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Plasma and tissue interleukin-2 receptor levels in inflammatory bowel disease

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SUMMARY

Plasma and tissue interleukin-2 receptor (IL-2R) levels were determined in patients with active ulcerative colitis and Crohn's disease. Compared with healthy controls (median 440 U/ml; range 240–900), significantly higher levels of plasma IL-2R were present in patients with active ulcerative colitis (median 1180 U/ml; range 580–7150; $P < 0.002$) and Crohn's disease (median 1340 U/ml; range 480–9000; $P < 0.002$). Compared with other laboratory parameters, plasma IL-2R levels were related most closely to clinical score of disease activity in Crohn's disease. Plasma IL-2R levels also reflected the clinical course and may provide a more accurate assessment of disease activity in Crohn's disease. In plasma of patients undergoing intestinal resection of active inflammatory bowel disease, raised levels of IL-2R were present in samples from mesenteric vein (draining inflamed intestine) compared with those from peripheral vein. In tissue homogenates of colonic biopsies, significantly higher levels of IL-2R were present in specimens from colons with active ulcerative colitis compared with healthy controls (median 230·2, range 20·7–581·5 *versus* 77·9, range 34·2–291·3; $P < 0.02$).

Keywords interleukin-2 receptors inflammatory bowel disease

INTRODUCTION

Interleukin-2 (IL-2) is a polypeptide which is synthesized and secreted by T lymphocytes after activation by antigen or mitogen. It acts as a growth factor for mature T cells and thymocytes, induces T lymphocyte cytotoxicity and stimulates natural killer cell activity (O'Garra, 1989). It exerts its effects by interacting with specific receptors which have been demonstrated on activated T cells, B cells and monocytes. Both high- and low-affinity receptors have been identified (Trowbridge, 1987). High-affinity receptors are composed of two polypeptide chains of molecular weight 55 kD (alpha chain) and 75 kD (beta chain). The alpha chain of the IL-2 receptor (IL-2R) can be shed from the cell surface and detected in the blood (Rubin *et al.*, 1984). It has proved to be useful in the assessment of *in vivo* immune activation (Duff, 1989). In rheumatoid arthritis (Wood, Symons & Duff, 1988) and atopic eczema (Colver, Symons & Duff, 1989) longitudinal studies have shown that blood IL-2R levels can predict improvement and exacerbations in clinical disease activity.

There is an increase in mucosal lymphocyte and macrophage populations in active ulcerative colitis and Crohn's disease. Increased number of intestinal cells expressing IL-2Rs have been demonstrated (Mahida, Patel & Jewell, 1988). Increased circulating numbers of T cells bearing activation antigens in active Crohn's disease have also been shown (Pallone *et al.*, 1987; Raedler *et al.*, 1985). We have therefore assessed the value of measuring IL-2R in the assessment of inflammatory bowel disease. Levels of IL-2R in plasma as well as in homogenates of colonic biopsies from patients with active inflammatory bowel disease were studied. The correlation of plasma IL-2R levels with the disease activity and clinical course has also been investigated.

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PATIENTS AND METHODS

Patients

Fourteen patients with active ulcerative colitis and 15 with active Crohn's disease (six ileal, five ileo-colonic and four colonic) were studied.

Eight patients with inflammatory bowel disease were on oral steroids, seven were on sulphasalazine or mesalazine and six were on azathioprine.

In three patients with Crohn's disease, samples were also obtained following treatment.

Seventeen healthy volunteers served as controls.

Plasma

Ten millilitres of venous blood were collected in EDTA and 0·3 ml aprotinin (Sigma), kept in ice, and within 10 min centrifuged at 400 *g* for 10 min. Supernatant fluid was then centrifuged at

2000 *g* for 10 min to remove platelets. Aliquots were frozen at −70°C until used for assay.

Blood was also obtained for full blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and α1 acid glycoprotein. CRP was measured by latex-enhanced immunoassay (O'Callaghan *et al.*, 1984). Alpha-1 acid glycoprotein levels were measured using PEG-enhanced immunoturbudimetric assay.

In 10 patients, peripheral and mesenteric venous blood, obtained at the time of intestinal resection for active ulcerative colitis (five) or Crohn's disease (five), was studied. All these patients were on intravenous steroids at the time of the operation.

