Clin. exp. Immunol. (1989) 77, 345–348

Serum interleukin-2-receptor in coeliac disease: response to treatment and gluten challenge

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(Accepted for publication 2 June 1989)

SUMMARY

Concentrations of the soluble interleukin-2 receptor (sIL-2R) in the serum of 33 patients with coeliac disease were measured by ELISA. The levels of sIL-2R were significantly raised in 15 patients with untreated coeliac disease compared with treated patients and age- and sex-matched symptomatic and non-symptomatic control groups. Longitudinal studies in individual coeliac patients showed that serum sIL-2R fell following commencement of a gluten-free diet. Gluten challenge of 16 treated coeliac patients for 1 week resulted in a significant increase in serum sIL-2R, which returned to pre-challenge levels within 4 weeks of recommencement of a gluten-free diet. We suggest that serum sIL-2R levels in patients with coeliac disease reflect specific immunological activation in response to gluten ingestion. Measurement of serum sIL-2R may therefore be useful in the assessment of response to treatment in patients with coeliac disease.

Keywords coeliac disease interleukin-2-receptor gluten T lymphocytes

INTRODUCTION

Activation of T lymphocytes with specific antigen or mitogens induces the synthesis of interleukin-2 (IL-2) and the expression of the specific cell surface receptors for this molecule (IL-2R) (Leonard *et al.*, 1983; Smith, 1984). The binding of IL-2 via its high-affinity receptor results in T lymphocyte proliferation and a cascade of immunological responses (Smith, 1984). Expression of IL-2R is not restricted to the T cell lineage. Although IL-2R have been demonstrated on activated macrophages (Hancock, Muller & Cotran, 1987; Mahida, Patel & Jewell, 1988) and activated B cells (Waldmann *et al.*, 1984), they occur at considerably lower densities than on activated T lymphocytes (Waldmann *et al.*, 1984).

The human receptor for IL-2 is composed of at least two polypeptide chains with different binding affinities (Wang & Smith, 1987). *In vitro* studies have shown that the low-affinity, smaller 55 000 M_r alpha chain of the IL-2R receptor (the 'Tac' protein) is shed from activated cells in soluble form (sIL-2R) (Rubin *et al.*, 1985a). sIL-2R is detectable in the serum of healthy individuals, but concentrations of circulating sIL-2R are elevated in certain disease conditions characterized by increased immune activation such as rheumatoid arthritis (Symons *et al.*, 1988), graft rejection (Colvin *et al.*, 1987), chronic dermatological inflammatory conditions (Colver, Symons & Duff, 1989) and lymphoreticular malignancies (Nelson, 1986). Recent studies have shown serum concentrations of sIL-2R to be an excellent monitor of disease activity in rheumatoid arthritis (Wood, Symons & Duff, 1988).

Coeliac disease is characterized by lymphocytic and plasma cell infiltrates in the small intestinal mucosa which resolve on gluten exclusion (Howdle & Losowsky, 1986). There is immunohistological (Malizia *et al.*, 1985; Kelly *et al.*, 1987) and functional (Ferguson *et al.*, 1975; Howdle, Bullen & Losowsky, 1982; Crabtree, Heatley & Losowsky, 1989) evidence of enhanced mucosal lymphocyte activity in patients with untreated coeliac disease, and several systemic immunological abnormalities have been reported (reviewed by Strober, 1985). As sIL-2R appears to be a marker of immune activation, we have examined patients with coeliac disease before and after gluten exclusion to observe whether changes in serum sIL-2R levels are an indicator of response to treatment. We have additionally examined the effect of gluten challenge on serum sIL-2R concentrations in patients with treated coeliac disease.

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MATERIALS AND METHODS

Patients

Thirty-three patients with coeliac disease were studied. Jejunal tissue was obtained using a Crosby capsule or a Quinton hydraulic multiple biopsy instrument. Peripheral blood samples were taken at the time of the investigative procedure and sera were stored at −70°C until assayed. Fifteen patients were untreated (eight women, seven men; mean age 41·4 years ± 3·5 s.e.m.; range 17–69) and on normal diets at the time of biopsy.

