Soluble interleukin 2 receptor in atopic eczema

Graham B Colver, Julian A Symons, Gordon W Duff

Abstract

Objective—To determine whether serum soluble interleukin 2 receptor concentrations are related to disease activity in atopic eczema.

Design—Single cohort longitudinal study with controls.

Setting—Outpatient and general medicine departments in secondary referral centre.

Patients—Of 15 patients aged 17-57 with severe atopic eczema, all with acute exacerbations of disease, 13 were admitted to hospital and two treated as outpatients until the skin lesions had resolved or greatly improved. Nineteen controls gave single blood samples.

Interventions—Daily skin dressing with beta-methasone valerate (0.025%) and ichthammol paste and tubular dressings.

End point—Resolution of or considerable improvement in skin lesions.

Measurements and main results—Enzyme linked immunosorbent assays (ELISA) were used to measure serum soluble interleukin 2 receptor concentrations in blood samples taken on admission, at intervals subsequently, and on discharge. Clinical scores of disease activity were also made. Median concentrations on admission were significantly higher (770 U/ml) in the patients than the controls (300 U/ml). Concentrations fell significantly during treatment. In 25 assessments made at different times in 13 patients serum soluble interleukin 2 receptor concentration correlated significantly ($R=0.73$) with clinical disease activity.

Conclusions—Cellular immunopathogenic mechanisms contribute to atopic eczema. Immune activation can be measured in atopic eczema by measurement of soluble interleukin 2 receptor, and this should facilitate assessment of response to treatment.

Introduction

Atopic eczema is a common skin disorder that usually runs a chronic course with acute episodes and is often associated with hay fever, asthma, or a family history of atopic disease. The pathogenesis has not been defined, but genetic, immunological, and biochemical factors have all been implicated. Though exacerbations may be related to allergens, drugs, and stress, it remains an unpredictable disease, and there are probably other unidentified stimuli.

The association of eczema with other atopic diseases in which IgE has been implicated suggests a role for hypersensitivity. Most patients with eczema have raised IgE titres,¹ but the presence of T cell infiltrates in skin with lesions suggests a cell mediated immune response.² Other studies have shown deficiencies in certain T cell subsets,³ decreased complement concentrations,⁴ and defective neutrophil chemotaxis.⁴ Though several such abnormalities are recognised, no test exists to monitor the underlying pathogenic mechanisms leading to clinical disease.

In vivo activation of the immune system can now be studied. T lymphocytes are central to cell mediated immunity, and, after activation by antigen, T cells express genes encoding T cell growth factors such as

interleukin 2 and its receptor.⁵ The interleukin 2 receptor is composed of at least two peptides,^{6,7} one of which (the α chain or Tac protein) is shed from the cell membrane in proportion to the amount of activation of T cells.⁸ This soluble form can be detected in the blood of normal people, and concentrations are raised in patients with immune mediated diseases such as rheumatoid arthritis^{9,10} and systemic lupus erythematosus.¹¹

We measured serum concentrations of soluble interleukin 2 receptor in patients with an exacerbation of atopic eczema throughout their treatment in hospital. These concentrations were compared with clinical scores of disease activity.

Patients and methods

We studied 15 patients with severe atopic eczema (mean age 30.5 (range 17-57) years; seven men). The diagnosis was based on a history of infantile eczema, clinical features, and, in some cases, positive intradermal responses to common allergens and raised blood titres of IgE. All the patients had acute exacerbation of disease, 13 being admitted to hospital and two being treated with daily dressings as outpatients. The first blood samples were taken and clinical assessments made on admission or at the start of dressings. The last sample was taken at discharge or when the dressings were stopped, when the skin lesions had resolved or were greatly improved. All patients had been using topical steroids (beta-methasone valerate 0.010-0.025% or fluocinolone acetonide 0.00625%) before admission, and all were treated with betamethasone valerate (0.025%), ichthammol paste, and tubular dressings during the study.

A control group comprised 19 normal people (mean age 24 (range 15-32); nine male) who had attended a pigmented lesion clinic. They were all apparently healthy and had no inflammatory or malignant skin disease.

The front and back of the body were each divided into six sections and scored from 0 to 3 for erythema, vesiculation, excoriation, and lichenification. In addition, the amount of skin affected was scored from 1-6. The maximum score for each section was 18 and that for the whole body 216. Clinical assessment and measurement of serum soluble interleukin 2 receptor concentrations were performed independently by different investigators.

