Complement Split Product C3d as an Indicator of Disease Activity in Systemic Lupus Erythematosus


Summary In order to investigate, if complement levels can be used as an indicator of clinical activity in systemic lupus erythematosus (SLE), levels of C3, C4, CH50, and C3d were measured in 79 patients, 41 with inactive, 31 with moderately active and 7 with severely active disease. Our study shows that C3d, and particularly the C3d/C3 ratio, provide sensitive markers for disease activity in SLE. Since C3d is a direct measurement of complement turnover, it reflects complement activation better than C3, C4 and CH50.

Key words Systemic Lupus Erythematosus, Complement C3 Split Product C3d, Disease Activity.

INTRODUCTION

Activation of the classical and alternative complement pathway via immune complex formation is thought to play a central role in the pathophysiology of active systemic lupus erythematosus (SLE). This activation leads to an enhanced complement turnover with reduced or normal serum complement levels together with an increase of complement split products. The extent of complement consumption is best reflected by its breakdown products (1,7). The third component of complement C3 is the most important and abundant protein of the system. C3 is cleaved by proteolytic enzymes into several conversion products (e.g., C3a, C3b, C3c, C3d). C3d has the longest in vitro half life of all C3 split products (3). The aim of our analysis was to evaluate, if total haemolytic complement (CH50) or single components such as C3, C4, C3d reflect the clinical disease activity in SLE.

PATIENTS AND METHODS

Patients

Seventy-nine SLE-patients from our outpatient clinic (73 women and 6 men) with a mean age of 45 years (range 12-76) were studied. Their disease manifestations satisfied the American Rheumatism Association revised criteria for SLE (23). To quantify disease activity we used a slightly modified lupus activity index (AI) established by Morrow 1982 (13). This AI index allows classification of patients with severely active, moderately active, or inactive SLE forms. Severe activity was assumed when three or more of the following symptoms were evident: arthralgia, myalgia, vasculitis, pleuritis, cerebral, skin or renal involvement, pericarditis. When only one or two of these criteria were present, the patient was considered to have moderately active disease. When none of these features were present or causing only minimal disturbance the disease was considered as being inactive. Activity was ascribed to renal disease in those patients with combinations of a diastolic blood pressure greater than 90 mm Hg, oedema requiring diuretic therapy, hypertension, proteinuria greater than 0.5 grams 24/hours, creatinine clearance less than 60 ml/minute, or a raised serum creatinine level. The criteria for the assessment of cerebral disease were vascular headaches, disturbance of mood or consciousness and neurological deficit of recent onset.

Scoring of lupus activity was performed by the same examiner at each visit without knowledge of the laboratory results of this day. Lupus activity was judged to be inactive in 41 (disease activity Grade 0), moderately active in 31 (Grade 1), and severely active in 7 patients (Grade 2).

Blood collection

Five milliliters of blood were collected in EDTA in a final concentration of 10 mmoles/liter; the blood was immediately centrifuged at 1,000g at 4°C for 15 min-
utes and plasma was stored at -70°C. Another 5 ml of blood were allowed to clot at room temperature for 2 hours; after centrifugation, the serum was stored at -70°C. Plasma was used to measure complement fragment C3d. Serum served for the measurement of antinuclear antibody (ANA) titers, CH50 and the intact components C3 and C4.

Determination of anti-nuclear antibodies

Anti-DNA antibodies were determined by indirect immunofluorescence using rat liver sections and an FITC labelled, polyvalent anti-human immunoglobulin antibody (Wellcome, Dartford, England).

CH50, C3 and C4 measurement

Serum concentrations of C4 and C3 were determined by laser nephelometry, using specific antisera, according to the manufacturer’s instructions (Behring Werke AG, Marburg, Germany). Results were expressed in g/l. The normal range for C3 was 0.5-1.2 g/l, and for C4 0.2-0.5 g/l.

The total haemolytic complement (CH50) was measured by standard complement binding reaction to antibody-coated sheep erythrocytes (5).

C3d

C3d levels were determined by a modified double-decker rocket immunoelectrophoresis (2,19). C3d can be separated from C3c and C3 by electrophoretic mobility and immunoprecipitation using specific antibodies (Dakopatts, Kopenhagen, Denmark). Following immunoelectrophoresis, the precipitation ‘rockets’ can be stained, measured and compared with standard ‘rockets’ of known C3d concentrations (C3d-Standard, Behring, Marburg, Germany). The C3d levels observed in more than 100 healthy controls ranged from 0 to 10 mg/l (mean 3.2 ± 3.3). The upper limit of normal values was set at the mean + 2 SD. Concentrations higher than 10 mg/l were considered to be elevated.

As the initial concentration of intact complement components may influence the levels of their fragments, the C3d/C3 ratio was calculated to obtain indices of complement activation independent of the concentration of parent molecules.

Statistical analysis

The mean values of complement components of the three SLE activity subgroups were compared among each other using an analysis of variance (ANOVA) method. Correlation coefficients were calculated by Kendall’s tau. Inferential statistics were calculated by the chi-square test. Statistical analysis was also carried out using the nonparametric computations Kruskal-Wallis test and the Wilcoxon rank test to replace the analysis of variance. All p values are 2-tailed. The results were reported as the mean ± SD, when appropriate.

RESULTS

C3d

The results are shown in Figures 1-3. Plasma concentrations of C3d in the 79 SLE patients ranged between 0 and 24 mg/l. The inactive patient group (disease activity Grade 0) (41 patients) had a mean C3d concentration of 7.4 ± 6.6 mg/l. The mean C3d concentration of the 31 patients with moderately active disease (disease activity Grade 1) was 9.6 ± 8.0 mg/l. The 7 patients with severely active disease (disease activity Grade 2) had markedly elevated plasma C3d levels with a mean of 16.0 ± 4.3 mg/l. C3d levels correlated significantly with disease activity (Kruskal-Wallis-ANOVA p<0.005, chi-square test = 10.42) (Fig. 1). Elevated C3d levels >10.0 mg/l were obtained in 34 of the 79 patients (43%). In the inactive patients (Group 0) only 10 out of 41 (24%) had an elevated C3d. Among