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Frequently relapsing Crohn's disease is characterized by persistent elevation in interleukin-6 and soluble interleukin-2 receptor serum levels during remission

C. Van Kemseke J. Belaiche E. Louis

Service de Gastroentérologie, CHU Sart Tilman, 4000 Liège, Belgium

Abstract

We examined immune and inflammatory activation during remission in patients with Crohn's disease who presented with various clinical profiles (prolonged remission vs. relapsing disease). Thirty-six patients with at least 3 years' follow-up starting from a remission period were studied retrospectively. Relapses were defined by a retrospective calculation of the Crohn's disease activity index or by the clinical judgement of the physicians in charge of the patients. Disease course over the study period was assessed by the mean number of annual relapses. Analysis used measurements during remission of the following: erythrocytes sedimentation rate, relative lymphocytosis, acid α_1-glycoprotein, interleukin-6 (IL-6), and soluble interleukin-2 receptor (sIL-2R) serum levels. During the study period 21 patients experienced at least one relapse and 15 did not. Mean serum levels of sIL-2R and mean relative lymphocytosis in remission significantly discriminated between relapsing and nonrelapsing patients. Only the mean sIL-2R serum level was selected by multivariate analysis, with a cutoff value of 82 pM/1 (sensitivity of 76% and specificity of 80%). The only features correlated with mean number of annual relapses in the relapsing patients were mean serum levels of sIL-2R ($r = 0.58$, $P = 0.015$) and IL-6 in remission ($r = 0,45$, $P = 0.039$). Multivariate analysis demonstrated statistical significance only for the mean serum level of IL-6 ($P = 0.014$). In Crohn's disease the persistent elevation in sIL-2R serum levels during remission corresponds to chronic active disease, while high serum levels of IL-6 in these patients is associated with a high frequency of relapse.

Keywords : Crohn's disease ; interleukin-6 ; soluble interleukin-2 receptor

INTRODUCTION

Crohn's disease (CD) is a chronic relapsing inflammatory bowel disease of unknown cause. The natural history of the disease varies considerably from one patient to another, with some patients experiencing frequent relapses while others have prolonged periods of remission. Several immune or inflammatory parameters have been shown to be correlated with disease activity [1, 2, 3, 4, 5, 6]. However, a few of these remain elevated in some patients despite clinical remission and may be useful in predicting clinical relapse [4, 7, 8, 9]. In particular we have found the interleukin-6 (IL-6) serum level, acid α_1-glycoprotein, soluble interleukin-2 receptor (sIL-2R) serum level, and relative lymphocytosis to be good predictors of relapse [4,7]. The state of immune and inflammatory activation during clinical remission of CD remains unclear. It may be different in prolonged remission as compared to frequently relapsing disease. In the latter, remission may reflect either complete immune and inflammatory inactivation interrupted by frequent reactivation or subclinical persistent disease with transient exacerbations giving rise to clinical manifestations. A recent study has shown that the risk of relapse is associated with a persistent increase in proinflammatory cytokines production at the mucosal level [10]. According to this hypothesis, patients with relapsing disease may have higher mean serum levels of immune and inflammatory parameters while in remission than patients with extinguished disease. It is important to determine the pathway of this possibly persistent immune and inflammatory activation, with parameters such as sIL-2R reflecting lymphocyte activation and IL-6 more macrophage activation.

The aims of our work were twofold. First, to compare repeated measures of immune and inflammatory parameters in remission of CD in patients characterized by either of two different clinical profiles: prolonged remission and relapsing disease. Secondly, in patients with relapsing disease to assess the correlation between these parameters and the frequency of relapse.

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PATIENTS AND METHODS

Patients and study design

We studied 36 CD patients with at least 3 years' follow-up at the same medical center (University Hospital of Liège), and starting with a period of clinical remission defined by a Crohn's disease activity index (CDAI [11]) lower than 150. These patients had previously been included in a prospective study on relapse of CD over a 1-year period [7]. Diagnosis of CD was based on standard criteria. The notes on these patients were analyzed retrospectively. Clinical relapses were defined either by a retrospective calculation of the CDAI (CDAI >150 with an increase greater than 100 compared to the CDAI at inclusion, or a CDAI >200) or by the judgement of the clinician in charge of the patients (J.B. or E.L.) found in the notes on the patient; this corresponded to a significant change in symptoms requiring a modification in treatment. Disease course was assessed in terms of the mean number of annual relapses.