To assay soluble interleukin 2 receptor in the serum an enzyme linked immunosorbent assay (ELISA) with two non-competing murine monoclonal antibodies to the α chain of the interleukin 2 receptor was used (T Cell Sciences) according to previously published methods.⁹ Briefly, microtitre plates with 96 wells (Dynatech Laboratories) were coated with an antibody to the interleukin 2 receptor (2R1.2). Non-specific binding was blocked with phosphate buffered saline, 0.2% (vol/vol) polysorbate 20, and 1% (wt/vol) bovine serum albumin. After the wells had been washed three times with phosphate buffered saline and 0.2% (vol/vol) polysorbate 20 freshly thawed samples were added in duplicate and incubated for two hours at 37°C. Samples were then discarded, the plate

Department of
Dermatology, University of
Edinburgh, Edinburgh
Graham B Colver, MRCP,
senior registrar

Department of Medicine,
Molecular Immunology
Group, Rheumatic
Diseases Unit, Northern
General Hospital,
Edinburgh EH5 2DQ
Julian A Symons, DPHIL,
research fellow
Gordon W Duff, PHD,
senior lecturer

Correspondence to:
Dr Duff.

Br Med J 1989;298:1426-8

washed, and an antibody to the interleukin 2 receptor conjugated with horseradish peroxidase (7G7/B6) was added to each well. After another two hours at 37°C plates were washed and incubated with O-phenylenediamine (0.5 g/l) in citrate phosphate buffer containing 0.01% (vol/vol) hydrogen peroxide. After 30 minutes at room temperature in the dark, colour was developed by the addition of 2N sulphuric acid and the plates were read at 490 nm with a microplate reader (Dynatech MR700). Units of soluble interleukin 2 receptor were calculated from a standard curve obtained with supernatant from peripheral blood mononuclear cells stimulated with phytohaemagglutinin (T Cell Sciences) designated 1000 U/ml. All samples were coded and read blind in the assay. The mean (SE) interassay variation was 10% (1.6%).

Results

On admission to the study the patients had a median concentration of soluble interleukin 2 receptor of 770 U/ml (n=15). After treatment concentrations fell to a median of 600 U/ml (n=14) (p<0.01, Wilcoxon paired rank sum test). Even after treatment concentrations remained significantly higher than those in normal people (n=19; median value 300 U/ml; p<0.001, Wilcoxon rank sum test). Figure 1 shows the details with 95% confidence intervals.

Thirteen patients were assessed by clinical score and concurrent measurement of serum soluble interleukin 2 receptor concentration. Six were graded clinically only on admission, but seven had two or more assessments during the study. Overall, in 25 assessments there was a significant correlation between the clinical score and serum soluble interleukin 2 receptor concentration (R=0.732; p<0.001, Spearman's test) (fig 2).

In 14 patients serum soluble interleukin 2 receptor concentrations were measured in samples taken at intervals during hospital treatment. The measurements were performed independently at the end of the study. Figure 3 shows the values in the four patients who had the highest concentrations on admission. Two showed rapid falls in concentrations with concomitant clinical improvement leading to early discharge. The other two showed delayed falls and required longer

FIG 1—Serum interleukin 2 receptor concentrations in patients with atopic eczema on admission to hospital or at start of daily dressings (n=15) and at time of discharge or stopping dressings (n=14) and in controls matched for age and sex (n=19). Median values and 95% confidence intervals are shown

FIG 2—Clinical scores of disease activity against soluble serum interleukin 2 receptor concentrations taken on same day (total assessments=25; R=0.732, p<0.001)

FIG 3—Soluble serum interleukin 2 receptor concentrations during treatment in hospital for four patients with highest concentrations on admission

treatment in hospital. Six more patients showed similar patterns, but the remaining four, who had the lowest concentrations and clinical scores on admission, showed no significant change.

One patient who had a slow reduction in the concentration of soluble interleukin 2 receptor from 950 U/ml also had a slow clinical improvement but was discharged from hospital. After two weeks her eczema flared, and on readmission her soluble interleukin 2 receptor concentration was again raised at 920 U/ml. Over the subsequent days it fell to 550 U/ml but three days later, on the day of discharge, had risen to 670 U/ml. She required yet another admission for treatment seven days later, suggesting that her previous rise before discharge was of some predictive value.

