Evaluation of Lymphocyte Migration to Induced Paederus Dermatitis: An Experimental Study in Rats

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Abstract
Background: Paederus dermatitis (PD) is a blistering disorder that is caused by a small insect of the genus Paederus, especially Paederus fuscipes. This study aimed to investigate the reaction of the adaptive immune system regarding the recruitment of CD3, CCR4, and CCR10 markers, which are specifically expressed on the surface of T lymphocytes.

Materials and Methods: In this experimental study, 24 female rats were divided into two groups: the test and the negative control. In the test group, PD was induced by making insects in contact with shaved rat skin. Biopsies were obtained 24, 72, and 120 h after induction. In the negative control group, physiological saline was applied. Specimens were evaluated by immunohistochemical staining method. Antibodies against CD3, CCR4, and CCR10 were used. Distribution and staining intensities of CD3, CCR4, and CCR10 markers were estimated by the H-score index and findings were analyzed using the Kruskal–Wallis and Wilcoxon statistical tests.

Results: Based on the results of immunohistochemistry, the expression of CD3, CCR4, and CCR10 in the test group at 24, 72, and 120 h compared to the control group showed significant increase (P = 0.0006, P = 0.001, and P < 0.0001), respectively. The peak of expression of all markers was at 72 h after exposure. Hematoxylin and eosin staining also confirmed the fact that the majority of the lymphocyte infiltration occurred at 72 h postexposure.

Conclusion: The expression of CD3, CCR4, and CCR10 on cells present in PD lesions could indicate that T-lymphocytes are recruited to the site of inflammation by chemokine–chemokine receptor interactions and hence provide evidence for the response by the adaptive immune system following a PD.

Key Words: CCR10, CCR4, CD3, immunohistochemistry, paederus dermatitis

Introduction
Paederus dermatitis (PD) is an inflammatory skin disorder; it is brought about following the crushing of Paederus beetles on the skin. After the crushing, pederin is released by coelomic fluid causing vesicles and pustules on an erythematous base as well as abrupt appearance of stinging and burning feeling. Afterward, a linear dermatitis composed of erythematobullous lesions by 24 h appears; it is identified by “burn-like” lesions, which could be associated with vesicles, bullae, or pustules. Several countries have reported instances of PD. Full healing of the lesions takes place within 10–12 days accompanied by transient postinflammatory hyperpigmentation.

Contact dermatitis (CD) is a common disease; it is divided into two main categories - the irritant CD (ICD) and allergic CD (ACD). ICD is a nonspecific inflammatory dermatosis caused by the chemical toxicity on the skin cells; they activate the innate immune system by triggering inflammations. ACD is a hypersensitive response prompted slowly. The antigen-specific T-cells mediate the skin inflammation. It is believed that PD could be a specific type of acute ICD brought about by pederin, but it is not clear how these skin reactions are triggered off.

T-lymphocytes play a critical role in inflammatory skin reactions. Chemokines regulate passing of inflammatory cells through the tissues. Chemokines are small molecules that make lymphocytes migrate by attaching

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to receptors located on the lymphocytes surface.\textsuperscript{\cite{14}} Thymus and activation-regulated chemokine (TARC) and cutaneous T-cell-attracting chemokine (CTACK) are the main chemokines in the skin-specific homing of T-cells. Cutaneous venules display the chemokine TARC (CCL17) and uniformly express the TARC receptor CC chemokine receptor CCR4, which is expressed mainly on skin homing, cutaneous lymphocyte antigen–positive (CLA\textsuperscript{+}) T-cells.

Cutaneous memory T-cells draw in chemokine CTACK (CCL27), which is believed to help assembling lymphocytes to the skin as well. CTACK is expressed, in terms of structure, in the epidermal keratinocytes of the skin; it is the CCR10 ligand as well.\textsuperscript{\cite{19}} CCR10 is expressed incompletely for CLA\textsuperscript{+} CD4\textsuperscript{+} cell. T,\_ homing to cutaneous sites is influenced by both CCR10 and CCR4. Both CCR4 and CLA are expressed by majority of the skin-penetrating lymphocytes in allergic delayed-type hypersensitivity and skin lesions; however, CCR10 is expressed by about 10%.\textsuperscript{\cite{20}} Considering the prevalence of PD in the world and in northern provinces and southern regions of Iran, many studies have been done to examine the symptoms of the disease and its therapeutic aspects. However, the exact mechanism of skin reaction has not been investigated. Previous studies showed that PD was considered as an ICD due to the importance of the chemokine receptors in the migration of T-lymphocytes to the inflamed skin, we evaluated the expression of these chemokine receptors in PD.