Patients were treated during remission daily with either 2-4 g 5-aminosalicylic acid (*n*=29) or 2 mg/kg azathioprine (*n*=17), sometimes associated with low-dose steroids (*n*=19). In 21 patients the treatment changed during follow-up. Patients were divided in two groups: those in group A showed no relapse during follow-up (*n*=15) and those in group B at least one relapse (*n*=21). As the treatment used, mainly azathioprine, may affect inflammatory parameters, we also analyzed the data in subgroups of patients depending on this parameter. Patients' characteristics are shown in Table 1.

Table 1 *Patients characteristics*

	All patients (*n*=36)	Group A (*n*=15)	Group B (*n*=21)
Age (years)	39.8 (21-84)	41.4 (21-84)	38.6 (25-74)
Women/men	25/11	9/6	16/5
Duration of follow-up (years)	4.7 (3-9)	4.6 (3-9)	4.7 (3-7)
Duration of disease (years)	11.6 (5-34)	13.8 (5-34)	10.4 (6-25)
Familial history (%)	11	13	9.5
Previous resection surgery (%)	55.5	60	52.4
Disease location (%)			
Ileal	8	7	10
Colonic	28	7	43
Ilecolonic	58	73	47
Multiple (including upper GI)	6	13	4
Anal lesions	47	60	33
Remission treatment (%)			
5-Aminosalicylic acid	80	80	81
Azathioprine	47	33	57
Corticoids	53	33	66
Number of annual relapses per year	0.30 ± 0.36	0	0.50 ± 0.30

Laboratory tests

The parameters studied were selected on the basis of our previous studies in which more than 30 clinical or biological parameters were tested [4, 7]. Erythrocyte sedimentation rate (ESR), relative lymphocytosis, and acid α_1-glycoprotein serum level were determined using standard procedures. Serum samples were stored at -20°C before use in measuring cytokines. The serum level of sIL-2R was measured using a commercial double-antibody sandwich-linked immunosorbent assay detecting the α-chain (p55) of the receptor (Boehringer-Mannheim). Serum level of IL-6 was measured using a commercial enzyme-amplified sensitivity immunoassay based on an oligoclonal system in which several monoclonal antibodies directed against distinct epitopes of the cytokine are used (Medgenix). The analysis used each measurement of these parameters performed in remission during the study period, at variable intervals according to the clinical requirement. The mean number of measures over the study period were 4.6 ± 3 for ESR (range 2-16), 7.8 ± 4.5 for relative lymphocytosis (range 3-18), 7.2 ± 4.6 for acid α_1-glycoprotein (range 2-18), 7.8 ± 4.4 for sIL-2R (range 3-18), and 4.4 ± 2.7 for IL-6 (range 2-12).

Statistics analysis

Mean values of biological parameters measured in remission were computed for each patient. A log transform was used to normalize the distribution of some variables. Results are expressed as mean ±SD. We first compared immune and inflammatory profiles between relapsing (group B) and nonrelapsing disease (group A).

Student's *t* test was used to compare the various parameters studied between groups A and B. A stepwise logistic regression was then used to select independent parameters discriminating between groups A and B. In group B we then evaluated the correlation between immune and inflammatory parameters during remission and the disease course as defined by the mean number of annual relapses. A stepwise multiple regression used the mean annual number of relapses as dependent variable and inflammatory markers as independent variables. Results were considered statistically significant at the level of $P < 0.05$). Statistical calculations were carried out using the SAS package (SAS Institute, Cary, N.C., USA).

RESULTS

The results of laboratory tests are shown in Table 2.

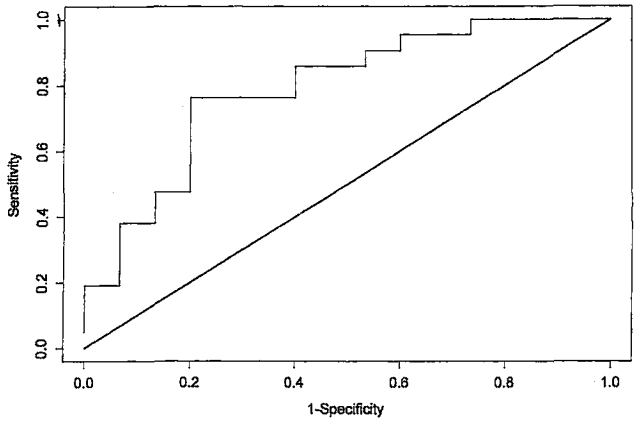
Table 2 *Biological profiles*

	All patients (*n*=36)	Group A (*n*=15)	Group B (*n*=21)	Normal values
ESR (mm/h)	14 ± 10	11 ± 8	17 ± 11	0-10
Relative lymphocytosis (%)	22.5 ± 7.0	25.3 ± 6.3	20.5 ± 6.9	11.0-53.0
Acid α_1-glycoprotein (g/l)	1.09 ± 0.34	0.95 ± 0.20	1.20 ± 0.39	0.4-0.9
sIL-2R (pM/l)	96 ± 37	77 ± 20	109 ± 41	50-100
IL-6 (pg/ml)	13.5 ± 12.0	11.1 ± 13.1	15.3 ± 11.0	0-8.5