The mucosa of 14 patients showed subtotal villous atrophy on histological examination and one patient had severe partial villous atrophy. Thirteen of the untreated patients have, to date, shown histological improvement on gluten-free diets. Two patients are yet to be rebiopsied. The results of one additional patient, who presented with subtotal villous atrophy and showed symptomatic but no histological improvement after 20 months on a gluten-free diet are included for comparative purposes. Four relapsed coeliac patients, with poor dietary compliance, were also examined (three women, one man; ages 15, 23, 41, and 58, respectively), who all presented with subtotal villous atrophy. These patients had previously shown improved histology on gluten exclusion. Twenty-four treated coeliac patients (15 women, nine men; mean age 39·2 ± 2·6; range 17–69) were studied. All had been on gluten-free diets for a minimum period of 2 months and had shown histological improvement following gluten exclusion. Biopsies from seven treated patients were histologically normal, 15 showed partial villous atrophy and two severe partial villous atrophy. Ten of the 24 treated coeliac patients were among those investigated while on a normal diet. The length of time on a gluten-free diet in the patients examined both before and after treatment varied from 2 to 14 months (mean duration 6·2 months).

Control subjects

Two groups of control subjects were examined. A symptomatic control group (mean age 42·6 ± 3·5) consisted of 15 patients age- and sex-matched to the untreated coeliac patients, who had a jejunal biopsy to exclude coeliac disease. The jejunal mucosa was normal on histological examination and no other intestinal inflammatory conditions were demonstrated on further investigation. Patients with rheumatoid arthritis, psoriasis and atopic eczema (Symons *et al.*, 1988; Colver *et al.*, 1989) were excluded. The eventual diagnoses of patients in the symptom control group were irritable bowel syndrome/motility disorder (10 subjects), lactase deficiency (one), diverticular disease (one), rectal polyp (one) and hypothyroidism (one). An additional control group of 15 sex- and age-matched, non-symptomatic healthy volunteers (mean age 42·7 ± 3·5) was also studied. The project was approved by the Clinical Research (Ethics) Committees of the Health Districts and informed consent was obtained from all patients and control subjects.

Gluten challenge of treated coeliac patients

Sixteen additional patients (mean age 48·6 ± 3·9; range 17–73) with histologically proven coeliac disease (an abnormal jejunal biopsy with morphological improvement on a gluten-free diet), who had been on gluten-free diets for a minimum of 6 months, were given 30 g of gluten powder (BDH, Poole, UK) daily for 7 days. Blood samples were taken immediately before commencement of gluten challenge, after 7 days of gluten ingestion and after a further 4 weeks following re-instatement of a gluten-free diet. This study was part of a wider research protocol, which was approved by the local Clinical Research (Ethics) Committee.

ELISA for serum sIL-2R

Serum sIL-2R levels were determined by an ELISA (T Cell Sciences Inc., Cambridge, MA) using two non-competing monoclonal antibodies (Rubin *et al.*, 1985b) to the alpha chain of the IL-2R, as previously described (Symons *et al.*, 1988). Briefly, microtitre plates (Nunc, Roskilde, Denmark) were coated with the monoclonal antibody 2R1.2, washed and blocked. Samples were assayed in duplicate for 2 h at 37°C. After washing, the plates were incubated for a further 2 h with horseradish peroxidase-conjugated 7G7/B6 monoclonal antibody. 7G7/B6 does not block IL-2 or anti-Tac binding to the IL2-R alpha chain (Rubin *et al.*, 1985b) and recognizes an epitope distinct from 2R1.2. After further washing, bound peroxidase-conjugated antibody was detected using the substrate *O*-phenylenediamine. The reaction was quenched after 30 min incubation at room temperature with 1 M H_2SO_4 and the absorbance was measured at 490 nm. Units of sIL-2R were calculated from a standard curve obtained by assaying serial dilutions of a supernatant from phytohaemagglutinin (PHA) stimulated peripheral blood mononuclear cells (T Cell Sciences) defined as 1000 U/ml. Mean inter-assay variability was 7·12%.

Statistical analysis

Data are expressed as means ± s.e.m. Analysis of variance was carried out using the Kruskall–Wallis test. Statistical comparisons between mean values of unpaired data were carried out using the Mann–Whitney *U*-test for non-parametric data. A Wilcoxon's signed rank test was used to analyse paired data.

