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Enzymatic alteration of C1q, the collagen-like subcomponent of the first component of complement, leads to cross-reactivity with type II collagen

Hans-Peter Heinz, Dieter Brackertz[+] and Michael Loos

Institute of Medical Microbiology, Johannes Gutenberg-University and [+]*St. Vincenz and Elisabeth Hospital, Mainz, FRG*

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Native serum C1q, the collagenous-like subcomponent of the first component of complement, is not recognized by polyclonal anti-collagen type II antibodies. However, when purified C1q was subjected to limited proteolysis by collagenase it showed antigenic cross-reactivity with collagen type II. The same cross-reactivity was observed with hemolytically active C1q in synovial fluids of patients with rheumatoid arthritis (RA), whereas C1q from synovial fluids of patients with osteoarthritis (OA), villo-nodular synovitis and ankylosing spondylitis was not recognized by this antibody. However, incubation of synovial fluid C1q of OA patients with synovial fluid leucocytes from RA patients led to an alteration of OA-C1q which was now recognized by the anti-collagen type II antibody.

Complement; C1q; Collagen; Collagenase; Rheumatoid arthritis

1. INTRODUCTION

C1q, a subcomponent of the first component of complement (C1), contains a collagen-like sequence of ~80 amino acid residues which imparts to this serum protein some of the biophysical and biochemical characteristics of collagen [1,2]. Biosynthesis studies on C1q and collagen have shown that both molecules undergo similar post-translational steps [3–5].

Under normal conditions serum C1q and collagen do not show any antigenic cross-reactivity, however under certain circumstances the structural similarity of collagen and C1q may result in biochemical and biological cross-reaction. The immunological aspects of the structural relationship are of great interest, since auto-immunity to collagen or to the collagen-like C1q may explain both the systemic nature and chronicity of the inflammation occurring especially in rheumatoid arthritis (RA) [6,7]. Since both molecules serve as substrates for collagenase, the relationship between activated collagenase and the pathology of RA is of particular interest. Since activated collagenase is only found in synovial fluid of RA patients, but not in the joint fluid of patients with osteoarthritis (OA) [8], one can emphasize the importance of this enzyme, which probably leads to an immunological cross-reactivity between C1q and collagen by limited proteolysis.

In this report we present evidence that limited proteolysis of serum C1q by collagenase induces an alteration of this molecule which leads to a cross-reactivity with collagen type II as measured by an anti-collagen type II antibody. Furthermore, we demonstrate that C1q in synovial fluids of patients with RA already contains a C1q which is recognized by the anti-collagen type II antibody. Leucocytes of the RA-synovial fluids were found to be responsible for the alteration of C1q.

Correspondence address: H.-P. Heinz, Institut für Medizinische Mikrobiologie, Hochhaus am Augustusplatz, 6500 Mainz, FRG

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2. MATERIALS AND METHODS

2.1. *Complement components and assays*

Sheep erythrocytes (E) sensitized with anti-E rabbit antibodies (A) were prepared as described in [9]. Cell intermediates EA, EAC4 and EAC1q4, as well as appropriate buffers, e.g. Veronal-buffered saline, VBS (ionic strength μ = 0.15; pH 7.3) and Veronal-buffered saline with sucrose, VBS-S (ionic strength μ = 0.065; pH 7.3) were prepared as described in [10]. Human C1q was purified according to Stemmer and Loos [11], using fast protein liquid chromatography (FPLC). Partially purified C1r and C1s were prepared as described in [12]. $C2^{EP}$ and $C4^{EP}$ were isolated by a slightly modified method [13]. Guinea pig serum with 2 mM EDTA (C-EDTA; 1:50) was used as source for the late acting components C3–C9 [10]. The rate of hemolysis, calculations and cell concentrations were used as described in [14].

2.2. *ELISA procedures*

Purified polyclonal rabbit IgG to bovine collagen type II and III were kindly provided by Professor Holborow, London. From these antibodies $F(ab')_2$ fragments were obtained after pepsin digestion in 0.1 M acetate and gel filtration on Superose TM12 (Pharmacia, Uppsala, Sweden) [15]. Monoclonal anti-C1q antibodies were raised as described in [16–18].

