IMMUNOMODULATING TREATMENT WITH LOW DOSE INTERLEUKIN-4, INTERLEUKIN-10 AND INTERLEUKIN-11 IN PSORIASIS VULGARIS

M.L. ROBERTI[1], L. RICOTTINI[2], A. CAPPONI[3], E. SCLAUZERO[4], P. VICENTI[5], E. FIORENTINI[6], C. SAVOIA[7], G. SCORNAVACCA[8], D. BRAZIOLI[9], L. GAIO[10], R. GIANNETTI[11], C. IGNAZZI[12], G. MELONI[13] and L.M. CHINNI[14]

[1]Private Practice, Rome, Italy; [2]Centro medico polispecialistico "Sinergheia", Rome, Italy; [3]Private Practice, Latina, Italy; [4]OSTEMDA, Organizzazione Strategie Terapeutiche Empowerment e Metodologie Diagnostiche Avanzate, Udine, Italy; [5]Private Practice, Altamura, Bari, Italy; [6]Ambulatorio di dermatologia, Aversa, Caserta, Italy; [7]Private Practice, Fino Mornasco, Como, Italy; [8]Private Practice, Catania, Italy; [9]Private Practice, Torino, Italy; [10]Private Practice, Caserta, Italy; [11]Poliambulatorio di medicina convenzionata"Aurelia", Rome, Italy; [12]Azienda Sanitaria Locale, Putignano, Bari, Italy; [13]Poliambulatorio "GEA Medica", Montebelluna, Treviso, Italy; [14]Istituto Dermopatico dell'Immacolata, Roma, Italy

Psoriasis is a chronic inflammatory skin disease affecting approximately 2-3% of the world population; it is characterised by hyperproliferation and hyperplasia of the superficial layers of the epidermis. Inappropriate signals released by the immune system determine an altered keratinocyte differentiation, resulting in the formation of desquamating, thickened, inflamed and erythematous plaques. The aim of this investigation was to study the pharmacological activity and safety of three low dose cytokines, Guna-Interleukin 4, Guna-Interleukin 10 and Guna-Interleukin 11 at the concentration of 10 fg/ml in patients affected by moderate to slight psoriasis vulgaris. The multicenter, double-blind, randomized, placebo-controlled clinical trial involved 48 patients who were enrolled and followed up according to a 8-month experimental project. All patients received, according to a cross-over model, either the experimental treatment or placebo, alternatively. Globally, in the 41 evaluated patients it was observed a PASI significant reduction (Friedman test: p=0.00960). The DLQI too decreased significantly in all subjects compared to baseline (Friedman test: p=0.00007). The safety of the treatment with three low dose cytokines administered simultaneously was proved; no adverse event was reported during the whole trial.

Psoriasis is a common chronic inflammatory skin disease, most often characterized by thickened erythematous scaly plaques, and appears in a variety of forms with distinct characteristics. The most common form, psoriasis vulgaris, affects 1-3% of the Caucasian population, usually persists, with 40% developing seronegative arthritis, and has a very negative impact on quality of life (1).

The existence of familial cases of psoriasis and the occurrence of the pathology in monozygotic twins demonstrates the role of genetic factors. The mode of inheritance of psoriasis is complex. In the multifactorial hypotesis environmental triggering factors (traumas, infections, stress, climate) interplays with genetic factors (2).

The pathogenesis of disease is still not clear, therefore psoriasis is generally regarded as a T-cell mediated autoimmune disease. Although psoriasis

Key words: Psoriasis vulgaris, interleukin-4, interleukin-10, interleukin-11, PASI score, DLQI

Mailing address:
Maria Luisa Roberti MD,
Private Practitioner,
Piazza di Villa Carpegna, 42/B,
00165 Roma, Italy
Tel.: +39 06 6634750
e-mail: mlroberti@gmail.com