Comparison of immune and inflammatory profiles between groups A and B

Univariate analysis showed only mean sIL-2R serum level and mean relative lymphocytosis to discriminate between the two groups ($P = 0.003$ and $P = 0.043$ respectively). The stepwise logistic procedure selected only the mean sIL-2R serum level ($P = 0.009$) as discriminating between relapsing and nonrelapsing patients. We determined the optimal cutoff value of mean sIL-2R serum level for discriminating between the two groups by means of a receiver-operating characteristic curve. This cutoff value was 82 pM/l and discriminated between the two groups with a sensitivity of 76% and a specificity of 80% (Fig. 1). Regarding only patients treated with azathioprin, sIL-2R was still significantly higher in group B than A (124 ± 68 pM/l versus 75 ± 19 pM/l, $P = 0.009$) and lymphocytosis was still significantly lower in group B than in group A (17.3 ± 6.3% versus 24.5 ± 5.6%, $P = 0.045$).

Fig. 1 *Receiver-operating characteristic curve for sIL-2R serum levels in remission of CD discriminating between relapsing and nonrelapsing patients*



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Correlation between immune and inflammatory parameters and evolutivity of the disease in patients with relapsing disease (group B)

The only parameters correlated with mean number of annual relapses were mean serum levels during remission of ΓL-6 ($r = 0.58$, $P = 0.015$) and sIL-2R ($r = 0.45$, $P = 0.039$; Table 3, Figs. 2, 3). The stepwise multivariate procedure selected only mean IL-6 serum level. This parameter explained 66% of the variance in mean number of annual relapse ($P = 0.014$).

Table 3 *Correlations between mean number of annual relapses and mean levels of immune and inflammatory parameters during remission in group B*

	n	*r*	*P*
sIL-2R	21	0.45	0.039
IL-6	18	0.58	0.015
Relative lymphocytosis	21	-0.35	NS
Acid α_1-glycoprotein	21	0.33	NS
ESR	21	0.01	NS

Fig. 2 *Correlation between mean number of annual relapses and mean sIL-2R serum level in remission in group B patients*

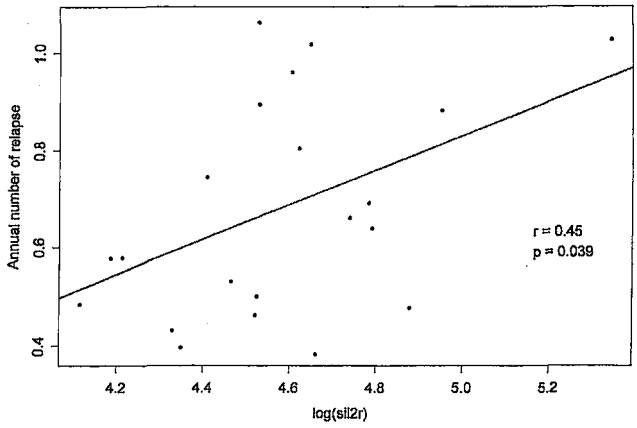
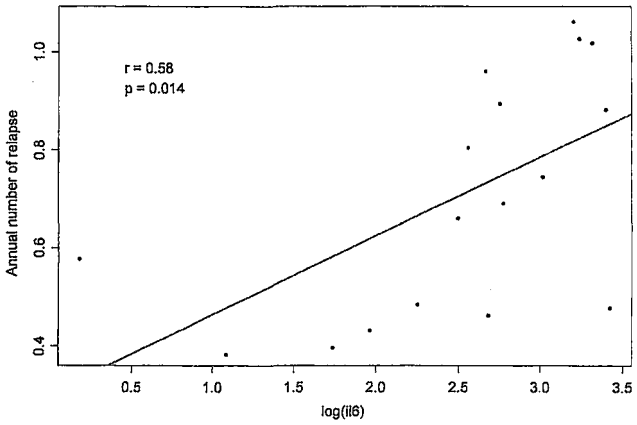


Fig. 3 *Correlation between mean number of annual relapses and mean IL-6 serum level in remission in group B patients*



