

T-lymphocyte cytokine profiles in Compositae airborne dermatitis

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Summary

Compositae airborne dermatitis is a well-recognized disorder characterized by erythematous lesions and papules on light-exposed areas. The presence of positive patch test reactions and the absence of specific serum IgE suggest delayed-type hypersensitivity, the murine model of which is characterized by a Th1 cytokine production profile [high amounts of interferon (IFN)- γ and interleukin (IL)-2; little or no IL-4 and IL-5]. The aim of this study was to evaluate the cytokine profile of T-cell lines and T-cell clones from peripheral blood in a 38-year-old non-atopic male woodcutter affected by seasonal airborne contact dermatitis. The patient showed positive patch test reactions to several Compositae extracts (*Achillea millefolium*, *Chamomilla recutita*, *Tanacetum parthenium*, *T. vulgare*) and sesquiterpene lactone mix. On prick testing with Compositae and other plants, serum-specific IgE levels and phototesting were negative or normal. Allergen-specific T-cell lines produced with Compositae extracts showed a good *in vitro* cell proliferation only to *C. recutita* extract. Serial cloning performed using the *C. recutita*-specific T-cell lines revealed an $\alpha\beta$ +CD4+ phenotype with high amounts of IFN- γ and IL-4 in T-cell clones. Thus, these cells expressed a preferential Th0 phenotype. These data suggest that in addition to IFN- γ , other T-cell derived cytokines, such as IL-4, may play a part in the immunopathogenesis of contact dermatitis.

Key words: airborne dermatitis, CD4⁺, Compositae, cytokines, interferon- γ , interleukin-4, Th1, Th2

Compositae (synonym Asteraceae) form the second largest botanical family in the world and include many troublesome weeds as well as ornamental annual, herbaceous perennial and edible species.¹ These plants may cause contact dermatitis, first described in 1919^{2,3} and generally reported as Compositae dermatitis.⁴

Compositae dermatitis is usually airborne⁵ and is more frequent in men over 40 years of age employed in farming and forestry activities. Its clinical aspects are characterized by erythematous patches and papules; however, during exacerbations, exudative acute eczema may be observed.⁶ The lesions are localized in light- and air-exposed areas, including the upper eyelids and the triangle of skin behind the earlobes.^{7,8} Positive patch test reactions to sesquiterpene lactones and/or plant extracts,^{9–12} together with a negative prick test and absence of specific-serum IgE,⁵ are diagnostic. This is suggestive of a delayed-type hypersensitivity (DTH) mechanism which in the murine model is

mediated by Th1 cells, characterized by production of interferon (IFN)- γ and interleukin (IL)-2. This Th1 cytokine production profile has been reported in nickel-specific T-cell clones (TCCs) obtained from peripheral blood.^{13,14} High levels of IL-4 and IL-5, in contrast, are indicative of Th2 cells, which are associated with atopic disorders, but these cytokines have recently been reported in nickel contact dermatitis using TCCs obtained from the site of positive patch tests to the metal.¹⁵

The functional type of the allergen-specific TCCs in a patient affected by Compositae airborne allergic contact dermatitis was investigated. Cloned peripheral allergen-specific T cells showed normal production of IFN- γ and increased secretion of IL-4. This is suggestive of a preferential Th0/Th2 cytokine profile.

Materials and methods

Patient characteristics

A 38-year-old non-atopic male farmer was referred to

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the Allergic and Environmental Dermatology Section for a suspected photodermatitis recurring over the last 12 years during the wood-cutting period from August to December. The eczematous and lichenified lesions involved light-exposed sites (face, neck, back of the hands, extensor aspects of the forearms), as well as shaded areas of exposed skin (upper eyelids, retroauricular and submental regions).