0393-974X (2014)

was initially classified as a Th-1-polarized disease, a clear role for CD4+ T cells that produce IL-2, IL-6, IL-17 and IL-22 has been demonstrated. The cytokines show a key role in the development and maintenance of psoriasic lesions. The hypothesis of a cytokine network in psoriasis proposes a central role of pro-inflammatory cytokines, including TNF-α. In this disease, three predominant cytokines seem to be at play: type I interferons, IFN-γ and TNF-α. In psoriasis, IFN-γ, the prototype Th-1 cytokine, interplays with TNF-α, IL-17, IL-22, and IL-6 to inflammatory process (3, 4). Therapies targeting T cells seem to be very effective in the treatment of skin lesions and arthritis involvement. Strategies for intervention into the cytokine network have included antagonism of pro-inflammatory cytokines (e.g. TNF-α, IL-1, IL-6, IL-8, IL-12, IL-18, IL-23) with neutralizing antibodies and soluble receptors; application of recombinant cytokines (e.g. IL-4, IL-10, IL-11, IFN-γ) to shift the cytokine balance; and administration of small molecules to modulate cytokine expression or signaling (5). The treatment with IL-4, IL-10 and IL-11 down-regulates type I cytokine pro-inflammatory pathways in psoriasis lesions. IL-11 therapy has demonstrated immunomodulatory activity to regulate both T cell and macrophage function (6).

Aims of this study were to assess the safety and efficacy of oral low-dose cytokines (IL-4, IL-10 e IL-11) in the treatment of psoriasis and to evaluate the effects of this therapy in the quality of life.

MATERIALS AND METHODS

Subjects

Forty-eight patients with moderate to slight psoriasis, according to the psoriasis area and severity index (PASI), were enrolled in this study, 41 (17 females and 24 males; mean age 46.2± std.dev. 11.7 years) were evaluated. Table I shows the patients' epidemiological and clinical features.

Patients with guttate psoriasis, palmoplantar, pustular and erythrodermic psoriasis and other skin diseases were excluded.

Patients who underwent topic or systemic therapies specific for psoriasis, respectively in 15 and 90 days prior the enrolment.

Other exclusion criteria: subjects under 18 years of age and subjects in therapy for other pathologies with steroid (topical or systemic), anti-TNF-α therapies, immunosuppressive agents (e.g., cyclosporine, tacrolimus or methotrexate).

Protocol inclusion criteria concerned also patients in more serious conditions, in order to assess whether patients not responding to phototherapy treatment could benefit from the studied therapy.

Study design

This is a multicenter, double-blind, randomized, placebo-controlled clinical trial, alternated according to a cross-over model, lasting eight months overall. Since the beginning of the trial, for a period of 3 months, all individuals enrolled in the study were treated with low dose cytokines or with placebo solution. After a wash-out period of 60 days, the administration of the experimental product was resumed for a further three-month period, thus implementing the cross-over experimental model. After signing an informed consent the patients were enrolled progressively in the different experimental sites and identified with their initials as well as through a serial number.

A randomization procedure performed using a tailored software has generated two equal parts, one containing cytokines and one containing placebo. These preparations were marked with a serial number and an identifying code of the three products. Each preparation was allocated to the related subject. The chief of the Guna Labs (GUNA Spa, Milan - Italy) was the only one aware of the product content. A yellow label identified the products prepared for the first period of treatment; a green label identified the products prepared for the second period of treatment.

One group was treated with Guna-Interleukin 4, Guna-Interleukin 10, Guna-Interleukin 11 at the concentration of 10 fg/ml in a hydroalcoholic solution 30% for oral use (GUNA Spa, Milan, Italy) administered at a dose of 20 drops twice daily, taken on an empty stomach from 8.00 to 10.00 and from 19.00 to 21.00. The other group was treated with placebo (hydroalcoholic solution at 30% containing no active ingredients) with the same mode of administration. Neither the doctor nor the patient were aware of the nature of the preparation assigned.

At the end of the first 3 months of treatment, patients underwent a new clinical control and after a wash-out period of 60 days, the 2 experimental groups were inverted (cross-over model). This in order to reduce the possible error factors in the evaluation, as well as to give the possibility to both groups to benefit from the therapy (Fig. 1).

