C1r Deficiency: an Inborn Error Associated with Cutaneous and Renal Disease

N. K. DAY, H. GEIGER, R. STROUD, M. deBRACCO, B. MANCADO, D. WINDHORST, and R. A. GOOD

From the Departments of Pathology and Pediatrics, University of Minnesota, Minneapolis, Minnesota 55455; the Department of Medicine, University of Alabama, Birmingham, Alabama 35233; and the Division of Dermatology, University of Chicago, Chicago, Illinois 60601

ABSTRACT The studies of sera from two siblings with C1r deficiency are described. The brother (18 yr old) has shown clinical manifestations resembling lupus erythematosus for 5 yr, and the sister (24 yr old) has had arthralgia and recurrent episodes of rhinobronchitis since early childhood. Three siblings have died: one brother died at age 12 with symptoms similar to the disease of the male patient studied here, and two other siblings died in infancy, probably from infection. The low hemolytic C1 activity of the patients could be restored by the addition of purified C1r to their sera. Bactericidal activity and immune adherence were found to be impaired. When alternate pathways of the complement system were studied, both sera permitted activation of terminal components with endotoxin and cobra venom factor. These findings support the view that an alternate pathway for activation of the terminal portion of the complement cascade exists which does not utilize the conventional pathway operating through the usual early components.

INTRODUCTION

In recent years, progress has been made in the purification of the subcomponents of C1, C1q, C1r, and C1s, and it is now possible to study the relationship of these subcomponents to the complement system and to each other, and to clarify their physiology and role in disease. Patients deficient in C1 have been described by several investigators. Among these, patients having deficiencies of each subcomponent of C1-C1q (1-3), C1r (4), and C1s (5) have been described.

Recently we have studied two patients, a brother and a sister, who have a deficiency of C1r.[1] The brother (18 yr old) has had clinical manifestations resembling lupus erythematosus for 5 yr. At the age of 13, he developed erythematosus, scaling atrophic lesions which slowly progressed to involve the skin of his nose, around his ears, and on the upper part of his chest and back and upper extremities. At the age of 16, he was hospitalized three times for acute episodes characterized by high fever, nausea, vomiting, exacerbation of skin lesions, and swelling and stiffness of wrists, elbows, and knees. He had normal renal function, negative lupus erythematosus (L.E.) tests and no antinuclear antibodies, but total serum complement was low. A kidney biopsy performed at the age of 17 is reported to have shown focal membranous glomerulitis in one of eight glomeruli, and was interpreted as early renal involvement with lupus erythematosus.

The sister (24 yr old) had recurrent attacks of otitis media and upper respiratory tract infections since infancy and early childhood, but now has arthralgia and recurrent episodes of rhinobronchitis. Three siblings have died. One brother died at age 12 with "lupus erythematosus," described by the mother as being similar to the disease of the male patient studied here. Two other siblings died in infancy, one with gastroenteritis and one of unknown cause. Studies of hemolytic serum complement and complement components in both patients indicated extremely low total hemolytic complement and C1. Extensive studies of the sera of these patients are reported in this paper. These studies show that C1r is undetectable in the serum immunochemically, and that upon addition of purified C1r to the serum,

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hemolytic C1 activity is restored. No evidence of a deficiency of hemolytic C1 was detectable in the sera of the investigated family members (parents and three siblings). The sera of these patients provide a useful basis for the study of the dependence of known complement biologic activities of C1r. It is shown that the later complement components can be activated in the absence of C1r. These findings support the view that an alternative pathway for activation of the terminal portion of the complement cascade exists which does not utilize the conventional pathway through the usual early components.

METHODS

Buffers for complement assays. The disodium salt of ethylenediaminetetraacetic acid (EDTA),[2] reagent grade Na_2H_2EDTA, was titrated to pH 7.4 at a stock concentration of 0.15 mole/liter. $Na_2MgEDTA$ (Geigy Chemical Corp., Ardsley, N. Y.) was also titrated to pH 7.4 at a stock concentration of 0.15 mole/liter. Gelatin Veronal buffer and glucose gelatin Veronal buffer with and without Ca^{++} and Mg^{++} (GGV^{++}, GGV^{--}) were prepared as described previously (6).

