Nephritic Factor of the Classical Pathway of Complement

IMMUNOGLOBULIN G AUTOANTIBODY DIRECTED AGAINST THE CLASSICAL PATHWAY C3 CONVERTASE ENZYME

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ABSTRACT A factor, functionally characterized by its capacity to stabilize the normally labile classical pathway C3-converting complex of the classical pathway of complement, has been isolated from the serum of one patient with a case of acute glomerulonephritis, subsequent to a cutaneous infection. The factor confers long-lived stabilization of classical pathway C3 convertase complexes formed both in the solid (sensitized sheep erythrocytes bearing activated $\overline{C1}$ and the classical pathway C3 convertase) and fluid phase. The half-life of such stabilized C3-cleaving enzymes extended beyond several hours at 37°C. The stabilizing activity was associated with a protein fraction immunochemically identified as immunoglobulin (Ig)G, a sizeable population of which exhibited a gamma chain of 60,000 daltons. The IgG-associated stabilizing activity was found to bind to the classical pathway C3 convertase enzyme via a fragment bearing the antigen-binding site of the molecule [$F(ab)_2$ and F(ab)]. Such binding was demonstrable for classical pathway and not for alternative pathway C3 convertase. Thus, the stabilizing factor behaves like an autoantibody to the C3-converting complex of the classical pathway of complement. The binding of the antibody to the enzyme affords protection of the latter against decay-degradation. By analogy with the nephritic factor of the alternative pathway situation where IgG autoantibodies specifically bind to alternative pathway C3 convertase enzymes and protect them from degradation, the functionally unusual IgG in our patient was designated as the nephritic factor of the classical pathway. Indirect evidence suggests that nephritic factor of the classical pathway-IgG might be of the IgG3 subclass.

INTRODUCTION

The complement system schematically comprises two major enzymatic complexes, both endowed with similar cleaving functions on the third (C3) and the fifth (C5) native complement proteins.

These C3/C5 convertases differ in their molecular composition according to the events that have initiated their formation: (*a*) Immune complexes, which activate the first component, C1, will lead to the formation of a classical pathway C3/C5 convertase, consisting in the active forms of the fourth and the second components bound together as classical pathway C3 convertase ($C\overline{4b2a}$)[1] complexes (1). (*b*) "Activators" of the alternative pathway will allow factor B and C3 to form alternative pathway C3 convertase ($C\overline{3b}B\overline{b}$) complexes endowed with C3/C5 convertase activity (2).

Both classical and alternative pathway bimolecular convertases are subjected in serum to potent homeo-

This work was presented in part at the Société Française de Néphrologie (May 1979), and at the Eighth International Complement Workshop (October 1979).

Dr. Leveillé was the recipient of a grant from the Collège de Médicne des Hôpitaux de Paris.

Received for publication 14 September 1979 and in revised form 27 November 1979.

[1] *Abbreviations used in this paper*: β-1H, β-1H globulin; $C\overline{3b}B\overline{b}$, alternative pathway C3 convertase; $C\overline{4b2a}$, classical pathway C3 convertase; DGVB, dextrose gelatin veronal buffer; $DGVB^{++}$, DGVB containing calcium and magnesium; $EAC\overline{14b2a}$ sensitized sheep erythrocytes bearing activated C1 and the complex $C\overline{4b2a}$; $EAC\overline{3b}\overline{B}$, sensitized sheep erythrocytes bearing the complex $C\overline{3b}B\overline{b}$; NF_A, Nephritic factor of the alternative pathway; NF_C, NF of the classical pathway; NHS, normal human serum; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

static control, and will only acquire an efficient C5 cleaving activity provided: (*a*) the respective $\overline{C4b2a}$ and $\overline{C3bBb}$ complexes are deposited and/or formed on a suitable solid surface, and not if they are present in a "fluid-phase" form (3, 4). (*b*) the complexes are not degraded by intrinsic decay dissociation and/or by the action of control proteins (5). These are the C4 binding protein (6), the βIH globulin (βIH) (7, 8), and the C3b inactivator, which inactivates both C3b (9, 10) and C4b (11).

