

Soluble interleukin-2 receptor in Crohn's disease: relation of serum concentrations to disease activity

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Abstract

Serum concentrations of soluble interleukin-2 receptor (sIL-2R) were measured as a marker of immune activation in a group of 30 patients with Crohn's disease. sIL-2R concentrations were determined by enzyme linked immunosorbent assay during periods of active and inactive disease and correlated with standard parameters of disease activity. Serum concentrations of sIL-2R were significantly raised in patients with active Crohn's disease compared with patients with inactive disease ($p < 0.001$) and control subjects. There was a significant correlation between serum sIL-2R concentrations and disease activity as assessed by the Harvey-Bradshaw index ($r = 0.42$, $p < 0.01$), platelet numbers ($r = 0.49$, $p < 0.01$), and orosomucoid ($r = 0.47$, $p < 0.01$), α_1 antitrypsin ($r = 0.44$, $p < 0.01$), and C reactive protein concentrations ($r = 0.48$, $p < 0.001$) but not with the erythrocyte sedimentation rate. Measurement of serum sIL-2R concentration is a simple and useful laboratory means of assessing disease activity. Raised concentrations in patients with active Crohn's disease is further evidence for in vivo immune activation occurring in this disease.

Although the aetiology of Crohn's disease is unclear, both cellular and humoral immune phenomena are considered to play a part in the pathogenesis of the condition.¹ The importance of T lymphocytes in regulating intestinal defences in Crohn's disease is well recognised.² Both changes in immunoregulatory T cell function^{3,4} and altered expression of molecular markers of T cell activation on peripheral and mucosal lymphocyte populations have been described.^{5,6}

Activation of resting T lymphocytes with specific antigen or mitogens results in the cell surface expression of interleukin-2 receptors (IL-2R).^{7,8} The binding of interleukin-2 to this high affinity receptor induces T cell proliferation and a chain of immunological responses.⁷ The IL-2R is composed of at least two interleukin-2 binding polypeptides⁹ and in vitro studies have shown that after activation the 55 000 M_r α chain, the Tac peptide, is released in a soluble form (sIL-2R).¹⁰ sIL-2R is detectable serologically in healthy people, and concentrations are raised in diseases associated with increased immune activation such as rheumatoid arthritis,¹¹ sarcoidosis,¹² parasitic infections,¹³ atopic eczema,¹⁴ and graft rejection.¹⁵ Recent studies have shown serum sIL-2R concentrations to be an excellent marker of gluten sensitivity in coeliac disease, with concentrations falling rapidly in response to gluten withdrawal.¹⁶

In Crohn's disease few well defined biochemical markers satisfactorily quantify disease activity, the disease being characterised by periods of activity and quiescence. As serum sIL-2R concentrations seem to indicate the extent of in vivo immune activation in various disease states, we investigated patients with Crohn's disease to determine whether serum sIL-2R concentrations reflect disease activity and examine the relation of sIL-2R to other standard assessments of disease activity.

Methods

PATIENTS AND CONTROL SUBJECTS

Thirty patients with Crohn's disease were studied (14 men, 16 women; mean (SE) age 33.9 (2.34) years). Twenty three of the patients were examined in both active and inactive phases of the disease. The diagnosis of Crohn's disease was based on standard clinical, endoscopic, histological, and radiological criteria. In 10 patients the disease affected both the ileum and the colon, in 10 patients the colon, and in 10 the small bowel. Twenty patients were receiving steroids (mean (SE) dose 14 (2.7) mg) and six azathioprine (mean (SE) dose 125 (9.5) mg).

A control group of 18 healthy volunteers (10 women, eight men; mean (SE) age 39.6 (3.4) years) was studied. A disease control group consisted of 13 patients (seven women, six men; mean (SE) age 43 (3.1) years), of whom 11 had an irritable bowel syndrome/motility disorder, one diverticular disease, and one rectal polyp.

Sera for sIL-2R measurement were stored at -70°C until assayed. Collection of blood for other laboratory tests (C reactive protein, erythrocyte sedimentation rate, platelet number, orosomucoid and α_1 antitrypsin concentrations) was performed concurrently according to standard procedures.

