Theses

Study of complement functions in patients with malignant lymphomas
based on experiences from SLE

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1 Introduction

The complement system is composed of more than 30 components: soluble factors, regulatory proteins, a series of receptors, and other regulatory membrane-proteins. This complex system is an essentially important effector both in the innate and in the adaptive immune defense mechanisms. Its activation caused by special molecules on surfaces of microorganisms and antigen-antibody complexes is appearing as chain reaction and strengthening as cascade. The complement system is able to increase and limit itself via co-operation of three activation-pathways and by participation of regulatory factors. The activation of any of three complement pathways causes the destruction of molecules or pathogens provoking the activation, while self-structures of organisms remain intact. The complement system has important role in the formation of immune complexes (IC-s) via determination of their structure, size, transport and elimination, as well as in the pathomechanism of IC-s provoked inflammations.

Nowadays the aim of researches is to explain the role of complement system in the course of the immunoregulation, in the uptake and presentation of antigens and in the development of auto-tolerance. Based on the effects on the whole immune response the complement system must be considered as an essentially important participant of immunoregulation; they work in a close collaboration. The role of the complement system in autoimmune diseases and in the IC-mediated inflammations has been explored extensively. However few data are known about the connection between the complement system and malignant lymphomas despite the clinical observations, including high susceptibility to infections, immunohematological disorders and paraneoplastic syndromes based on the IC pathomechanisms.

The examination of the complement functions has a novel importance in diagnostic of malignant lymphomas, because of newly adopted procedures in therapy of these malignancies, like autologous blood stem cell transplantation (auto-BSCT) and therapy with monoclonal antibodies.

The aim of this work was to study the alterations of complement functions in malignant lymphomas, as well as their connection with changes in its clinical courses via using the experiences and adopting the methods proved in SLE. Studying the complement system is also important because of the spreading of novel, more and more effective therapies.
My experimental work was done between 1978-96 in the University of Medical School of Debrecen (now: University of Debrecen, Medical and Health Science Center, Institute for Internal Medicine), 3rd Department of Medicine.

I was commissioned to the attendance of lymphoma patients in 1987 and from that time I started to investigate the complement functions in lymphomas too. This work was continued from 1996 in the National Institute of Haematology and Immunology (now: National Medical Center) Budapest, Department of Immunology.
2 Objects of the study

2.1 To develop and adopt novel methods for study of complement functions and characterization of the structure of circulating immune complexes. To use these methods in SLE which can be regarded as a prototype of autoimmune diseases.

2.1.1 To work out new, modified methods on the basis of ELISA microtechnique for analysis of components of circulating IC-s and to study the correlation between the clinical symptoms of SLE and the components of complexes as well as the complement parameters.

2.1.2 To measure the complement mediated immune complex solubilization and the precipitation inhibition as functions of complement system in immunopathological diseases.

2.2 To search the connections between the changes of complement parameters and the clinical courses in malignant lymphomas via adoption of methods developed for investigation of SLE. To follow up the changes of complement parameters caused by treatments.

2.2.1 To explore the possible defects in complement functions and characterize them by determination of serum levels of complement components and by applying functional complement tests.

2.2.2 To characterize the connection between changes in complement functions and remission, relapse as well as prognosis of patients with malignant lymphomas.

2.3 To characterize the changes of serum levels of complement components and circulating immune complexes during autologous blood stem cell transplantation of patients with malignant lymphomas.

2.3.1 To determine the changes of complement parameters in transplant cases without complications.

2.3.2 To study the influence of septic and/or toxic transplant complications on complement parameters. To evaluate prognostic values of follow up investigation of complement levels during transplantation in cases of relapse and for further prognosis of disease.
3 Patients and methods

3.1 Patients

Participants of investigations were included from two institutes I attended. SLE cases were selected from 500 SLE patients treated at the 3rd Dept. of Medicine, University of Debrecen. Patients with malignant lymphomas were chosen from 265 lymphoma cases treated and followed-up at University of Debrecen between 1987-96, and from 234 patients attended at the Department of Immunology in the National Institute of Haematology and Immunology, Budapest.

3.2 Methods

3.2.1 Methods based on ELISA microtechnique:

- ELISA microtechnique was modified and prepared as novel method for measuring serum levels of antibodies against single(ss)- and double(ds)-stranded DNA considering their distribution in the three main immunoglobulin classes. Standardization of the measurements was carried out by use of immunoglobulin calibration curves.