RESULTS

Serum concentrations of sIL-2R in patients with untreated and treated coeliac disease and symptomatic and non-symptomatic control groups are shown in Fig. 1. The mean serum concentration of sIL-2R in untreated coeliac patients (1324 ± 223) was significantly greater ($P < 0.001$) than that of age-matched control patients who had normal jejunal histology (249 ± 22·4) and non-symptomatic controls (186 ± 25·9). The mean serum levels of sIL-2R in treated coeliac patients (519 ± 60·3) following gluten exclusion was significantly lower ($P < 0.001$) than that of untreated patients, but greater than that of both the symptom controls and healthy control group ($P < 0.001$). There was no significant difference between the sIL-2R levels in the two control groups. The mean serum sIL-2R value in the group of four relapsed coeliac patients (515 ± 59), whose jejunal histology showed subtotal villous atrophy following previous histological improvement, was significantly lower ($P < 0.05$) than that of

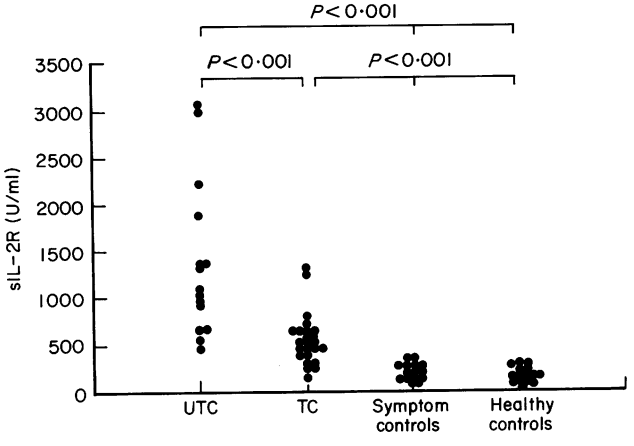

Fig. 1. Serum sIL-2R levels in untreated (UTC) and treated (TC) coeliac patients and symptomatic and healthy controls.

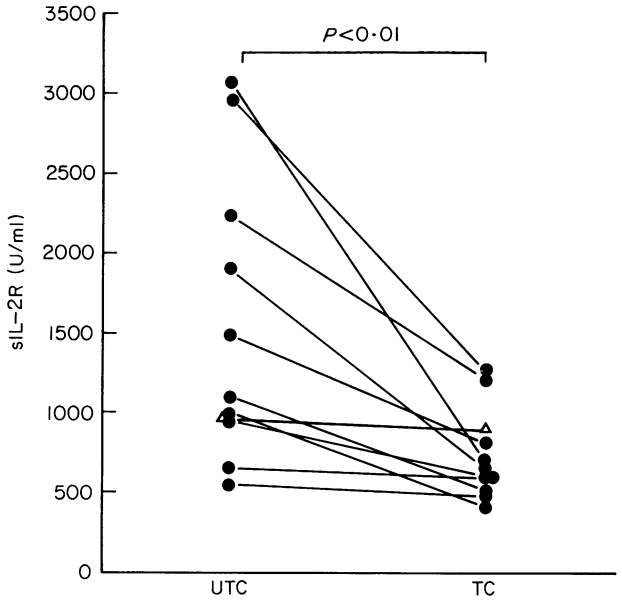

Fig. 2. Serum sIL-2R in patients with coeliac disease before (UTC) and after (TC) gluten exclusion. △, the patient with subtotal villous atrophy who showed no histological response to a gluten free diet.

newly presenting coeliac patients, but was greater than both control groups ($P < 0{\cdot}01$).

The serum sIL-2R values of coeliac patients examined before and after gluten exclusion are shown in Fig. 2. The serum sIL-2R concentration fell significantly on treatment ($P < 0{\cdot}01$). One patient showed a 56% reduction in serum sIL-2R values after only 2 months on a gluten-free diet. In one patient who initially presented with subtotal villous atrophy but showed no histological improvement over a 20-month period on a gluten-free diet, no reduction in serum sIL-2R values was observed (Fig. 2).

