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Elevated Levels of Circulating Intercellular Adhesion Molecule-3 (cICAM-3) in Psoriasis

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Intercellular adhesion molecule (ICAM)-3 is important in regulating leukocyte function and T-lymphocyte-antigen presenting cell interactions. Soluble, circulating forms of ICAM-3 and ICAM-1 (cICAM-3, cICAM-1) exist in serum, and levels are elevated in a variety of autoimmune diseases. Two types of soluble circulating tumour necrosis factor receptor (cTNF-R1, cTNF-R2) are found in the sera of healthy people. cTNF-R1 binds TNF-α and is important in regulating TNF-α-mediated inflammation. Psoriasis is a T-lymphocyte-mediated disease, characterized by cutaneous expression of ICAM-1, ICAM-3 and TNF-α. As it is unknown whether cICAM-3 is increased in sera of patients with psoriasis, we measured serum levels of cICAM-3 and compared them to levels of cICAM-1, cTNF-R1 and clinical severity of psoriasis.

Sera was taken from 112 healthy controls and 32 patients with psoriasis. Clinical severity of psoriasis was assessed using the psoriasis area severity index (PASI). cICAM-1, cICAM-3 and cTNF-R1 in serum were quantitated using a dual antibody, solid phase ELISA. Levels of cICAM-3, cICAM-1 and cTNF-R1 were significantly increased in sera of patients with psoriasis as compared with controls, and these elevated levels correlated with clinical severity of psoriasis as assessed by the PASI. Also, there were good correlations between serum levels of cICAM-3, cICAM-1 and cTNF-R1 in psoriasis.

These results demonstrate, for the first time, that circulating levels of cICAM-3 are increased in psoriasis and that these levels correlate both with disease severity and with elevated levels of cICAM-1 and cTNF-R1. The exact physiologic roles of circulating, soluble adhesion molecules and cTNF-R1 are unknown, but it is hypothesised that elevation of their circulating levels, as observed in psoriasis, may play a role in modulating the inflammatory reactions occurring in this disease. ***Key words: cICAM-1; cTNF-receptor; psoriasis severity.***

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Intercellular adhesion molecule (ICAM)-3 (ICAM-R, CD50) is one of three ICAM ligands for lymphocyte function-associated antigen-1 (1, 2). Soluble ICAM-3 (cICAM-3) exists in the sera of healthy people (3), and levels are significantly elevated in certain autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis (3). Whether cICAM-3 is increased in sera of patients with inflammatory skin disease, such as psoriasis, is unknown.

Intercellular adhesion molecules are fundamental to leukocyte function and migration (4). ICAM-3 is: 1) strongly and constitutively expressed on resting lymphocytes (5) and Langerhans' cells (6); 2) an important costimulatory or accessory molecule in Langerhans' cell-T-lymphocyte interactions; 3) and not expressed on keratinocytes (7). ICAM-1 is: 1) constitutively expressed on dermal endothelial cells (8, 9); 2) inducible on keratinoeytes by interferon-γ and tumour necrosis factor (TNF)-α (9, 10); and 3) expressed by activated lymphocytes (1). Previous work has demonstrated the importance of ICAM-1 in lymphocyte trafficking integral to inflammatory dermatoses (11, 12). Serum levels of soluble or circulating ICAM-1 (cICAM-1) are elevated in patients with psoriasis (13–15) and atopic eczema (15, 16).

The biologic response of tumour necrosis factor (TNF), an important early cytokine released in cutaneous inflammation (17), is mediated by two distinct cell surface receptors (TNF-R) of molecular weight 55 kD (TNF-R1) and 75 kD (TNF-R2) (18, 19). Both TNF-Rs are expressed on a variety of cell types, especially dermal endothelium, in normal and psoriatic skin (20). Two soluble or circulating forms of TNF-Rs, (cTNF-R1 and cTNF-R2) exist in the sera of healthy people (19), and cTNF-R1 can neutralise circulating TNF-α (21).