Assessment of disease activity

Clinical score of disease activity was obtained for patients with inflammatory bowel disease. For Crohn's disease, modified Harvey–Bradshaw index was used (Harvey & Bradshaw, 1980) and for ulcerative colitis, a scoring system modified from Powell-Tuck, Bown & Lennard-Jones (1978) was used. For both, active disease was defined as a clinical score of 5 or more and/or raised levels of CRP and α1 acid glycoprotein.

Colonic biopsies

Colonic biopsies (obtained at colonoscopy) from patients with active ulcerative colitis (13) or normal colons (eight) were also studied. Ulcerative colitis colonic mucosa had grade 2–3 inflammation (Baron, Connell & Lennard-Jones, 1964) and the presence of inflammation was confirmed by routine histology on a biopsy obtained at the same time. Normal colonic biopsies were obtained from patients undergoing investigations for gastrointestinal symptoms in whom no endoscopic or histological abnormality of the colon was found. The biopsies were homogenized (for 1 min in ultra turrax homogenizer) and supernatant obtained by centrifugation. The amount of IL-2R present in the supernatants was assayed and expressed as units of IL-2R per mg tissue.

Assay of IL-2R

IL-2R level were measured using a commercial double-antibody sandwich ELISA (T Cell Sciences). The microtitre plates were coated with mouse monoclonal antibody (IgG1) to IL-2R. IL-2R in samples was bound by a second, horseradish peroxidase-conjugated, mouse monoclonal anti-IL-2R antibody (IgG2a). Units of IL-2R in samples were calculated from a standard curve constructed using supernatant from PHA-stimulated peripheral blood mononuclear cells.

In our laboratory, inter- and intra-assay coefficient of variation was less than 10%.

Statistical analysis

The Mann–Witney *U*-test and Wilcoxon paired test were used for analysis. For correlation, Spearman's rank correlation (r_s) was used.

RESULTS

Plasma levels

Compared with controls (median 440 U/ml, range 240–900), significantly higher levels of IL-2R were present in plasma of patients with active ulcerative colitis (median 1180 U/ml, range 580–7150; $P<0{\cdot}002$) and Crohn's disease (median 1340 U/ml, range 480–9000; $P<0{\cdot}002$) (Fig. 1).

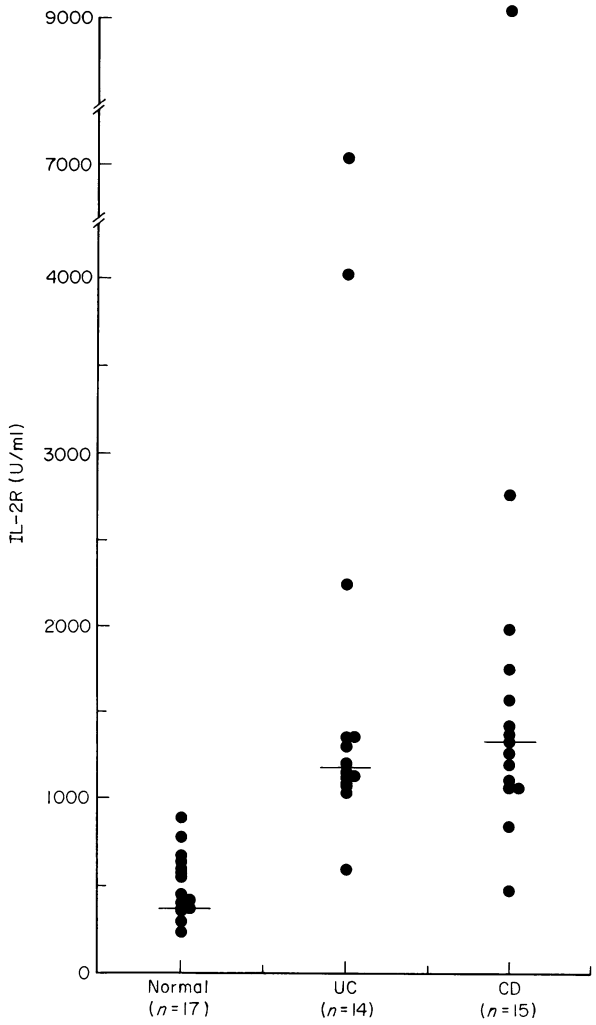

Fig. 1. Plasma IL-2R levels in normal controls and patients with active ulcerative colitis (UC) and Crohn's disease (CD).

Relation to disease activity

Ulcerative colitis. There was significant correlation between plasma IL-2R levels and CRP ($r_s=0{\cdot}82$; $P=0{\cdot}004$; Fig. 2). There were no significant correlations between plasma IL-2R levels and clinical score ($r_s=0{\cdot}33$; $P=0{\cdot}25$) or α1 acid glycoprotein levels ($r_s=0{\cdot}39$; $P=0{\cdot}26$).

