PROTEIN A FROM *STAPHYLOCOCCUS AUREUS** CONVERSION OF COMPLEMENT FACTOR C3 BY AGGREGATES BETWEEN IgG AND PROTEIN A

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1. Introduction

Protein A from *Staphylococcus aureus* reacts with the Fc fragment (**) of all mammalian IgG's that have been tested thus far [1–4]. Protein A elicits Arthus reactions in rabbits pretreated with normal human immunoglobulin [5], anaphylaxis in normal guinea pigs [6] and erythema-wheal reactions in man [7]. The Arthus reaction is known to be mediated by complement [8]. Whether the complement system is involved in anaphylactic reactions is uncertain, but it is known to generate vasoactive substances from tissues [9]. The effect of protein A–IgG complexes on the complement system has been investigated and it was found that such complexes inactivate complement in a way similar to that of an antigen–antibody complex [10–12].

The complement factor existing in highest concentration in serum is $C3^a$ [9]. During activation of the complement system, this protein is split into two fragments by C3 convertase, $\overline{C4,2}$ [9]. The smaller fragment released, $C3^a$, has anaphylatoxic activity. The larger fragment has a higher electrophoretic mobility than C3 at neutral pH. Conversion of C3 proves that enzymatic activity has been induced in serum provided that the material under consideration does not have enzymatic activity in itself. Protein A added to human serum gives rise to C3 conversion. It does not affect purified C3, however, and the conversion in whole serum is not a result of enzymatic action of protein A on factor C3.

* Part XII of a series.

** Abbreviations for immunoglobulins, complement and complement factors conform with the rules suggested in [22] and [23].

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2. Materials and methods

Protein A was purified from lysozyme-digested *S. aureus* as previously described [12].

Fresh individual human sera, stored at −20°, were used.

Anti-C3 serum was produced in rabbits by immunisation against zymosan-absorbed human factor C3. The antiserum was kindly supplied by Dr. K.-E. Fjellström, Dept. of Medicine, Uppsala Akademiska Sjukhus. The antiserum was stored at 4° with 0.01% NaN_3 as preservative.

Human IgG was a Cohn fractionated preparation (Kabi AB, Stockholm).

Immunoelectrophoresis was performed on microscope slides [13] in one percent agarose (l'Industrie Biologique Française S.A.) 0.01 M tris-acetic acid, pH 8.0. The electrophoresis was run for 90 min at 50 V. Antiserum, 50 µl, was added and the precipitates formed were examined and photographed after 24 hr. Human complement factor C3 (β1C) was prepared as described by Nilsson and Müller-Eberhard [14], omitting the last step, the preparative block electrophoresis. C3 was purified from the euglobulin fraction of human serum by chromatography on TEAE cellulose and hydroxyl apatite columns, in that order. This partially purified C3 gave only one precipitation line in immunoelectrophoresis with goat antiserum against human serum proteins (Kabi AB, Stockholm). In polyacrylamide disc electrophoresis, 7% gel, pH 8.9 [15], three bands appeared, one strong band and one weak band on each side of this.

Trypsin digestion of purified C3 was performed according to Bokisch et al. [16] using 2 mg C3 per ml.

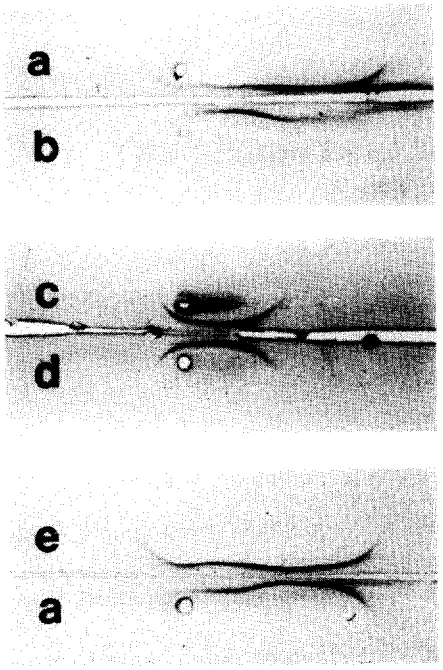

Fig. 1. Conversion of factor C3. Equal volumes of a) human serum and 0.04% protein A; b) human serum and VBS; c) 0.35% purified human C3 and 0.025% protein A; d) 0.35% purified human C3 and VBS. All mixtures were incubated at 37° for 60 min prior to electrophoresis; e) purified human C3 (0.2% concentration) treated with trypsin. Soybean trypsin inhibitor was subsequently added. Immunoelectrophoresis developed with rabbit antiserum against human C3. Anode to the right. For details see text.

3. Results

To 50 μl aliquots of human serum, 1–1000 μg protein A was added in 50 μl veronal buffered saline (VSB) [17]. In a control, 50 μl VBS was added to 50 μl serum. The samples were incubated at 37° for 60 min. EDTA was then added to a final concentration of 0.01 M to prevent nonspecific conversion. After centrifugation, 5 μl of each sample was used in immunoelectrophoresis (fig. 1). Material migrating faster than the serum control appeared in all cases. This indicated that conversion had taken place even following the addition of 1 μg protein A.