- By modification of the method a procedure was worked out to characterize the composition of IC-s without splitting them. Precipitate with higher content of IC-s was prepared and not only the immunoglobulin content but the C3 fragment concentration were also determined in these complexes using three immunoglobulin- and C3- calibration curves at the same time.

3.2.2 Measurement of the effects of patients’ sera on artificial, isotope-labeled immune complexes:

- The complement mediated immunocomplex solubilizing capacity (CMSC) as function of the alternative complement pathway was measured on artificial, J^{125} isotope labeled BSA - anti-BSA complexes.

- The complement mediated immunoprecipitation inhibition capacity (IPIC) as function of the classical complement activation pathway was measured as prevention of formation artificial complexes from J^{125} labeled BSA and anti-BSA antibodies.

3.2.3 Activity of classical complement pathway (CH50) was measured.
3.2.4 Measurement of serum level of C3, C4 complement components using radial immunodiffusion.

3.2.5 Circulating immune complexes in patients’ sera was measured using PEG precipitation assay or complement consumption method.
4 Results

(Based on the 9 publications reported in the topic of the study)

4.1 By the adaptation of methods based on the principle of micro-ELISA technique, new method was developed to measure the serum levels of anti-ssDNA in patients suffered from SLE. An IgG calibration curve was made for the quantitation of measured data, as a result, the anti-ssDNA content of serum samples was given in μg/ml. The comparison of our results with the values measured by radioimmunoassay in the same samples led to a correlation coefficient of 0.99.

4.2 A modified method has been developed using the principle of ELISA technique for measuring serum levels of antibodies against dsDNA. For the adsorption of the native (ds) DNA on the microtitrator plate, its polistirene surface was treated with methylated BSA. The serum level of anti-dsDNA was measured in each main immunoglobulin class. Their quantity could be given using standard immunoglobulin calibration curves. In sera of 60 SLE patients it was found that anti-dsDNA IgG- and IgM-type antibody levels were significantly higher compared with levels in healthy control sera. In active periods of SLE the IgG-type antibody levels were significantly elevated than in the inactive stage of the disease.

4.3 The new method developed by modification of ELISA microtechnique proved to be suitable for qualitative and quantitative analysis of composition of immune complexes separated from the circulation. Measurements were made in serum samples of 68 SLE patients considering the active period of the disease as well as the nephropathy. Our results obtained by this method were comparable with data of literature, which were received with splitting of complexes. Firstly in the literature, the C3 fragment components of the intact immune complexes were studied and measured. We found that C3 split products content was significantly lower in active periods of the disease and in patients with nephropathy compared with inactive periods and in SLE patients without renal involvement (p < 0.05 and p < 0.01). The sera of patients with active SLE provide a more hypocomplementaemic environment for forming of immune complexes. The observations confirm that the complexes, formed in hypocomplementaemic sera, can be incompletely solubilized and they are potentially able to provoke more complement activation.
The complement mediated IC solubilizing capacity was studied in sera of SLE patients on the artificially produced, $^{125}$ isotope labeled BSA - anti-BSA complexes. 65 patients with SLE were grouped on the basis of showing activity and renal involvement. The solubilizing capacity of patients’ sera was significantly lower than the healthy controls ($p < 0.001$). The CMSC values were characteristically low also in patients in active stage and in cases with SLE nephropathy compared to those without complaints ($p < 0.001$). Comparing the solubilizing capacity to the other complement parameters, correlation was found with the elevated level of circulating IC-s and with decrease of C3 level in sera.

My study includes the accumulated observations about IC formation, working up and elimination mechanism and the role of the complement system in physiological and pathological events of IC formation. Discussing the clinical significance of the inflammations caused by immune complexes in the autoimmune diseases I presented other inflammatory syndromes based on the IC pathomechanism, too. I reviewed also the possible roles of complement system and IC-mediated inflammations in pathophysiology of malignant lymphomas.

The complement parameters and functions were evaluated in sera of patients with non-Hodgkin’s lymphomas, including the complement mediated immune complex solubilizing capacity and the immunoprecipitation inhibition capacity. The patients were grouped by Kiel’s nomenclature; 14 patients with high-grade malignancy and 14 other with low-grade malignancy were examined. The CMSC and IPIC values were significantly lower in sera of lymphoma patients either with high-grade malignancy or with low-grade malignancy compared to the values of the healthy controls ($p < 0.001$ and $p < 0.05$). Decrease in the solubilizing capacity and in the immunoprecipitation inhibition capacity showed correlation with elevated level of circulating immune complexes, moreover the IPIC values showed correlation with decreased levels of CH50 and C3 complement.

