KAWASAKI MED. J. VOL. 8 : NO. 1, 19-24 (1982)

Crossed Immunoelectrophoresis of Human C3 : Electrophoretical Heterogeneity among Purified Molecules

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Accepted for Publication on January 29, 1982

ABSTRACT. One component of complement system, C3, is detected in β region electrophoretically. Purified C3 has a molecular weight of 187500, and has been considered to have a homogeneous physicochemical structure. The authors intend to mention in this paper about the observations which suggest the heterogeneity among molecules of human C3 in solution. Crossed immuoelectrophoresis, using anti-human C3 serum (rabbit or goat), detected at least 5 precipitation arcs in β1c globulin (C3), and this pattern of electrophoresis was observed in repeated experiments. When human serum was used as the source of C3, the same electrophoretic pattern as that of purified C3 was detected.

Recently, correlation between some conformational changes of C3 molecules and the appearance of C3b-like functional properties was reported by some investigators. The relationship between our data and these conformational changes is unclear and requires further investigation.

Our results suggest that standard semi-quantitative calculation of C3 conversion ratios could be mistakenly high because of the existence of several sub-arcs in the β1c arc, and that such calculation require quantitative consideration of the sub-arcs.

C3 is a serum protein which plays an important role in host defense and the inflammatory functions of complement, i.e., C3 is the precursor of several physiologically occurring fragments as anaphylatoxin or chemotactic factor for neutrophils. The protein has a molecular weight of 187500[1] and is composed of the polypeptide chains, the molecular weights of which are approximately 115000 (α chain) and 75000 (β chain). Upon complement activation, the enzyme C3 convertase cleaves peptide bond 77 of the α chain of C3, thereby producing the fragments C3a and C3b[2]. C3a constitutes one of the three anaphylatoxins of the complement system. C3b is cleaved by C3b inactivator (C3b INA) to the fragment of C3c and C3d. Electrophoretically C3, C3c and C3d are detected in the region of β1c, β1a and α2D respectively[3].

It is the purpose of this paper to demonstrate that purified human C3 or C3 in human serum show electrophoretical heterogeneities studied with antigen-

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antibody crossed immunoelectrophoresis.

Recently, a novel form of C3 has been described which, although intact with regard to its peptide structure, is hemolytically inactive and displays C3b-like functional property[4]. C3 with C3b-like property is directly responsible for the initiation of the alternative complement pathway, and the conformational changes are detected by spectroscopic methods[5].

It remains unknown if our data suggest some conformational changes of C3 molecules in liquid phase or not.

MATERIALS AND METHODS

Reagent : Purified human C3 was purchased from Cordis Lab. (France). Human sera were collected from healthy donors. Within half an hour of collection the samples were centrifuged, and the sera were frozen in small aliquots at -70°C.
Anti-sera : Rabbit anti-human C3, goat anti-human C3, rabbit anti-human C4, and goat anti-human C5 were purchased from MBL (Nagoya, Japan). Rabbit anti-human C3 activator was obtained from Behring Berke (Germany).
Crossed immunoelectrophoresis : Antigen-antibody crossed electrophoresis was performed in a 1% agarose gel (Nakarai Chem. Japan) with barbital buffer, 0.05M, pH 8.6. The first dimension run was performed with a potential drop of 20V/cm using a constant power supply (LKB, type 2197) for 45 min. The second dimension electrophoresis was run at 10V/cm for 1-4 hr against 1% agarose gel containing 2.4% anti-serum.
Immunoelectrophoresis and micro-Ouchterlony double diffusion : Identification of antigenic determinants was performed using immunoelectrophoresis and micro-Ouchterlony double diffusion. Immunoelectrophoresis was carried out in 1% agarose with Veronal buffer at 20V/cm for 1 hr. Micro-Ouchterlony double diffusion was performed in 1% agarose with Veronal buffer, pH 8.6.