The nephritic factor of the alternative pathway of complement (NF_A) was first reported in 1969 (12) in a patient with chronic glomerulonephritis, and described as a factor that would lead to the formation of a fluid-phase, magnesium-dependent C3-cleaving enzyme when added to normal human serum. Over the next decade the occurrence of this factor was unequivocally established in dense deposit disease membranoproliferative glomerulonephritis (type II) (13) and partial lipodistrophy (14, 15).

NF_A has been identified as an autoantibody specifically directed against the $\overline{C3bBb}$ enzyme, (16–19) which confers a prolonged half-life upon this labile complex. The enhanced longevity of NF_A stabilized $\overline{C3bBb}$ complex is caused by retardation of Bb factor B release from the complex and protection against β1H-mediated accelerated decay dissociation (20, 21).

The existence of an analogue in the classical pathway for the NF_A has not yet been reported.

In this paper, we describe a serum factor functionally characterized by its unique capacity to stabilize $\overline{C4b2a}$ complexes in both solid and fluid phase. This factor is indistinguishable from immunoglobulin (Ig)G, and it is shown to act as an antibody. It was isolated from the serum of a patient with a case of acute postinfectious glomerulonephritis.

METHODS

Buffers and reagents. Isotonic phosphate-buffered saline (PBS), pH 7.2; half isotonic veronal-buffered saline, pH 7.2, containing 2.5% dextrose, 0.5 mM magnesium, 15 mM calcium, and 0.1% gelatin ($DGVB^{++}$); the same buffer containing EDTA 0.02 M (DGVB-EDTA); CM-cellulose (Whatmann Biochemicals, Ltd., Maidstone, Kent, England); Sephadex G-100 and G-200, CNBr-activated Sepharose 4B (Pharmacia Fine Chemical, Ltd., Uppsala, Sweden); Papain (Boehringer, Mannheim, West Germany); Pepsin (Sigma Chemical Co., St. Louis, Mo.); and human purified IgG (Centre de Transfusion Sanguine, Paris, France).

Sera

R.A.M. serum. The studies to be reported were performed on serum and EDTA plasma from a patient (R.A.M.) with postinfectious acute glomerulonephritis. The case summary and the complement profile are reported elsewhere (22). In brief, patient R.A.M. presented acute glomerulonephritis after a protracted cutaneous infection (impetiginized scabies), all of which subsided in 6 wk. Low C3 and low C5 were the main serological abnormalities together with the finding of a circulating C3 cleaving complex. All the blood samples obtained from the patient contained similar levels of stabilizing factor.

Normal human serum (NHS). This was a pool obtained from normal healthy blood donors. Normal and patient's sera were separated after clotting 2 h at room temperature, and kept frozen at −80°C.

C2-deficient serum. These sera were obtained from patients with demonstrated homozygous C2 deficiency.

Purified components. Purified $\overline{C1s}$, the enzymatically active subcomponent of $\overline{C1}$, was obtained by the method of Takahashi et al. (23). Purified C4 was kindly donated by Dr. M. G. Colomb, Laboratoire de Biochimie, Centre d'Études Nucléaires, Grenoble, France. C2 was purified by the method of Kerr and Porter (24) in which the CNBr-Sepharose step was replaced by a chromatography on CM-cellulose in 0.01 M PO_4 0.005 M EDTA, pH 5.9. C2 hemolytic activity was titrated according to Lachmann et al. (25). In our conditions, a normal human serum pool contains 2.5×10^4 C2 hemolytic U/ml. Factor B and factor D were purified according to Lachmann et al. (25) and Lesavre et al. (26), respectively.

NF_A was purified according to Daha et al. (21) from the serum of one patient with membranoproliferative glomerulonephritis (type II).

Preparation of $EAC\overline{14b2a}$. $EAC\overline{14b}$, sensitized sheep erythrocytes bearing activated C1 and C4 were prepared as described by Lachmann et al. (25); 25×10^8 EA in $DGVB^{++}$ were incubated with 400 μl of C2-deficient serum for 30 min at 37°C, then washed several times in $DGVB^{++}$. $EAC\overline{14b2a}$ were prepared by incubating 10^9 $EAC\overline{14b}$ with 2,000 hemolytic U of C2 for 5 min at 30°C in 2 ml $DGVB^{++}$. They were immediately used for the stabilization assay.

