

COMPLEMENT ACTIVATION PRODUCTS IN PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY

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Received after revision August 2006

Abstract

Complexes of antigen and antibody participate in activation of the complement system.

The goal of our study was to determine whether decrease in serum immunoglobulin levels in patients with hypogammaglobulinemia led to abnormalities in complement activation.

The levels of complement activation products C4d and the C5-9 complex were determined by ELISA in 30 patients with the most frequent primary hypogammaglobulinemia - common variable immunodeficiency (CVID) and in a control group of 60 (for C4d) and 40 healthy persons (for the C5-9 complex). All patients with CVID were treated with intravenous immunoglobulin in a dose of 250-500 mg/kg every 3-4 weeks. The patients had no symptoms of acute inflammatory process at the time of blood collection. The levels of the complement activation products were correlated with the serum levels of IgG (at the time of diagnosis and at the time of blood sampling) and also with the number of acute infections during the year prior to the study.

No significant difference in serum levels of C4d in CVID patients and healthy controls was observed. When evaluating the serum level of the C5-9 complex, a highly significant decrease in serum levels of this complement product was observed in patients with CVID. We did not observe any correlations between serum IgG levels, the number of infections, and levels of the complement activation products measured. Also, no correlation was observed between the clinical state of the patients and the levels of the activation complement products.

Key words

Hypogammaglobulinemia, Common variable immunodeficiency, Complement system, Complement activation

Abbreviations used

CVID, common variable immunodeficiency; ELISA, enzyme-linked immunosorbent assay

INTRODUCTION

The complement system belongs to major non-specific mechanisms of the immune system. It can be activated by three pathways - classical, lectin, and alternative. The classical one is activated by complexes of antigen and antibody, while the alternative and the lectin ones are antibody-independent.

The most frequent symptomatic hypogammaglobulinemia, common variable immunodeficiency (CVID), is characterized by failure of B cell differentiation leading to defective immunoglobulin production. Patients with markedly decreased production of immunoglobulins (hypogammaglobulinemia) suffer from bacterial infections, particularly of the respiratory tract, but also other systems may be affected by infectious as well as non-infectious pathological processes (for review see 1). Immunoglobulin substitution is the only effective treatment at present. Patients with antibody production deficiencies, even on substitution treatment, have low levels of serum immunoglobulins, so it is supposed that the activation of the complement system may be abnormal. Two possible consequences may be considered: Low levels of antibodies may cause decreased activation of the classical pathway. On the other hand, a high burden of antigens may activate the complement system by the lectin or alternative pathways.

Several studies investigating complement components in hypogammaglobulinemic patients were published. These studies showed that the levels of C3 and C4 complement component in CVID patients were not affected (2,3). Untreated patients had decreased levels of C1q, whose level increased after initiation of immunoglobulin replacement (2,4,5,6).

Investigation of complement activation/split products seems to be a better marker than determination of intact components (classically C3 and C4) in laboratory assessment of the complement activation (7,8). Determination of some of those products in CVID patients was performed only in one study (9) showing increased levels of some complement activation products (C3a, C4a, C5a) in CVID patients on immunoglobulin substitution therapy, while the C5-9 complex was not affected.

In this study we determined serum levels of complement activation products C4d and C5-9 in patients with CVID and in a control group.

MATERIALS AND METHODS

Thirty patients (mean age 42 years, range 12-78, 20 females, 10 males) with well diagnosed common variable immunodeficiency according to ESID criteria (10) were involved in the study. All patients were treated by regular immunoglobulin substitution in a dose of 250-500 mg/kg/3-4 weeks.

The clinical state of the patients was evaluated by the physician; the patients were scored as well compensated (group 1, n = 14), compensated (group 2, n = 10), and non-compensated (group 3, n=6).

Healthy volunteers were used as a control group. For statistical evaluation of C4d levels and C5-9 complex levels, 60 volunteers (mean age 36 years, range 9-74, 37 females, 23 males), and 40 healthy donors (mean age 34 years, range 21-47, 32 females, 8 males) were used, respectively.

Serum was collected in patients without symptoms or signs of acute infection before immunoglobulin infusion was initiated. Sera were separated from the blood clot one hour after blood collection and stored at -80 °C before complement components were determined. Subsequent ELISA kits were used: C5-9 and C4d Fragment (both Quidel, San Diego, CA, USA). IgG levels were evaluated by nephelometry (BM-II, Behring Diagnostica GmbH, Newark, DE, USA). Antisera from Behring Diagnostica GmbH were used.