Human serum. Blood was allowed to clot for 1 hr at room temperature. The serum was removed after centrifugation at 4°C, aliquoted, and stored at −70° until used.

Human C1 subcomponents C1q, C1r, C1s, and antisera. C1q, C1r, and C1s and their respective antisera were prepared and purified as described previously (7–10).

Guinea pig C2. Partially purified C2 was prepared from guinea pig serum (Texas Biological Laboratory Inc., Fort Worth, Tex.) according to the method described by Nelson et al. (11).

EAC1, EAC1,4, EAC4. Cell intermediates with C1 were prepared according to methods described previously (12, 13).

Assays of total complement (CH50) and of the complement components. Sensitization of erythrocytes from sheep (6) and the measurement of total complement in 50% hemolytic units (CH50) were carried out as previously described (14).

Assays of human C1, C4, C2, and C3 complex were determined according to methods described before (12).

Assay of human C3, C5, C6, C7, C8, and C9. Functionally pure complement components for the assays of these components and EAC1gp4-7 human for use in assay of C8 and C9 were obtained from Cordis Laboratories and the assays were carried out according to the method described by Nelson, Jensen, Gigli, and Tamura (11). The percentage experimental error of the C components 1–9 ranged between 5% and 10%.

Assay of C1 subcomponents, C1q, C1r, and C1s and C1-esterase inhibitor. C1q was assayed by the Mancini technique (15). Antibody to this component was prepared by the method described by Morse and Christian (10) or by the method of Yonemasu and Stroud (7) with equivalent results.

C1r was measured by Ouchterlony immunodiffusion in 1% agarose, using monospecific antiserum (8).

C1s was measured according to the method of Nagaki and Stroud (16).

C1-esterase inhibitor was assayed by the Mancini method. The results are expressed as a percentage of the value in a normal serum pool.

Endotoxin lipopolysaccharide (LPS). *Salmonella typhosa* batch 225323 from Difco was dissolved in isotonic saline. A dose response experiment was previously set up to determine the optimum amount of LPS required to fix a maximum amount of hemolytic complement. To a constant volume of normal serum, varying concentrations of endotoxin were added, and the CH50 and C3 determined after incubation. This amount of endotoxin was then added to the test serum. Controls consisting of serum and saline and a normal pool of serum and LPS were included. The tubes were incubated at 37°C, for 15 min and centrifuged at 16,000 rpm, and then C1q, C1, C4, C2, C3, C5, C6, C7, C8, and C9 were assayed.

Purified cobra venom factor. Purified cobra venom factor (CVF) was prepared and assayed as described earlier (17). Test serum and control sera were incubated with equal volumes of CVF at 37°C for 1 hr and all the complement components were then measured.

Bovine serum albumin (BSA) anti-BSA complexes. BSA rabbit anti-BSA complexes were prepared at equivalence as described (18). The experiment was set up with one volume of the complex suspended in saline to two parts of the patient's serum. Controls consisted of patients' serum and saline. Normal serum and immune complex and normal serum with saline were also set up. All the complement components were then measured.

Immune adherence was measured according to the method of Nishioka (19).

Bactericidal activity was measured according to the method of Muschel and Treffers (20), using a rough (Lilly) strain of *Escherichia coli.*

RESULTS

Table I represents comparisons of total complement (CH-50) and each of the separate complement components of the deficient brother and sister and of all the other family members with a series of values from 40 healthy adults 20-40 yr of age. While total hemolytic complement activity was not detectable at any concentration in the sera of the two patients, the rest of the family members (parents, one brother, and two sisters) had elevated levels. C1 was markedly depressed in the brother and sister (37 and 2,533 CH50 U/ml, respectively, compared to the normal 430,000 U/ml). C4 was markedly elevated in the sister's serum. The remaining C components in sera from the rest of the family members were variable. However, C8 seemed to be constantly elevated in all the family members. Because of the marked deficiency of C1 in the patients' sera, further analysis of the subcomponents of C1 were undertaken. Table II represents the results of C1q, C1r, C1s, and C1-esterase inhibitor measured by immunodiffusion using monospecific antisera. The values of C1q expressed as micrograms per milliliter were normal in both the brother and sister. Radial immunodiffusion assay of recently isolated C1r (9) cannot yet be expressed in units, but by the Ouchterlony method

[2] *Abbreviations used in this paper:* CVF, Cobra venom factor; EDTA, ethylenediaminetetraacetic acid; EA, sensitized erythrocytes; BSA, bovine serum albumin; LPS, lipopolysaccharide.