$EAC\overline{14b2a}$ stabilization assay. 200 μl of $EAC\overline{14b2a}$ cells (5×10^8/ml) in $DGVB^{++}$ were mixed with 50 μl of twofold dilutions of the patient's serum, starting at 1/10, or dilutions of the purified factor in DGVB-EDTA 0.02 M, and incubated for 30 min at 37°C. 10 μl of normal guinea pig serum were then added as a source of C3–C9 and, after further incubation at 37°C for 1 h, the reaction was stopped by the addition of 2 ml cold $DGVB^{++}$. After centrifugation, lysis was measured by OD at 415 nm. The amount of lysis was expressed as a percentage of a control exhibiting maximal $\overline{C4b2a}$ hemolytic sites (100%). We defined one "stabilizing unit" as the amount of R.A.M. serum or purified fractions maintaining 50% of the maximal $\overline{C4b2a}$ sites in the face of a challenge decay of 30 min at 37°C.

$EAC\overline{3bBb}$ stabilization assay. It was performed according to Daha et al. (21).

Antisera. Monospecific goat antisera to human C3, factor B, and alpha 1 antitrypsin (Atlantic antibodies, Westbrook, Maine), rabbit anti-human IgG, IgA (Behringwerke, AG, Marburghone, West Germany). Rabbit anti-IgA antiserum was made monospecific by adsorption on glutaraldehyde insolubilized IgA-deficient serum (27). Goat anti-Fc gamma antiserum was a gift of Dr. Druet, INSERM U 28, Hôpital Broussais, Paris.

Purification of the $\overline{C4b2a}$ stabilizing activity. The first steps consisted of an euglobulin precipitation by dialysis against PO_4 5 mM, EDTA 2.5 mM, pH 5.9, and filtration of the supernate on a CM-cellulose in the same buffer. The stabilizing activity, was eluted with a linear salt gradient between 7 and 14 mmhos/cm. In other preparations DEAE-cellulose was used, equilibrated with PO_4 0.02 M, pH 7.0. The $\overline{C4b2a}$ stabilizing activity came out in the exclusion peak, together with IgG.

G-200 chromatography. After concentration, the CM-cellulose active pool (7 ml) was applied to a 2.5 × 100-cm

Sephadex G-200 column in PBS. IgG elution was followed by electroimmunodiffusion after carbamylation of samples of each fraction (28). The column had been precalibrated with normal human serum and the elution positions of IgG, factor B and alpha 1 antitrypsin determined by electroimmunodiffusion.

Adsorption on anti-Ig immunoadsorbents. The monospecific (affinity chromatography) antibodies were insolubilized on CNBr-4B Sepharose as described (29). The immunoadsorption of the G-200 active pool was carried out according to Davis et al. (15). The adsorbed material was eluted with 4M $MgCl_2$, pH 6.8, and immediately dialyzed against PBS. After concentration to the initial volume, both the excluded and the eluted fractions were tested for IgG or IgA by electroimmunodiffusion after carbamylation, and for EAC$\overline{14b2a}$ stabilizing activity.

Pepsin digestion. This was carried out according to Lachmann et al. (30): 1 ml of NF_C (G-200 pool) at 4.5 mg/ml was incubated for 2 h at 37°C and pH 3.0 with 0.18 mg pepsin, and after neutralization, applied to a 1.5 × 60-cm precalibrated G-100 Sephadex column. The concentrated protein peak did not react with anti-Fc on Ouchterlony. Normal IgG at the same concentration were treated in the same way as a control. Another control was obtained by incubating NF_C at 37°C and pH 3.0, as described above, but without pepsin.

Papain digestion. This was performed in the absence of cystein, as described by Swanworth and Turner (31); 1 ml of NF_C (2.5 mg/ml) was incubated with 25 μg papain in PBS (EDTA 0.002 M) for 4 h at 30°C. The reaction mixture was then applied to an anti-Fc Sepharose immunoadsorbent. The exlcusion peak and the $MgCl_2$ eluted peak were concentrated to the initial volume and tested for stabilizing activity. As for the pepsin digestion, a control was made with normal IgG treated in the same way.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide gel electrophoresis was performed in a Tris 0.025 M glycine 0.192 M buffer, pH 8.3, according to Laemmli (32).