Since our primary goal was to assess the activity of the three interleukins, the sample size was calculated considering the possible reduction of PASI score within treated patients by means of a crossover design. On the other hand, from our previous experiences, we suspected a possible long lasting carry-over effect exerted by the

interleukins (so interfering with the crossover results), and therefore we chose a sample size as we were measuring the PASI score in a "before-after" design. Assuming to recruit 20 subjects, in which the average reduction of PASI score at end of protocol should have been 1.5, with a standard deviation of reduction equal to 2, a sample of 20 patients would have reached a 87% power (with an error α=0.05) to detect the expected difference (before-after) within the group starting with interleukins. Then, the same sample size was chosen also for the other group, bearing in mind that our focus would have been on group firstly treated with interleukins.

Clinical assessment

Disease severity was assessed using the psoriasis area and severity index (PASI). The PASI is a dermatological index conventionally used, through which are evaluated extension, erythema, infiltration and desquamation in the areas of the head, arms, trunk and legs. The degree of severity of psoriatic manifestations provides a score ranging from 0 (no psoriasis) to 72 (maximum value, severe psoriasis). Higher scores represent greater degrees of disease severity (7). Severe psoriasis is defined as a PASI score >12 (8).

For the evaluation of individual distress it was used a Dermatology Life Quality Index (DLQI). This questionnaire fulfils reliability, validity, repeatability, and internal consistency requirements. It is meant for adults older than 16 years. It is self-administered and consists of 10 questions and measures 6 categories: symptoms and feelings (questions 1 and 2), daily activities (questions 3 and 4), leisure (questions 5 and 6), work and school (question 7), personal relationships (questions 8 and 9), and treatment (question 10). All questions relate "to the last week." Each question is answered using a tick box, with not at all, a little, a lot or very much, being scored from 0 to 3. The DLQI is calculated by summing the scores of each question, resulting in a minimum score of 0 (no impairment) to a maximum score of 30 (maximum impairment) in order to measure Heath Related Quality of Life (HRQoL) in patients. Higher scores represent greater degrees of HRQoL impairment (14). Severe impact on HRQoL by psoriasis is defined as a DLQI >10 (9).

Statistical analysis

All data were analyzed with usual descriptive statistics. D'Agostino-Pearson test, together with visual inspection of the histogram, were used to check the variable distributions: mean and standard deviation were used for the normally distributed variables, while median and IQR (InterQuartile Range) were used for non-normal ones. The Friedman test was used to analyze the within-group repeated measure variation of both PASI and DLQI scores; the post-hoc test according to Conover was used to check the pairwise comparisons. The Mann-Whitney U test was used to evaluate the difference of the scores between groups at baseline. Statistical significance was assumed with $p<0.05$.

RESULTS

Forty-eight patients were enrolled in the study in a period of 12 months; of these, 41 patients completed the entire experimental design and could be evaluated (Fig. 2). 34 subjects had a PASI score at enrollment <10; 7 had a PASI score at enrollment <30. All the recruited patients were divided in two groups, group A and group B. Group A, consisting of 23 individuals, performed the experimental design taking at time T0-T3 active preparation and at time T3-T6 the placebo. Group B, consisting of 18 subjects, conversely began with placebo in the first time and then took the cytokines in the second time (Fig. 1).

The global PASI score for the 41 patients at baseline (T0) ranged from a minimum of 1 to a maximum of 21 (median=5). Considering the 2 groups separately, the PASI of group A (n=23) is 5 (1-18, IQR 2-8). In group B (n=18) the PASI at baseline (T0) is 5 ranged (1-21, IQR 2-8). No significant differences are observed at baseline between two groups (Mann-Whitney U test: $p>0.9999$).

Globally, in the 41 evaluated patients, who completed the study, it is observed a PASI significant reduction (Friedman test: $p=0.0096$). According to the Conover's post-hoc test, significant difference ($p<0.05$) is evident between values at time T0 and at time T3 and between values at time T0 and at time T6, but not between T3 and T6 time values (Table II).

Group A (n=23), treated during the first period, shows a PASI significant reduction (Friedman test: $p=0.0275$) (Table III). A significant difference according to Conover ($p<0.05$) was found only between values at T0 and at T6, whereas variations at T3 are not significant.