TABLE I

Total Hemolytic C and C Components in C1r-Deficient Patients and Healthy Family Members

	CH50	C1	C4	C2	C3	C5	C6	C7	C8	C9
Patients										
Brother	<12*	37*	401,800§	876	4,500‖	9,000‖	9,400‖	9,000‖	92,000§	28,000‖
Sister	<12*	2,533*	2,460,000‖	1100	7,800‖	9,800‖	7,200	6,600	78,000	21,000§
Family										
Mother	106‖	512,000	105,128‡	1960§	3,100	3,400	7,200	5,600	160,000‖	15,000
Father	109‖	318,000	133,333‡	1560	2,700	6,000‖	6,400	11,000‖	115,000‖	13,000
Brother, E.	95‖	390,000	262,564	1300	3,700‖	3,800	8,800‖	7,200§	110,000‖	28,000‖
Sister, R. M.	95‖	350,000	148,718	1600	3,800‖	3,100	5,500	6,600	190,000‖	20,000
Sister, M.	78‖	360,000	207,692	1320	2,400	2,600	4,800‡	5,400	128,000‖	14,000
Normal										
$\bar{X}$¶	45	430,000	246,000	1350	2,600	3,415	6,150	5,529	65,889	15,795
1S**	37–53	286,000–574,000	144,500–347,500	850–1850	2,068–3,132	2,595–4,235	5,059–7,241	4,094–6,964	46,311–85,467	9,946–20,644
2S‡‡	29–61	145,000–721,000	43,000–449,000	350–2350	1,536–3,664	1,775–5,055	3,968–8,332	2,659–8,399	26,733–105,045	4,097–27,493

* Below second standard deviation.
‡ Below first standard deviation.
§ Above first standard deviation.
‖ Above second standard deviation.
¶ $\bar{X}$ Mean values of 40 healthy adults.
** 1S Values representing first standard deviation.
‡‡ 2S Values representing second standard deviation.

of immunodiffusion and by immunoelectrophoresis no bands were produced with patient serum against monospecific anti-C1r Fig. 1). C1s expressed as micrograms protein per milliter was reduced to 30–40% of normal in the serum of both patients. C1-esterase inhibitor activity was greater than normal in the sister.

Reconstitution of hemolytic C1 by purified C1r. To determine whether or not purified C1r would restore the hemolytic function, C1r was added to the patients' sera and incubated, and then C1 activity was assayed. The reconstitution experiments are based on the C1r assay method and use highly purified C1r as described by deBracco and Stroud (8). The assay cannot be done in whole serum if macromolecular C1 is detectable. In both the patients' sera, no C1 was detectable at a dilution of 1:100 after incubation for 45 min at 30°C (the specified

TABLE II

Component Protein Concentration in C1r-Deficient and Normal Human Serum

Patient	C1q*	C1r‡	C1s§	C1s Inhibitor‖
	μg N/ml		*μg protein/ml*	*% of normal pool*
Brother	16	NB¶	11.7	280
Sister	20.8	NB	12.8	178
Normal	17–20	B**	30	100

* C1q was measured by the Mancini method of immunodiffusion using antisera prepared by the method of Morse and Christian (10).
‡ C1r was determined by the immunodiffusion method of Ouchterlony using prepared antisera according to the method of deBracco and Stroud (8). Quantitative determinations were not obtained at this stage.
§ C1s was measured by radial immunodiffusion according to the method of Nagaki and Stroud (16).
‖ C1s inhibitor was assayed by the Mancini method. The results are expressed as a percentage of the value in a normal serum pool.
¶ NB, no band.
** B, band.