DETERMINATION OF DISEASE ACTIVITY

Disease activity was assessed by the Harvey-Bradshaw index, which is based on a patient's well being, abdominal pain, number of liquid stools, presence of an abdominal mass, and extraintestinal manifestations, such as arthralgia and aphthous ulcers.¹⁷ This index correlates well with other more complex indices of disease activity.¹⁷

ELISA FOR sIL-2R

Serum sIL-2R concentrations were determined by an enzyme linked immunosorbent assay (ELISA) (T Cell Sciences, Cambridge MA) using two non-competing monoclonal anti-

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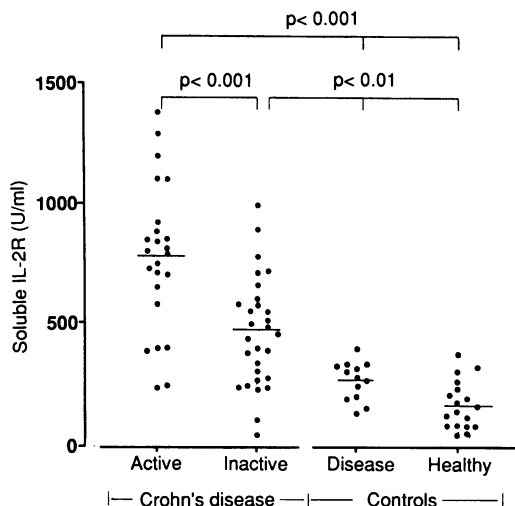


Figure 1: Serum sIL-2R concentrations in patients with active and inactive Crohn's disease, disease control subjects, and healthy control subjects.

bodies¹⁸ to the alpha chain of IL-2R, as described previously.¹¹ Microtitre plates (Nunc, Roskilde, Denmark) were coated with the monoclonal antibody 2R1.2, washed, and blocked. All samples were assayed in duplicate for 2 h at 37°C. After washing, plates were incubated for 2 h with horseradish peroxidase-conjugated 7G7/B6 monoclonal antibody, which does not block IL-2 or anti-Tac binding to the IL-2R alpha chain.¹⁸ Bound antibody was detected using the substrate *O*-phenylenediamine and units of sIL-2R were determined from a standard curve of serial dilutions of supernatant from phytohaemagglutinin stimulated peripheral blood mononuclear cells (T Cell Sciences) defined as 1000 units/ml. Interassay variability was 7.12% and the limits of sensitivity 50 units/ml.

The laboratory parameters of platelet number and erythrocyte sedimentation rate were determined using standard techniques. C reactive protein, orosomucoid, and α_1 antitrypsin concentrations were measured by radial immun assay.¹⁹

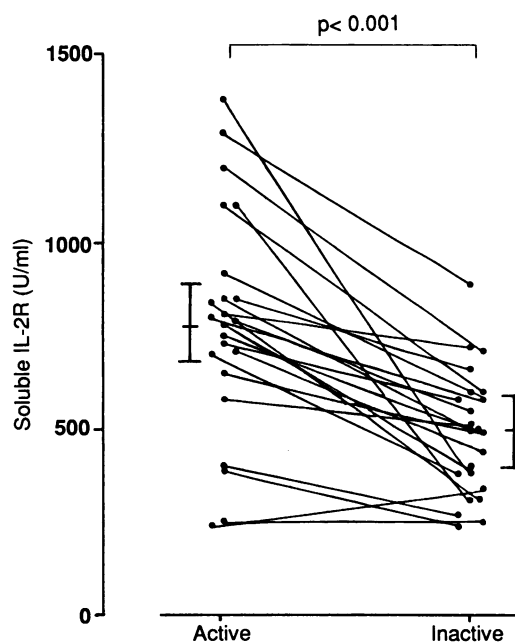


Figure 2: Serum sIL-2R concentrations in patients with Crohn's disease during active and inactive phases.

STATISTICAL ANALYSIS

Data are expressed as medians (SEM). Statistical comparisons were carried out using the Kruskal-Wallis test, Mann-Whitney U test, Wilcoxon's signed rank test for paired data, and Spearman rank correlation coefficient.

Results

Serum concentrations of sIL-2R in patients with active (Harvey-Bradshaw index 5–13) and inactive (0–4) Crohn's disease and control groups are shown in Figure 1. The median (SEM) serum concentration of sIL-2R in the group of 24 patients with active Crohn's disease (785 (61.9)) was significantly greater ($p < 0.001$) than that of healthy subjects (160 (22.9)) and disease control subjects (280 (21.5)). Five patients with active disease, however, had serum sIL-2R concentrations within the control range. The median serum sIL-2R concentrations in 29 patients with inactive Crohn's disease (460 (40.3)) was significantly lower ($p < 0.001$) than in patients with active disease, but greater than both control groups ($p < 0.01$). The serum sIL-2R concentrations in the disease control group were significantly greater ($p < 0.01$) than in the healthy control group.