Patch testing

The patient was patch tested with the Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali standard series, the farmer series (Firma Diagent, Florence, Italy) and Compositae mix (Chemotecnique, Malmö, Sweden). The last is a mixture of the most common weeds (*Achillea millefolium* 1%, *Arnica montana* 0.5%, *Chamomilla recutita* 2.5%, *Tanacetum parthenium* 1%, *T. vulgare* 1%). The allergens were applied on the upper back with Van der Bend square chambers and removed after 2 days. The readings were carried out on days 2 and 4, and the reactions evaluated according to the International Contact Dermatitis Research Group criteria.

After 1 month, sesquiterpene lactone mix (Chemotecnique), containing equimolar concentrations of three purified lactones (alantolactone 0.033%, costunolide 0.033% and dehydrocostus lactone 0.033%) and extracts of fresh samples of the Compositae mix plants with the exception of *A. montana*, were patch tested. The plants were supplied by the Department of Plant Biology, University of Perugia. The extracts were prepared according to the method of Bleumink *et al.*,¹⁶ modified by Campolmi *et al.*¹⁷ Finely chopped flowers, leaves and stems of each plant examined were added to 95% ethyl alcohol for 16 h at 20 °C (ratio plant/alcohol: 1 g/10 mL). After filtration, the insoluble matter was treated again with ethyl alcohol. The global alcoholic solution (about 20 mL) was reduced to a volume of 1 mL by vacuum rotatory evaporator and dissolved in petrolatum at the same concentration as each component of Compositae mix (Table 1).

Prick testing and serum IgE determination

Water-based extracts of pollens of Compositae (*Artemisia vulgare*, *Helianthus annuus*, *Iva axillaris*, *Solidago virgaurea*, *Xanthium strumarium*) and a battery of common allergens (Graminaceae mix, *Parietaria* mix, Plantaginaceae Poligonaceae mix, *Ambrosia* mix, Betulaceae mix, *Olea europea*, *Cupressus sempervirens*, *Corylus avellana*,

Table 1. Patch test results

	%	Day 2	Day 4
Compositae mix	6	+++	+++
<i>Achillea millefolium</i>	1	++	+++
<i>Chamomilla recutita</i>	2.5	++	++
<i>Tanacetum parthenium</i>	1	+++	+++
<i>Tanacetum vulgare</i>	1	++	++
Sesquiterpene lactone mix	0.1	+++	+++

All in petrolatum.

Dermatophagoides pteronyssinus, *D. farinae*, cat and dog epithelium), saline solution and histamine hydrochloride (Bracco, Milan, Italy) were prick tested on the volar forearm. Weal reactions were read at 10 and 20 min and judged positive when the test side was equal to or greater than the histamine reaction. The patient was reassessed 1 and 2 days after skin prick testing to evaluate possible delayed reactions.

The serum total and specific IgE levels were detected by an enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's procedure (DPC, Los Angeles, CA, U.S.A.). Subsequently, prick tests were carried out using fresh extracts of Compositae (*A. millefolium*, *C. recutita*, *T. parthenium*, *T. vulgare*), all 1% of the described alcoholic extract in water (v/v) according to the European Guidelines and the European Pharmacopoeia monograph on allergen products.¹⁸

Phototesting

The minimal erythema doses (MEDs) for ultraviolet (UV) A and UVB were determined using the Waldmann UV 800 K (Waldmann Medical Division, Schwenningen, Germany) as a light source. This instrument was equipped with a bank of six Philips TLK 40 W/09 fluorescent tubes for UVA (emission spectrum 315–395 nm, maximum at 355–365 nm) and four Philips TL 20 W/12 fluorescent tubes for UVB (emission spectrum 285–350 nm, maximum at 310–315 nm). The irradiance was measured with the Waldmann UV meter. The test readings were carried out 24 h after the irradiation of six small buttock areas (2 cm²) with increasing doses of UVA (5, 7.5, 10, 12.5, 15, 17.5 J/cm²) and UVB (30, 45, 60, 75, 90, 105 mJ/cm²).

