

Study of pro- and anti-inflammatory cytokine profile in the patients with parthenium dermatitis

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Background: Parthenium dermatitis is a common airborne allergic contact dermatitis induced by exposures to the weed *Parthenium hysterophorus*. The disease manifests as itchy erythematous papules, papulovesicular and plaque lesions on exposed areas of the body.

Objectives: The aim of this study was to show the alterations in pro/anti-inflammatory cytokines in parthenium dermatitis.

Methods: The study included 50 patients with parthenium dermatitis confirmed by patch testing using aqueous extracts of *P. hysterophorus* and 50 age-matched healthy controls. The levels of pro-inflammatory [tumour necrosis factor- α (TNF- α), interleukin (IL)-6, IL-8, and IL-17] and anti-inflammatory (IL-4 and IL-10) cytokines were estimated by commercially available high sensitivity enzyme-linked immunosorbent assay (ELISA) kits.

Results: All the dermatitis patients showed significantly ($P < 0.001$) elevated levels of TNF- α , IL-6, IL-8, and IL-17 levels as compared to healthy controls. In contrast, the anti-inflammatory cytokine IL-4 showed an insignificant decrease ($P < 0.217$) and a decrease in level of IL-10 was statistically significant (0.001) compared with controls.

Conclusions: The present study suggests the involvement of pro-inflammatory cytokines in the pathogenesis of parthenium dermatitis. A decrease in levels of anti-inflammatory cytokines was demonstrated, which could not downregulate pro-inflammatory cytokines in parthenium dermatitis.

Key words: anti-inflammatory; cytokines; delayed-type hypersensitivity; IL-4; IL-6; IL-8; IL-10; IL-17; parthenium dermatitis; *Parthenium hysterophorus*; pro-inflammatory; TNF- α . © John Wiley & Sons A/S, 2010.

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Airborne allergic contact dermatitis is a skin inflammation caused by a delayed-type hypersensitivity mediated by T-lymphocytes (1). In India and its neighbouring countries, most such cases are caused by the weed *Parthenium hysterophorus*. It is an alien weed with its origin in the Caribbean region, Mexico, and United States and has prolifically spread in central Queensland, Australia, Africa, and Asia (2, 3). The allergens responsible for the contact dermatitis are sesquiterpene lactones, mainly parthenin, present in the oleoresin fraction of the leaf, the stem, and the flower and also in pollen of *P. hysterophorus* (Fig. 1). Parthenium dermatitis

usually presents as itchy, erythematous papules and plaques primarily affecting the exposed areas and the flexures. This classic pattern may change to chronic actinic dermatitis or a mixed pattern dermatitis (4, 5). The dermatitis is usually worse during the summer and the rainy season, when the plants grow more profusely. The disease normally persists with variable remissions and relapses causing great distress and morbidity (6).

Allergic contact dermatitis is a delayed hypersensitivity in which the allergen first induces a sensitization phase and then, after re-exposure to the allergen a later elicitation phase (7). The response

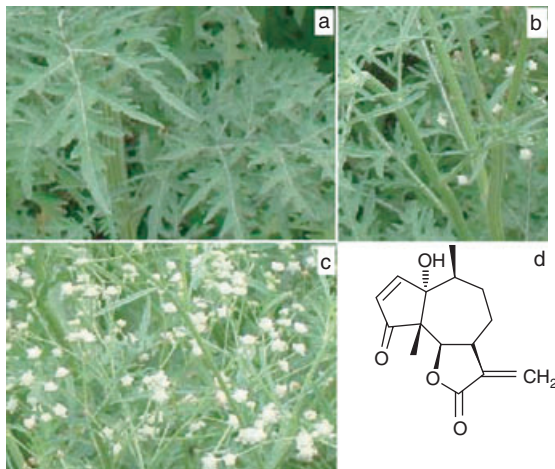


Fig. 1. Different parts of *Parthenium hysterophorus*. (a) Leaves, (b) mature plant, (c) flowers, and (d) chemical structure of parthenin: major allergen from parthenium plant responsible for allergic contact dermatitis.

is mediated by a series of cellular and molecular mechanisms. Epidermal Langerhans' cells and other cutaneous dendritic cells transport the allergen from the skin to regional lymph nodes, where it is presented to T-lymphocytes (8–10). The reaction is characterized by the infiltration of T-lymphocytes into challenged skin sites and the development of cutaneous inflammation (10).