**Materials and Methods**

**Animals**

Faculty of Medicine, Mazandaran University of Medical Sciences, provided the present study with 24 adult albino rats (150 ± 30 g); the rats were transferred to wire cages for further experiments. They were kept under the standard conditions and had access to drinking water and food freely in a temperature-controlled room with 12-h lightness and 12-h darkness.

**Induction of dermatitis and skin specimen collection**

To collect skin tissue specimens, the rats were shaved on their dorsal skin and then divided into two equal groups. In Group A, 12 rats were contacted with two insects for 2 min, and in Group B, which served as the control group, 12 rats were treated with saline water for 2 min. Skin specimens (punch biopsies) from two groups were collected 24, 72, and 120 h after the contact and then fixed in 10% neutral buffered formalin and embedded in paraffin for immunohistochemical detection. Ascending grades of alcohol were applied to dehydrate the samples; afterward, the following steps were taken: clearing in xylene and embedding in paraffin wax, mounting and sectioning, and staining with hematoxylin and eosin (H and E). Finally, they were studied by light microscopy.

**Immunohistochemistry staining**

Formalin-fixed and paraffin-embedded tissues of skin biopsies were cut into 2–3 µm sections and mounted on to poly-L-lysine-coated slides. After removing paraffin and rehydration, slides were immersed into 10 mmol citrate buffer (pH 6.0) to perform heat-induced epitope retrieval; boiling the buffer was done for 10 min in a pressure cooker.

Endogenous peroxidase activity was quenched by 3% H\textsubscript{2}O\textsubscript{2}, for 10 min at room temperature (RT). All slides were blocked with normal goat serum (Santa Cruz, USA) for 60 min at RT in a humid chamber. Following blocking, all sections were subsequently incubated overnight at 4°C with Anti-CD3 Mouse Monoclonal Primary Antibody (1:100 diluted, Santa Cruz, USA), Anti-CCR10 Mouse Monoclonal Primary Antibody (1:200 diluted, Santa Cruz, USA), and Anti-CCR4 Rabbit Polyclonal Primary Antibody (1:100 diluted, Abnova, Taiwan). After four times washing, the sections were incubated with biotinylated corresponding Secondary Antibody Goat anti-mouse (1:200 diluted, Santa Cruz, USA) for CD3 and CCR10 and goat anti-rabbit (1:200 diluted, Santa Cruz, USA) for CCR4 for 30 min at RT. The Santa Cruz avidin–biotin complex (ABC) staining system was used for the ABC method according to the manufacturer’s instructions. The sections were counterstained with hematoxylin, dehydrated through ethanol series, cleared in xylene, and then mounted. All slides were analyzed by a pathologist, and the semi-quantitative H-score system analysis was used to assess staining intensity and percentage of the positive stained cells.

The following equation was used to determine the H-score:

\[
H\text{-score} = \sum_{i=0}^{3} Pi (i) (i = 0, 1, 2, 3, Pi = 0 \sim 100\%) 
\]

Pi indicates the percentage of stained cells ranging from 0 to 100. Thus, the H-score varies from 0 to 300. H-score >0 is set as positive staining and H-score = 0 is set as a complete negative staining, weak staining = 1, moderate staining = 2, and strong staining = 3.\textsuperscript{\cite{21}}

**Statistical analysis**

All statistical analyses were performed using SPSS software version 16 for Windows (SPSS Inc. Chicago, USA). Wilcoxon test was used to compare the statistical difference between two groups and Kruskal–Wallis test was applied to compare differences between groups. P < 0.05 was considered statistically significant.
Results

**CD3 expression**
Comparison of the expression of CD3 at 24, 72, and 120 h showed that it had significantly higher expression than in the control group ($P = 0.0006$) [Figure 1]. The data also showed that the expression level at 72 h after induction of disease, was more than 24 and 120 h ($P < 0.001$).