Psoriasis is a common inflammatory dermatosis in which ICAM-1 and ICAM-3 expression are prominent in involved skin (7–9) and cutaneous levels of TNF-α protein (20, 22) and biologic activity (23) are increased. Furthermore, circulating soluble forms of ICAM-1, E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and cTNF-R1 are increased in psoriasis (13–15, 22, 24) and in the case of cICAM-1 correlate with disease severity (13). Using a "sandwich" enzyme-linked immunosorbant assay (ELISA), we measured levels of cICAM-3, cICAM-1 and cTNF-R1 in sera of control subjects in patients with psoriasis.

PATIENTS AND METHODS

Patients

Sera were taken from 32 patients with untreated, active psoriasis (19 males, 13 females; age range 16–73 years; mean 42). Severity of psoriasis was assessed using the psoriasis area and severity index (PASI), a well established grading system (25) that takes into account erythema, induration and scaling of psoriatic plaques and skin surface area affected by psoriasis. The PASI scale runs from 0: no psoriasis to 72: maximum severity. In the patients studied the PASI scores ranged from 5 to 41; mean 15 ± 1.6.

Control sera were taken from 112 healthy volunteers (62 males, 50 females; age range 18–70 years, mean 28). Serum samples collected from patients and controls were frozen at $-70°C$ until use.

ELISA assays

ICAM-3. Two different monoclonal antibodies (ICR-4 and ICR-8, ICOS Corporation, Bothell, WA) directed to the extracellular domains of ICAM-3 and truncated recombinant, soluble ICAM-3 expressed in Sf9-cells were used to develop a sandwich ELISA assay (3). Affinity-purified monoclonal antibody ICR-8 was diluted to 5 μmg/ml in phosphate-buffered saline (PBS), pH 7.4, and used to coat a 96-well

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microtiter plate (1 h at 37°C). After washing with PBS, non-specific binding sites were blocked by 2% bovine serum albumin (BSA) in PBS for 30 min at 37°C. Dilutions of recombinant ICAM-3 in 1% BSA-PBS were used over a range of 2 μg to 16 ng/ml. Human serum was used at a dilution of 1:2. Samples of 100 ml were added to wells and incubated for 30 min at 37°C. After washing, bound control ICAM-3 or human serum cICAM-3 were reacted with a second biotinylated antibody against ICAM-3 (ICR-4, 2 μg/ml diluted in 1% BSA-PBS) for 30 min at 37°C. After washing, avidin horse-radish peroxidase (50 ml of a 1:2000 dilution in 2% BSA-PBS; Zymed, San Francisco, CA) was added for 30 min at 37°C and staining performed with ATBS substrate buffer (Zymed). Adsorption at 414 nm was measured using a Titertek ELISA reader and mean OD readings calculated. Each sample was tested in duplicate and the serum standard chosen to contain cICAM-3 equivalent to 100 ng/ml of recombinant ICAM-3 standard.

cICAM-1 and cTNF-R1. Analyses of cICAM-1 and cTNF-R1 were performed using a commercial sandwich ELISA assay (Bender Medsystems, Vienna, Austria). The colour reaction was developed using tetramethylbenzidine and adsorbance recorded at 450 nm wavelength. The lower detection limits were 0.5 ng/ml for cICAM-1 and 80 pg/ml for cTNF-R1.

All serum assays were run in a blinded, coded fashion and levels expressed as ng/ml.

Statistical analysis

The unpaired *t*-test was used to compare psoriasis and control serum levels. The relations between PASI and cICAM-1, cICAM-3 or cTNF-R1 and the inter-relations between cICAM-1, cICAM-3 and cTNF-R1 were assessed with the Pearson product-moment correlation analysis. All *p* values were two-sided and summary data are expressed as means ± SEM.

RESULTS

Control volunteers

Low, but detectable, levels of cICAM-1, cICAM-3 and cTNF-R1 were present in the sera of healthy controls, as previously reported (3, 21).