The human immune response is regulated by a highly complex and intricate network of control elements; a dynamic balance exists between pro-inflammatory cytokines and anti-inflammatory components (11, 12). Polarization of T-helper (T_H) lymphocytes into functional T_H1 and T_H2 subsets is one of the main factors that determine the direction of the balance between pro- and anti-inflammatory cytokines (13, 14). A number of cytokines, known collectively as pro-inflammatory cytokines because they accelerate inflammation, also regulate inflammatory reactions either directly or by their ability to induce the synthesis of cellular adhesion molecules or other cytokines in certain cell types. The major pro-inflammatory cytokines, predominantly produced by activated macrophages and T_H1 lymphocytes, involved in the upregulation of inflammatory reactions are interleukin (IL)-1- α , IL-1- β , TNF- α , IL-6, IL-8, and IL-18. T_H2 type and other cells produce a variety of cytokines, including IL-4, IL-5, IL-6, IL-10, and IL-13 (15). IL-17 is an important pro-inflammatory cytokine produced by activated T_H17 cells and has major implication in allergic and autoimmune diseases (16). Regulation of T-cell activation by the anti-inflammatory cytokines is a crucial early control element in the process of inflammation of skin.

The present study is a first attempt to evaluate and compare the pro-inflammatory and anti-inflammatory cytokine secretion patterns in parthenium-induced allergic contact dermatitis and non-allergic healthy individuals.

Materials and Methods

Methods

The serum samples from 100 age-matched study subjects (50 with parthenium-induced allergic contact dermatitis and 50 healthy controls) were studied. The samples for study was collected in summer and rainy seasons (March–October), when the plant grows profusely and there is increased severity of the dermatitis. Blood samples were taken just after diagnostic patch test readings (4 days) and prior to any immunosuppressive treatment from a total of 50 patients with parthenium dermatitis attending the outpatient department of Department of Dermatology and Venereology, All India Institute of Medical Sciences (AIIMS), New Delhi. All these patients had been patch tested with a standardized aqueous extract of *P. hysterophorus* to confirm the diagnosis and were tested further with serial dilutions of the antigens to determine the titre of contact hypersensitivity (17). Patients with other eczematous conditions or any other inflammatory skin diseases, rather than parthenium dermatitis, were excluded from the study. Fifty healthy individuals, who gave their consent for this study, were enrolled from different departments of AIIMS. All the healthy controls were also patch tested.

The ethical institutional committee approved the study and all participants gave their informed consent.

Ten millilitres of venous blood was taken from each subject. All blood samples were drawn into tubes free of endotoxins. The tubes were centrifuged for 10 min at 3000 rpm and serum was separated and stored at -70°C .

Patch testing

Patch testing was done with Indian Standard Series (ISS), which include extracts of plant allergens of the Compositae family. The compositae mix includes Xanthium and Chrysanthemum other than Parthenium allergens. Individual standardized aqueous extracts of parthenium allergens were used separately for the patch test to confirm the parthenium induced allergy (18). The allergens were applied on the upper back using 8 mm internal diameter aluminium chambers (Systopic Pharmaceuticals (P) Ltd., New Delhi, India). The readings were at time of removal of chambers on day 2 and two days later on day 4, and the reactions evaluated according to

the International Contact Dermatitis Research Group criteria. Only those reactions that persisted at day 4 were considered to be positive.