RESULTS

Crossed immunoelectrophoresis : As it has been called as β1c globulin, purified C3 shows a clear arc in a β electrophoretic position against rabbit or goat specific antiserum to human C3 (β1c/β1a). However, the arc of C3 contains at least 5 different sub-arcs, and this pattern was always observed after the repeated experiments (Fig. 1). When human sera were used as the source of C3, the same electrophoretic mode as in purified C3 was detected as shown in Fig. 2&3. But there was a difference in the electrophoretic mode between in purified C3 and C3 in fresh serum. Another small peak of more slowly or cathodally migrating substance than β1c can be seen in the case of purified C3, which could be artificial break down products of purified C3, and unclear in the case of serum C3. The electrophoretic feature of purified C3 and C3 in fresh serum was the same in either case, when anti-human C3 (β1c/β1a) in the crossed immunoelectrophoresis was derived from rabbit or goat. Since electrophoretic

sub-arcs of β1c globulin mentioned above lowered and disappeared altogether when the C3 conversion occurred, there might be some possibility of the contamination of other component in complement system, e.g., C4, C5 or factor B (C3 proactivator). To discriminate electrophoretic peaks of C3 from C4, C5 or factor B, experiments were performed as shown in Fig. 2, 3 & 4. Fig. 2 shows the electrophoretic pattern of C5 and C3 in the fresh serum at the same time, and Fig. 3 shows that in the serum iucubated with zymosan (1mg/50μ)

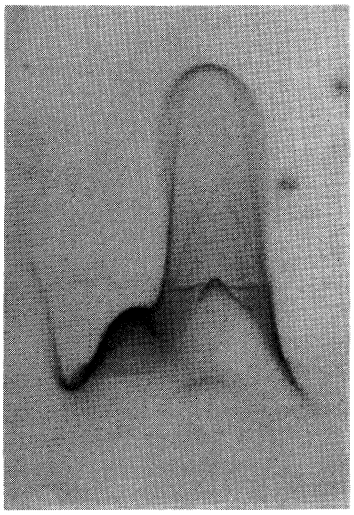

Fig. 1. Immunoelectrophoresis of purified human C3. Agarose gel (1%) containing 2.4% of anti-human C3.

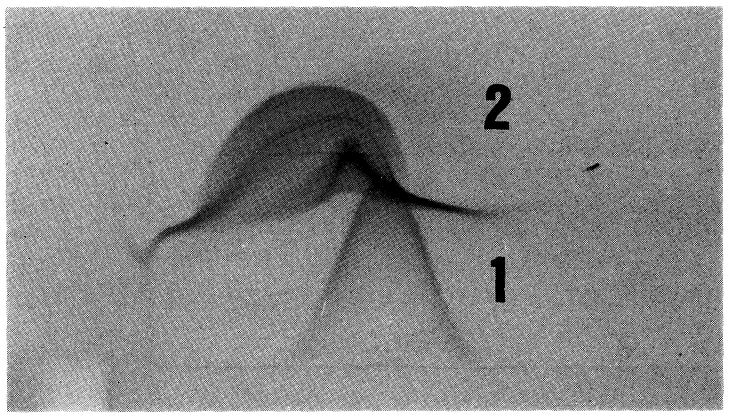

Fig. 2. Immunoelectrophoresis of fresh human serum. One per cent agarose gel containing 2.4% of anti-sera,
1 : anti-human C5
2 : anti-human C3+C5

for 1 hr respectively. The sub-arcs of C3 and that of C5 show the different pattern as seen in Fig. 2, and the possibility of the contamination of C5 or anti-human C5 antibody could be deniable. When the serum was incubated with zymosan, only the precipitation line of C3 could be in upper layer and none in lower layer, what means that the fragments of C5, C5a or C5b, situate in other electrophoretic position than β1c β1a and α2D. Fig. 4 demonstrate