The clinical score of activity correlated with CRP ($r_s=0{\cdot}77$; $P=0{\cdot}01$) and α1 acid glycoprotein ($r_s=0{\cdot}68$; $P=0{\cdot}031$) levels but not platelet count or ESR.

All patients, except one with active ulcerative colitis, had plasma IL-2R levels of > 1000 U/ml. The one patient with low IL-2R level (580 U/ml) had normal CRP and α1 acid glycoprotein levels. In one patient with active ulcerative colitis, the CRP, platelet count and α1 acid glycoprotein levels were normal but the IL-2R level was 1040 U/ml.

Crohn's disease. Correlation between IL-2R levels and clinical score of activity fell short of statistical significance ($r_s=0{\cdot}46$; $P=0{\cdot}08$). There were no significant correlations

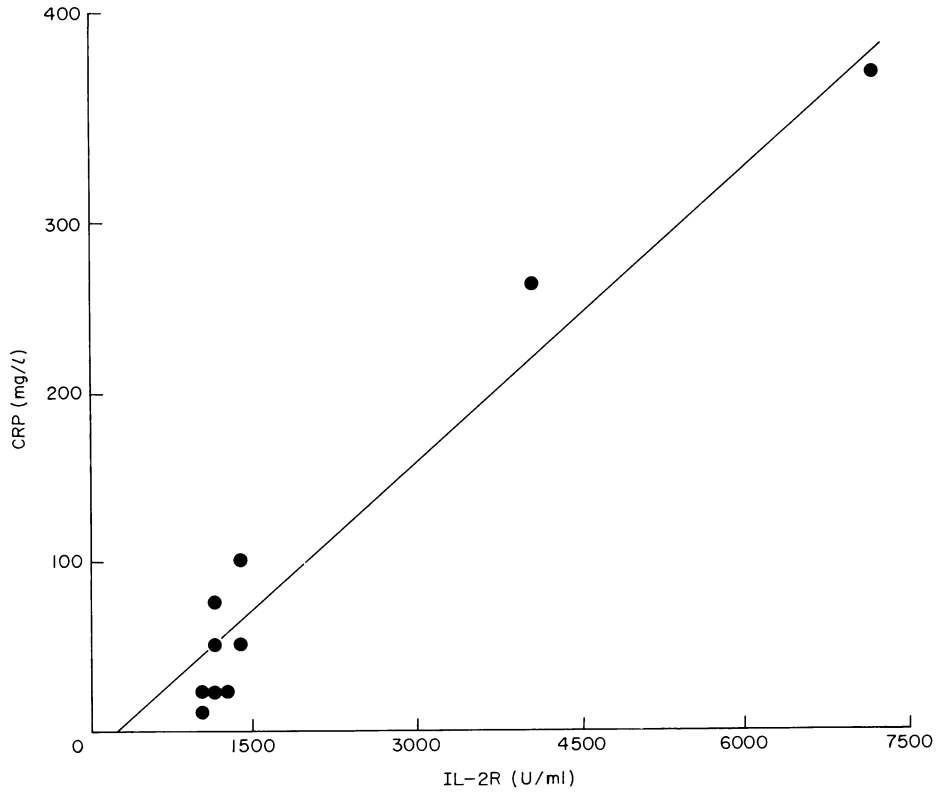

Fig. 2. Correlation between plasma IL-2R levels and C-reactive protein (CRP) ($r_s = 0{\cdot}82$; $P = 0{\cdot}004$).

Table 1. Correlations between clinical score of activity and plasma IL-2R levels and laboratory parameters in patients with active Crohn's disease

	r_s	P
Plasma IL-2R	0·46	0·08
ESR	0·19	0·51
CRP	0·24	0·39
α1 acid glycoprotein	0·15	0·65
Platelet count	0·38	0·16
Total leucocyte count	−0·32	0·25
Serum albumin	0·43	0·14

between plasma IL-2R levels and CRP, α1 acid glycoprotein levels, platelet count, or total leucocyte count. The clinical score of activity also did not correlate with these parameters (Table 1).

Of the patients with active Crohn's disease and IL-2R levels > 1000 U/ml, three had normal ESR (< 10), three had normal CRP (< 20 mg/l) and two had normal α1 acid glycoprotein levels (< 1·4 mg/*l*).