**CCR4 expression**
The expression of CCR4 was significantly higher in the test group in all internal times ($P = 0.001$).

In addition, CCR4 expression was higher at 72 h after the induction of the disease ($P < 0.001$) [Figure 2].

**CCR10 expression**
The comparison of four groups (24, 72, 120, and control group) showed that the expression of CCR10 increased significantly at different hours compared to that of control group. In addition, 72 h after induction of the disease, the highest level of expression was seen in comparison with other groups ($P < 0.001$) [Figure 3].

**Macroscopic findings**
The results of the present study showed that after approximately 24 h of the insect contact, redness of skin and erythematous papules were observed in Group A. After 3 days of application, vesicles and marginal plaques, burn-like features and pustules, a perifollicular and intrafollicular inflammatory infiltrate (mainly of lymphocytes), edema, microvesiculation, and necrosis appeared in rats of Group A. After 5 days, the edema reversed, accompanied by desquamation of the epidermal cells; afterward, the lesions started to dry, and dark scales were observed and recovery progressed.

Discussion
The results showed that the expression of CD3, CCR4, and CCR10 were increased at 24 and 72 h post- *Paederus* contact in comparison with the control group, and the highest level of protein expression of all markers was at 72 h after exposure to *Paederus* [Figure 4].

The increased expression of CD3, CCR4, and CCR10 in PD suggests the recruitment of T-lymphocytes to the site of inflammation by the chemokine–chemokine receptor interactions. Consistent with our findings, previous studies also showed infiltration of lymphocytes to the inflammation site. In 2002, Uslular et al. reported the infiltration of lymphocytes, mononuclear cells, histiocytes, and eosinophils in biopsy specimens of PD by H and E staining.$^{[22]}$ In addition, another previous study examined histopathologic changes in induced dermatitis in rat.

According to the results, increased infiltration of eosinophils, polymorphs, and lymphocytes led to slight necrotic changes of the dermis. Edematous, macrophages, and granulocytes changed slightly at
the same time.[3] However, it should be mentioned that the type of leukocytes infiltrated to the site of inflammation was not completely identified in the present study.[16] Of note, the results obtained from 5 days after Paederus contact was remarkable. After 5 days, dryness and scaling were observed which showed that the lesions were recovering clinically. Immunohistochemistry (IHC) findings also showed a decrease in the expression of CD3, CCR4, and CCR10 markers on the lymphocyte level as compared to the 3rd day, which, according to the symptoms of the disease, was characterized by the onset of a disease progression [Figure 5].

Generally, since the presence of T-lymphocytes is an indicator of ACD, this study is not consistent with the results of the previous studies of PD.[3-8] However, due to the lack of examination of the specificity of T-cells by IHC, the determination of the type of T-cells and the specificity of these cells are recommended in future studies.

Conclusion
This study showed that the expression of CD3, CCR4, and CCR10 markers on the surface of T-lymphocytes in biopsy specimens suggested the presence of T-lymphocytes in the PD lesions. The specific recruitment of these lymphocytes to the site of inflammation, such as ACD to toxic ivy, put this type of dermatitis in the category of ACD, which causes lymphocytes to be involved without memory cells in the first contact. This is, of course, the first step in examining the mechanism of PD, and further studies are needed.

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Conflicts of interest
There are no conflicts of interest.