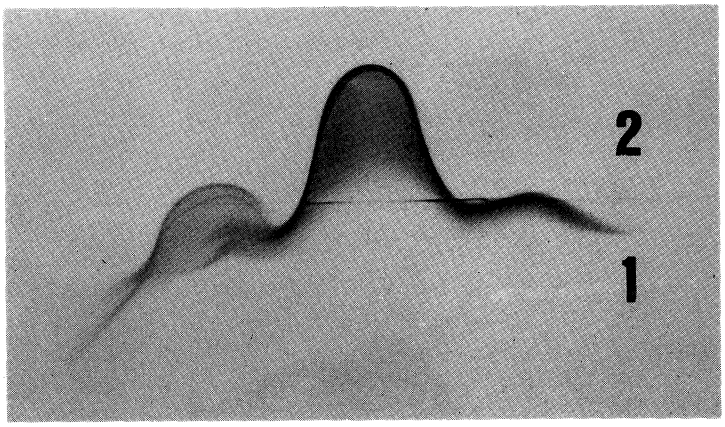

Fig. 3. Immunoelectrophoresis of human serum incubated with zymosan at 37°C for 1 hr (1mg/50μl). Agarose gel containing 2.4% anti-sera,
1 : anti-human C5
2 : anti-human C3+C5

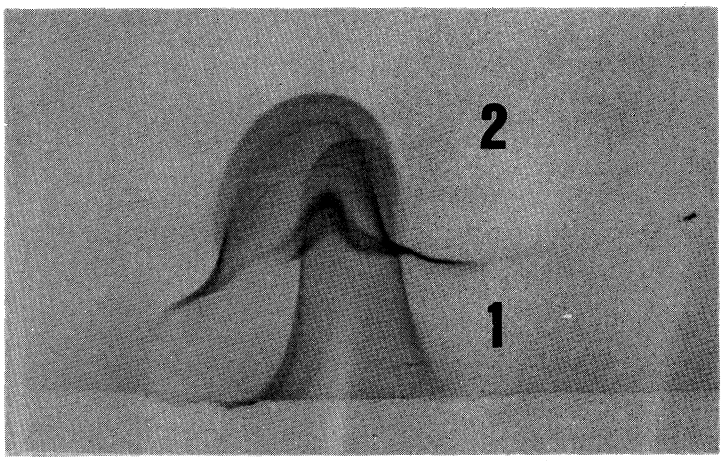

Fig. 4. Immunoelectrophoresis of fresh human serum. Agarose gel containing 2.4% of anti-sera,
1 : anti-human C4
2 : anti-human C3+C4

the electrophoretic pattern of C4 and C3 in fresh human serum. Precipitation line of C3 could be detected in upper gel, and that of C4 in lower gel. Here also the contamination of C4 and anti-C4 antibody might be negligible. Fig. 5 shows the precipitaion lines of C3 and C3 activator in the serum after the incubation with zymosan (1 mg/50μl). In upper gel the fragments of C3, β1c, β1a and α2D, can be seen, and in lower gel the fragment of C3 activator could be detected in the different pattern from that of C3 moieties.

Immunoelectrophoresis : Immunoelectrophoresis of purified C3 and C3 in human serum was performed, and precipitation curve in β region was seen in each sample.

Micro-Ouchterlony double diffusion : Double diffusion method in agarose gel was performed and one precipitation line was recognized in each sample.

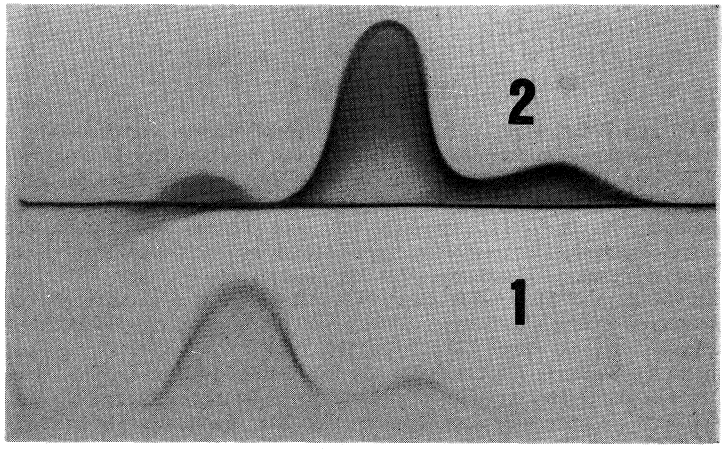

Fig. 5. Immunoelectrophoresis of human serum incubated with zymosan at 37°C for 1 hr (1mg/50μl). One per cent agarose gel containing 2.4% of anti-sera,
1 : anti-human C3 activator
2 : anti-human C3+C3 activator

