Acta Derm Venereol 2002; 82: 245–248

Taylor & Francis
healthsciences

INVESTIGATIVE REPORT

Soluble CD30 Plasma Concentrations Correlate with Disease Activity in Patients with Atopic Dermatitis

REGINA FÖLSTER-HOLST[1], TILO HENSELER[1], JÖRG WEHDE[2], HILMAR LEMKE[2], MICHAEL WEICHENTHAL[1], ENNO CHRISTOPHERS[1] and HINRICH P. HANSEN[2]

Departments of [1]Dermatology and [2]Biochemistry, University of Kiel, Germany

The levels of soluble CD30 in 79 patients with atopic dermatitis were compared with those found in 54 patients with psoriasis and 36 control individuals (no psoriasis, no atopic dermatitis). In relation to the control group, patients with atopic dermatitis were found to exhibit an increased concentration of sCD30 of at least 1.5-fold ($p < 0.001$). In addition, sCD30 concentrations were shown to correlate with the severity of the disease as measured by the score index for atopic dermatitis and different stages of disease activity, such as acute, subacute, or chronic forms, and localized or generalized distribution of atopic dermatitis. The application of topical glucocorticoid therapy for a period of 2 weeks resulted in a decrease in the level of sCD30 by 46% in 8 patients, especially in the acute, generalized form of atopic dermatitis. Psoriasis patients showed no significant differences in sCD30 levels in relation to the control group. This study demonstrates a correlation between sCD30 concentration and the activity of the disease and therefore suggests sCD30 as a prognostic marker, being superior to predictions from measurements of IgE or eosinophil cationic protein. ***Key words: atopic eczema; CD30; Th2 phenotype; SCORAD.***

(Accepted April 8, 2002.)

Acta Derm Venereol 2002; 82: 245–248.

Regina Fölster-Holst, Department of Dermatology, Christian Albrechts University of Kiel, Schittenhelmstr. 7, DE-24105 Kiel, Germany. E-mail: rfoelsterholst@dermatology.uni-kiel.de

Atopic dermatitis (AD) is a chronic inflammatory skin disease commonly associated with early infancy. The disease is characterized by at least 4 features: pruritus, typical morphology and distribution, a tendency towards chronicity and a positive personal and/or positive family history for atopic diseases (1).

Both AD and psoriasis exhibit inflammatory skin lesions. However, it has been shown that different Th-cell subsets are involved in the pathogenesis of the two diseases. The cytokine profile in patients with AD is characterized by Th2 cells yielding the production of IL4, IL5 and IL10 (2, 3). In contrast, patients with psoriasis typically demonstrate a predominance of IFNγ, IL2 and IL12 due to Th1 overrepresentation (4).

Elevated plasma levels of soluble CD30 (sCD30) have been found in patients with AD but not in those with psoriasis (5). The 120 kD protein CD30 (Ki-1) is a member of the tumour necrosis factor receptor family and is mainly expressed by Th0 and Th2 T-helper cell clones (6). Specific activation of CD30 positive cells leads to stimulation of endoproteolytic cleavage of membrane-anchored CD30 and thus enhanced release of the soluble ectodomain of CD30 (7–9).

The score index for atopic dermatitis (SCORAD) (10) is frequently used as an outcome measure in studies of AD, but it is prone to observer bias and does not sufficiently take into account the activity of the disease. Since the acute and chronic stages may well reflect different aspects of the inflammatory process of AD, we sought to investigate whether sCD30 might be a useful marker in these different stages.

MATERIAL AND METHODS

Patients

A total of 169 subjects were investigated. The 79 patients with AD had a median age of 30 years and a female:male ratio of 56:23 (71% vs 29%) (Table I).

The diagnosis of AD was established according to the criteria of Hanifin & Rajka (1). The severity of atopic dermatitis in patients was determined by SCORAD (10). This

Table I. *Characteristics of patients with atopic dermatitis (AD), psoriasis and healthy controls. Median values are shown with 95% confidence intervals in parentheses*

	AD	Psoriasis	Controls
Patients (*n*)	79	54	36
M/F	23/56	23/31	14/22
Age (years)	30 (23, 37)	49 (33, 60)	31 (24, 44)
IgE (kU/l)	1,400*** (247, 3908)	47.5 (23.5, 142)	23.5 (9.5, 50.5)
ECP (μg/l)	26*** (17, 51)	12 (9, 19.5)	12 (6, 16.5)
EOS (%)	6.0*** (4, 8)	1.0 (0, 3)	1.0 (0, 2)
sCD30 (U/ml)	9.0*** (7, 17)	6.0 (5, 9)	6.0 (4, 8)

ECP: eosinophil cationic protein; EOS: eosinophil granulocytes.
***$p < 0.001$ versus psoriasis or controls.

severity grading takes into account the extent applying the rule of nines and the severity of erythema, oedema/papulation, oozing/crust, excoriation, lichenification and dryness. The score also includes evaluation of pruritus and sleep loss.