Evaluation of C3 conversion. C3 conversion was measured by two-dimensional antigen-antibody crossed electrophoresis (33) in 1% agarose with a 0.037 M barbital buffer containing 0.045 M Tris, 0.1 M glycine, and 0.02 M EDTA at pH 8.6. A voltage of 20 V/cm was applied for 1 h for the first dimension, and 3 h for the second dimension; 0.5% anti-C3 was used in the second dimension.

RESULTS

Capacity of R.A.M. whole serum to stabilize cell-bound C$\overline{4b2a}$ sites. EAC$\overline{14b2a}$, prepared as described in Methods, were mixed with a 1/50 dilution of the patient's serum or normal human serum in DGVB-EDTA 0.02 M, and incubated at 37°C; at timed intervals, samples were drawn from the reaction mixture and the residual C$\overline{4b2a}$ sites revealed as described in Methods.

Fig. 1 shows that C$\overline{4b2a}$ sites exhibited no decay on cells incubated with R.A.M. serum after 5 h at 37°C.

Fig. 2 shows that the stabilizing effect was linearly dose-related to the input of R.A.M. serum when the residual C$\overline{4b2a}$ sites were evaluated after a 30-min "decay" time at 37°C.

Purification of the stabilizing factor and its characterization as IgG. All stabilizing activity was found in the pseudoglobulin fraction of serum and was adsorbed on a CM-cellulose column, pH 5.9, from which it was eluted after application of a salt gradient. The concentrated active fractions were filtered on a precalibrated Sephadex G-200 column, from which it eluted in the early ascending limb of the IgG peak, with an apparent 300,000 mol wt (Fig. 3). The active pooled fractions were applied to an anti-Fc Sepharose column, on which the activity was absorbed and from which it could be desorbed with a high ionic strength buffer (Table I). The IgG molecules eluted from the anti-Fc immunoabsorbent showed a pattern of migration in SDS-PAGE identical to that of normal IgG molecules; specifically

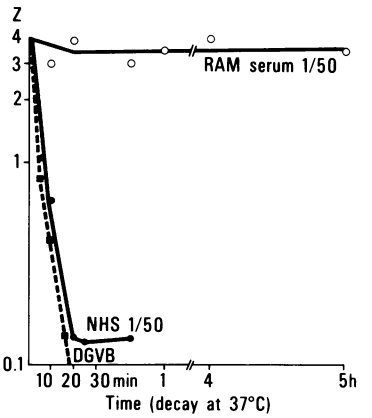

FIGURE 1 Rate of decay at 37°C of EAC$\overline{4b2a}$ in the presence of R.A.M. or NHS. EAC$\overline{4b2a}$ in DGVB-EDTA were incubated at 37°C in the presence of R.A.M. serum 1/50 (○), NHS 1/50 (●) or DGVB-EDTA alone (■). At timed intervals, samples were removed and added to 10 μl of guinea pig serum to assess residual C$\overline{4b2a}$ hemolytic activity. Z, average number of hemolytic sites per cell.

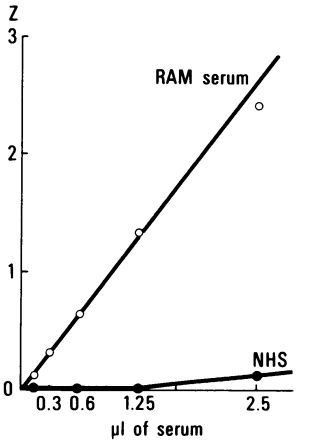

FIGURE 2 Dose-dependent stabilization of EAC$\overline{4b2a}$ by R.A.M. serum. EAC$\overline{4b2a}$ residual hemolytic activity was measured after 30 min incubation at 37°C in the presence of dilutions of R.A.M. serum (○) or NHS (●) in DGVB-EDTA. Z, average number of hemolytic sites per cell.