Psoriasis patients

cICAM-3. Mean serum values of cICAM-3 were significantly increased in patients with psoriasis as compared with normal controls ($p<0.0005$), and these serum levels correlated with psoriasis severity as assessed by PASI ($r=0.35$; $p=0.05$; Figs. 1 and 2).

cICAM-1. As previously observed (13–15), mean serum values of cICAM-1 were significantly increased in patients with psoriasis as compared with normal controls ($p=0.001$), and these levels correlated with psoriasis severity ($r=0.40$; $p=0.02$; Figs. 1 and 2).

cTNF-R1. Mean serum levels of cTNF-R1 in patients with psoriasis were significantly greater than those in normal controls ($p<0.0005$) and the levels of cTNF-R1 correlated with psoriasis severity ($r=0.39$; $p=0.03$, Figs. 1 and 2).

Correlation of cICAM-1, cICAM-3 and cTNF-R1. There were significant correlations between serum levels of cICAM-1 and cICAM-3 ($r=0.48$; $p=0.005$); cICAM-1 and cTNF-R1 ($r=0.77$; $p<0.001$) and cICAM-3 and cTNR-R1 ($r=0.63$; $p<0.001$) in patients with psoriasis (Fig. 3).

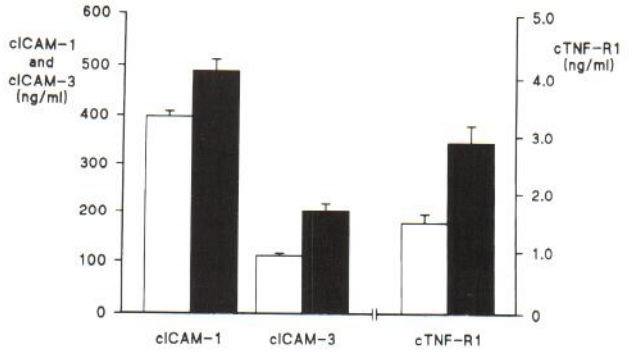

Fig. 1. Serum levels of cICAM-1, cICAM-3 and cTNF-R1 are significantly increased in psoriasis patients as compared with normal controls, $p=0.001$; $p=<0.0005$ and $p<0.0005$, respectively. Controls, *open bars*; psoriasis, *closed bars*. Bars are means ± SEM; $n=32$ for psoriasis and $n=99$, 112 and 98 for cICAM-1, cICAM-3 and cTNF-R1, respectively, in normal controls.

DISCUSSION

This study demonstrates, for the first time, that circulating levels of cICAM-3 are significantly increased in the sera of patients with psoriasis and that these levels correlate both with elevated levels of cICAM-1 and cTNF-R1 and with clinical severity of disease as measured by PASI. Although relatively little is known about the cutaneous expression and function of ICAM-3, it is constitutively and strongly expressed on resting T-lymphocytes and Langerhans' cells (5, 6) and probably plays an important role in initial Langerhans' cell-T-lymphocyte interactions and Langerhans' cell-driven T-lymphocyte proliferation (6). As ICAM-3 is only rarely expressed on endothelial cells it would appear that, in distinction to other soluble adhesion molecules, the high levels of cICAM-3 observed in psoriasis serum are derived mainly from cells of leukocyte or antigen-presenting cell lineage. Levels of cICAM-1 and cICAM-3 in sera from psoriasis patients correlated highly with each other; such a correlation appears unique to psoriasis, or at least cutaneous inflammation, as cICAM-3 elevation in other autoimmune diseases, e.g. multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus is not associated with a concomitant rise in cICAM-1 (3).

Previous investigators have observed increased serum levels of cICAM-1 in psoriasis patients (13–15) and that these levels correlate with disease severity (13). Elevated serum levels of cICAM-1 are not unique to psoriasis but are a marker of inflammation, whether it is cutaneous or otherwise (26). In psoriasis, cICAM-1 is probably shed into the circulation from inflamed dermal endothelium as well as from keratinocytes, activated mononuclear cells and dermal dendrocytes (15). Other endothelial cell-derived, circulating adhesion molecules are increased in sera of patients with psoriasis (15, 24) and include cVCAM-1 and cE-selectin. Serum levels of cICAM-1 and cE-selectin correlate with each other in erythrodermic psoriasis (15), but in less extensive, chronic plaque psoriasis such a correlation is not apparent (24). As we demonstrate in this study, other investigators have shown that cICAM-1 levels correlate with psoriasis severity as assessed by PASI (13) and that cE-selectin levels also appear to correlate with PASI (24). Although serum levels of cICAM-1 and cE-selectin are increased in psoriasis, these levels do not fall subsequent to