Two patients with active Crohn's disease had IL-2R levels < 1000 U/ml. Both these patients had predominantly obstructive symptoms due to small bowel strictures and had been on oral prednisolone for many months (one was also on azathioprine). CRP and α1 acid glycoprotein levels, ESR and platelet count were also normal in these two patients.

Changes with treatment

In three patients with Crohn's disease plasma IL-2R levels before and after treatment with steroids were studied (Table 2). In two of them, levels of IL-2R fell soon after institution of treatment. This coincided with the clinical response reflected in a declining clinical activity score. In the third patient (patient C) there was no significant clinical response to high dose oral prednisolone or elemental diet and his levels of plasma IL-2R remained high until resection of diseased terminal ileum. This patient had normal ESR, platelet count, CRP and α1 acid glycoprotein levels throughout the period of study despite marked symptoms and severe inflammation in the resected specimen.

Peripheral and mesenteric venous blood samples

Blood from a branch of the mesenteric vein draining the inflamed part of the intestine to be resected, as well as from a peripheral vein was obtained from 10 patients at operation (Table 3). Five patients were undergoing colectomy for ulcerative colitis and five had ileal resection for Crohn's disease. All the patients had been on high-dose prednisolone for various lengths of time before operation. Patients 1 and 5 (with ulcerative colitis) had only mild inflammation at the time of the operation. All those with Crohn's disease (patients 6–10) had tight strictures at operation. Initial studies showed small

Table 2. Plasma IL-2R levels, clinical score of activity, and laboratory parameters in three patients with active Crohn's disease, before and after treatment

Patient A							
Day	1	2	3	5	6		
Clinical score	6	4	4	3	3		
IL2R (U/ml)	1100	680	700	630	610		
CRP (mg/l)	94	54	29	27	27		
α1 acid glycoprotein (mg/l)	2·4	2·4	2·4	1·7	1·5		
Platelet count ($\times 10^9$/l)	860	847	845	902	850		
ESR (mm/h)	44	45	36	27	23		
Treatment	Nil	Prednisolone from day 2					
Patient B							
Day	1	2	3	6	8	35	
Clinical score	10		5	6	4	3	
IL-2R (U/ml)	9000		2780	2400	3260	1640	
CRP (mg/l)	172		54	69	60	25	
α1 acid glycoprotein (mg/l)	>2·4		>2·4	>2·4	>2·4	1·9	
Platelet count ($\times 10^9$/l)	510		845	879	890	470	
ESR (mm/h)	67		49	40	50	35	
Treatment	Sulphasalazine	Prednisolone and Sulphasalazine from day 2					
Patient C*							
Day	1	2	7	13	17	37	120
Clinical score	11	11	11	10	7	8	3
IL-2R (U/ml)	1760	1590	1420	1430	1740	1920	940
CRP (mg/l)	<20	<20	<20	<20	<20	<20	<20
α1 acid glycoprotein (mg/l)	1·2	1·0	1·4	1·1	0·9	1·2	0·9
Platelet count ($\times 10^9$/l)	390	388	333	356	311	400	350
ESR (mm/h)	12	14	5	4	10	7	8
Treatment	Nil	Prednisolone and elemental diet from day 2					

* Resection on day 48.

Table 3. Plasma IL-2R levels in samples from peripheral and mesenteric vein (draining diseased intestine) from 10 patients undergoing intestinal resection

Patient no.	Peripheral	Mesenteric	Diagnosis	Site of Inflammation	Indication for operation
1	530	600	UC	Distal, mild	Chronic disease
2	2270	2525	UC	Total	Toxic megacolon
3	675	917	UC	Total	Chronic disease
4	910	850	UC	Distal, severe	Chronic disease
5	860	920	UC	Total	Chronic disease
6	400	425	CD	Terminal ileum	Intermittent obstruction
7*	1770	1960	CD	Ileal	Chronic active disease
8	825	955	CD	Terminal ileum	Intermittent obstruction
9	505	640	CD	Terminal ileum	Chronic active disease +enterovesical fistula
10	700	735	CD	Terminal ileum	Intermittent obstruction

UC, ulcerative colitis; CD, Crohn's disease.
* Patient C in Table 2.

differences between pairs of samples and therefore the assays were performed on at least two separate occasions (each time in duplicate) and the mean derived. Modest but significantly elevated levels of IL-2R were seen in mesenteric venous blood compared with peripheral blood.