The data of follow up investigations in patients with B cell chronic lymphocytic leukemia (B-CLL) were presented in our collaborative work. Complement parameters were evaluated in serum samples of 46 patients. The starting values were obtained in the time of diagnosis. Eight years later the clinical documents of these patients were again collected and two different groups were established: a
group of short survivors dying in 4 years after the first complement examinations and a group of longer survivors living over 4 years. As regards the starting values, the CH-50 indicating the deficiency of classical complement pathway and the C3 complement contents of sera were significantly lower among the shorter survivors compared to the values obtained in sera of longer survivors ($p < 0.002$ and $p < 0.05$ in case C3). These findings indicate the prognostic values of complement parameters in B-CLL.

4.8 Serum levels of CH50, C3, C4 and circulating immune complexes were measured in parallel with the events of autologous blood stem cell transplantation of patients with malignant lymphomas during the remission of their disease. 17 lymphoma patients were included in the follow-up investigations. The auto-BSCT was complications-free in 14 cases and complications appeared in 3 cases. The day of transplantation was determined as day “0”. CH50, C3 and C4 levels decreased significantly in the 14 complications-free patients on the -2nd day, caused by conditioning chemotherapy and/or total body irradiation ($p < 0.05$). These decreased levels returned to the starting values measured before the conditioning treatment from the day +7. In contrast, the level of circulating IC-s significantly increased between day +7 and +14 after the transplantation. Despite the changes the values in complication-free group remained within the wide normal ranges. Septic shock syndrome appeared in two patients and they died on the +8th and +11th days. In both cases the values CH50, C3, C4 measured on the day +7 showed a significant elevation compared to their starting levels. In one case early post-transplant relapse was signed by decreasing complement parameters on the day +21 but definitely low on the day +35 in association with increasing IC level preceding the clinical symptoms of relapse by some weeks. By determining the pattern of complement changes of complication-free cases in curses of auto-BSCT the alterations from these values can be considered as signs of prospective complications.

4.9 Data of literature and our experiences were discussed in this chapter of the book about the angioimmunoblastic lymphadenopathy and its transformation into malignant lymphoma. In the early period the disease suggests clinical signs of severe acute life-threatening infection. The immunoserological tests suggest an inflammation with immunopathomechanism associated with a characteristic
lymphadenopathy. There are patients recovering as a result of immunosuppressive treatments while other patients develop malignant lymphomas with poor prognosis. Our knowledge about the pathomechanism of angioimmunoblastic lymphadenopathy, the different changes in the autoimmune diseases and also the initial lymphoma proliferation suggest some similarities as the sign of defected immunoregulation.
5 Conclusion and Summary of results

5.1 Principles of the novel and prosperous techniques of the literature were adapted in my experimental work by developing new and suitable methods for examinations of complement functions, autoimmune diseases as well as of malignant lymphomas. These were the methods based on the ELISA microtechnique and the measurements of complement mediated immune complex solubilizing capacity and immunoprecipitation inhibition capacity.

- A procedure using immunoglobulin and C3 complement calibration curves was successfully added to the method based on principle of micro-ELISA technique for quantitation of serum parameters. This step made possible to develop a new modified method for studying the structure of immune complexes. Correlation has been observed with decreased C3 fragments content of IC-s and the active stage as well as with renal involvement in SLE. This finding strengthens the theory suggested by applying other methods, that incompletely solubilized immune complexes exist in sera of SLE patients able to provoke further complement activation and inflammation.

- The complement mediated immune complex solubilizing capacity (CMSC) and the immunoprecipitation inhibition capacity (IPIC) were found decreased in sera of SLE patients. The degree of decreasing showed correlation with activity and renal involvement in SLE. These parameters changed parallel with activity of symptoms during the course of disease. The CMSC showed correlation with activation of alternative complement pathway and the IPIC with activation of classical complement pathway, but correlation was not close. Both parameters showed correlation with elevated concentration of circulating IC-s.