In group B (n=18) no significant differences between values at T0 and at T3 and between values at T0 and at T6 are observed (Friedman test: $p=0.23348$) (Table III).

The DLQI for all patients (n=41) at baseline is 5 (0-23, IQR 2-10) (Table II). In group A, the DLQI

Table I. *Epidemiological and clinical features of patients with psoriasis*

Sex (female/male)	17/24
Age, years	46.2 ±11.7
Disease duration <5 years	9
Disease duration 5>years<15	13
Disease duration >15 years	18
Smokers, n (%)	8 (19.5%)
Patients with intermittent psoriasis, n (%)	21 (51%)

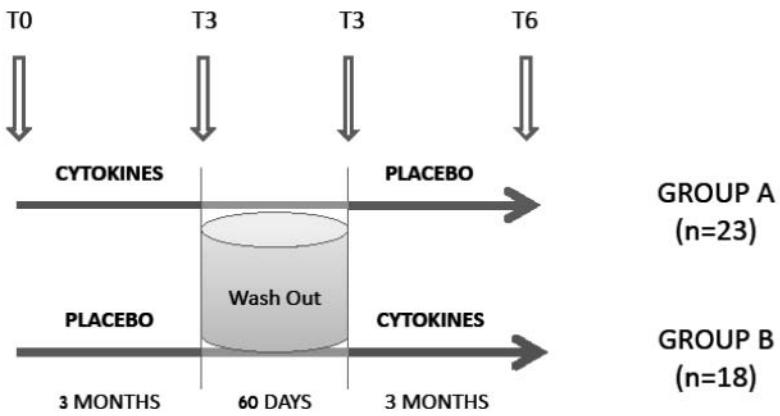

Fig. 1. *Study design*

is 4 (0-14, IQR 1.25-10) (Table III). In group B, the DLQI is 6 (0-23, IQR 3-10) (Table IV). The DLQI at baseline did not show any difference between the 2 groups (Mann-Whitney U test: p=0.2688).

The DLQI decreased significantly in all subjects (n=41) compared to baseline (p<0.0001). A significant difference according to Conover (p<0.05) was observed between values at T0 and at T3 and between values at T0 and at T6, but not between T3 and T6 (Table II).

In group A (n=23) we observed a DLQI significant reduction (p=0.0134) between values at T0 and at T6. A significant pairwise difference (p<0.05) was found between baseline and values at 6 months, but

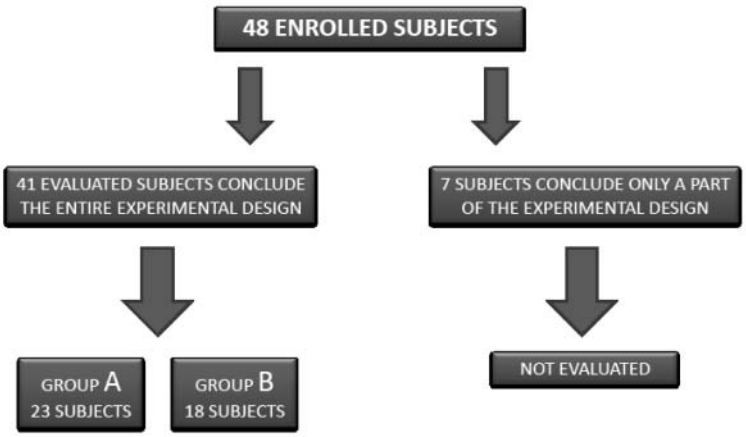

Fig. 2. *Flow chart of enrolled patients.*

Table II. *The PASI and DLQI in all subjects. Data are expressed in median with range (minimum and maximum).*

N=41	Basal	3 months	6 months	p
PASI	5 (1-21)	3 (0-11)	3 (0-12)	p<0.05 (bas. vs 3/ 6 m.)
DLQI	5 (0-23)	3 (0-14)	3 (0-18)	p<0.05 (bas. vs 3/ 6 m.)