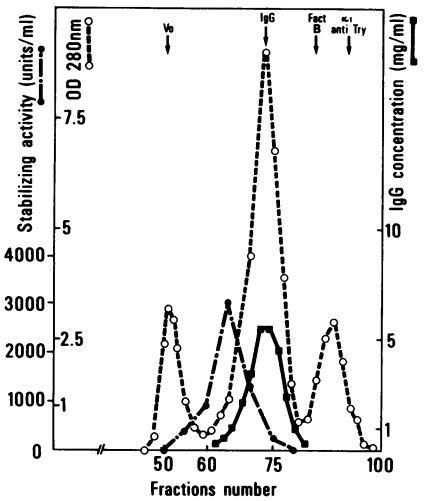

FIGURE 3 Sephadex G-200 gel filtration of the EAC$\overline{4b2a}$ stabilizing activity. 7 ml of concentrated CM-cellulose active fractions were applied to a 2.5 × 10-cm Sephadex G-200 column. A description of the EAC$\overline{4b2a}$ stabilizing activity assay and the unit definition are given in Methods. IgG concentration was measured by electroimmunodiffusion. The elution volumes of marker proteins are shown by arrows.

no large component (in excess of 160,000 mol wt) could be seen on the unreduced gels. The apparent incongruence in molecular weight estimations of the stabilizing factor by gel filtration in relation to SDS-PAGE will be discussed later. After reduction, this NF_C-enriched IgG fraction (representing <3% of the total R.A.M. IgG) showed that in addition to the expected IgG heavy and light chain bands, a third band was apparent (Fig. 4) corresponding to IgG molecules bearing a relatively large heavy chain (~60,000 mol wt). This population comprised ~30% of the NF_C-enriched fraction and thus ~1% of the total R.A.M. IgG.

Persistence of the stabilizing activity in F(ab)'$_2$ and Fab fragments of IgG. Whether the IgG-associated stabilizing factor in R.A.M. serum behaves like an antibody was assayed by the capacity of F(ab)'$_2$ and Fab fragments of R.A.M.-IgG to stabilize solid-phase C$\overline{4b2a}$ sites (Table II).

Upon pepsin treatment, the C$\overline{4b2a}$ stabilizing activity which was excluded from Sephadex G-100 when undigested, eluted after the void volume, at the same position as F(ab)$_2$ obtained by pepsin digestion of normal IgG. The specific activity of the F(ab)$_2$ fragments was 50% that of the intact parent IgG. However, acid treatment without pepsin also led to an approximate 50% diminution in stabilizing activity as compared to untreated R.A.M. IgG.

After papain hydrolysis, NF_C was applied to the anti-Fc gamma Sepharose column. In contrast with the results obtained with undigested NF_C (*vide supra*), the stabilizing activity was retrieved in the breakthrough protein peak, indicating that the stabilizing activity resides in the Fab fragment.

Samples of the so purified F(ab)'$_2$ and F(ab) fragments were run on SDS-PAGE. These preparations gave one unique visible band after coloration, corresponding to the F(ab)'$_2$ and Fab molecular weight, respectively (not shown).

Specificity of the interaction of NF_C with cell-bound C$\overline{4b2a}$ complex. To study its specificity, NF_C was added to different complement intermediates: (*a*) EAC$\overline{14b2a}$ (experimental) and EAC$\overline{14b}$ (control) cells were incubated with NF_C for 30 min at 4°C and then washed; C2 was added and the stability of the resulting C$\overline{4b2a}$ sites was assessed as above. Whereas >90% of

TABLE I
Elution of the Stabilizing Activity (G-200 Pool) from Anti-IgA and Anti-Fc-IgG Immunoadsorbents

	IgG concentration*	Stabilizing‡ activity	Activity
	nmol	*μ*/ml	nmol/IgG
NF_C	13.6	1,600	118
Anti-IgA Sepharose			
Breakthrough peak	4.3	567	132
Salt eluate	0	0	0
Anti-Fc-IgG Sepharose			
Breakthrough peak	0	0	0
Salt eluate	6.1	336	56

The Ig fixed on the immunoadsorbent were specifically eluted with 4 M $MgCl_2$, pH 6.8.
* Ig concentrations were measured by electroimmunoassay.
‡ Definition of one stabilizing activity unit is given in the text.