The effects of ingestion of 30 g gluten/day for 1 week on serum sIL-2R levels in 16 patients with treated coeliac disease are illustrated in Fig. 3. The mean serum sIL-2R level after gluten ingestion for 1 week ($498{\cdot}8 \pm 64{\cdot}7$) was significantly

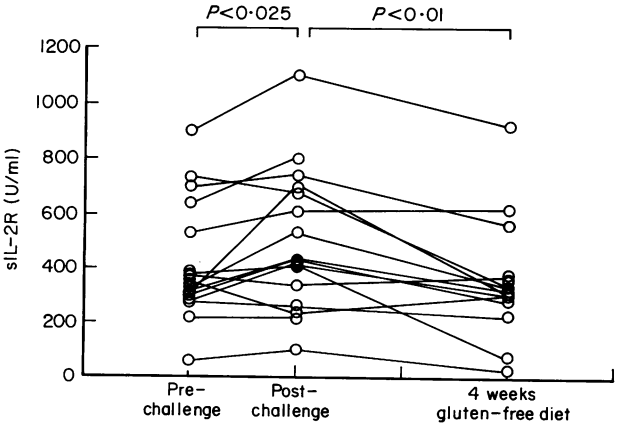

Fig. 3. Serum sIL-2R levels following gluten challenge in treated coeliac patients.

greater ($P < 0{\cdot}025$) than that at the commencement of the study ($423 \pm 54{\cdot}5$). Ten of the 16 patients showed an increase in sIL-2R levels greater than 10% of pre-challenge values and in only one patient was there a comparable reduction in sIL-2R value. Following the 1 week of gluten challenge, reversion to a gluten-free diet for 4 weeks resulted in a significant reduction ($P < 0{\cdot}01$) in serum sIL-2R values (Fig. 3), with the mean 5 week sIL-2R level ($367 \pm 58{\cdot}8$) being lower, but not significantly different than the pre-challenge value.

DISCUSSION

Although the pathogenesis of coeliac disease is unclear, there is evidence of altered immunological responses in untreated patients (reviewed in Strober, 1985). It is not known whether such changes are primary or secondary phenomena in coeliac disease but recent studies with fetal intestinal tissue suggest that T lymphocytes may have a primary role in enteropathy (MacDonald & Spencer, 1988). A central event in antigen-induced activation of T lymphocytes is the secretion of IL-2 and the expression of cell surface IL-2R to permit clonal expansion of antigen specific T cells. The shedding of the IL-2R (Rubin *et al.*, 1985a), which occurs in proportion to their cell surface expression, permits evaluation of *in vivo* immune activation by serological measurement of the sIL-2R.

The results of this study show that untreated coeliac patients have significantly elevated serum sIL-2R concentrations. The raised concentrations in untreated patients clearly differentiated such patients from treated patients and control groups. The significant reduction in serum sIL-2R following treatment in patients with coeliac disease, examined over time, which mirrored histological improvement of the jejunal mucosa, suggests that measurement of sIL-2R levels following gluten exclusion may be a useful adjunct in the monitoring of treatment.

To establish whether the raised levels observed in untreated patients in this study result from long-term immune stimulation and to permit kinetic analysis of cellular activation *in vivo* to a specific antigen, we examined the effects of gluten challenge on serum sIL-2R levels in a group of treated coeliac patients. After only 1 week of gluten ingestion there was a significant increase in sIL-2R levels. However, the values did not increase to those observed in the untreated coeliac patients, suggesting that the high levels of sIL-2R observed in untreated patients are a result of long-term immunostimulation. Clearly, if T cell-mediated events are a primary phenomenon in inducing coeliac disease as recently postulated (MacDonald & Spencer, 1988), variable levels of T cell activation as measured by serum sIL-2R concentrations result in the pathological changes characteristic of coeliac disease. The significant decrease in sIL-2R values following a 4-week period on a gluten-free diet in the challenge patient group demonstrates a rapid response to gluten exclusion. The reduction to below pre-challenge values could be a result of improved dietary compliance following closer medical supervision (Anon., 1988).

In vitro studies on the kinetics of IL-2R expression by T lymphocytes have shown that receptor expression occurs more rapidly in primed cell populations (Cantrell & Smith, 1983). A rapid *in vivo* response to immune stimulation with gluten by pre-sensitized lymphocytes, therefore, would account for the serological increase in IL-2R after gluten ingestion for 1 week.