DISCUSSION

Crossed immunoelectrophoresis permits semi-quantification of C3 split products by measuring the peaks area[6,7]. This technique is simple to perform, and used to demonstrate and estimate the rate of C3 conversion. Our findings in this paper suggest that electrophoretic arc of β1c globulin (C3) in crossed immuno-electrophoresis is composed ot several sub-arcs, and that semi-quantification should be performed taking it into consideration. The quantity of C3 should be calculated as total value of the sub-peak areas, otherwise the rate of C3 conversion could be unreasonably highly decided.

A number of physicochemical changes are associated with conversion of C3

to C3b, and the observed differences reflect cleavage-induced allosteric changes in conformation or exposure of residues initially sequested from solvent by the C3a domain. The factors which initiate the conversion of C3 to C3b are well known, e.g., alterations in the environment of certain aromatic residues, a decreased susceptibility of disulfides to reduction, and increase in surface hydrophorbicity. The slow thawing process which results in inactivation of C3 and formation of C3b-like substance was presumably mediated by water hydrolysis of the thioeser bond, although no peptide bonds were broken in the process.[8,9]

Our data detected that there are several sub-peaks of C3 in crossed immunoelectrophoresis with anti-human C3 serum, and decrease altogether with the C3 conversion.

It remains to be clarified what kinds of molecular structures could be exist between β1c globulin molecules, and what is the reason to initiate the electrophoretic heterogeneity between C3 molecules.

REFERENCES

1) Tack, B.F. and Prahl, J.W. : Third component of human complement : purification from plasma and physicochemical characterization. Biochemistry **15** : 4513-4521, 1976
2) Hugli, T.E., and Müller-Eberhard, H.J. : Anaphylatoxins : C3a and C5a. Adv. Immunol. **26** : 1-53, 1978
3) Brandslund, I., Siersted, H.C., Svehag, S.E. and Teisner, B. : Double-decker rocket immunoelectrophoresis for direct quantitation of complent C3 split products with C3d specificities in plasma. J. Immunological Methods **44** : 63-71, 1981
4) Pangburn, M. K. and Müller-Eberhard, H.J. : Relation of a putative thioester boud in C3 to activation of the alternative pathway and the binding of C3b to biological targets of complement. J. Exp. Med. **152** : 1102-1114, 1980
5) Isenman, D. E., Kells, D. I. C., Cooper, N. R., Müller-Eberhard, H. J. and Pangburn, M.K. : Nucleophilic modification of human complement protein C3 : Correlation of conformational changes with acquisition of C3b-like functional properties. Biochemistry **20** : 4458-4467, 1981
6) Teisberg, P. : In vitro activation of C3 revealed by crossed immunoelectrophoresis as a parameter of immunological activity in disease. Clin. Chim. Acta **62** : 35-41, 1975
7) De Vecchi, A., Montaguino, G., Massaro, L., Constantino, A., Bencini, P. L. and Tarantino, A. : Comparison between immunofixation and crossed immunoelectrophoresis for the detection of C3 activation products. Clin. Chim. Acta **97** : 27-32, 1979
8) Von Zabern, I., Nolte, R. and Vogt, W. : Studies on amine treated human complement components C3 and C4. J. Immunol. **124** : 1543, 1980
9) Janatova, J., Tack, B.F. and Prahl, J.W. : Third component of human complement : Structural requirements for its function. Biochemistry **19** : 4479-4485, 1980