Tissue homogenates

In homogenates of colonic biopsies from normal and inflamed colonic biopsies, significantly higher levels of IL-2R were present in samples from colons with active ulcerative colitis (median 230·2, range 20·7–581·5 U/mg tissue) compared with

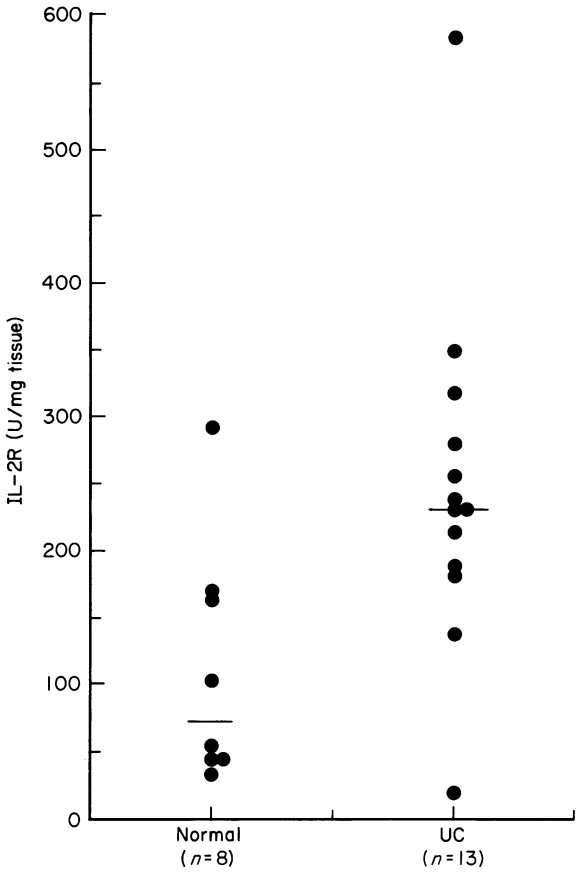

Fig. 3. Levels of IL-2R (expressed as U/mg tissue) in tissue homogenates of colonic biopsies from healthy controls and patients with active ulcerative colitis (UC).

those from normal colons (median 77·9, range 34·2–291·3 U/mg tissue; $P < 0{\cdot}02$; Fig. 3).

DISCUSSION

In active inflammatory bowel disease, there is an increase in the mucosal population of lymphocytes and macrophages. Increased proportion of intestinal mononuclear cells expressing IL-2R has been demonstrated (Mahida *et al.*, 1988). These mononuclear cells comprise lymphocytes and macrophages. In active ulcerative colitis and Crohn's disease, macrophages expressing IL-2R are able to release oxygen radicals upon triggering, and therefore appear to be activated (Mahida *et al.*, 1988). Increased circulatory numbers of lymphocytes bearing activation antigens has been demonstrated in inflammatory bowel disease (Pallone *et al.*, 1987).

In this study, increased circulating levels of IL-2R were present in patients with active ulcerative colitis and Crohn's disease. For ulcerative colitis, plasma levels of IL-2R correlated with CRP. For Crohn's disease, the closest correlation of clinical score of activity was with plasma IL-2R levels. In one patient with active Crohn's disease, IL-2R levels remained high but all other laboratory indices of inflammation remained normal. In this patient, symptoms continued and his IL-2R levels remained high despite high-dose steroids and an elemental diet. In two other patients with Crohn's disease, IL-2R levels fell early in response to treatment with corticosteroids. This suggests that IL-2R levels are related to disease activity and that cells expressing these receptors may be involved in the disease pathogenesis. In Crohn's disease, circulating IL-2R levels may also be more accurate in assessing and monitoring disease activity than other laboratory parameters.

Unlike other investigators (Andre *et al.*, 1981), we did not find a significant correlation between the clinical score of activity of Crohn's disease and CRP levels. This may be due to the fact that we studied only patients with active disease.

In some patients undergoing intestinal resection, plasma obtained from a branch of the mesenteric vein draining inflamed intestine was compared with that obtained from the peripheral venous circulation. Small but consistently higher levels of IL-2R were detected in plasma from the mesenteric venous blood, suggesting at least that there is significant contribution by the intestinal mononuclear cells to the circulating IL-2R levels. It is likely that both circulating as well as intestinal cells expressing IL-2R are contributing to the circulating levels but their degree of contribution is unknown.