Generation of allergen-specific T-cell lines

Peripheral blood mononuclear cells (PBMCs) were collected by density gradient centrifugation with Ficoll-Hypaque (Lymphoprep, Oslo, Norway). Allergen-specific

T-cell lines (TCLs) were obtained from 0.5×10^6 PBMCs/mL stimulated with Compositae extracts (*C. recutita*, *A. millefolium*, *T. parthenium*, *T. vulgare*; all $10 \mu\text{g/mL}$ according to the method of Bradford¹⁹) for 5 days in 24-well flat-bottomed culture plates containing RPMI 1640 medium supplemented with 2 mmol/L L-glutamine (Life Technologies, Chagrin Falls, U.S.A.), penicillin (100 U/mL), streptomycin ($50 \mu\text{g/mL}$) and human AB⁺ serum (10%). Subsequently, 25 U/mL of recombinant IL-2 (Immunotech SA, Marseille, France) were added and cells were kept in culture for a further 7 days. To obtain non-allergen-specific autologous TCLs, the same procedure without allergen stimulation but with phytohaemagglutinin (PHA) (Seromed, Berlin, Germany) (final dilution 1:20) was used.

After a 12-day culture, in each batch the cell viability was tested by Trypan Blue exclusion and the antigen specificity by the proliferative response with ³H-thymidine incorporation. Briefly, 0.5×10^6 /mL Compositae-stimulated PBMCs with 0.5×10^6 /mL irradiated autologous PBMCs as antigen-presenting cells, and $10 \mu\text{g/mL}$ of Compositae extract, were cultured for 72 h in 96-well round-bottom plates. When the stimulation index (ratio between the mean c.p.m. obtained in triplicate wells with autologous irradiated PBMCs plus allergen and the mean c.p.m. obtained in triplicate wells containing PBMCs alone) was plus 10, responses were considered positive.

Generation of allergen-specific T-cell clones

Allergen-specific and control TCCs were obtained from TCLs using a limiting dilution technique and expanded at weekly intervals with 10^5 irradiated feeder cells and recombinant IL-2 as previously described in detail.²⁰ The TCC phenotype was detected on a FACStar analyser (Becton Dickinson, Mountain View, CA, U.S.A.) using fluorescein isothiocyanate- or phycoerythrin-conjugated anti-CD3, -CD4, -CD8, - $\alpha\beta$ and - $\gamma\delta$ monoclonal antibodies (MoAbs) (Serotech, Oxford, U.K.).

Detection of cytokines in T-cell clone supernatants

To obtain optimal stimulation, viable control T-cell blasts and allergen-specific TCCs were extensively washed and stimulated for 24 h with phorbol myristate acetate (PMA) (10 ng/mL) and anti-CD3 MoAb ($50 \mu\text{g/mL}$). The supernatants were collected, filtered through a $0.22 \mu\text{m}$ filter and stored at -70°C until use. Detection of IFN- γ , IL-2 and IL-4 was performed by an ELISA system (Amersham International, Little Chalfont, U.K.),

according to the manufacturer's instructions. IFN- γ and IL-2 measurements were used as a selective Th1 marker and IL-4 as a Th2 marker. When all these cytokines were found in the same clones, they were classified as Th0.²¹

Results

Patch testing showed positive reactions only to Compositae and sesquiterpene lactone mix (Table 1), while skin prick tests were negative and levels of serum total and specific IgE within the normal range. Patch tests with ethanolic extracts of Compositae mix components in 20 controls were negative. Phototesting showed an UVA and UVB MED within the normal range (12.5 J/cm^2 for UVA, 105 mJ/cm^2 for UVB).