To test whether protein A has any direct effect on factor C3, partially purified C3 was used. One volume of 0.35% C3 in phosphate buffer pH 7.0, $\mu = 0.1$, was mixed with one volume 0.025% protein A. The mixture was incubated at 37° for 60 min and 5 μl was used for immunoelectrophoresis (fig. 1). This treatment did not cause any change in the electrophoretic mobility of C3. Aggregates of protein A and purified human IgG were used in one experiment. Equal volumes of 1% human IgG and 0.05% protein A were mixed and incubated at 37° for 90 min and at 4° overnight. The precipitate formed was spun down and suspended in 100 μl VBS. To 50 μl 0.35% C3, 50 μl of this suspension was added. After incubation at 37° for 60 min no conversion of C3 was detected by immunoelectrophoresis against anti-C3 serum. When the same amount of IgG–protein A aggregates was added to human serum conversion occurred. Treatment of factor C3 with trypsin for 60 sec at 20° resulted in a product with the same electrophoretic mobility as that after treatment of serum with protein A (fig. 1).

4. Discussion

In earlier investigations it has been found that protein A from *S. aureus* is able to elicit hypersensitivity reactions. At least in the Arthus reaction, activation of complement is known to be involved. It has also been shown that protein A–IgG complexes fix complement in vitro. There are a number of substances such as zymosan [17], NH_3 [17], NH_2OH [18] endotoxin [19] and cobra venom factor [20] which affect the complement system but not in the same way as an antigen–antibody complex.

To obtain information as to how protein A–IgG complexes act on the complement system, their effect on factor C3 was studied. When protein A is added to human serum, C3 is converted. However, protein A or aggregates of protein A and IgG do not bring about the conversion of purified C3. The most probable explanation for the C3 conversion observed is that $\overline{C1}$ acts on factors C4 and C2, resulting in formation of C3 convertase, $\overline{C4,2}$. C1 is probably activated by fixation to the protein A–IgG complex. This complex, which is formed by the reaction of protein A with the Fc part of IgG, would then have the same effect on the complement system as an ordinary antigen–antibody complex. This interpretation would explain the results presented by Kronvall and Gewurz [11], who observed a decrease of all complement

factors after addition of protein A to serum.

It is possible, however, that the C3 conversion observed is mediated by the action of the protein A–IgG complex on serum components outside the complement system. This seems to occur with complexes of guinea pig IgG1 and homologous antigen [21]. However, the results of Kronvall and Gewurz [11] would seem to reduce the likelihood of this possibility.

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References

[1] A. Forsgren and J. Sjöquist, J. Immunol. 97 (1966) 822.
[2] A. Forsgren and J. Sjöquist, J. Immunol. 99 (1967) 19.
[3] A. Forsgren, J. Immunol. 100 (1968) 927.
[4] G. Kronvall, U.S. Seal, J. Finstad and R.C. Williams, J. Immunol. 104 (1970) 140.
[5] G.T. Gustafson, J. Sjöquist and G. Stålenheim, J. Immunol. 98 (1967) 1178.
[6] G.T. Gustafson, G. Stålenheim, A. Forsgren and J. Sjöquist, J. Immunol. 100 (1968) 530.
[7] R.R. Martin, J.G. Crowder and A. White, J. Immunol. 99 (1967) 269.
[8] C.G. Cochrane in: The Inflammatory Process, eds. B.W. Zweifach, L. Grant and R.T.McCluskey (Academic Press, New York, London, 1965).
[9] H.J. Müller-Eberhard, Ann. Rev. Biochem. 38 (1969) 389.
[10] J. Sjöquist and G. Stålenheim, J. Immunol. 103 (1969) 467.
[11] G. Kronvall and H. Gewurz, Clin. Exptl. Immunol. 7 (1970) 211.
[12] G. Stålenheim, to be published.
[13] J.J. Scheidegger, Intern. Arch. Allergy 7 (1955) 103.
[14] U.R. Nilsson and H.J. Müller-Eberhard, J. Exptl. Med. 122 (1965) 277.
[15] H.R. Maurer, Diskelektrophorese, Theorie und Praxis der Diskontinuierlichen Polyakrylamidgel Elektrophorese (Walter de Gruyter, Berlin, 1968).
[16] V.A. Bokisch, H.J. Müller-Eberhard and C.G. Cochrane, J. Exptl. Med. 129 (1969) 1109.
[17] M.M. Mayer, in: Experimental Immunochemistry, eds. E.A. Kabat and M.M. Mayer, 2nd ed. (Thomas, Springfield, Ill., 1967).
[18] D.B. Budzko and H. Müller-Eberhard, Science 165 (1969) 506.
[19] H. Gewurz, H.S. Shin and S.E. Mergenhangen, J. Exptl. Med. 128 (1968) 1049.
[20] C.G. Cochrane, H.J. Müller-Eberhard and B. Aikin, J. Immunol. 105 (1970) 55.
[21] A.L. Sandberg, A.G. Osler, H.S. Shin and B. Oliveira, J. Immunol. 104 (1970) 329.
[22] World Health Organization, Bull. W.H.O. 30 (1964) 447.
[23] World Health Organization, Bull. W.H.O. 39 (1968) 935.