In accordance with the definitions proposed by Hamid et al. (11), our patients were characterized as belonging to a subgroup defined as acute phase (in which the main criterion is erythema lasting for less than 3 days) or chronic phase (in which the main criterion is scaling and lichenification lasting for more than 2 weeks). In addition, we have defined a third group of patients, the subacute phase, characterized mainly by mild erythema and pruritic papules which persist for more than 3 days. In addition to stage of AD, we considered the extent of the lesions. Patients with generalized and localized forms were included. In the latter group, skin lesions covered only small areas of the body, e.g. the antecubital or popliteal areas, the face, or the palms.

For comparison, 54 patients with psoriasis and 36 control subjects were included in this study. The median age was 49 years in the psoriasis patients and 31 years in the control group (Table I).

Psoriasis patients had a median age of onset of 24 years, and usually presented with type I psoriasis (12).

sCD30, and relative counts of peripheral blood eosinophils were measured in all subjects. Total serum IgE and eosinophil cationic protein (ECP) were determined in all of the patients with AD and the controls. In the psoriasis patients these parameters were investigated only in a subgroup of 12 subjects, owing to technical reasons.

Furthermore, 15 patients with AD participated in a longitudinal analysis to determine sCD30 during therapy.

All patients gave informed consent to participation in this study.

Immunological analyses

The concentrations of IgE and ECP were measured by CAP-FEIA and RIA commercial kits, respectively, according to the manufacturer's instructions (Pharmacia, Freiburg, Germany). All assay measurements were performed in duplicate.

Plasma levels of sCD30 were determined by RIA as described elsewhere (7). The following anti-CD30 mAbs were used: Ki-2(α1, κ, serological cluster A, capture antibody), and Ki-3 (α2b, κ, serological cluster C). Ki-3 was radio-labelled with [125] sodium iodide (Amersham Bioscience, Freiburg, Germany) and served as detection antibody (13). In brief, Ki-2 mAb coated microtitre plates were used to extract sCD30 from 100 µl samples. The plates were calibrated according to the international standard. A serial dilution of an extract of 10^6 HUT 102 cells equivalent to 1000 U per ml was used. After incubation for 1 h, the plates were washed and the bound sCD30 was specifically labelled with Ki-3 mAb. Radioactivity of the wells was determined using a γ-counter.

Statistics

Owing to non-normal data distribution non-parametric statistical analyses were performed. The medians and 95% confidence intervals (CI) are presented. Comparisons between groups of patients were made using the Mann–Whitney U-test. Dissimilarities in sCD30 level before/after treatment were proved by Wilcoxon's matched pair test. Correlations were tested by Spearman's rank test.

RESULTS

Patients with AD demonstrated a median SCORAD of 30 (95% CI: 26–39). In 24% of patients the severe form of the disease was found (SCORAD > 50), in 47% the moderate form, and in 29% the mild form of the disease (SCORAD < 20). In psoriasis patients a median of 30% of body surface was affected, all patients manifesting a plaque-type psoriasis.

Patients with AD demonstrated significantly increased levels of IgE, ECP, and elevated eosinophils compared with the levels in patients with psoriasis and in controls ($p < 0.001$).

Overall, patients with AD presented with an increased median sCD30 level of 9.0 U/ml as compared to patients with psoriasis and controls (6.0 for both; $p < 0.001$) (Table I).

In patients with AD a clear correlation between SCORAD and sCD30 level was detected ($r = 0.447$, $p < 0.00001$). Furthermore, SCORAD demonstrated a significant and positive correlation with IgE ($r = 0.541$, $p < 0.0001$) and relative counts of eosinophils in peripheral blood ($r = 0.345$, $p < 0.05$). In these patients sCD30 was also significantly correlated with serum IgE levels ($r = 0.31$, $p < 0.01$).

Patients with AD were clustered according to the stage of disease activity, distinguishing between acute ($n = 9$), subacute ($n = 25$), and chronic lesions ($n = 45$). In addition, we made a further classification according to whether skin eruptions were localized ($n = 56$) or generalized ($n = 23$).

In patients with the generalized form of AD, markedly elevated sCD30 levels (32 U/ml) were found in the acute stage of the disease ($p < 0.05$) as compared to the subacute (12 U/ml) and chronic stages (14 U/ml). In contrast, the localized form of the acute AD showed no comparable elevation of sCD30 (Fig. 1).