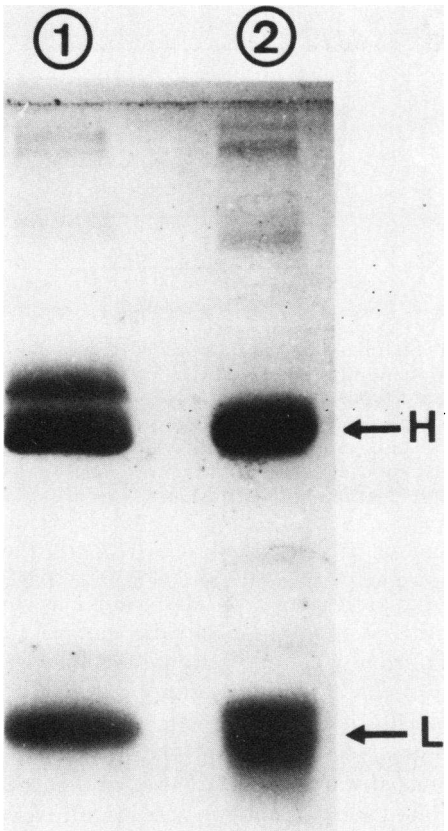

FIGURE 4 SDS-PAGE electrophoresis in 10% gel of NF_C (1) and normal IgG (2) after reduction. The positions of normal IgG heavy and light chains are indicated by arrows.

the $C\overline{4b2a}$ sites borne by control cells were lost after decay, no diminution in the number of these sites could be appreciated with the experimental cells. Furthermore, when 5 μl of NF_C (4,000 stabilizing U/ml) were incubated with various cellular intermediates, for 5 min at 30°C and 2 h at 4°C, the titer of the stabilizing activity was significantly lowered in the supernate of the mixture of NF_C with $EAC\overline{14b2a}$ but not in that of the mixture of NF_C with EA or $EAC\overline{14b}$ cells. Thus, NF_C binds specifically to the $C\overline{14b2a}$ complex and not to $C\overline{14b}$ alone (Table III). (*b*) NF_C eluted from the anti-Fc Sepharose column (where it separated from properdin) did not stabilize the alternative pathway C3 convertase in the $C\overline{3bBb}$ stabilizing assay (Methods) (Table IV). Conversely, NF_A did not stabilize cell-bound C42 sites in our assay system. Thus, NF_C and NF_A antibodies do not share their respective specificity for either of the two complement C3 converting enzymes.

Stabilization of fluid-phase $C\overline{4b2a}$ complex prepared from purified classical pathway components. 5 μg of C4 and 10,000 C2 hemolytic units in PBS-Mg 3 mM were mixed at 37°C and 3 μg of $\overline{C1s}$ added. After 5 min at 37°C, 0.04 M EDTA, and 5 μl of NHS-EDTA 20 mM were sequentially added to the mixture which was further incubated for 1 h at 37°C. Under these conditions, 80% of the serum C3 was converted. The fluid-phase $C\overline{4b2a}$ enzyme so created is however labile, with a half-life of about 3–4 min (Fig. 5). When 5 U of NF_C were present in the mixture of C4, C2, and $\overline{C1s}$, the enzyme converted 100% of the serum C3, and no decay was observed after 1 h at 37°C. This C3 cleaving activity required each of the components $\overline{C1s}$, C4, C2, Mg, and NF_C, and did not require other factors. Normal IgG at the same concentration did not prevent the decay of the enzyme.

Lack of C3 activation in NHS incubated with R.A.M. IgG. The incubation of up to 10 μg of NF_C with 10 μl of NHS for 2 h at 37°C did not produce any measurable activation of C3.