Challenge with gluten has been shown to induce a rapid infiltration of T lymphocytes into the jejunal epithelium in patients with treated coeliac disease (Freedman *et al.*, 1987). Following antigen exposure, the receptor for IL-2R is only transiently expressed and the shedding of the receptor is considered to be an important mechanism by which the specificity of the immune response is maintained (Reske-Kunz *et al.*, 1984; Rubin *et al.*, 1985a).

The source of the high serum levels of sIL-2R in untreated coeliac patients is unclear. Although increased immunolabelling of cells with anti-Tac (a monoclonal antibody specific for IL-2R) has been observed in jejunal mucosa of untreated coeliac patients relative to control patients (Kelly *et al.*, 1987), the levels of expression on T lymphocytes are low, relative to the Ta-1 marker of immune activation (Kelly *et al.*, 1987) and the CD7 marker of stimulation (Malizia *et al.*, 1985). A possible explanation for the low expression of IL-2R on mucosal cells is that with chronic immunostimulation, the rapid release of IL-2R is a protective mechanism to prevent excessive T cell proliferation. One possible source of the serum sIL-2R could be the mesenteric lymph nodes. In patients with sarcoidosis, thoracic lymph node cells label strongly with anti-Tac (Hancock *et al.*, 1986).

Whatever the source of sIL-2R in patients with coeliac disease, the results of this study are supportive evidence for the role of T lymphocytes in the pathogenesis of coeliac disease (Ferguson *et al.*, 1975) and measurement of serum sIL-2R appears a simple, non-invasive means of assessment of immunological activation to gluten in patients with this disorder.

ACKNOWLEDGMENTS

This work was carried out with funding from the Medical Research Council. We would particularly like to thank Dr A. Manning, Dr J. Findlay, Sister M. Edwards and the staff of the Gastroenterology Department of Bradford Royal Infirmary and Dr A. T. R. Axon of Leeds General Infirmary for their cooperation, Dr L. K. Trejdosiewicz for valuable discussions and Miss Sarah Walker-Merangh for her excellent technical assistance.

REFERENCES

ANON. (1988) Growing up with coeliac disease. *Lancet*, **ii,** 1231.

CANTRELL, D.A. & SMITH, K.A. (1983) Transient expression of interleukin 2 receptors, consequences for T cell growth. *J. exp. Med.* **158,** 1895.

COLVIN, R.B., FULLER, T.C., MACKEEN, L., KUNG, P.C., IP, S.H. & COSIMI, A.B. (1987) Plasma interleukin-2 receptor levels in renal allograft recipients. *Clin. Immunol. Immunopathol.* **43,** 273.

COLVER, G., SYMONS, J.A. & DUFF, G.W. (1989) Soluble interleukin-2 receptor in atopic eczema. *B. med. J.* **298,** 142.

CRABTREE, J.E., HEATLEY, R.V. & LOSOWSKY, M.S. (1989) Immunoglobulin secretion by isolated intestinal lymphocytes: spontaneous production and T-cell regulation in normal small intestine and in patients with coeliac disease. *Gut*, **30,** 347.

FERGUSON, A., MACDONALD, T.T., MCCLURE, J.P. & HOLDEN, R.J. (1975) Cell mediated immunity to gliadin within the small intestinal mucosa in coeliac disease. *Lancet*, **i,** 895.

FREEDMAN, A.R., MACCARTNEY, J.C., NELUFER, J.M. & CICLITIRA, P.J. (1987) Timing of infiltration of T lymphocytes induced by gluten into the small intestine in coeliac disease. *J. clin. Pathol.* **40,** 741.

HANCOCK, W.W., KOBZIK L., COLBY, A.J., O'HARA, C.J., COOPER, A.G. & GODLESKI, J.J. (1986) Detection of lymphokines and lymphokine receptors in pulmonary sarcoidosis: immunohistologic evidence that inflammatory macrophages express IL-2 receptors. *Am. J. Pathol.* **123,** 1.

HANCOCK, W.W., MULLER, W.A. & COTRAN, R.S. (1987) 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.* **138,** 185.

HOWDLE, P.D. & LOSOWSKY, M.S. (1986) Coeliac Disease. In *Gut Defences in Clinical Practice* (ed. by M.S. Losowsky & R.V. Heatley) p. 243. Churchill Livingstone, Edinburgh.