Generally, the levels of IL-2R obtained at operation were less than 1000 U/ml (except for one patient who underwent colectomy for toxic megacolon and another with active Crohn's disease). The reason for the relatively lower levels may be that four out of the five patients with ulcerative colitis had colectomy for chronic active disease which had been treated with high-dose steroids for long periods and by the time they came to operation, the degree of inflammation was not as severe as it had been. For the patients with Crohn's disease undergoing resection, operation was usually for strictures and all the patients had been on high-dose steroids which would have reduced the degree of active inflammation present.

Homogenates of colonic biopsies from patients with ulcerative colitis confirmed the presence of IL-2Rs in tissue. This is likely to be derived from receptors on the surface of lymphocytes and macrophages in the mucosa and also mononuclear cells in the blood vessels. Somewhat surprisingly, colonic biopsies from healthy individuals also had detectable IL-2R levels. This may indicate low level of IL-2R expression by mononuclear cells in the lamina propria of the normal colonic mucosa, which is not detected by routine immunohistochemistry. There may also be some contribution by cells expressing IL-2R in lymphoid aggregates of the normal mucosa.

The function of circulating IL-2R levels is unknown. They may play an immunoregulatory role by competing with IL-2Rs on cell surfaces for IL-2 and therefore down-regulating the immune response. This may also be responsible for low levels of IL-2 detected (by bioassay) after stimulation (with mitogen) of peripheral (Ebert *et al.*, 1984) and intestinal mononuclear cells (Fiocchi *et al.*, 1984), from patients with inflammatory bowel disease.

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REFERENCES

ANDRE, C., DESCOS, L., LANDAIS, P. & FERMANIAN, J. (1981) Assessment of appropriate laboratory measurements to supplement the Crohn's disease activity index. *Gut*, **22**, 571.

BARON, J.H., CONNELL, A.M. & LENNARD-JONES, J.E. (1964) Variation between observers in describing mucosal appearances in proctocolitis. *Br. med. J.* **1,** 89.

COLVER, G.B., SYMONS, J.A. & DUFF, G.W. (1989) Soluble interleukin-2 receptor in atopic eczema. *Br. med. J.* **298,** 1426.

DUFF, G.W. (1989) Peptide regulatory factors in non-malignant disease. *Lancet*, **i,** 1432.

EBERT, E.C., WRIGHT, S.H., LIPSHUTZ, W.H. & HAUPTMAN, S.P. (1984) T-cell abnormalities in inflammatory bowel disease are mediated by interleukin 2. *Clin. Immunol. Immunopathol.* **33,** 232.

FIOCCHI, C., HILFIKER, M.L., YOUNGMAN, K.R., DOERDER, N.C. & FINKE, J.H. (1984) Interleukin-2 activity of human intestinal mucosa mononuclear cells. Decreased levels in inflammatory bowel disease. *Gastroenterology*, **86,** 734.

HARVEY, R.F. & BRADSHAW, J.M. (1980) A simple index of Crohn's disease activity. *Lancet*, **i,** 514.

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.

O'CALLAGHAN, C., FRANKLIN, P., ELLIOT, T.S.J., DEVERILL, I., RICHARDS, N. & POWELL, R.J. (1984) C reactive protein concentrations in neonates: determination by a latex enhanced immunoassay. *J. clin. Pathol.* **37,** 1027.

O'GARRA, A. (1989) Interleukins and the immune system 1. *Lancet*, **i,** 943.

PALLONE, F., FAIS, S., SQUARCIA, O., BIANCONE, L., POZZILLI, P. & BOIRIVANT, M. (1987) 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*, **27,** 745.

POWELL-TUCK, J., BOWN, R.L. & LENNARD-JONES, J.E. (1978) A comparison of oral prednisolone given as single or multiple daily doses for active proctocolitis. *Scand. J. Gastroenterol.* **13,** 833.

RAEDLER, A., FRAENKEL, S., KLOSE, G., SCYFARTH, K. & THIELE, H.G. (1985) Involvement of the immune system in the pathogenesis of Crohn's disease. Expression of T9 antigen on peripheral immunocytes correlates with the severity of the disease. *Gastroenterology*, **88,** 978.

RUBIN, L.A., KURMAN, C.C., FRITZ, M.E., BIDDISON, W.E., BOUTIN, B., YARCHOAN, R. & NELSON, D.L. (1984) Soluble interleukin-2 receptors are released from activated human lymphoid cells *in vitro*. *J. Immunol.* **135,** 3172.

TROWBRIDGE, I. (1987) Interleukin-2 receptor proteins. *Nature* **327,** 461.

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.