Fifteen patients were monitored during therapy. The median of the SCORAD changed from 78 to 34 as a result of a treatment with topical glucocorticosteroids over 2 weeks in 8 patients. As shown in Fig. 2, sCD30

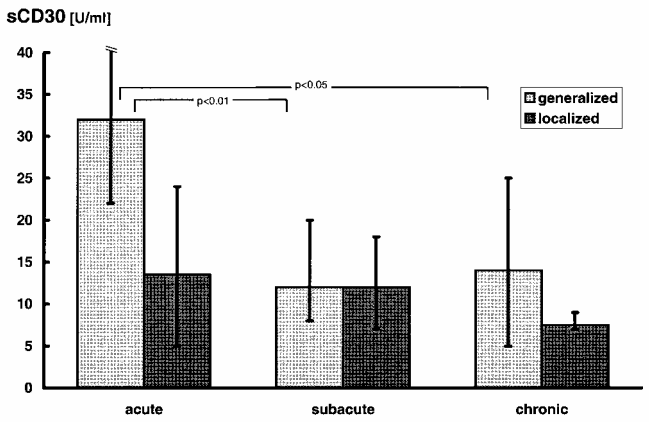

Fig. 1. Soluble CD30 (sCD30) plasma levels in different stages of disease activity of atopic dermatitis (AD). Median values in acute ($n = 9$), subacute ($n = 25$) and chronic (n = 45) forms of the disease are compared using U-test statistics. Error bars denote the 95% confidence intervals of the median.

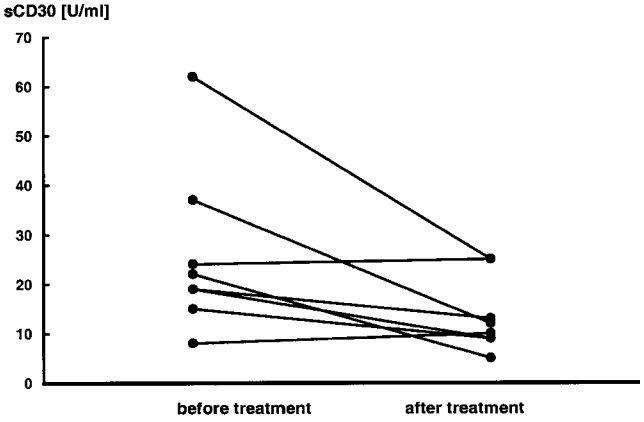

Fig. 2. Significant reduction in sCD30 plasma levels in 6 (acute/subacute generalized form) of 8 patients with atopic dermatitis after topical glucocorticosteroid therapy from median 20.5 U/ml (baseline) to 11.0 U/ml ($p < 0.05$; Wilcoxon). SCORAD was reduced from 78 (before therapy) to 34 (after therapy) ($p < 0.05$; Wilcoxon).

was reduced to 46% of the initial concentration (from 20.5 to 11 U/ml) ($p < 0.05$). In 6 patients characterized by the acute/subacute generalized form, the sCD30 level decreased ($p < 0.05$). In contrast, 2 patients also receiving topical glucocorticosteroids, but showing a chronic generalized and an acute localized form, demonstrated no significant change in the levels of sCD30. Furthermore, SCORAD and sCD30 levels were not significantly reduced in the remaining 7 patients receiving only emollients (results not shown).

DISCUSSION

In this study we evaluated the usefulness of several criteria to assess clinical disease activity parameters compared to the most frequently used clinical score (SCORAD). A significant correlation between sCD30 level and disease activity was established in patients with AD, as shown by other research groups (14, 15).

In contrast, others could not find this correlation (5, 16). The disparity between these results may be related to differences in system of selection and classification of patients. Our patients with AD exhibited a generalized or localized distribution and were classified according to the activity stage of AD as acute, subacute and chronic stage. In contrast, others have studied patients with moderate to severe AD, without considering the activity stage of AD separately (5).

Several studies investigated eosinophil- and T-cell products as markers for disease activity. For example Czech et al. (17) suggested a correlation between disease activity and deposition of eosinophil granule content in affected skin. In their study ECP levels were found to correlate with the disease activity. Clinical improvement was associated with a decrease of clinical score and serum ECP levels. However, the authors were unable to observe a significant correlation between serum IgE and clinical score.