References
1. Davoodi S, Bakhtiari P, Khoob Del M. Determination of signs and symptoms of Paederus dermatitis in Behshahr hospital in 2000. J Mil Med 2008;10:29-34.
2. Gnanaraj P, Venugopal V, Mozhi MK, Pandurangan CN. An outbreak of Paederus dermatitis in a suburban hospital in South India: A report of 123 cases and review of literature. J Am Acad Dermatol 2007;57:297-300.
3. Ahmed MS, Boraei HA, Rakha OM. Histopathological characterization of induced Paederus dermatitis caused by Egyptian rove beetles (Paederus alfieri). Beni Suef Univ J Basic Appl Sci 2013;2:108-13.
4. Al-Dhalimi MA. Paederus dermatitis in Najaf Province of Iraq. Saudi Med J 2008;29:1490-3.
5. Fakoorziba MR, Eghbal F, Azizi K, Moemenbellah-Fard MD. Treatment outcome of Paederus dermatitis due to rove beetles (Coleoptera: Staphylinidae) on guinea pigs. Trop Biomed 2011;28:418-24.
6. Heo CC, Latif B, Hafiz WM, Zhou HZ. Dermatitis caused by Paederus fuscipes curtis, 1840 (Coleoptera: Staphilinidae) in student hostels in Selangor, Malaysia. Southeast Asian J Trop Med Public Health 2013;44:19720
7. Banney LA, Wood DJ, Francis GD. Whiplash rove beetle dermatitis in central Queensland. Australas J Dermatol 2000;41:162-7.
8. Mammino JJ. Paederus dermatitis: An outbreak on a medical mission boat in the Amazon. J Clin Aesthet Dermatol 2011;4:44-6.
9. Nasir S, Akram W, Ahmed F. The population dynamics, ecological and seasonal activity of Paederus fuscipes curtis (Staphylinidae; Coleoptera) in the Punjab, Pakistan. APCBEE Procedia 2012;4:36-41.
10. Nikookar SH, Moosa-Kazemi SH, Haj Haydari Z, Davari B. Comparison of therapeutic and anti-inflammatory effects of topical triamcinolon with placebo in the treatment of Paederus dermatitis in guinea pig. Sci J Kurdistan Univ Med Sci 2011;15:10-8.
11. Khan TM, Hassali MA, Gillani SW, Hameed MA. Clinical presentation of Rove beetle dermatitis. Australas Med J 2009;2:19-24.
12. Singh G, Yousuf Ali S. Paederus dermatitis. Indian J Dermatol Venereol Leprol 2007;73:13-5.
13. Ghoneim KS. Human dermatosis caused by vesicating beetle products (Insecta), cantharidin and paederin: An overview. World J Med Med Sci 2013;1:1-26.
14. Zargari O, Kimyai-Asadi A, Fathalikhani F, Panahi M. Paederus dermatitis in Northern Iran: A report of 156 cases. Int J Dermatol 2003;42:608-12.
15. Tončić RJ, Lipozenčić J, Martinac I, Gregurić S. Immunology of allergic contact dermatitis. Acta Dermatovenerol Croat 2011;19:51-68.
16. Nosbaum A, Vocanson M, Rozieres A, Hennino A, Nicolas JF. Allergic and irritant contact dermatitis. Eur J Dermatol 2009;19:325-32.
17. Wang X, Fujita M, Prado R, Tousson A, Hsu HC, Schottelius A, et al. Visualizing CD4 T-cell migration into inflamed skin and its inhibition by CCR4/CCR10 blockades using in vivo imaging model. Br J Dermatol 2010;162:487-96.
18. Hijnen D, De Bruin-Weller M, Oosting B, Lebre C, De Jong E, Bruijnzeel-Koomen C, et al. Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell-attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. J Allergy Clin Immunol 2004;113:334-40.
19. Reiss Y, Proudfoot AE, Power CA, Campbell JJ, Butcher EC. CC chemokine receptor (CCR) 4 and the CCR10 ligand cutaneous T cell-attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. J Exp Med 2001;194:1541-7.
20. Soler D, Humphreys TL, Spinola SM, Campbell JJ. CCR4 versus CCR10 in human cutaneous TH lymphocyte trafficking. Blood 2003;101:1677-82.
21. Zhuang X, Zhang X, Xia X, Zhang C, Liang X, Gao L, et al. Ectopic expression of TIM-3 in lung cancers: A potential independent prognostic factor for patients with NSCLC. Am J Clin Pathol 2012;137:978-85.
22. Uslular C, Kavukçu H, Alptekïn D, Acar MA, Denli YG, Memiçioglu HR, et al. An epidemicity of Paederus species in Cukurova region. Cutis 2002;69:277-9.