Wells of the microtiter plates were coated with purified rabbit anti-collagen type II IgG-$F(ab')_2$ by addition of 20 μl of a 1:100 dilution in 10 mM Tris-HCl, 0.13 M NaCl, pH 9.0. After 1 h the plates were washed in the same buffer containing 0.015% Tween 20, 1% BSA to block remaining binding sites. Purified human C1q, treated with different amounts of collagenase (type III, Sigma, FRG) or buffer was added for 1 h. The reaction was blocked by the addition of 10 mM EDTA. The samples were diluted 1:100 in 10 mM Tris-HCl, 0.13 M NaCl, pH 7.4, prior to use. After washing twice, the bound material was detected with a mixture of three different biotinylated monoclonal anti-C1q antibodies in a ratio of 1:1:1. Biotinylation was performed as described in [19]. The binding of the biotinylated antibodies was determined after addition of avidin coupled to alkaline phosphatase.

2.3. *Patients and synovial fluids*

Synovial fluid samples were collected from the knees of the patients at the time of a clinically indicated diagnostic puncture. Some of the patients were diagnosed as having classic RA according to the American Rheumatism Association classification criteria [20]. The others were diagnosed as having unilateral or bilateral OA, psoriatic arthritis, villo-nodular synovitis or ankylosing spondylitis. The samples were tested immediately after collection.

3. RESULTS

Purified C1q was subjected to an enzymatic alteration by collagenase. In order to measure the influence of the enzyme on cell-bound C1q, which enables us to wash out the enzyme at any given time, the following assay was performed: EAC1q4 was prepared by incubation of EAC4 with purified

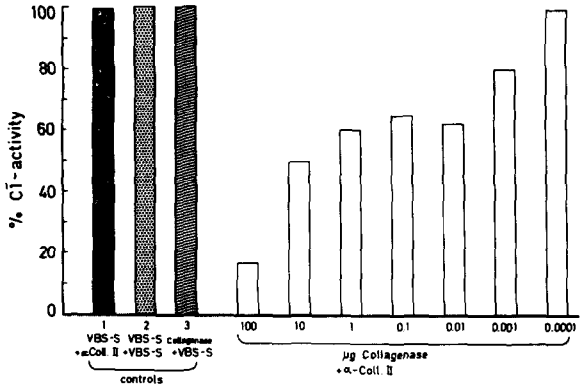

Fig.1. Dose-dependent inhibition of $C\bar{1}$ (C1q) hemolytic activity after treatment with collagenase: EAC1q4 preparations (100 effective C1q molecules per cell) were treated with different amounts of collagenase. Control 1 was treated with VBS-S instead of collagenase and anti-collagenase II diluted 1:100 in the same buffer. Control 2 was treated with buffer alone. Control 3 was treated with 100 μg/ml collagenase and VBS-S instead of anti-collagenase II. After washing twice, purified anti-collagen type II IgG (1:100 in VBS-S) was added. Again the cells were washed and after addition of $C1\bar{r}$, C1s, C2 and the late acting complement components, $C\bar{1}$ activity was determined.

C1q (100 effective molecules per cell) for 10 min at 30°C in VBS-S. The cells were washed, resuspended with VBS-S and incubated with different amounts of collagenase for 2 h at 30°C. Controls were treated with buffer. After washing twice the cells were incubated with the purified anti-collagen type II IgG (1:100 diluted in VBS-S, additional controls were treated with buffer) for 30 min at 30°C. Again, the cells were washed twice and the hemolytic C1q activity was determined. Fig.1 shows that the anti-collagen type II antibody does not inhibit the activity of bound C1q. However, when bound C1q was pretreated with collagenase then inhibition of C1q activity by the anti-collagen type II antibody was observed. The extent of inhibition was dependent upon the concentration of collagenase used. Collagenase by itself had no effect on C1q activity. In additional experiments it was found that purified anti-collagen type III antibodies had no effect on collagenase treated bound C1q (not shown; see also fig.3).

The recognition of collagenase-altered C1q with the anti-collagen type II antibody could also be demonstrated in a biotin-avidin ELISA system. Plates covered with anti-collagen type II $F(ab')_2$ antibody fragments (50 μg/ml) were incubated

with collagenase (0.01 to 100 μg/ml) pretreated C1q (30 min at 30°C). Binding of C1q was monitored with biotinylated monoclonal anti-C1q antibodies (fig.2). Again only collagenase-treated C1q was recognized by anti-collagen type II antibodies. The use of the F(ab')$_2$ fragments excluded Fc-mediated binding of C1q of the anti-collagen antibody.