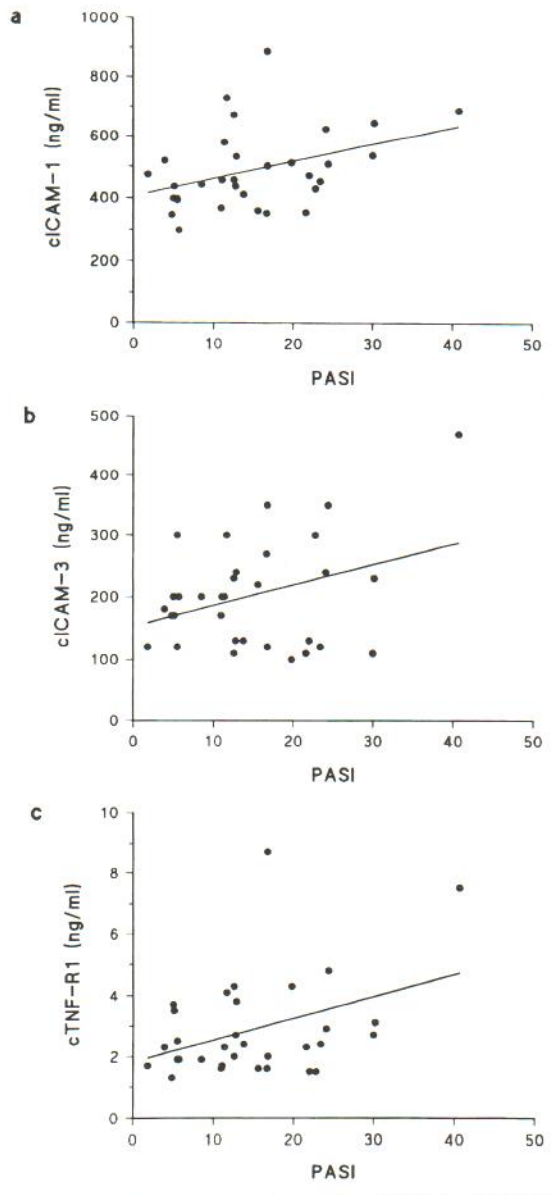

Fig. 2. Relationship of serum levels of cICAM-1, cICAM-3 and cTNF-R1 to psoriasis severity as assessed by the PASI. (*a*), cICAM-1 and PASI ($r=0.4$; $p=0.02$); (*b*), cICAM-3 and PASI ($r=0.35$; $p=0.05$); and (*c*), cTNF-R1 and PASI ($r=0.39$; $p=0.03$). $n=32$.