5.2 As a result of examining complement parameters in sera of patients with malignant lymphomas, alterations have been proved in the complement functions CH50, CMSC, IPIC and complement levels C3, C4 as well as in concentration of circulating IC-s. All these alterations suggest the relationship with the changes in symptoms of disease and with the effects of therapies.
• In courses of non-Hodgkin’s lymphomas the traditionally measured complement parameters were evaluated in the histological groups of patients. The complement abnormalities were more frequent and severe in lymphomas with high-grade malignancies. Deficiencies of complement functions could also been demonstrated in the low-grade lymphomas but these were milder and less frequent. We found decreases in CMSC and IPIC values and elevated levels of circulating immune complexes parallel.

• The defective complement functions of patients with B-CLL showed correlation with a poorer prognosis and a shorter survive. In cases of high-grade lymphomas, deficiency of complement functions was sharp in periods of progression and the values returned into the normal ranges in remission.

5.3 Autologous peripheral blood stem cell transplantation of patients with malignant lymphomas was monitored by evaluating the complement function CH50, the serum levels of complement components C3, C4 and the circulating immune complexes.

• In complications-free transplantation, the evaluation of CH50, C3, C4 and circulating IC level was realized at the time points considered critical regarding the clinical events, they were days –7, -2, +7, +21 +35. Consideration of the typical changes of the averages in time rendered possible for us to determine the basic patterns of changes.

• In case of serious septic and/or toxic complications the complement functions and parameters showed elevation on the day +7, despite of acute complement activation, possibly caused by the balance effect of increase in the production of factors provoked by the simultaneous infection. Furthermore the decrease in complement functions and serum levels of parameters can be the indicator of the early relapse. The follow up investigation of complement parameters seems to be suitable to predict infectious complications or relapse of malignant lymphomas, and allows starting prevention the soonest possible.
6 Summary

The complement system has essentially important effector function in the immune defense mechanism. Complement can be activated by surface molecules of microorganisms and antigen-antibody complexes. Complement system has also an important role in formation of immune complexes (IC) via determination of their structure, size, transport, elimination as well as role in pathomechanism of IC provoked inflammations. Connection between IC disease, defective complement functions and SLE has been explored extensively. However, few data are known about connection between complement system and malignant lymphomas despite clinical observations including high susceptibility to infections and paraneoplastic syndromes with IC pathomechanism.

The aim of our work was to develop methods for characterization of function and components of complement system and circulating IC in SLE in order to study connections between activity of disease and changes of parameters. Using experiences and adopting the methods obtained in SLE connection between clinical course and complement alterations was studied in malignant lymphomas, too.

On the basis of ELISA microtechnique new modified method was developed for study of IC composition in SLE. Quantification of results was determined by using calibration curves for immunoglobulins and C3. Decreased C3 content of IC was observed in active stage of SLE and in cases with renal involvement suggesting incomplete IC solubilization resulting in further complement activation and inflammation. In malignant lymphomas defective complement functions and decreased levels of complement components were observed. Complement abnormalities were more frequent in high-grade malignancies. However, hypocomplementemia was observed in low-grade cases, too. Decrease of complement mediated immune complex solubilization and immune precipitation inhibition was also determined. Study and follow-up of complement functions, serum levels of complement components C3, C4, and circulating IC during autologous blood stem cell transplantation in malignant lymphoma patients were published by our group as novel findings. Results of complications-free cases were regarded as standard and alterations from these values were suggested as signs of different complications.
7 Publications reported in topic of the study.


8 Related publications


    Haematologia (Budap). 1996;27(2):99-105. – **IF:0,143**

12. Illés Á, **Bányai A**, Jenei K, Bacskó Gy, Kovács J, Szakáll Sz, Szegedi G. Terhesség során felismert kétoldali, pimer emlőlymphoma.

13. **Bányai A**, Illés Á, Nemes Z, Vadász Gy, Dévényi K, Szegedi Gy: A gastrointestinalis tractus másodlagos lymphomatous polyposisa nyirokcomó lymphomában szenvedő betegekben

    Haematologia (Budap). 1997;28(3):117-122. – **IF:0,098**

    Transplant Proc. 1998 Dec;30(8):4130-4131. – **IF:0,740**

    Leuk Lymphoma. 2000 Nov;39(5-6):661-665. – **IF:1,252**

    Acta Haematol. 2001;106(3):100-105. – **IF:0,796**


Haematologia (Budap). 2002;32(4):519-527. – **IF:0.293**

Bone Marrow Transplant. 2002 Mar;29(5):449-452. – **IF:2.378**


Rheumatol Int. 2004;24(6):359-361. – **IF:1.000**

Impact factors: **21,735**

Impact factors of the publications in topic of the study: **8,543**