Auto-antibodies against collagen type II are well documented in the literature and are thought to contribute to the pathogenic mechanisms causing RA [6,7]. The generation of auto-antibodies could be due to enzymatic alterations of collagen or collagen-like material. One of the candidates causing such an alteration is collagenase, which occurs in a high concentration and in the activated form within the synovial fluids during the acute phase of inflammation [8]. Since we were able to show in vitro the recognition of collagenase-treated human C1q by an anti-bovine collagen type II antibody,

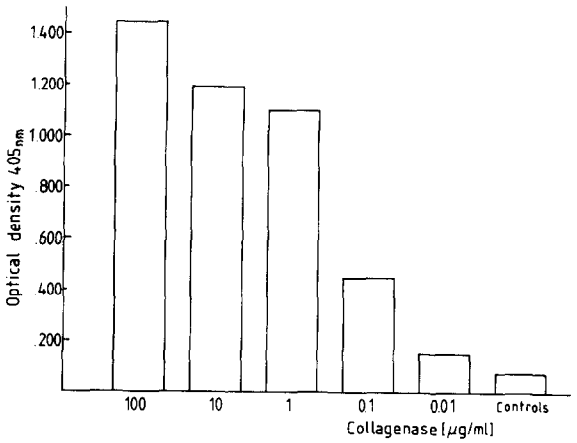

Fig.2. Recognition of collagenase-treated C1q by the anti-collagen type II F(ab')$_2$-fragments. Collagenase-treated C1q was bound by the anti-collagen type II F(ab')$_2$ coated on wells. Binding was detected with biotinylated anti-C1q monoclonal antibodies. Buffer-treated C1q as well as anti-collagen type III antibody (IgG-fraction) served as control.

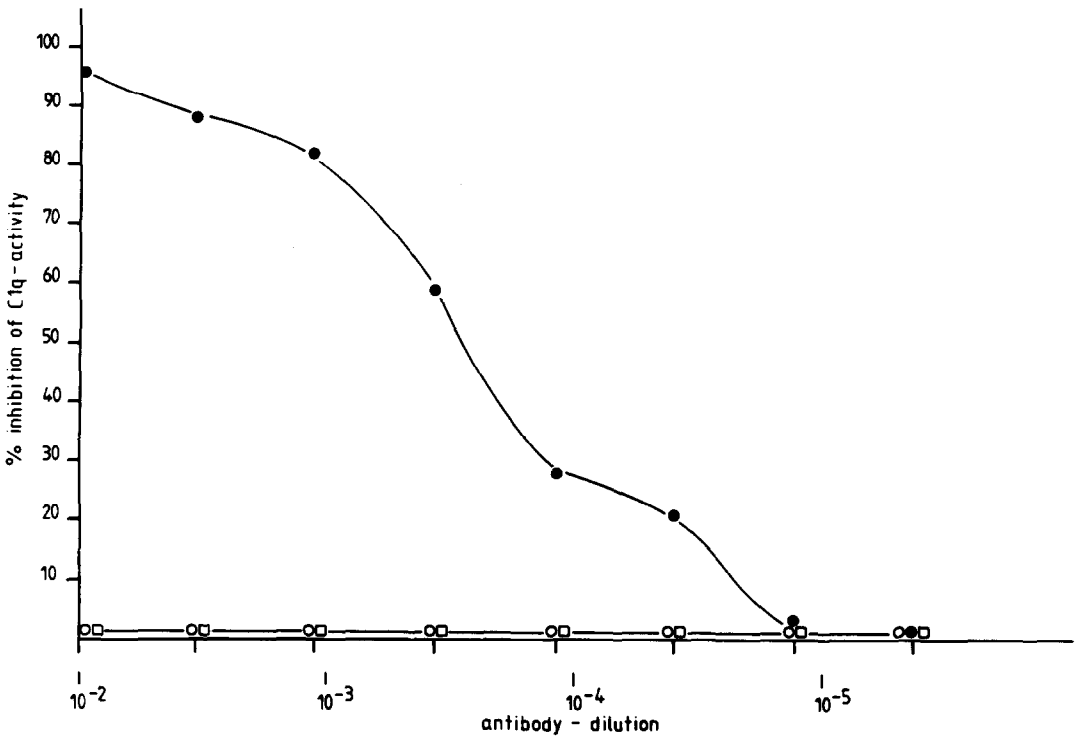

Fig.3. Inhibition of $C\bar{1}$ (C1q) hemolytic activity by the anti-collagen type II antibody in an RA-synovial fluid. C1q within the EAC1-complex from the synovial fluid of patients with RA (●—●), OA, ankylosing spondylitis, and villo-nodular synovitis (○—○) were tested for their reactivity with the anti-collagen type II antibody in the hemolytic assay. As an additional control all samples were tested for their reactivity with an anti-collagen type III antibody (□—□).

we investigated the influence of this antibody on C1q in macromolecular C1 from the synovial fluid of patients with RA, OA, psoriatic arthritis, villonodular synovitis and ankylosing spondylitis.