Discussion

A laboratory test to monitor disease activity in atopic eczema would be useful. Previously it was thought that the serum IgE titre might be informative because it is raised in most patients with atopic eczema.¹ Some investigators have found a correlation between IgE titre and clinical disease,¹² but others have reported that it is often unchanged after successful topical treatment.^{4,13}

The lymphokine interleukin 2 has a major role in the activation of T cells, resulting in further lymphokine production and cellular proliferation. It also acts on other leucocytes including B cells,¹⁴ natural killer cells,¹⁵ and macrophages.¹⁶ These cellular responses are mediated by a surface receptor complex of which three separate peptide chains have been described.^{7, 17, 18} The α chain of the receptor (Tac protein) is released from the cell surface⁸ and in its soluble form retains affinity for interleukin 2.¹⁹ The rate of release is related to the degree of surface expression (T cell activation). Soluble interleukin 2 receptor concentration is raised in diseases such as graft versus host disease,²⁰ rapidly progressive scleroderma or systemic lupus erythematosus,²¹ lymphoreticular malignancy,²² and systemic but not localised parasitic infection.²³ An excellent correlation exists between serum soluble interleukin 2 receptor concentration and the Ritchie articular index in rheumatoid arthritis, in which falls in receptor concentration predict clinical improvement and rises are seen before clinical relapse.¹⁰

We found that on admission to hospital patients with atopic eczema had higher concentrations of serum soluble interleukin 2 receptor than controls matched for age. Also, within the group concentrations fell significantly with treatment. At the time of discharge, however, when most patients had a distinct clinical improvement, there was still a significant difference in soluble interleukin 2 receptor concentration between patients and controls. Clinical score correlated with the concentrations at all stages of treatment.

Steroids are known to suppress synthesis of interleukin 2 and its receptor²⁴ and inhibit T cell proliferation.²⁵ Production of interleukin 2 in lymphocytes from patients resistant to steroids is also fairly resistant to steroids.²⁶ Hydrocortisone suppresses not only production of interleukin 2 receptor but also the number of receptors already on the cell surface.²⁷ These findings suggest that falling receptor concentrations in our patients could have been related to topical steroids. Before admission, however, when their soluble interleukin 2 receptor concentrations were highest, all our patients were using a steroid preparation of similar potency to that used in hospital.

As well as being a useful marker of immune activity soluble interleukin 2 receptor, by binding interleukin 2, may have an immunoregulatory role with inhibitory effects on T cell activation.^{9, 28} This could contribute to defective cell mediated immunity in atopic eczema²⁹ and other inflammatory diseases. The cellular events that give rise to the lesions of atopic eczema remain unknown, but we suggest that raised soluble interleukin 2 receptor concentrations reflect activation of immunopathogenic mechanisms contributing to exacerbation of the disease. Clinically, the concentration seems potentially useful for assessing responses to new treatments and may even have prognostic value.

- 1 Barnetson RStC, Merrett TG, Ferguson A. Studies on hyperimmunoglobulinemia E in atopic disease with particular reference to food allergens. *Clin Exp Immunol* 1981;44:54-60.
- 2 Braathen LR, Forre O, Natvig JB, EEG-Larsen T. Predominance of T lymphocytes in the dermal infiltrate of atopic dermatitis. *Br J Dermatol* 1979;100:511-9.
- 3 Zachary CB, MacDonald DM. Quantitative analysis of T lymphocyte subsets in atopic eczema using monoclonal antibodies and flow cytometry. *Br J Dermatol* 1983;108:411-22.