IgE has been shown to be a poor marker for assessing disease strength (18, 19). Furthermore, serum IgE levels were found to be present at normal concentrations in nearly 20% of patients with AD (19, 20). However, a positive correlation between extent and severity of the disease commonly associated with respiratory atopic disease has been reported (21). Thus, IgE can be seen to be associated with a severe form of AD in general, but it does not reflect flares/exacerbations in the course of the disease, particularly through exposure to trigger factors. Consequently, it is not surprising that in patients demonstrating clinical improvement as a result of therapy, there was no transition noted in the concentration of IgE levels; these remained elevated (17).

In the present study, we found that plasma sCD30 levels showed a significant and positive correlation with serum IgE levels. This was also found by other authors (14, 22, 23), while in some studies such correlations could not be found (5, 16). Elimination of both sCD30 and IgE follows different metabolization and elimination pathways. sCD30, a 85 kD protein, has been demonstrated to be eliminated via the urinary tract and we could confirm the presence of sCD30 in the urine of patients with AD (results are not shown). On the other hand, the IgE molecule is 190 kD of weight. IgE – regulated by different immunological processes – appears to accumulate and to persist, especially in the severe form of AD.

CD30 has been shown to be associated with T cells generated mainly from Th2 and Th0 clones in consequence of a predominance of Th2 cytokines (IL-4, IL-5) (6, 24).

However, CD30 does not appear to be an intrinsic marker for Th2 cells *per se*. Nakamura et al. (25) found that CD30 is induced by IL-4 in activated CD4+T cells, and demonstrated that this induction could be antagonized by IFN-γ. Furthermore, Bengtsson et al. (26) observed that Th1 clones also have the ability to express CD30, but there was a higher expression in the clones defined as Th2 cells.

The significance of sCD30 reflecting immunopathogenic activity in AD is further supported by the strong reduction of sCD30 plasma levels after therapy (Fig. 2). sCD30 was reduced to 46% of the initial concentration in patients characterized by the acute/subacute generalized form. However, in two patients with the chronic generalized and acute localized forms sCD30 levels did not change significantly. These results are in line with those of Dummer et al. (27), who found increased CD30 expression in acute AD but rarely in patients with chronic AD, and also with studies (23, 28, 29) showing an association between successful treatment of AD and downregulation of sCD30.

In conclusion, sCD30 shows significantly higher plasma concentrations in patients with acute forms of AD as compared to subacute and chronic disease expressions. Furthermore, significant differences were seen when comparing generalized and more locally restricted

disease forms, with significantly increased sCD30 levels in the generalized forms.

These data suggest that the production of sCD30 in atopic skin disease is a measure of the extent of skin involvement as well as of the activity of the disease. Thus, sCD30 concentrations reflect the acute phase of inflammation characterized by a predominance of Th2 cells, while in chronic ongoing disease associated with Th1 cells (23, 30) this serologic marker for T-cell activation appears to be of minor significance.

ACKNOWLEDGEMENTS

We thank all our patients and the healthy controls for their friendly collaboration. This work was supported by grants from the Hensel Stiftung, University of Kiel, Germany.