DISCUSSION

We found that patients with relapsing CD are characterized by persistently higher sIL-2R serum levels during remission than are patients with prolonged quiescent disease. Furthermore, in patients with relapsing CD the frequency of relapse is well correlated with the mean serum level of IL-6 during remission. Several studies have shown the persistence of biological and functional abnormalities in some patients with clinically quiescent CD. In particular, a persistent elevation in inflammatory markers such as C-reactive protein [9], α_2-globulin [8], ESR [8], and acid α_1-glycoprotein [8] have been shown. We also found a persistent elevation in BL-6 and sIL-2R serum levels. These findings may reflect either remaining lesions [12], activation of the mucosal immune system [10], or abnormal intestinal permeability [13]. These biological or functional abnormalities have also been reported to be associated with an increased risk of relapse assessed over a short to medium period of time (usually 1 year) [4,7, 8, 9,10,13,14].

The present study considered disease course as defined by the mean number of annual relapse over a longer period of time. Our working hypothesis was that patients with no relapse over a period of several years would have an extinguished immune and inflammatory activity while patients with frequent relapses would have ongoing immune and inflammatory reaction even when in clinical remission. Multivariate analysis revealed the only parameter to discriminate between relapsing and nonrelapsing CD was mean sIL-2R serum level measured during remission. This most probably reflects an ongoing activation of T lymphocytes characterizing the chronic relapsing disease. Indeed the measured sIL-2R corresponds to the α-chain of cellular interleukin-2 receptor (α-chain + β-chain) released in a soluble form in proportion to its cell surface expression [15]. It is therefore related to the activation of cell population that express this receptor, i.e., mainly activated T lymphocytes.

The origin of this circulating sIL-2R must be discussed. In normal subjects the sIL-2R serum level is correlated with sIL-2R production by lamina propria mono-nuclear cells in culture [16]. In CD the sIL-2R serum level is increased in active disease [3, 17, 18, 19], is correlated with sIL-2R level in the supernatants of lamina propria mononuclear cells in culture [16], and is higher in mesenteric vein than in peripheral veins [17]. In a previous study we have shown both the lack of correlation between sIL-2R serum level and cIL-2R positive cells in the blood and a negative correlation between sIL-2R serum level and blood lymphocytosis [4]. Together with previous data, these findings suggest that sIL-2R serum levels reflect mucosal rather than peripheral blood T lymphocyte activation.

It is noteworthy that in univariate analysis the presence of relative lymphocytopenia discriminated as well between relapsing and nonrelapsing disease. Lymphocytopenia may be due in part to mucosal migration of T lymphocytes. Lymphocytopenia has previously been described as a predictive marker of relapse after curative surgery [20]. None of the other parameters - IL-6, acid α_1-glycoprotein, and ESR - discriminated between relapsing and nonrelapsing disease. This may be because these parameters are less specific for mucosal immune and inflammatory activation in CD, being elevated also in other ordinary circumstances. Overall these results suggest that chronic relapsing disease differs from extinguished disease by a persistent activation and probably migration of T lymphocyte to the gut mucosa.

Considering only patients with relapsing disease, the mean IL-6 serum level during remission was the only parameter in the multivariate analysis that was correlated with the number of annual relapses. Mean sIL-2R serum level, however, was also selected in univariate analysis. This suggests that in frequently relapsing disease the ongoing immune activation involves not only sIL-2R but also IL-6 secreting cells and thus probably both lymphocytes and other cells such as macrophages or intestinal epithelial cells [21, 22, 23,24,25].

Both the mean number of annual relapses and the serum levels of immune and inflammatory parameters may have been affected by the treatment during remission, particularly immunosuppressive treatments. Azathioprine, which was used in almost one-half of our patients, may have induced a decreased both in lymphocytosis and in circulating sIL-2R or IL-6. However, when considering only patients under azathioprine during remission, mean serum level of sIL-2R was still higher in relapsing (n=5) than nonrelapsing (n=9) patients, and mean lymphocytosis was still lower in relapsing than in nonrelapsing patients.

CONCLUSION

These results do not imply that sIL-2R and IL-6 are the main actors in the mucosal immune reactions. Verifying such a conclusion would require study of the cytokine profile at the mucosal level [10]. However, at this stage we can confirm that a persistent elevation in sIL-2R serum levels during remission is associated with frequently relapsing CD, and that in relapsing patients high serum levels of IL-6 during remission reflect a particularly high

frequency of relapse. Further studies must clarify whether these serum levels are associated only with persistent mucosal or systemic immune activation or also with persistent subclinical abnormalities such as increased intestinal permeability or even persistent mucosal macroscopic lesions.

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