Serum sIL-2R concentrations in patients with Crohn's disease examined during both active and inactive phases of disease activity are shown in Figure 2. Serum sIL-2R concentrations fell in all patients with high index scores of disease activity and raised sIL-2R concentrations who responded to treatment. In only two patients with a high clinical index, who were biochemically inactive (according to C reactive protein and erythrocyte sedimentation rate) was there no reduction in sIL-2R after reversion to a clinically inactive disease state.

There was a significant correlation between the serum sIL-2R concentrations and disease activity as assessed by the Harvey-Bradshaw index ($r = 0.42$, $p < 0.01$, $n = 53$) (Fig 3). In addition, there was a significant correlation between serum sIL-2R concentrations and platelet number ($r = 0.495$, $p < 0.01$, $n = 36$) and orosomucoid ($r = 0.474$, $p < 0.01$, $n = 45$), α_1 antitrypsin ($r = 0.44$, $p < 0.01$, $n = 44$), and C reactive protein concentrations ($r = 0.485$, $p < 0.001$, $n = 46$), but not with the erythrocyte sedimentation rate ($r = 0.23$, $n = 29$). The Table shows the range and median values for C reactive protein, α_1 antitrypsin, and orosomucoid in the patients with active and inactive Crohn's disease.

Discussion

There is considerable evidence that Crohn's disease is associated with immune activation both in the intestinal mucosa^{5, 20, 21} and systemically.⁶ Activation of T lymphocytes is accompanied by the cellular expression of the IL-2R (the Tac antigen) and, after immune stimulation in vitro, this receptor is shed from activated cells.¹⁰ The release of the IL-2R alpha chain, which occurs in proportion to its cell surface expression, allows assessment of in vivo immune activation by serological measurement of soluble IL-2R.

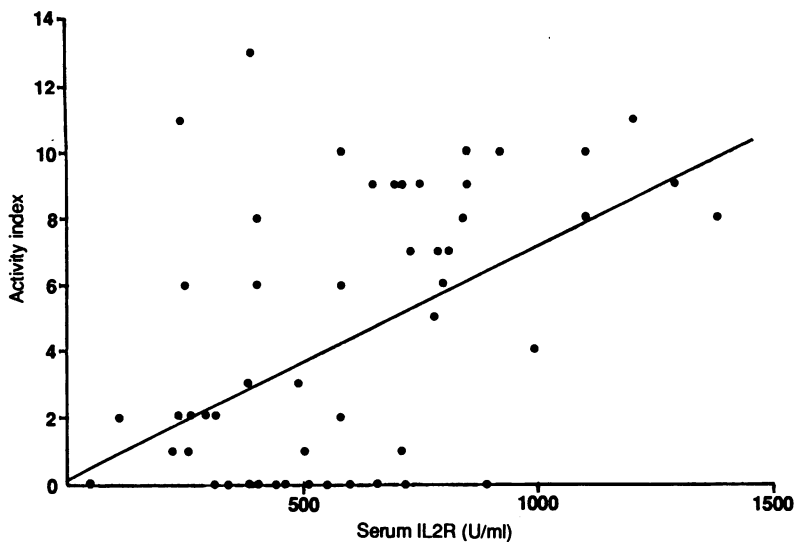


Figure 3: Relation between serum sIL-2R concentrations and disease activity as assessed by the Harvey-Bradshaw index ($r=0.42$, $p<0.01$).

The results of this study show that patients with active Crohn's disease have significantly raised serum sIL-2R concentrations and the circulating levels correlated well with disease activity as assessed by the Harvey-Bradshaw index. Even during inactive phases, sIL-2R values were raised in patients with Crohn's disease compared to control subjects. Although some studies have shown that clinical improvement in Crohn's disease is accompanied by a decrease in activated peripheral T cells expressing T9 (the transferrin receptor) and HLA/DR antigens,⁶ others have found persistence of early activation antigens on peripheral and intestinal mononuclear cells in quiescent phases of disease.⁵ The median serum sIL-2R concentration of the disease control patients was significantly greater than that of the healthy control group; however, no one in the former group had serum sIL-2R concentrations greater than 400 U/ml, the values being in the upper ranges of those of the healthy controls. The diagnosis in the disease control group was largely irritable bowel syndrome reached by excluding other serious disease.

