukopenic animals consistently showed a weaker inflammatory response associated with a significant diminution in the dermal cellular infiltrate at the site of croton oil dermatitis, whereas decomplemented animals developed strong responses like the control animals.

Inflammatory lesions contain numerous metabolites and degeneration products that might be chemotactic for leukocytes. Our present study (Fig. 1) showed that croton oil

dermatitis abounded with leukocytes even in the decomplemented animals, as in the controls. The recent study by Patrick et al. (10) on the effect of CVF on ear thickness response of mice to croton oil also demonstrated no significant difference between CVF-treated and control groups. Therefore, the role played by complement cleavage products, C5a, for migration of leukocytes seems to be only a minor one.

Our time-course investigation of the dermal infiltrates showed early mononuclear infiltrate in the dermis that preceded the infiltration of PMNs. The arachidonate lipoxygenase metabolite, leukotriene B_4, is another endogenous potent PMN chemotactic factor (11). Monocytes can produce leukotriene B_4 (12), and also generate a PMNs chemotactic factor with a molecular weight of 10000 which is distinct from leukotriene B_4 and C5a (13). However, the role of these factors in croton oil dermatitis is still undetermined.

In summary, it is clear from the results of the present study that leukocytes play a key role in skin inflammation caused by croton oil. However, we have obtained negative results about the role of C5a as primary chemoattractant in croton oil dermatitis. Further studies are required for the characterization of primary chemotactic factor(s) for the clarification of the pathogenesis of pustular irritant dermatitis.

REFERENCES

1. Fisher AA. The role of patch testing in allergic contact dermatitis. In: Fisher AA, ed. Contact Dermatitis. Philadelphia: Lea & Febiger, 1967; 30–31.
2. Tagami H, Ogino A. Kerosine dermatitis: Factors affecting skin irritability to kerosine. Dermatologica 1973; 146: 123–131.
3. Skoog M-L. Measurement and differentiation of the cellular infiltrate in experimental toxic contact dermatitis. Acta Derm Venereol (Stockh) 1980; 60: 239–244.
4. Anderson C, Groth O. The effect of cyclophosphamide on the toxic contact reaction to croton oil in guinea pig. Acta Derm Venereol (Stockh) 1985; 65: 287–290.
5. Fernandez HN, Henson PM, Otani A, Hugli TE. Chemotactic response to human C3a and C5a anaphylatoxins. I. Evaluations of C3a and C5a leukotaxis in vitro and under simulated in vivo conditions. J Immunol 1978; 120: 109–115.
6. Lim HW, Novotny H, Gigli I. Role of complement and polymorphonuclear cells in demethylchlortetracycline-induced phototoxicity in guinea pigs: Inhibition by decomplementation in vivo. J Clin Invest 1983; 71: 1326–1335.
7. Eaglstein WH, Sakai M, Mizuno N. Ultraviolet radiation-induced inflammation and leukocytes. J Invest Dermatol 1979; 72: 59–63.
8. Groth O, Skoog M-L. Measurement and differentiation of the cellular infiltrate in experimental allergic contact dermatitis. Acta Derm Venereol (Stockh) 1979; 59: 129–134.
9. Anderson C. The effect of selected immunomodulating agents on experimental contact reactions. Acta Derm Venereol (Stockh) 1985 suppl 116; 1–48.
10. Patrick E, Burkhalter A, Maibach HI. Recent investigations of mechanisms of chemically induced skin irritation in laboratory mice. J Invest Dermatol 1987; 88: 24s–31s.
11. Camp R, Jones RR, Brain S, Woollard P, Greaves MW. Production of intraepidermal microabscesses by topical application of leukotriene B_4. J Invest Dermatol 1984; 82: 202–204.
12. Rosenbach T, Grabbe J, Moller A, Schwanitz HJ, Czarnetzki BM. Generation of leukotrienes from normal epidermis and their demonstration in cutaneous disease. Br J Dermatol 1985; 113 suppl 28: 157–167.
13. Kownatzki E, Kapp A, Uhrich S. Novel neutrophil chemotactic factor derived from human peripheral blood mononuclear leukocytes. Clin Exp Immunol 1986; 64: 214–222.