$EAC1_{lim}$ ($z = 1$) preparations were prepared by incubating the individual synovial fluids with EA for 10 min at 30°C. 100 μl of each $EAC1_{lim}$ preparation were incubated with different amounts of anti-purified collagen type II IgG for 30 min at 30°C. After washing twice, the hemolytic C1 activity was determined. Fig.3 shows that only the hemolytic $\overline{C1}$ activity from the synovial fluid of the patient with RA could be inhibited by the antibody. C1 from NHS as well as from the synovial fluids from the other patients were not affected by the anti-collagen type II antibody. As an additional control, all samples were tested for their reactivity with purified anti-collagen type III IgG. No reaction could be observed (fig.3).

The cellular composition in the synovial fluids of RA patients plays a crucial role in diagnosis: in the acute phase the ratio of granulocytes versus lymphocytes is found to be at least 8:1. Since granulocytes are described as secreting collagenase [8], we considered these cells to cause the alteration

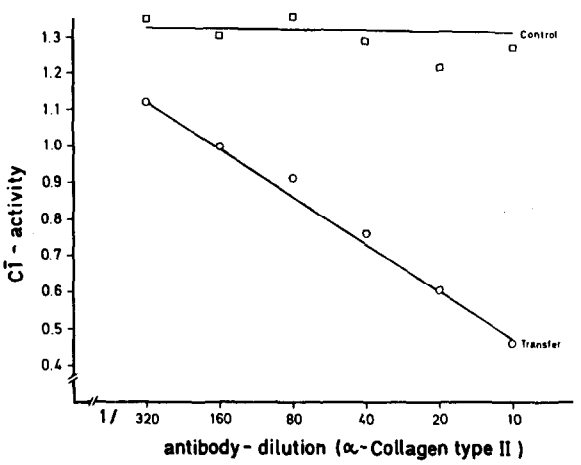

Fig.4. Influence of the leucocytes from an RA-synovial fluid on the reactivity of C1q from an OA joint fluid with the anti-collagen type II antibody. Leucocytes were transferred from RA- to OA-synovial fluid and incubated for 3 h at 37°C. The cells were spun down and the supernatant was tested in the hemolytic assay. Control was performed by OA leucocytes in OA-joint fluid for 3 h at 37°C. (□—□) Control; (○—○) transfer. A dose-dependent decrease of the $\overline{C1}$ (C1q) hemolytic activity can only be observed in the transfer experiment.

of synovial fluid C1q. To test this hypothesis, the following experiment was performed: cells from synovial fluid of RA patients were spun down. After discharging the supernatant, the cells were washed three times and adjusted to a concentration of 1.0×10^7/ml. These cells were incubated with the OA-synovial fluid (as source of C1 or C1q) from which the cells had been removed by centrifugation for 3 h at 37°C. After centrifugation, the supernatant was incubated with EA to form EAC1. Equal volumes of EAC1 were incubated with different dilutions of purified anti-collagen type II IgG. After washing the remaining C1 activity was determined. Fig.4 shows that incubation of OA-synovial fluid with granulocytes from the RA patient led to a recognition of the OA-C1 (C1q) by purified anti-collagen type II IgG resulting in an inhibition of its hemolytic activity.

4. DISCUSSION

Native C1q, the collagen-like subcomponent of the first component of complement, is not recognized by an anti-collagen type II antibody. In this report we raised the question of whether enzymatic treatment of C1q with collagenase alters its antigenic behaviour in such a way that C1q now shares epitope(s) with collagen type II. Treatment of cell-bound C1q (EAC1q4) or fluid-phase C1q with different concentrations of collagenase resulted in a dose-dependent recognition of C1q by an anti-collagen type II antibody (figs 1 and 2). Collagenase (100 μg/ml, 3 h at 37°C) by itself had no effect on the hemolytic activity of cell-bound C1q. The hemolytic activity of collagenase-treated C1q was blocked by the anti-collagen type II antibody depending on the concentration of collagenase used for C1q treatment.