Cellular expression of IL-2R is not restricted to cells of the T cell lineage, as activated B cells²² and activated macrophages²³ also express IL-2R. The cellular origin of the raised circulating sIL-2R concentrations in Crohn's disease is unclear. There seems to be no imbalance of immunoregulatory T cell populations in the intestinal mucosa in Crohn's disease^{24,25}; however, it has recently been shown that intestinal macrophages in inflammatory bowel disease express IL-2R.²⁶ Whether macrophages release IL-2R in a similar manner to activated B and T

cells²⁷ is unknown. The median (SEM) sIL-2R concentration in active Crohn's disease (785 (61.9)) was lower than that observed in untreated patients with coeliac disease (1090 (223)),¹⁶ but clearly both of these intestinal inflammatory conditions are associated with increased immune activation, as assessed by serum sIL-2R concentrations.

The lack of correlation of serum sIL-2R concentrations with the erythrocyte sedimentation rate is not altogether surprising since this is sometimes normal in patients with inflammatory bowel disease even when the disease is active.²⁸ Serum sIL-2R concentrations were correlated, however, with other laboratory indices of disease activity including platelets, C reactive protein, α_1 antitrypsin, and orosomucoid. This suggests that these parameters are directly related to the extent of immune activation and are not simply a reflection of the acute phase response. Recent studies have shown interleukin-6 to be a major mediator of the acute phase response.^{29,30} A relation between serum sIL-2R concentrations and platelet counts has also been observed in rheumatoid arthritis where sIL-2R concentrations were found to be an excellent monitor of clinical disease activity and to predict disease exacerbation.³¹

The results of this study suggest that serum sIL-2R measurements in patients with Crohn's disease may be a useful marker of disease activity, although current use is likely to be limited to clinical trials or research. Longitudinal studies could have a predictive value in relation to the onset of active phases of the disease.

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- Heatley RV, Smart CJ, Danis VA. Inflammatory bowel disease. In: Losowsky MS, Heatley RV, eds. *Gut defences in clinical practice*. Edinburgh: Churchill Livingstone, 1986: 255-77.
- Ferguson A. Why study T cell subsets in Crohn's disease? *Gut* 1983; 24: 687-91.
- Elson CO, Graeff AS, James SP, Strober W. Covert suppressor T cells in Crohn's disease. *Gastroenterology* 1981; 80: 1513-21.
- James SP, Neckers LN, Graeff AS, Cossman J, Balch CM, Strober W. Suppression of immunoglobulin synthesis by lymphocyte subpopulations in patients with Crohn's disease. *Gastroenterology* 1984; 86: 1510-8.
- Pallone F, Fais S, Squarcia O, Biancone L, Pozzilli P, Biorivanti M. Activation of peripheral blood and intestinal lamina propria lymphocytes in Crohn's disease. In vivo state of activation and in vitro response to stimulation as defined by the expression of early activation antigens. *Gut* 1987; 28: 745-53.
- Raedler A, Fraenkel S, Klose G, Seyfarth K, Thiele HG. Involvement of the immune system in the pathogenesis of Crohn's disease. *Gastroenterology* 1985; 88: 978-83.
- Smith KA. Interleukin-2. *Ann Rev Immunol* 1984; 2: 319-33.
- Leonard WJ, Depper JM, Robb RJ, Waldmann TA, Greene WC. Characterization of the human receptor for T cell growth factor. *Proc Natl Acad Sci USA* 1983; 80: 6957-61.
- Wang HM, Smith KA. The interleukin-2-receptor: functional consequences of its bimolecular structure. *J Exp Med* 1987; 166: 1055-69.
- 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.
- Symons JA, Wood NC, Di Giovine FS, Duff GW. Soluble IL-2 receptor in rheumatoid arthritis: correlation with disease activity, IL-1 and IL-2 inhibition. *J Immunol* 1988; 141: 2612-8.
- Lawrence EC, Berger MB, Brousseau KP, et al. Elevated serum levels of soluble interleukin-2 receptors in active pulmonary sarcoidosis: relative specificity and association with hypercalcemia. *Sarcoidosis* 1987; 4: 87-93.
- Josimovic-Alasevic O, Feldmeier H, Zwingerberger K, et al. Interleukin 2 receptor in patients with localized and systemic parasitic diseases. *Clin Exp Immunol* 1988; 72: 249-54.