Estimation of cytokines

The levels of pro-inflammatory cytokines (TNF- α , IL-6, IL-8, and IL-17) and anti-inflammatory cytokines (IL-4 and IL-10) were estimated by high sensitivity sandwich enzyme-linked immunosorbent assay (ELISA). The samples that were shown to have high concentrations of cytokines were diluted and measured in triplicate. The ELISA kit for TNF- α and IL-6 was supplied by Diaclone (Besoncon Cedex, France), IL-8 by Invitrogen Corporation (Camarillo, CA, USA), IL-17 by Bio Scientific Corporation (Todd Lane Suite, Austin), and IL-4 and IL-10 by BD Biosciences (San Diego, CA, USA).

A monoclonal antibody specific for different cytokines (TNF- α , IL-6, IL-8, IL-17, and IL-4, IL-10) had been coated onto the wells of the microtiter strips provided. During the first incubation, TNF- α /IL-6/IL-8/IL-17 and IL-4/IL-10 present in the sample or standard and a monoclonal anti-TNF- α , IL-6, IL-8, and IL-17 antibody conjugated to biotin were simultaneously incubated in respective microtiter plates. Following incubation, unbound TNF- α /IL-6/IL-8/IL-17 and IL-4/IL-10 were removed during a wash step. Streptavidin horseradish peroxidase (HRP) was added and bound to the biotinylated anti-TNF- α /IL-6/IL-8/IL-17 and IL-4/IL-10. After incubation and wash step, a substrate solution reactive with HRP was added to the wells. A coloured product was formed in proportion to the amount of TNF- α /IL-6/IL-8/IL-17 and IL-4/IL-10 present in the sample. The reaction was terminated by adding sulfuric acid and absorbance was measured at 450 nm. The absorbance measured by an ELISA reader at 450 nm was proportional to the concentration of respective cytokine present in the sample. A reference curve was obtained by plotting the cytokine concentration of several dilutions of standard samples versus absorbance. The cytokine levels of the samples tested were determined by comparing their absorbance with the absorbance of the known amounts of respective cytokine provided with the standards.

Statistical analysis

Data are expressed as mean $\pm$ SD and comparisons between groups were performed by the Mann–Whitney *U*-test. Correlation between inter-parameter in the sample was obtained by Pearson correlation tests. *P* values lower than 0.05 were considered to be significant.

Table 1. Characteristics of study subjects

	Patients (<i>n</i> = 50)	Controls (<i>n</i> = 50)
Age (years), mean $\pm$ (SD) (range)	52.28 $\pm$ 12.36 (28–75)	49.24 $\pm$ 10.56 (31–73)
No. of males	34	35
No. of females	16	15

Results

Our study included 50 patients with confirmed parthenium dermatitis and 50 matches controls (Table 1). The majority (90%) of the patients were farmers and most of the patients (78%) had exacerbations during summer and rainy season.

The levels of pro-inflammatory cytokines TNF- α (144.17 pg/ml), IL-6 (43.07 pg/ml), IL-8 (5.39 pg/ml), and IL-17 (76.23 pg/ml) were increased significantly (*P* < 0.001), as compared to healthy controls as shown in Table 2. In contrast to pro-inflammatory cytokines, the mean values of anti-inflammatory cytokines were decreased in patients when compared with healthy controls. The decline in the levels of regulatory cytokine IL-10 (4.16 pg/ml) was statistically significant (*P* = 0.001) compared with controls (7.86 pg/ml). IL-4 levels showed an insignificant decline (*P* < 0.217) in the patients as compared to controls as shown in Table 3. Pearson correlation between each parameter in the patients group was done, which showed significant correlation for TNF- α and IL-6 and between IL-6 and IL-4 and IL-10 and IL-4 as shown in Table 4.

Discussion

Cytokines are soluble signals of importance in mediating cell–cell communication during inflammatory and immune responses (19). A complex network of cytokines is generated in response to a systemic immune challenge. It is the net effect of interactions between pro-inflammatory and anti-inflammatory molecules that determines the nature of the immune response in individual patients (20–22).