HOWDLE, P.D., BULLEN, A.W. & LOSOWSKY, M.S. (1982) Cell mediated immunity to gluten within the small intestinal mucosa in coeliac disease. *Gut*, **23,** 115.

KELLY, J., O'FARRELLY, C., O'MAHONY, C., WEIR, D.G. & FEIGHERY, C. (1987) Immunoperoxidase demonstration of the cellular composition of the normal and coeliac small bowel. *Clin. exp. Immunol.* **68,** 177.

LEONARD, W.J., DEPPER, J.M., ROBB, R.J., WALDMANN, T.A. & GREENE, W.C. (1983) Characterization of the human receptor for T cell growth factor. *Proc. natl. Acad. Sci. USA*, **80,** 6957.

MACDONALD, T.T. & SPENCER, J. (1988) Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. *J. exp. Med.* **67,** 1341.

MAHIDA, Y.R., PATEL, S., WU, K. & JEWELL, D.P. (1988) Interleukin-2 receptor expression by macrophages in inflammatory bowel disease. *Clin. exp. Immunol.* **74,** 382.

MALIZIA, G., TREJDOSIEWICZ, L.K., WOOD, G.M., HOWDLE, P., JANOSSY, G. & LOSOWSKY, M.S. (1985) The microenvironment of coeliac disease: T cell phenotypes and expression of the T2 'T blast' antigen by small bowel lymphocytes. *Clin. exp. Immunol.* **60,** 437.

NELSON, D.L. (1986) Expression of a soluble form of the interleukin-2 receptor in normal and neoplastic states, pp 565. In (Greene, W.C., moderator) The human interleukin-2 receptor: normal and abnormal expression in T cells and in leukemias induced by the human T-lymphotrophic retroviruses. *Ann. intern. Med.* **105,** 560.

RESKE-KUNZ, A.B., VON STELDERN, D., RUDE, E., OSAWA, H. & DIAMANTSTEIN, T. (1984) Interleukin 2 receptors on an insulin-specific T cell line: dynamics of receptor expression. *J. Immunol.* **133,** 1356.

RUBIN, L.A., KURMAN, C.C., FRITZ, M.E., BIDDISON, W.E., BOUTON, B., YARCHOAN, R. & NELSON, D.L. (1985a) Soluble interleukin-2 receptors are released from activated human lymphoid cells *in vitro*. *J. Immunol.* **135,** 3172.

RUBIN, L.A., KURMAN, C.C., BIDDISON, W.E., GOLDMAN, N.D. & NELSON, D.L. (1985b) A monoclonal antibody 7G7/B6, that binds to an epitope on the human IL2 receptor distinct from that recognised by IL-2 or anti-Tac. *Hybridoma*, **4,** 91.

SMITH, K.A. (1984) Interleukin-2. *Annu. Rev. Immunol.* **2,** 319.

STROBER, W. (1985) Gluten sensitive enteropathy, an abnormal immunologic response of the gastrointestinal tract to a dietary protein. In *Gastrointestinal Immunity for the Clinician* (ed. by R.G. Shortner & J.B. Kirsner) p. 75. Grove & Stratton, Orlando.

SYMONS, J.A., WOOD, N.C., DI GIOVINE, F.S. & DUFF, G.W. (1988) Soluble IL-2 receptor in rheumatoid arthritis: correlation with disease activity, IL-1 and IL-2 inhibition. *J. Immunol.* **141,** 2612.

WALDMANN, T.A., GOLDMAN, C.K., ROBB, R.J., DEPPER, J.M., LEONARD, W.J., SHARROW, S.O., BONGIOVANNI, K.F., KORSMEYER, S.J. & GREENE, W.C. (1984) Expression of interleukin-2 receptors on activated B cells. *J. exp. Med.* **160,** 1450.

WANG, H.M. & SMITH, K.A. (1987) The interleukin-2 receptor: functional consequences of its bimolecular structure. *J. exp. Med.* **166,** 1055.

WOOD, N.C., SYMONS, J.A. & DUFF, G.W. (1988) Serum interleukin-2-receptor in rheumatoid arthritis: a prognostic indicator of disease activity? *J. Autoimmunity*, **1,** 353.