There was a good *in vitro* T-cell proliferation only to *C. recutita* extract: after a 12-day culture to obtain specific TCLs, a good cell viability was observed in the *C. recutita*-stimulated and in the control PHA-stimulated batches (Fig. 1). The Trypan Blue dye exclusion test showed that in bulk cultures from *A. millefolium*, *T. parthenium* and *T. vulgare* extracts there was a significant loss of viable cells within the first 2 days. The allergen specificity of the recovered TCLs obtained after *C. recutita* stimulation was confirmed by ³H-thymidine incorporation assay, as shown in Fig. 2.

The further expansion of all TCCs obtained from *C. recutita*-specific TCLs in medium conditioned with IL-2 and their staining with anti-CD3, -CD4, -CD8, - $\alpha\beta$ and - $\gamma\delta$ MoAbs showed that most clones had an $\alpha\beta$ +CD4+ phenotype (Table 2) and only one had a CD8+ phenotype.

Stimulation with anti-CD3 MoAb and PMA for the functional study of *in vitro* cytokine production revealed

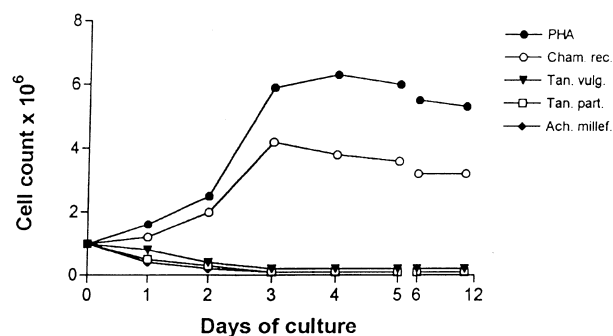


Figure 1. *In vitro* cell viability stimulated by allergens and measured by Trypan Blue dye exclusion test after 12-day culture: only phytohaemagglutinin (PHA)- and *Chamomilla recutita* (Cham. rec.)-stimulated samples yielded appreciable amounts of viable cells. Tan. vulg., *Tanacetum vulgare*; Tan. part., *T. parthenium*; Ach. millef., *Achillea millefolium*.

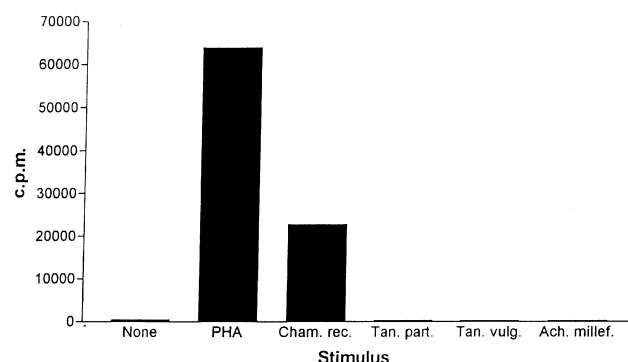


Figure 2. Allergen specificity of cultured cells: after 12-day culture, viable cells were harvested and re-exposed to the allergens (10 µg/mL). Viable cells without antigen and with phytohaemagglutinin (PHA: 2.4 µg/mL) were used as negative and positive controls, respectively. Only PHA and *Chamomilla recutita* (Cham. rec.) induced significant ³H-thymidine incorporation. Tan. part., *Tanacetum parthenium*; Tan. vulg., *T. vulgare*; Ach. millef., *Achillea millefolium*.

that most allergen-specific TCCs (eight of 13) (Table 2) had a Th0 cytokine production pattern. Very few TCCs (two of 13) produced IL-4 alone and, therefore, may be considered as truly Th2 type. Three others showed a clear Th1 cytokine profile, characterized by predominance of IFN-γ production, with no detectable amounts of IL-4 (Table 2).

Discussion

The history, clinical findings and patch test results suggest that the aetiological agent of the occupational airborne contact dermatitis was Compositae family, in particular *C. recutita*. *In vitro* experiments with the patient's circulating mononuclear cells demonstrated the survival only of cultures stimulated by *C. recutita*.