TABLE II
Recovery of Stabilizing Activity after Pepsin and Papain Digestion

	Protein concentration	Stabilizing activity	Stabilizing activity
	nmol	*μ/ml*	*nmol*
R.A.M. $(Fab)_2$	10	443	44
Acid-treated R.A.M.-IgG (pepsin control)	14	825	59
R.A.M.-Fab§	20	725	36
R.A.M.-IgG	13.6	1,600	118

Proteolysis conditions are described in Methods.
*IgG, $(Fab)_2$, and Fab concentrations were determined by Folin titration.
‡ $(Fab)_2$ was purified on Sephadex G-100.
§ Fab fragment was purified by passing through an anti-Fc Sepharose immunoadsorbent.

TABLE III
Specific Binding of NF_C to $\overline{C4b2a}$

Cellular intermediates* preincubated with NF_C	Residual‡ stabilizing activity
4,000 U/ml	*U/ml*
$EAC\overline{14b2a}$ (5×10^8)	400
$EAC\overline{14b2a}$ (0.75×10^8)	800
$EAC\overline{14b}$ (5×10^8)	3,755
EA (5×10^8)	4,000

* The different cellular intermediates were preincubated with 5 μl NF_C (4,000 U/ml) for 5 min at 37°C and 2 h at 4°C.
‡ The supernates were tested for $EAC\overline{14b2a}$ stabilization as described in Methods.

DISCUSSION

The results reported in this paper describe a unique factor functionally able to stabilize the normally labile $\overline{C4b2a}$ enzyme of the classical pathway of complement and identified as the IgG autoantibody to the enzymatic complex $\overline{C4b2a}$.

This factor enhances the half-life of cell-bound $\overline{C4b2a}$ sites from 4 to 300 min or even more in our test system (Fig. 1). This enhancing effect is proportional to the amount of the patient's serum used in the assay input (Fig. 2), and is also conferred upon $\overline{C4b2a}$ complexes formed in the fluid phase with purified C4 and C2 in the presence of an excess purified $\overline{C1s}$ (Fig. 5).

Such activity is associated to a serum protein that cannot be distinguished from IgG, according to the following data: (*a*) On SDS-PAGE it has a size and chain composition compatible with that of an IgG molecule. (*b*) After pepsin digestion, the stabilizing activity is found in a G-100 fraction containing molecules whose weights are similar to that of a $F(ab)_2$ IgG. This was confirmed by SDS-PAGE to which the G-100 pepsin digested active fractions were submitted, showing one single stainable band of an ~100,000 mol wt. (*c*) The stabilizing activity is associated to a material antigenically related to IgG, as shown by the specific adsorption on anti-Fc gamma immunoadsorbent, from which the activity, together with IgG, could be recovered after appropriate salt elution (Table I). Thus, this stabilizing factor has the physicochemical characteristics of an IgG.

TABLE IV
Specificity of Nephritic Factors for their Respective C3 Convertase

	Stabilizing activity
	U/nM IgG
$EAC\overline{14b2a}$ + NF_C*	109.5
$EAC\overline{14b2a}$ + NF_A‡	0
$EAC\overline{3bBb}$ + NF_C	0
$EAC\overline{3bBb}$ + NF_A	376

* IgG fraction from patient R.A.M. purified by affinity chromatography.
‡ IgG preparation from one patient with proven type II glomerulonephritis, purified according to Daha (21).

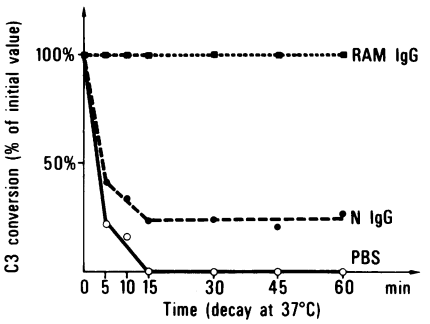

FIGURE 5 Stabilization of fluid-phase $\overline{C4b2a}$ by NF_C. Decay of $\overline{C4b2a}$ prepared from purified $\overline{C1s}$, C4, and C2 in the presence of PBS (○), normal IgG (●), or NF_C (■) at 37°C. After different incubation times at 37°C, the residual C3 convertase activity was tested by incubation with NHS-EDTA. The amount of C3 conversion after 1 h at 37°C was measured by two-dimensional electroimmunodiffusion.