In our study, the levels of pro-inflammatory cytokines TNF- α , IL-6, IL-8, and IL-17 were significantly elevated in patients as compared to healthy controls, which might suggest that pro-inflammatory cytokines may have involvement in the pathogenesis of parthenium dermatitis. Allergen-specific T-cells produce cytokines that activate macrophages and promote inflammatory mediators in skin lesions (10); this might be involved in parthenium dermatitis too.

Our study also showed that anti-inflammatory cytokine IL-10 levels were decreased statistically significantly and might have involvement in parthenium dermatitis, which in turn could not

Table 2. Levels of pro-inflammatory cytokines in patients with parthenium dermatitis and healthy subjects

Parameters		Patients (<i>n</i> = 50)	Controls (<i>n</i> = 50)	Significance (<i>P</i>)
Pro-inflammatory cytokines Mean ± SD (range)	TNF-α (pg/ml)	144.17 ± 88.64 (44–414)	12.29 ± 4.88 (5–25)	0.001
	IL-6 (pg/ml)	43.07 ± 31.16 (14.3–138.2)	3.58 ± 1.52 (2.1–7.6)	0.001
	IL-8 (pg/ml)	5.39 ± 1.99 (2.5–9.6)	3.39 ± 0.93 (2.3–5.6)	0.001
	IL-17 (pg/ml)	76.23 ± 18.37 (47.5–127)	31.47 ± 6.36 (20.8–42.4)	0.001

IL, interleukin; SD, standard deviation; TNF- α , tumour necrosis factor- α .

Table 3. Levels of anti-inflammatory cytokines in patients with parthenium dermatitis and healthy subjects

Parameters		Patients (n = 50)	Controls (n = 50)	Significance (P)
Anti-inflammatory cytokines	IL-4 (pg/ml)	5.26 $\pm$ 1.66 (2.10–8.40)	5.89 $\pm$ 2.28 (2.2–13.6)	0.217
	Mean $\pm$ SD (range)	IL-10 (pg/ml)	7.86 $\pm$ 2.20 (4.10–12.40)	0.001

IL, interleukin; SD, standard deviation.

Table 4. Pearson correlation between interparameters in the patients group

Parameters	TNF- α	IL-6	IL-8	IL-17	IL-4	IL-10
TNF- α	1.00	0.326*	0.204	0.167	–0.238	–0.120
IL-6	0.326*	1.00	0.181	0.009	–0.410**	–0.239
IL-8	0.204	0.181	1.00	0.222	0.147	0.200
IL-17	0.167	0.009	0.222	1.00	0.117	0.028
IL-4	–0.238	–0.410**	0.147	0.117	1.00	0.904**
IL-10	–0.120	–0.239	0.200	0.028	0.904**	1.00

IL, interleukin; TNF- α , tumour necrosis factor- α .

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

downregulate pro-inflammatory cytokines (TNF- α , IL-6, IL-8, and IL-17). These findings are in concordance with clinical manifestation in the patients, which would usually be considered as an expression of delayed-type hypersensitivity reaction.

The T_H1 type cells preferentially secrete pro-inflammatory cytokines that play an important role in cell-mediated immunity and chronic inflammation (13). TNF- α is a prominent cytokine that seems to be important in allergic contact dermatitis, is recognized as a powerful mediator of inflammatory reaction, and might induce these alterations either directly, as it can react with a wide range of cell types (23), or indirectly by the activation of several other cells.

IL-6 exerts stimulatory effects on T- and B-cells, thus favouring chronic inflammatory responses and is histologically associated with the presence of mononuclear cells, such as macrophages and lymphocytes (24, 25). IL-6 levels are elevated in several inflammatory diseases including rheumatoid arthritis. Our results have shown that IL-6 is important in the development of inflammation in parthenium dermatitis. The pathogenesis of contact allergy is complex and likely to involve the interaction of a number of genes. Recent study suggests that polymorphisms in the gene coding for TNF- α and IL-6 may also be associated with increased pro-inflammatory cytokines (26).