Normality of the obtained values was tested by the Kolmogorov-Smirnov test. As the observed distribution of the results significantly differed from normal distribution, non-parametric tests were used. For comparing values of C4d and the C5-9 complex, the Mann-Whitney test was used. For correlation between IgG levels, the number of infections during the year prior to the study and activation

fragment levels, the Pearson coefficient was used. For statistical evaluation of the differences in the activation complement product levels between certain groups of patients according to the clinical state, the Kruskal-Wallis test was used.

RESULTS

No difference was observed in the serum levels of C4d in CVID patients and in healthy controls (*Tab. 1, Fig. 1*). When evaluating serum levels of the C5-9 complex, a highly significant decrease in the serum levels of this complement complex was observed in patients with CVID (*Tab. 1, Fig. 1*). All patients had normal C3 and C4 serum levels at the time of blood collection. On correlating levels of C4d or the C5-9 complex with the serum IgG level before the start of immunoglobulin treatment and also serum IgG levels at the time of blood collection, no correlation was observed (in all cases $p > 0.05$). Also, no significant correlation was observed between the number of infections and the levels of complement activation fragments. No significant difference was observed when subdividing patients according to their clinical state (*Fig. 2*) (see Materials and methods).

DISCUSSION

In this study we showed that serum levels of the complement activation product C5-9 were decreased in CVID patients compared to the control group, while C4d levels were not affected. These results show that complement activation in CVID patients is, at least partly, affected in hypogammaglobulinemic patients. The low levels of the C5-9 complex may be explained by low complement activation through the classical pathway, which is activated by complexes of antibody and antigen. These results correspond with our previous observation that, although on immunoglobulin substitution, serum levels of immune complexes in hypogammaglobulinemic patients were low, even during apparent infections (*11*).

Our results differ from the only one comparable study (*9*), which showed increased levels of some complement fragments (C3a, C4a, C5a) in CVID patients, while the difference between C5-9 levels in CVID patients and healthy control people was not significant. One of the explanations for the different results may be relatively insufficient treatment of Czech patients, who are probably treated with lower doses of immunoglobulins than are Norwegian patients. In our patients pre-infusion serum IgG levels do not usually exceed 5 g/l, as recommended in the 90s (*12*), while at present the recommended dosage is higher with suggested pre-infusion levels of about 7 g/l (*13*). On the other hand, the fact of no correlation between pre-infusion levels of IgG and complement activation products in our patients does not support this explanation.

Another possible explanation of the different results in our study and that mentioned above may be different serum half-lives of the measured activation products, because we have measured C4d while in the study mentioned above (*9*) another

Table 1

	CVID patients (n = 30)	Control group (C4d: n = 60) (C5-9: n = 40)	p
C4d	4.5 (4.1)	3.0 (1.7)	0.1254
C5-9	694.6 (492.4)	1332.6 (54.6)	0.0003

Serum levels of C4d and C5-9 complex in patients with CVID and in a control group. Data are given as a median (standard deviation). For statistical evaluation Mann-Whitney test was used.

Table 2

	C4d	C5-9
Group 1 (n = 14)	4.9 (3.0)	548 (371)
Group 2 (n = 10)	3.3 (3.1)	709 (493)
Group 3 (n = 6)	4.2 (7.0)	1005 (529)

Serum levels of C4d and C5-9 complex in patients with CVID subdivided according to the clinical state (see Materials and methods). Data are given as a median (standard deviation). No difference between the groups was significant (Mann-Whitney test was used).

product of C4 activation, C4a, was measured. Also the methods used were different: ELISA in our study, flow cytometry in the study mentioned above (9). All complement activation products are short-lived molecules (7), but to our knowledge there are no exact data allowing us to compare the half-lives of the products measured by us and by the others.

Our study did not show any correlation between serum complement activation products and the clinical state or IgG pre-infusion levels of the investigated patients. Although several studies showed that the clinical state in hypogammaglobulinemic patients to some extent reflects pre-infusion levels of IgG (14, 15), there are more influences that may result in levels of activation of the complement system and consequent levels of the activation products. These include mainly immune complex formation which, as mentioned previously (11), does not reflect the presence of infection in CVID patients.

Obtaining more data on complement activation products is necessary for elucidating the exact function of the complement system in patients with hypogammaglobulinemia. Our results show that the study of complement activation in hypogammaglobulinemic patients deserves more interest in the future.