The unusual biological properties of this IgG (NF_C) are associated with the antigen binding site: the pepsin digestion experiment indicate that the activity is associated with the $F(ab)_2$ fragment of IgG. After papain treatment, the activity is linked to a smaller fragment of the size of Fab no longer reacting with the anti-Fc immunoadsorbent. That monovalent Fab fragment of NF_C could confer stabilization of $\overline{C4b2a}$ sites is compatible with this IgG-like factor acting as an antibody, and indicates that its function requires only one active binding site.

This antibody physically binds to $EAC\overline{14b2a}$, and not to $EAC\overline{14b}$ cells; it stabilizes the fluid-phase C3 convertase generated by the reaction of $\overline{C1s}$ with purified C4 and C2 in the presence of magnesium. Because $\overline{C1s}$ has never been shown to be associated to such a fluid-phase $\overline{C4b2a}$ complex, either antigenically or functionally, it is reasonable to assume that the antigenic determinant, specifically recognized by NF_C, is indeed borne by $\overline{C4b2a}$. Finally, because NF_C has no effect on cell-bound $\overline{C3bBb}$ enzyme of the alternative pathway (Table IV), it is distinct from the NF_A despite their other analogies.

From what is known of NF_A on the stabilization of the $\overline{C3bBb}$ complex, NF_C could provide this extraordinary functional longevity to the $\overline{C4b2a}$ complexes by one or both of the following two mechanisms: (*a*) Prevention of $\overline{C4b2a}$ intrinsic decay dissociation. (*b*) Inhibition of a speculated regulatory protein of the classical pathway convertase, functionally analogous to

β1H. Relevantly it was of interest that NF_C added to NHS did not lead to C3 conversion. This absence of C3 conversion in the presence of NF_C in NHS does not support the hypothesis of a "C1 tickover" (34) spontaneously able to generate $\overline{C4b2a}$ enzymes in vitro.

The pathophysiology of anticomplement autoimmunisation (immunoconglutinin) is that of a sustained immunogenic presentation of complement components to the immune system (35, 36). In our patient the protracted cutaneous lesions (scabies) might have served as foci of "alexination" of superinfecting microorganisms. Alternatively, a sustained intravascular activation of the classical pathway could conceivably lead to anti-C3 convertase autoimmunisation. Indeed circulating immune complexes are regular findings in acute postinfectious glomerulonephritis. As to the pathogenic role of NF_C in disease, the absence of survey on its occurrence precludes any definite statement on this point. A preliminary report by others, (37) however, suggests that it might be found in lupus erythematosus patients, a disease in which sustained classical pathway activation is known to occur.

The IgG molecules endowed with the autoantibody activity exhibited peculiar physico-chemical characteristics: (*a*) Their hydrodynamic behaviour on gel filtration (elution as "heavy" IgG) contrasted with a molecular weight not in excess of 160,000 daltons as judged by SDS-PAGE; a discrepancy possibly relatable in terms of molecular configuration and/or carbohydrate content. (*b*) The stabilizing activity was associated with a population of IgG molecules bearing a heavy chain slightly larger (60,000 mol wt) than the average 53,000 mol wt of the major IgG subclasses of man. In keeping with our findings, Scott et al. (17) and Schreiber et al. (19) have reported similar data on the heavy chain of IgG bearing the alternative pathway NF_A. In fact, such physico-chemical characteristics of nephritic factor IgG are quite reminiscent of that of IgG3: (*a*) The apparently "heavy" molecular weight of IgG3 is related to an unusually carbohydrate-rich hinge region (39). (*b*) In relation to this carbohydrate contents, the γ3-chain has a 60,000-mol wt (40). These analogies suggest but do not prove that NF_C-IgG may be an IgG3. If this can be positively confirmed, one might interrogate the relative prevalence of IgG3[2] as the subclass of immunoglobulin endowed with anticomplement activity (16, 40, 41) and whether such prevalence also exists in other autoantibody systems.

ACKNOWLEDGMENTS

We acknowledge the help of M. Netter and M. Lillié in preparing the manuscript.

[2] Preliminary data indicates that NF_C does not bind *Staphylococcus aureus* protein A.

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