Table III. *The PASI and DLQI in group A subjects. Data are expressed in median with range (minimum and maximum).*

N=23	Basal	3 months	6 months	p
PASI	5 (1-18)	4(1-11)	3 (0-12)	p<0.05 (bas. vs 6 m.)
DLQI	4 (0-14)	4 (0-14)	3 (0-17)	p<0.05 (bas. vs 6 m.)

Table IV. *The PASI and DLQI in group B subjects. Data are expressed in median with range (minimum and maximum).*

N=18	Basal	3 months	6 months	p
PASI	5 (1-21)	2 (0-11)	3.5 (0-7)	p>0.05 (bas. vs 3/ 6 m.)
DLQI	6 (0-10)	3 (0-9)	2.5 (0-18)	p<0.05 (bas. vs 3/ 6 m.)

not between baseline and values at 3 months, nor between 3 and 6 months (Table III).

In group B (n=18) the variation is still significant (p=0.0005) and the significant difference is kept between baseline and 3 months and between baseline and 6 months (Table IV).

DISCUSSION

In this study we demonstrated that therapy with low dose of cytokines (IL-4, IL-10 and IL-11) was safe and reduced severity of psoriasis vulgaris. In addition, this therapy improved the quality of life.

No adverse event was reported during the study in patients with psoriasis vulgaris. At our knowledge this is the first study who demonstrated the safety of low dose cytokine.

In this study the low dose cytokine ameliorate the PASI score significantly (p=0.00960) between baseline and 3 months and between baseline and 6 months. These results confirmed the hypothesis that anti-inflammatory cytokines (IL-4, IL-10, IL-11)

showed a key role in the pathogenesis of psoriasis. A network of pro-inflammatory cytokines is a central feature in the pathophysiology of cutaneous inflammatory diseases. While anti-TNF therapeutics have proven to be effective for the treatment of psoriasis, clinical investigations have now begun with other cytokine-directed therapies, such as those targeting IFN-γ, IL-12, and IL-18.

Since the IL-4 and IL-10 have reduced psoriatic plaques, we can suppose that the administration of these low dose cytokines restores the normal balance of cytokine network (10).

Ghoreschi et al. demonstrated that in patients with psoriasis IL-4 therapy was well tolerated and within six weeks all patients showed decreased clinical scores. IL-4 therapy can induce Th2 differentiation in human CD4+ T cells and is promising as a potential treatment for psoriasis (11).

IL-10 is an important immunoregulatory cytokine produced by many cell populations. Its main biological function seem to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, natural killers, antigen-presenting cells, mast cells, and granulocytes. Recombinant human IL-10 has been produced and is currently being tested in clinical trials. This includes rheumatoid arthritis, inflammatory bowel disease, psoriasis, organ transplantation, and chronic hepatitis C (12, 13). A study involving 28 patients with moderate-to severe psoriasis showed that treatment with rhIL-10 resulted in only temporary clinical improvement in psoriasis, despite sustained systemic decreases in pro-inflammatory and type 1 cytokine production (14).

IL-11, a multifunctional cytokine, modulates macrophage and type 1 T-lymphocyte function in cell culture and shows anti-inflammatory activity in animal models. Trepicchio et al. evaluated the effect of subcutaneous delivery of rhIL-11 in patients with psoriasis. Seven of 12 patients responded well to rhIL-11 treatment. Amelioration of disease by rhIL-11, as shown by reduced keratinocyte proliferation and cutaneous inflammation, was associated with decreased expression of products of disease-related genes, including K16, iNOS, IFN-γ, IL-8, IL-12, TNF-α, IL-1β, and CD8+, and with increased expression of endogenous IL-11 (6). The ability of rhIL-11 to modulate cytokine production from activated CD4+ T cells provides a mechanism through which rhIL-11 may ameliorate such inflammatory diseases as psoriasis (15). Conversely, in comparison with normal controls, bone marrow stromal cells from patients with psoriasis showed no alteration in the levels of GM-CSF, IL-11, IL-7 (16).

Our data confirm that most patients with psoriasis have poor quality of life prior to treatment, and that improvement in psoriasis lesion and symptoms (e.g. pruritus, restlessness) with treatment significantly correlates with improvement in DLQI.