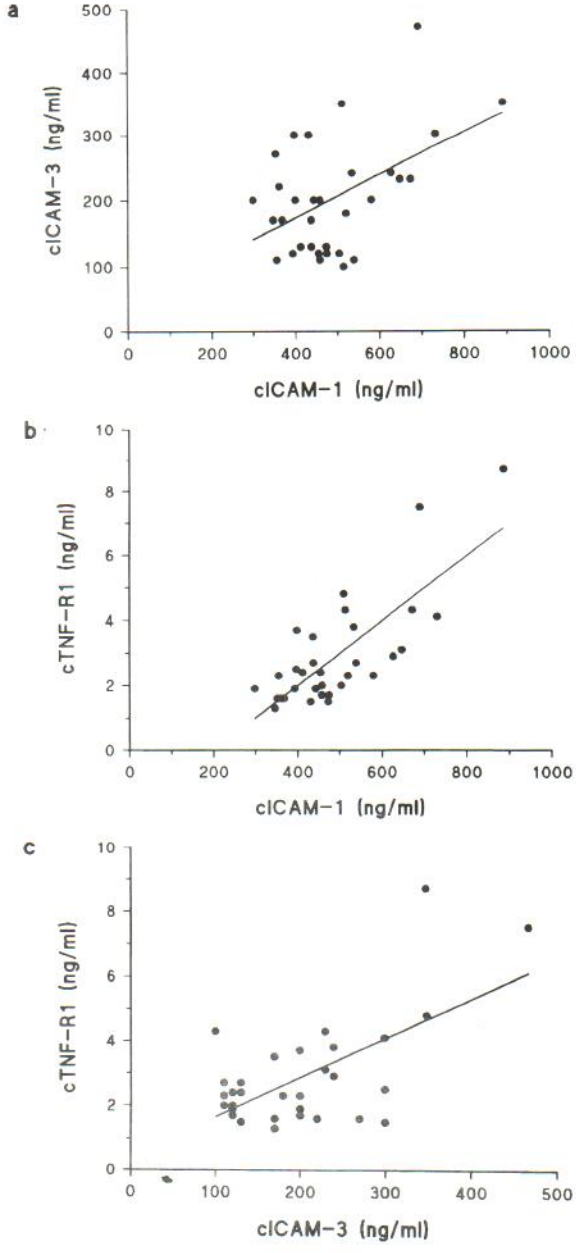

Fig. 3. Relationship between serum levels of cICAM=1, cICAM-3 and cTNF-R1 in patients with psoriasis. (*a*), cICAM and cICAM-3 ($r=0.48$;$p=0.005$; (*b*), cICAM-1 and cTNF-R1 ($r=0.77$; $p<0.001$); and (*c*), cICAM-3 and TNF-R1 ($r=0.63$; $p<0.001$). $n=32$.

clinical improvement following successful treatment with UVB/anthralin therapy (14, 24).

The raised serum levels of cTNF-R1 in psoriasis patients underscore the relative importance of TNF-α to the initiation and maintenance of psoriasis plaques; indeed both protein (20, 22) and biological activity (23) for TNF-α are elevated in skin involved by psoriasis, and TNF-R1 and TNF-R2 expression is upregulated on dermal blood vessels in psoriasis plaques (20). cTNF-R1 binds and neutralizes TNF-α and TNF-β (21) and in particular is believed to play a down-regulating role in control of TNF-α. High serum levels of cTNF-R1 in multiple sclerosis are associated with stable as compared to active disease, i.e. raised serum levels of cTNF-R1 appear to "damp down" the inflammatory effects of TNF-α (27). cTNF-R1 serum levels are increased in patients with advanced solid cancers, and higher levels are indicative of a poor prognosis as they correlate with cancer progression (28).

Concomitant increases in serum levels of soluble adhesion molecules and cTNF-R1 observed in psoriasis probably represent a down-regulating, protective mechanism (29) against orchestrated ICAM-1, ICAM-3 and TNF-α-mediated events observed in this form of cutaneous inflammation. Although levels of cICAM-1, cICAM-3 and cTNF-R1 correlate with psoriasis severity, as assessed by PASI, this correlation is only modest—a reflection of the relative crudity of clinical measurement of psoriasis. It is possible that levels of these soluble, circulating factors are a truer representation of intrinsic disease activity than is the clinical appearance of psoriasis. This

supposition, that such circulating factors may represent intrinsic activity of psoriasis, is supported by the failure to demonstrate any reduction in levels of cICAM-1 and cE-selectin subsequent to clinical improvement following UVB/anthralin therapy (14, 24). However, it would be of interest to compare this treatment with other modes of psoriasis therapy, such as methotrexate, cyclosporine or PUVA, in their ability to reduce levels of cICAM-3. If such a reduction did occur with therapy it would indicate inhibition of an integral component of the pathways that lead to psoriasis.

The increasing awareness of immunologic events pertinent to psoriasis and the consequent use of immunopharmacologic approaches in its treatment indicate that synthetic forms of cICAM-1, cICAM-3 and cTNF-R1 may be useful therapeutic innovations in this disease.

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