We cannot completely rule out a direct toxic effect of *T. parthenium*, *T. vulgaris* and *A. millefolium* extracts on the patient's bulk cultures. However, only the encounter with specific allergen(s) can directly stimulate responsive cells, whereas cross-reactive or unrelated allergens can induce a tolerogenic and/or unresponsive state leading to a failure in the *in vitro* cell proliferation assay.²²

The present study also suggests a Th0/Th2-type lymphocyte involvement in the pathogenesis of the skin lesions, because allergen-specific TCCs were CD4⁺ and able to produce IFN-γ and IL-2 and also large amounts of IL-4. This finding contrasts with the clinical picture in our patient, which would usually be considered as an expression of a DTH reaction (positive patch test, negative prick test, specific IgE within normal range). Our findings, however, confirm the results of a previous investigation¹⁵ where nickel-reactive TCCs derived from the patch test site in a patient with nickel contact dermatitis were characterized. These clones responded to nickel with the production of high levels of IL-5 and variable amounts of IFN-γ and IL-4, resembling a Th2- or Th0-like cytokine secretion pattern. None of the nickel-reactive TCCs showed a clear Th1 profile. Therefore, it is possible that in addition to IFN-γ, Th2 cytokines (IL-4 and IL-5) are involved in the immunopathogenesis of allergic contact dermatitis. If other studies on cytokine profiles of TCCs from the peripheral blood and skin lesions confirm this hypothesis, the currently perceived pathogenetic differences between allergic contact dermatitis and atopic dermatitis will become less definite. Some experimental evidence on atopic dermatitis suggests good IL-4 production and a high rate of IFN-γ secretion, which may downregulate

Clones	Phenotypes	IL-2 (U/mL)	IL-4 (pg/mL)	IFN-γ (pg/mL)	Th type
1	CD4	2.6	1540	5	Th0
2	CD4	0.8	20,010	0	Th2
3	CD4	5.6	0	5	Th1
4	CD4	1.5	1360	13	Th0
5	CD4	0.8	440	629	Th0
6	CD4	6.9	0	102	Th1
7	CD8	1.5	3690	10	Th0
8	CD4	2.1	4590	5	Th0
9	CD4	1.2	7220	5	Th0
10	CD4	0.9	14,980	25	Th0
11	CD4	0.01	14,960	0	Th2
12	CD4	0.8	910	154	Th0
13	CD4	5.3	0	16	Th1

Table 2. Phenotypes and cytokine production by *C. recutita*-specific T-cell clones

IL, interleukin; IFN, interferon.

IgE production and annual reactions to prick tests.²³ Recently, a role for both Th2 cytokines IL4 and IL5 and the Th1 cytokine IFN- γ in a murine model of allergic dermatitis has been demonstrated.²⁴ Therefore, sequential activation of Th2 and Th1 type cells in atopic dermatitis has been proposed to explain the persistence of skin lesions.²⁵ In conclusion, our case report seems to demonstrate that in airborne contact dermatitis also, a clear dichotomy between Th1 and Th2 cells, responsible for separate immune-mediated diseases, can no longer be accepted. This hypothesis, however, should be confirmed in a larger study group.