Range and medians of acute phase reactants in patients with active and inactive Crohn's disease

	Inactive (n=24)	Active (n=23)
Orosomucoid (g/l)	0.67-3.45 (1.33)	0.39-4.58 (2.33)*
α_1 antitrypsin (g/l)	1.95-5.77 (3.78)	1.99-10.3 (4.68)*
C reactive protein (mg/l)	<10-13 (10)	<10-137 (22)†

* $p<0.01$; † $p<0.001$.

- 14 Colver GB, Symons JA, Duff GW. Soluble interleukin 2 receptor in atopic eczema. *Br Med J* 1989; 298: 1426-8.
- 15 Adams DH, Wang L, Hubscher SG, Elias E, Neuberger JM. Soluble interleukin-2-receptors in serum and bile of liver transplant recipients. *Lancet* 1989; i: 469-71.
- 16 Crabtree JE, Heatley RV, Juby LD, Howdle PD, Losowsky MS. Serum interleukin-2-receptor in coeliac disease: response to treatment and gluten challenge. *Clin Exp Immunol* 1989; 77: 345-8.
- 17 Harvey RF, Bradshaw JM. A simple index of Crohn's disease activity. *Lancet* 1980; i: 514.
- 18 Rubin LA, Kurman CC, Biddison WE, Goldman ND, Nelson DL. A monoclonal antibody 7G7/B6, that binds to an epitope on the human IL-2 receptor distinct from that recognised by IL-2 or anti-Tac. *Hybridoma* 1985; 4: 91-102.
- 19 Ingild A. Single radial immunodiffusion. *Scand J Immunol* 1983; 17: 41-56.
- 20 Mahida YR, Wu K, Jewell DP. Enhanced production of interleukin 1-beta by mononuclear cells isolated from mucosa with active ulcerative colitis or Crohn's disease. *Gut* 1989; 30: 835-8.
- 21 Mahida YR, Patel S, Gionchetti P, Vaux D, Jewell DP. Macrophage subpopulations in lamina propria of normal and inflamed colon and terminal ileum. *Gut* 1989; 30: 826-34.
- 22 Waldmann TA, Goldman CK, Robb RJ, et al. Expression of interleukin-2 receptors on activated B cells. *J Exp Med* 1984; 160: 1450-66.
- 23 Hancock WW, Muller WA, Cotran RS. Interleukin-2 receptors are expressed by alveolar macrophages during pulmonary sarcoidosis and are inducible by lymphokine treatment of normal human lung macrophages, blood monocytes, and monocyte cell lines. *J Immunol* 1987; 138: 185-91.
- 24 Selby WS, Janossy G, Bofill M, Jewell DP. Intestinal lymphocyte subpopulations in inflammatory bowel disease: an analysis by immunohistological and cell isolation techniques. *Gut* 1984; 25: 32-40.
- 25 James SP, Fiocchi C, Graeff AS, Strober W. Phenotypic analysis of lamina propria lymphocytes: predominance of helper-inducer and cytolytic T-cell phenotypes and deficiency of suppressor-inducer phenotypes in Crohn's disease and control patients. *Gastroenterology* 1986; 91: 1483-9.
- 26 Mahida YR, Patel S, Wu K, Jewell DP. Interleukin 2 receptor expression in inflammatory bowel disease. *Clin Exp Immunol* 1988; 74: 382-6.
- 27 Nelson DL, Rubin LA, Kurman CC, Fritz ME, Boutin B. An analysis of the cellular requirements for the production of soluble interleukin-2 receptors in vitro. *J Clin Immunol* 1986; 6: 114-9.
- 28 Bouchier IAD, Allen RN, Hodgson HJF, Keighley MRB. *Textbook of gastroenterology*. London: Baillière Tindall, 1984: 940-6.
- 29 Ramadori G, Van Damme J, Rieder H, Meyer zum Buschenfelde KH. Interleukin 6, the third mediator of acute phase reaction, modulates hepatic protein synthesis in human and mouse. Comparison with interleukin 1 beta and tumor necrosis factor-alpha. *Eur J Immunol* 1988; 18: 1259-64.
- 30 Andus T, Geiger T, Hirano T, Kishimoto T, Heinrich PC. Action of recombinant human interleukin 6, interleukin 1 beta and tumor necrosis factor alpha on the mRNA induction of acute-phase proteins. *Eur J Immunol* 1988; 18: 739-46.
- 31 Wood NC, Symons JA, Duff GW. Serum interleukin-2-receptor in rheumatoid arthritis: a prognostic indicator of disease activity? *J Autoimmunity* 1988; 1: 353-61.