- 4 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;116:651-60.
- 5 Meuer SC, Hussey RE, Cantrell DA, et al. Triggering of the T3-Ti antigen-receptor complex results in clonal T cell proliferation through an interleukin 2 dependent autocrine pathway. *Proc Natl Acad Sci USA* 1984;81:1509-13.
- 6 Wang HM, Smith KA. The interleukin 2 receptor: functional consequences of its bimolecular structure. *J Exp Med* 1987;166:1055-64.
- 7 Szollosi J, Damjanovich S, Goldman CK, et al. Flow cytometric resonance energy transfer measurements support the association of a 95KDa peptide termed T27 with the 55KDa Tac peptide. *Proc Natl Acad Sci USA* 1987;84:7246-50.
- 8 Rubin LA, Kurman CC, Fritz ME, et al. Soluble interleukin 2 receptors are released from activated human lymphoid cells in vitro. *J Immunol* 1985;135:3172-7.
- 9 Symons JA, Wood NC, Di Giovine FS, Duff GW. Soluble interleukin 2 receptor in rheumatic diseases: correlation with disease activity, interleukin 1 and interleukin 2 inhibition. *J Immunol* 1988;141:2612-8.
- 10 Wood NC, Symons JA, Duff GW. Serum interleukin 2 receptor in rheumatoid arthritis: a prognostic indicator of disease activity. *Journal of Autoimmunity* 1988;1:353-61.
- 11 Wolf RE, Brelsford WG. Soluble interleukin-2 receptors in systemic lupus erythematosus. *Arthritis Rheum* 1988;31:729-35.
- 12 Byrom MA, Timlin DM. Immune status in atopic eczema: a survey. *Br J Dermatol* 1979;100:491-8.
- 13 Juhlin L, Johansson GO, Bennich H, Hogman C, Thyresson L. Immunoglobulin E in dermatoses. *Arch Dermatol* 1969;100:12-6.
- 14 Mingari MC, Gerosa F, Carra G, et al. Human interleukin 2 promotes proliferation of activated B cells via surface receptors similar to those of activated T cells. *Nature* 1984;312:641-3.
- 15 Henney CS, Kuribayashi K, Kern DE, Gillis S. Interleukin-2 augments natural killer cell activity. *Nature* 1981;291:335-8.
- 16 Malkovsky M, Loveland B, North M, et al. Recombinant interleukin 2 directly augments the cytotoxicity of human monocytes. *Nature* 1987;325:262-5.
- 17 Leonard WJ, Depper JM, Robb RJ, Waldmann TA, Greene WC. Characterisation of the human receptor for T cell growth factor. *Proc Natl Acad Sci USA* 1983;80:6957-61.
- 18 Tanaka T, Saiki O, Doi S, Negoro S, Kishimoto S. Interleukin 2 functions through novel interleukin 2 binding molecules in T cells. *J Immunol* 1988;140:470-3.
- 19 Rubin LA, Jay G, Nelson DL. The released interleukin 2 receptor binds interleukin 2 efficiently. *J Immunol* 1986;137:3841-4.
- 20 Volk HD, Brocke S, Osawa H, Diamantstein T. Suppression of the local graft-vs-host reaction in rats by treatment with a monoclonal antibody specific for the interleukin 2 receptor. *Eur J Immunol* 1986;16:1309-13.
- 21 Charley M, Deng JS. Elevated levels of soluble interleukin 2 receptors in the serum of scleroderma and SLE patients. *J Invest Dermatol* 1988;124:2828-33.
- 22 Schirmacher V, Josimovic O, Osawa H, Diamantstein T. Determination of cell-free interleukin 2 receptor level in the serum of normal animals and of animals bearing IL-2 receptor positive tumours with high or low metastatic capacity. *Br J Cancer* 1987;55:583-8.
- 23 Josimovic O, Feldmeier H, Zwingerberger K, et al. Interleukin 2 receptor in patients with localised and systemic parasitic diseases. *Clin Exp Immunol* 1988;72:249-54.
- 24 Reed JC, Abidi AH, Alpers JD, Hoover RG, Robb RJ, Nowell PC. Effect of cyclosporin A and dexamethasone on interleukin 2 receptor gene expression. *J Immunol* 1986;137:150-4.
- 25 Larsson E-L. Cyclosporin A and dexamethasone suppress T cell responses by selectively acting at distinct sites of the triggering process. *J Immunol* 1980;124:2828-33.
- 26 Walker KB, Potter JM, House AK. Interleukin 2 synthesis in the presence of steroids: a model of steroid resistance. *Clin Exp Immunol* 1987;68:162-7.
- 27 Horst H-J, Flad H-D. Corticosteroid-interleukin 2 interactions: inhibition of binding of interleukin 2 to interleukin 2 receptors. *Clin Exp Immunol* 1987;68:156-61.
- 28 Robb RJ, Kutny RM. Structure-function relationships for the IL-2-receptor system. 4. Analysis of the sequence and ligand-binding properties of soluble Tac protein. *J Immunol* 1987;139:855-62.
- 29 McGeady SJ, Buckley RH. Depression of cell mediated immunity in atopic eczema. *J Allergy Clin Immunol* 1975;56:393-8.

(Accepted 21 March 1989)

Correction

Transcervical resection of endometrium in women with menorrhagia

An error occurred in the paper by Mr Adam L Magos and others (6 May, p 1209). The fourth sentence of the second paragraph of the discussion should have read:

"Our results show a consistent and considerable reduction in the duration of menstrual bleeding, the amount of flow per cycle, and period pains in all women and amenorrhoea in over half the women after total resection."