References

- 1 Lovell CR. Compositae (Asteraceae). In: *Plants and the Skin* (Lovell CR, ed). Oxford: Blackwell Scientific Publications, 1993; 147–81.
- 2 Hannah L. Ragweed dermatitis: a new treatment, with preliminary report of a case. *JAMA* 1919; **72**: 853–4.
- 3 Sutton RL. Ragweed dermatitis. *JAMA* 1919; **73**: 1433–5.
- 4 Warshaw EM, Zug KA. Sesquiterpene lactone allergy. *Am J Contact Dermatitis* 1996; **7**: 1–23.
- 5 Paulsen E. Compositae dermatitis: a survey. *Contact Dermatitis* 1992; **26**: 76–86.
- 6 Benezra C, Ducombs G, Sell Y, Foussereau JL. *Plant contact Dermatitis*. New York: BC Decker Inc., 1985; 105–14.
- 7 Paulsen E, Andersen KE. Compositae dermatitis in a Danish dermatology department in 1 year. (II). Clinical features in patients with Compositae contact allergy. *Contact Dermatitis* 1993; **29**: 195–201.
- 8 Stingeni L, Lisi P. Dermatite allergica da contatto aerotrasportata da Compositae. *Ann Ital Dermatol Clin Sper* 1996; **50**: 170–3.
- 9 Wrangsjö K, Ros AM, Wahalberg JE. Contact allergy to Compositae plants in patients with summer-exacerbated dermatitis. *Contact Dermatitis* 1990; **22**: 148–54.
- 10 Paulsen E, Andersen KE, Hausen BM. Compositae dermatitis in a Danish dermatology department in one year. (I). Results of routine patch testing with the sesquiterpene lactone mix supplemented with aimed patch testing with extracts and sesquiterpene lactones of Compositae plants. *Contact Dermatitis* 1993; **29**: 6–10.
- 11 Hausen BM. A 6-year experience with Compositae mix. *Am J Contact Dermatitis* 1996; **7**: 94–9.
- 12 Stingeni L, Lisi P. Airborne contact dermatitis from Compositae: clinical, epidemiological and diagnostic evaluation. 3^a Reunião latina de Dermatites de contacto. Coimbra, 24 e 25 de Outubro de 1997.
- 13 Sinigaglia F, Scheidegger D, Garotta G *et al.* Isolation and characterization of Ni-specific T cell clones from patients with Ni-contact dermatitis. *J Immunol* 1985; **135**: 3929–32.
- 14 Kapsenberg ML, Wieranga EA, Stiekema FEM *et al.* Th1 lymphokine production profiles of nickel-specific CD4⁺ T-lymphocyte clones from nickel contact allergic and non-allergic individuals. *J Invest Dermatol* 1992; **98**: 59–63.
- 15 Probst P, Küntzlin D, Fleischer B. Th2-type infiltrating cells in nickel-induced contact dermatitis. *Cell Immunol* 1995; **165**: 134–40.
- 16 Bleumink E, Mitchell JC, Nater JP. Contact dermatitis to chrysanthemums. *Arch Dermatol* 1973; **108**: 220–2.
- 17 Campolmi P, Lotti T, Francalanci S, Sertoli A. Contact sensitivity to oak. *Contact Dermatitis* 1986; **14**: 314.
- 18 European Pharmacopoeia. *Allergenic products—European Pharmacopoeia*. 3rd edn. Strasburg, 1997.
- 19 Bradford MM. A rapid and sensitive method for quantitation of micrograms quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248–54.
- 20 Agea E, Bistoni O, Bini P *et al.* Co-stimulation of CD3/TcR complex with either integrin and non-integrin ligands protects CD4⁺ allergen-specific T cell clones from programmed cell death. *Allergy* 1995; **50**: 677–82.
- 21 Romagnani S. Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994; **12**: 227–54.
- 22 Newell MK, Haughn LJ, Maroun CR *et al.* Death of mature T cells by separate ligation of CD4 and the T cell receptor for antigen. *Nature* 1990; **347**: 286–9.
- 23 Kimura M, Tsuruta S, Yoshida T. Unique profile of IL-4 and IFN- γ production by peripheral blood mononuclear cells in infants with atopic dermatitis. *J Allergy Clin Immunol* 1998; **102**: 238–44.
- 24 Spergel JM, Mizoguchi E, Oettgen H, Bhan AK. Roles of Th1 and Th2 cytokines in a murine model of allergic-dermatitis. *J Clin Invest* 1999; **103**: 1102–11.
- 25 Grewe M, Bruijnzeel-Koomen CAFM, Schöpf E *et al.* A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. *Immunol Today* 1998; **19**: 359–61.