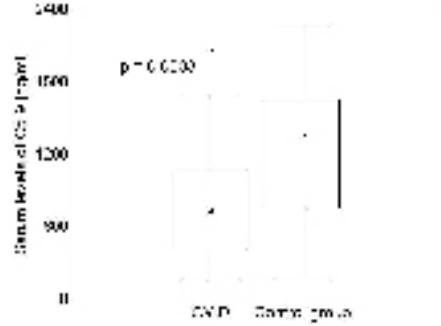
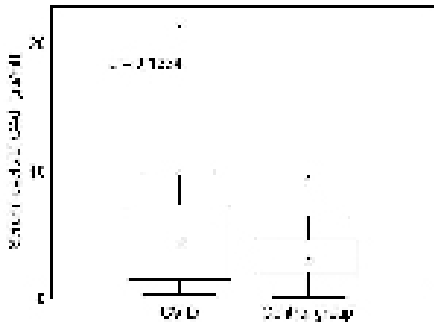


Fig. 1

Serum levels of C4d and C5-9 complex in patients with CVID and in a control group. For statistical evaluation Mann-Whitney test was used.

Legend: Median 25 %-75 % Non-Outlier Range Outliers

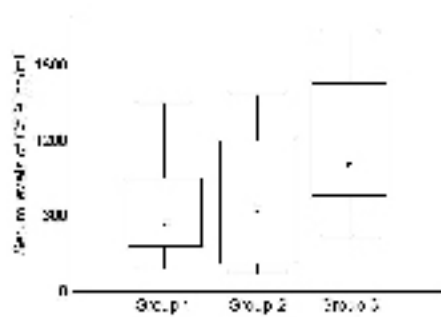
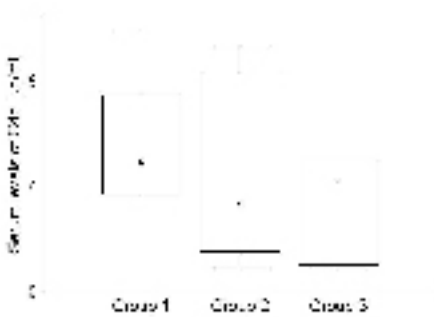


Fig. 2

Serum levels of C4d and C5-9 complex in certain groups of CVID patients, subdivided according to the clinical state.

Legend: Median 25 %-75 % Non-Outlier Range Outliers

Acknowledgement

This work was supported by the grant No. NI/7921-3 of the Czech Ministry of Health. We would like to thank Tereza Kamenická for her excellent technical assistance.

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AKTIVACE KOMPLEMENTU U NEMOCNÝCH S BĚŽNÝM VARIABILNÍM IMUNODEFICITEM (CVID)

Souhrn

V aktivaci komplementového systému se významně uplatňují komplexy antigen-protilátka. Málo je známo, jak tento proces probíhá za abnormálních okolností, například u nemocných s hypogamaglobulinémiemi. Modelem takové situace může být primární hypogamaglobulinémie označovaná jako běžný variabilní imunodeficit (CVID). Měřením hladiny štěpných produktů komplementu jsme zjišťovali, zda dochází ke změně aktivity komplementu u těchto nemocných. Hladiny aktivační složky komplementové kaskády C4d a C5-9 komplexu byly stanoveny u 30 nemocných s CVID; jako kontrolní skupina sloužilo 60 (C4d), resp. 40 (C5-9) zdravých osob. Všichni nemocní byli léčeni intravenózním imunoglobulinem v dávce 250-500 mg/kg/3-4 týdny. Žádný z nemocných neměl v době odběru známky akutní infekce. Hladiny štěpných produktů komplementu jsme srovnávali s hladinami IgG (před zahájením imunoglobulinové terapie a v době odběru vzorku) a dále s počtem infekcí v průběhu jednoho roku. Nenalezli jsme statisticky významné rozdíly mezi hladinami C4d u nemocných s CVID a u kontrolních osob. Při vyšetření hladin C5-9 komplexu jsme prokázali statisticky významné snížení u nemocných s CVID oproti kontrolním osobám. Nezjistili jsme korelace mezi hladinami štěpných produktů komplementu a hladinami IgG, ani počtem infekcí v průběhu jednoho roku. Stejně tak nebyla prokázána korelace mezi klinickým stavem nemocných a hladinami štěpných produktů komplementu.

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