Comparing C1q from synovial fluids of different rheumatic diseases revealed that only C1q from synovial fluids of RA patients was recognized by the anti-collagen type II antibody (fig.3). This indicated that within the synovial fluid of RA patients an alteration of C1q takes place resulting in cross-reactivity with collagen type II. The proteolytic alteration of C1q could be achieved by the leukocytes separated from the synovial fluids of an RA patient (fig.4). Since granulocytes represent the predominant cell population within synovial fluids of RA patients, the granulocyte-derived col-

lagenase must be considered to cause an alteration in the collagenous portion of the C1q molecule. This interpretation is in agreement with the observations that 'activated' collagenase derived from granulocytes is present in the joint fluids of RA patients [8]. To confirm this assumption experiments are planned using purified granulocyte-derived collagenase.

So far, there is only one report showing cellular immune response after intradermal immunisation of guinea pigs with the collagen-like C1q fragments [21]. In this report a cross-reactivity to collagen type I was observed.

Our in vitro and in vivo observations show that limited proteolysis of C1q with collagenase reveals common epitope(s) with collagen type II. Even when an antibody against bovine collagen type II was used in this study, the observed cross-reactivity with human C1q after limited proteolysis indicates that within the collagen-like portion of C1q common epitopes with collagen type II are present. There are several reports in the literature describing the induction of a collagen-induced arthritis using heterologous collagen type II preparations [22]. The observations described in this report raise the question as to whether collagenase-altered C1q is involved in the pathomechanism of rheumatoid arthritis.

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REFERENCES

[1] Reid, K.B.M. (1976) Biochem. J. 155, 5–17.
[2] Reid, K.B.M. and Porter, R.R. (1976) Biochem. J. 155, 19–23.
[3] Müller, W., Hanauske-Abel, H. and Loos, M. (1978) FEBS Lett. 90, 218–222.
[4] Loos, M. (1982) Prog. Allergy 31, 135–192.
[5] Prockop, D.J., Berg, R.A., Kivirikko, K.I. and Uitto, J. (1976) in: Biochemistry of Collagen (Ramachandran, G.N. et al. eds) pp.163–273, Plenum, New York.
[6] Andriopoulos, N.A., Mestecky, J., Wright, G.P. and Miller, E.J. (1976) Arthritis Rheum. 19, 613–617.
[7] Stuart, J.M., Cremer, M.A., Townes, A.S. and Kang, A.H. (1982) J. Exp. Med. 155, 1–16.
[8] Harris, E.D. jr and Krane, S.U. (1974) New Engl. J. Med. 291, 557–563; 291, 605–609; 291, 652–660.
[9] Mayer, M.M. (1961) in: Experimental Immunochemistry (Kabat, E.A. et al. eds) pp.133–240, Thomas, Springfield, IL.
[10] Rapp, H.J. and Borsos, T. (1970) Molecular Basis of Complement Action, Appleton-Century-Crofts, New York.
[11] Stemmer, F. and Loos, M. (1984) J. Immunol. Methods 74, 9–16.
[12] Schultz, D.R., Loos, M., Bub, F. and Arnold, P.J. (1980) J. Immunol. 124, 1251–1257.
[13] Nelson, R.A. jr, Jensen, J., Gigli, T. and Tamura, N. (1966) Immunochemistry 3, 111–135.
[14] Ringelmann, R., Opferkuch, W., Röllinghoff, M. and Loos, M. (1969) Z. Med. Mikrobiol. Immunol. 154, 329–343.
[15] Stanworth, D.R. and Turner, W. (1978) Handbook of Experimental Immunology (Weir, D.M. ed.) 3rd edn, Blackwell, Oxford.
[16] Golan, M.D., Burger, R. and Loos, M. (1982) J. Immunol. 129, 445–447.
[17] Heinz, H.-P., Burger, R., Golan, M.D. and Loos, M. (1984) J. Immunol. 132, 804–808.
[18] Heinz, H.-P. and Loos, M. (1984) Behring Inst. Mitt. 76, 42–58.
[19] Holmdahl, R., Nordling, C., Rubin, K., Tarkowski, A. and Klareskog, L. (1986) Scand. J. Immunol. 24, 197–203.
[20] Ropes, M.W., Bennett, G.A., Cobb, S. et al. (1958) Bull. Rheum. Dis. 9, 175–176.
[21] Menzel, E.J., Smolen, J.S. and Reid, K.B.M. (1981) Mol. Immunol. 18, 765–771.
[22] Trentham, D.E. (1982) Arthritis Rheum. 25, 911–916.