We can conclude that low dose cytokines are safe and effective in the treatment of psoriasis vulgaris. Considering the initial suspect of carry over effect, the results of this exploratory study seemed to highlight a long time action of interleukins. The latter is shown also after stopping the treatment. Further trials with a longer observational period are necessary to confirm our preliminary data.

AKNOWLEDGEMENTS

We wish to thank M.Assirati, B.Fiorentino, A.Crippa, A Nazari, B.Arsuffi, G.Tomasini, M.Passafaro, S.Cangemi, R.Malvicini, M.Siffredi, C.Gremita, F.Mazzola, L.Morlando, L.Cendret for their valuable clinical contribution to the achievement of this work. We wish to thank V.Miranda (Clinical Research Unit Guna S.p.a Milan-Italy) for his helpful scientific discussion and critical revision of the manuscript. We also thank M.Nichelatti and C.Mattana for the bioinformatic analysis, M.Campanella (Clinical Research Unit Guna S.p.a Milan-Italy) for her scientific and technical support in data management, F.Melzi (Guna S.p.a) for her linguistic assistance.

REFERENCES

1. Kurd SK, Gelfand JM. The prevalence of previously diagnosed and undiagnosed psoriasis in US adults: results from NHANES 2003-2004. J Am Acad Dermatol 2009; 60:218-24.
2. Krueger GG, Duvic M. Epidemiology of psoriasis: clinical issues. J. Invest Dermatol 1994; 102:14-18S.
3. Gudjonsson JE, Johnston A, Sigmundsdottir H et al.

Immunopathogenic mechanisms in psoriasis. Clin Exp Immunol 2004; 135:1-8.
4. Nestle FO, Kaplan DH, Barker J. Psoriasis. N Engl J Med 2009; 30(361):496-509.
5. Numerof RP, Asadullah K. Cytokine and anti-cytokine therapies for psoriasis and atopic dermatitis. BioDrugs 2006; 20:93-103.
6. Trepicchio WL, Ozawa M, Walters IB. Interleukin-11 therapy selectively downregulates type I cytokine proinflammatory pathways in psoriasis lesions. J Clin Invest 1999; 104:1527-37.
7. Fredriksson T, Petersson U. Severe psoriasis oral therapy with a new retinoid. Dermatologica 1978; 157: 238-44.
8. Schmitt J, Wozel G. The Psoriasis Area and Severity Index Is the Adequate Criterion to Define Severity in Chronic Plaque-Type Psoriasis. Dermatology 2005; 210:194-9
9. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)-a simple practical measure for routine clinical use. Clin Exp Dermatol 1994; 19:210-6.
10. Numerof RP, Dinarello CA, Asadullah K. Cytokines as potential therapeutic targets for inflammatory skin diseases. Eur Cytokine Netw 2005; 16:101-3.
11. Ghoreschi K, Thomas P, Breit S, Dugas M, Mailhammer R, van Eden W, et al. Interleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. Nat Med 2003; 9:40.
12. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy – review of a new approach. Pharmacol Rev 2003; 55:241.
13. Asadullah K, Sabat R, Friedrich M, Volk HD, Sterry W. Interleukin-10: an important immunoregulatory cytokine with major impact on psoriasis. Curr Drug Targets Inflamm Allergy 2004; 3:185-92.
14. Kimball AB, Kawamura T, Tejura K, et al. Clinical and immunologic assessment of patients with psoriasis in a randomized, double-blind, placebo-controlled trial using recombinant human interleukin 10. Arch Dermatol 2002; 138:41.
15. Bozza M, Bliss JL, Dorner AJ, Trepicchio WL. Interleukin-11 modulates Th1/Th2 cytokine production from activated CD4+ T cells. J Interferon Cytokine Res 2001; 21:21-30.
16. Zhang K, Liu R, Yin G, Li X, Li J, Zhang J. Differential cytokine secretion of cultured bone marrow stromal cells from patients with psoriasis and healthy volunteers. Eur J Dermatol 2010; 20:49-53.