IL-8 is the best-known member of the α -chemokines with chemotactic properties predominantly for neutrophils (27, 28). Recently, in chronic inflammatory skin diseases such as psoriasis and eczema, IL-8 was shown to be involved (29–32). Our observations clearly show that IL-8 is a characteristic finding in inflammatory skin diseases and most probably involved in the pathogenesis of parthenium dermatitis.

IL-17 plays an important direct role in creating pro-inflammatory and chemotactic environment, enhances TNF- α , IL-8, and IL-6 expression, supporting their role in chronically inflamed skin disease (33). Our findings might suggest some important implications in parthenium dermatitis, which is in concordance with Almkhazangy et al. (34) recent report of involvement of T_H17-mediated inflammation in psoriasis and in human allergic contact dermatitis (35). Recently, a study by Watkins et al. (36) showed that pro-inflammatory cytokines, such as TNF- α , IFN- γ , IL-6, and IL-8, which home naive T-lymphocytes to the skin, have been shown to be elevated in irritant contact dermatitis, which is in support to our findings that substantially enhanced our understanding of the inflammation in parthenium dermatitis.

Pro-inflammatory agents such as TNF- α , IL-6, and IL-1 modulate lymphocyte recruitment via upregulation of endothelial adhesion molecules and chemokines (37, 38). The resulting activation of the T-cells produces cytokines such as chemokines for macrophages, other T-cells and, to a lesser extent, neutrophils, as well as TNF- α and IFN- γ . The consequence is a cellular infiltrate in which mononuclear cells, such as T-cells, and macrophages tend to predominate that mediate inflammation in the skin as reported by Rios et al. (7). Our findings correlate with this mechanism and might play a crucial role in the inflammation of parthenium dermatitis.

The T_H2 type cells produce a variety of cytokines, including IL-4, IL-5, IL-10, and IL-13. In our study,

anti-inflammatory cytokine, IL-10, exhibited significant changes in the patients with parthenium dermatitis. As reported by Woods et al. (39), IL-4 functions as an anti-inflammatory cytokine, and it has ability to suppress the infiltration of T cells, including T_H1 cells, that secrete IFN- γ . IL-4 or IL-12 drives antigen-stimulated naive CD4 $^+$ T cells towards the T_H2 or the T_H1 phenotype, respectively. IL-4 and IL-10 lead to the inhibition of T_H1 -type immune responses by downregulating the production of macrophage-derived IL-12 (40). Spergel et al. (41) showed an anti-inflammatory role of IL-4 in atopic dermatitis, by virtue of its ability to downmodulate T-cell infiltration into the skin.

IL-10 is the most important anti-inflammatory cytokine and a potent deactivator of monocyte/macrophage proinflammatory cytokine synthesis and inhibits leukocyte infiltration, inflammation, and tissue damage associated with immunological responses that can injure the skin (42, 43). IL-10 provides a regulatory mechanism for T_H1 memory CD4 $^+$ T-cell responses and IL-10 and transforming growth factor- β (TGF- β) naturally downregulate the inflammation (44, 45). In the pathogenesis of atopic allergic disorders, in contrast to our result, Ohmen et al. (46) showed that the increase in level of IL-10 shows involvement of T_H2 cells. Our findings collectively suggest that the decrease in level of IL-10 could not limit the production of pro-inflammatory cytokines and chemotactic factors for granulocytes, which might show autoregulatory loop in cytokines production in parthenium dermatitis. A recent study on atopic dermatitis (49), associated with a T_H2 phenotype, showed dominance of IL-4, and IL-10 secretion (47). This is in contrast to our findings in parthenium dermatitis, which is a cell-mediated (T_H1) delayed hypersensitivity disease.

P. hysterothorus poses a serious health risk. The problem is now being more widely acknowledged. Our study provides understanding of the pathogenic mechanisms of parthenium dermatitis thereby possibly identifying therapeutic targets for the treatment of this disease.

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