REFERENCES

1. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980; Suppl 92: 44–47.
2. van Reijsen FC, Bruijnzeel-Koomen CAFM, Kalthoff FS, Maggi E, Romagnani S, Westland JK, et al. Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. J Allergy Clin Immunol 1992; 90: 184–192.
3. Leung D. Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic disease. J Allergy Clin Immunol 1995; 96: 302–319.
4. Schlaak JF, Buslau M, Jochum W, Hermann E, Girndt M, Gallati H, et al. T cells involved in psoriasis vulgaris belong to the Th1 subset. J Invest Dermatol 1993; 101: 701–705.
5. Bengtsson A, Holm L, Bäck O, Fransson J, Scheynius A. Elevated serum levels of soluble CD30 in patients with atopic dermatitis (AD). Clin Exp Immunol 1997; 109: 533–537.
6. Romagnani S, Del Prete G, Maggi E, Chilosi M, Caligaris-Cappio F, Pizzolo G. CD30 and type 2 T helper (Th2) responses. J Leuk Biol 1995; 57: 726–730.
7. Wang G, Hansen HP, Tatsis E, Csernok E, Lemke H, Gross WL. High plasma levels of the soluble form of CD30 activation molecule reflect disease activity in patients with Wegener's granulomatosis. Am J Med 1997; 102: 517–523.
8. Hansen HP, Dietrich S, Kisseleva T, Mokros T, Mentlein R, Lange HH, et al. CD30 shedding from Karpas 299 lymphoma cells is mediated by TNF-alpha-converting enzyme. J Immunol 2000; 165: 6703–6709.
9. Hansen HP, Barth S, Matthey B, Kisseleva T, Mokros T, Davis SJD, et al. Inhibition of metalloproteinases enhances the internalization of anti-CD30 antibody Ki-3 and the cytotoxic activity of Ki-3 immunotoxin. Int J Cancer 2002; 98: 210–215.
10. Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. Dermatology 1993; 186: 23–31.
11. Hamid O, Boguniewicz M, Leung DYM. Differential *in situ* cytokine gene expression in acute versus chronic atopic dermatitis. J Clin Invest 1994: 870–876.
12. Henseler T, Christophers E. Psoriasis of early and late onset: characterization of two types of psoriasis vulgaris. J Am Acad Dermatol 1985; 13: 450–456.
13. Horn-Lohrens O, Tiemann M, Lange H, Kobarg J, Hafner M, Hansen H, et al. Shedding of the soluble form of CD30 from the Hodgkin-analogous cell line L540 is strongly inhibited by a new CD30-specific antibody (Ki-4). Int J Cancer 1995; 60: 539–544.
14. Frezzolini A, Paradisi M, Ruffelli M, Cadoni S, Depita O. Soluble CD30 in pediatric patients with atopic dermatitis. Allergy 1997; 52: 106–109.
15. Caproni M, Bianchi B, D'Elios M, De Carli M, Amedei A, Fabbri P. In vivo relevance of CD30 in atopic dermatitis. Allergy 1997; 52: 1063–1070.
16. Dummer W, Bröcker EB, Bastian BC. Elevated serum levels of soluble CD30 are associated with atopic dermatitis, but not with respiratory atopic disorders and allergic contact dermatitis. Br J Dermatol 1997; 137: 185–187.
17. Czech W, Krutmann J, Schöpf E, Kapp A. Serum eosinophil cationic protein (ECP) is a sensitive measure for disease activity in atopic dermatitis. Br J Dermatol 1992; 126: 351–355.
18. Chiarelli F, Canfora G, Verrotti A, Amerio P, Morgese G. Humoral and cellular immunity in children with active and quiescent atopic dermatitis. Br J Dermatol 1987; 116: 651–660.
19. Juhlin L, Johannson GO, Bennich H, Hogman C, Thyresson N. Immunoglobulin E in dermatoses. Levels in atopic dermatitis and urticaria. Arch Dermatol 1969; 100: 12–16.
20. Wüthrich B. Clinical aspects, epidemiology, and prognosis of atopic dermatitis. Ann Allergy Asthma Immunol 1999; 83: 464–470.
21. Wüthrich B, Benz A, Skvaril F. IgE and IgG4 levels in children with atopic dermatitis. Dermatologica 1983; 166: 299–335.
22. Latza U, Davis S, Wilhelm D, McKnight B, Seyfarth M, Stein H. Soluble cytokine receptor CD30 in atopic disorders: a case control study. Clin Exp Allergy 1999; 29: 97–104.
23. Katoh N, Hirano S, Suehiro M, Ikenaga K, Yamashita T, Sugawara N, et al. Soluble CD30 is more relevant to disease activity of atopic dermatitis than soluble CD26. Clin Exp Immunol 2000; 121: 187–292.
24. Del Prete G, De Carli M, Almerigogna F, Daniel CK, D'Elios MM, Zancuoghi G, et al. Preferential expression of CD30 by human CD4+T cells producing Th2-type cytokines. FASEB J 1995; 9: 81–86.
25. Nakamura T, Lee RK, Nam SY, Al-Ramadi BK, Koni PA, Bottomly K, et al. Reciprocal regulation of CD30 expression on CD4+T cells by Il-4 and IFN-γ. J Immunol 1997: 2090–2098.
26. Bengtsson A, Johansson C, Tengvall Linder M, Hallden G, van der Ploeg I, Scheynius A. Not only Th2 but also Th1 and Th0 cells express CD30 after activation. J Leukoc Biol 1995; 58: 683–689.
27. Dummer W, Rose C, Bröcker EB. Expression of CD30 on T helper cells in the inflammatory infiltrate of acute atopic dermatitis but not of allergic contact dermatitis. Arch Dermatol Res 1998; 290: 598–602.
28. Caproni M, Salvatore E, Cardinali C, Brazzini B, Fabbri P. Soluble CD30 and cyclosporine in severe atopic dermatitis. Int Arch Allergy Immunol 2000; 121: 324–328.
29. Holm L, Öhman S, Bengtsson A, Hage-Hamsten M, Scheynius A. Effectiveness of occlusive bedding in the treatment of atopic dermatitis – a placebo-controlled trial of 12 months' duration. Allergy 2001; 56: 152–157.
30. Grewe M, Gyufko K, Schöpf E, Krutman J. Lesional expression of interferon-gamma in atopic dermatitis. Lancet 1994; 343: 25–26.