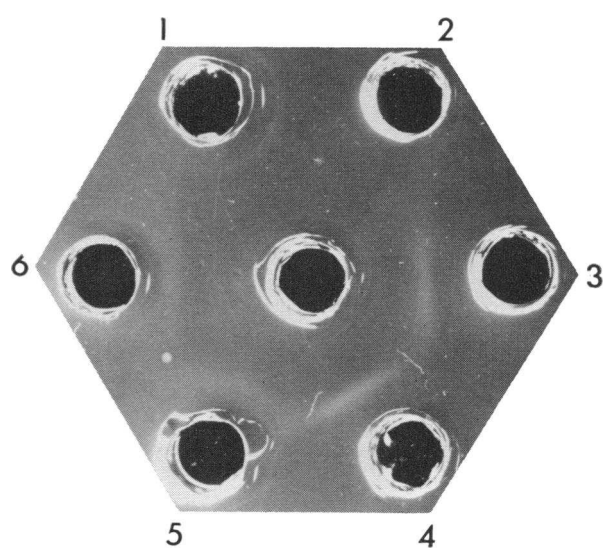

FIGURE 1 Immunodiffusion pattern by Ouchterlony of C1r-deficient patients using anti-C1r antiserum prepared against purified C1r. Well 1, undiluted serum from the brother; Well 2, undiluted serum from the sister; Well 3, normal serum; Well 4, C1 (kindly provided by R. Nelson); Well 5, normal serum diluted 1:2; Well 6, normal serum diluted 1:4.

TABLE III
Reconstitution of C1 with Purified C1r

	Without C1r	C1r added
	units/ml	*units/ml*
Brother	0	2200
Sister	0	5000

Purified C1r, obtained as described (8), was added to 1:100 dilution of the patients' sera and incubated for 45 min at 30°C. C1 titers were then determined, but the EAC4 were washed before the addition of C2. C1r has no acitivity by itself in this assay, and is the limiting component. The units are C1 site forming units. C1r values were comparable to the values expected from the measured levels of C1q and C1s in the sister (see text).

time for C1r assays). It should be noted that after the serum dilutions were added to EAC4 and incubated, the cells were washed twice in low ionic strength buffer solution but before C2 is added in order to remove free C1s. C1s alone has hemolytic activity (accounting for the routine C1 titers [Table I] in these patients), but it is not bound to EAC4 at low ionic strength. The fact that there are zero titers in the C1r assay indicates that all the activity is due to C1s and is not macromolecular C1.

As noted in Table III, significant activity could be restored in both sera. The amount of C1r activity added was also added to equivalent amounts of highly purified C1q and C1s, corresponding to the amount in the patients' serum. The titer in the female patient is essentially the same as found in these control mixtures. The regenerated activity was not as high in the patient with higher C1s inhibitor levels in spite of comparable C1s and C1q concentrations.

Bactericidal and immune adherence activity. Studies of the bactericidal activity and immune adherence function of patients' sera deficient in C1r are presented in Table IV. The bactericidal activity against a rough strain of *E. coli* (Lilly) was markedly impaired as compared to normal in both the patients' sera. Immune adherence function was also reduced when the patients' sera were used with sensitized erythrocytes (EA). However, when sensitized cells carrying C1 EAC1 were used, immune adherence activity showed normal values, suggesting the inability of these sera to form EAC142.

Activation of the alternate pathway. Recently it has been emphasized that the complement system can be activated by alternate pathways (21-24). Sera deficient in C1r provided a useful new reagent with which to study the concept of the alternate pathways.

Table V represents data obtained when the patients sera and normal sera were treated with purified cobra venom factor, endotoxin, and immune complexes. As is shown in the table, no reduction in the concentration of C1q protein was demonstrable in either the CVF- or endotoxin-treated sera. C1q was utilized when antigen-antibody complexes were employed suggesting that the immunoglobulin binding site of C1q is functional and occurs in the absence of C1r.

The utilization of C4 and C2 with CVF was less than 10% (Table V). However, the consumption of C3 was approximately 90%. To a variable degree consumption of terminal components other than C3 was observed with the patients' sera and cobra venom factor. When endotoxin was used (Table V) minimal consumption of earlier components in both normal and patients sera occurred. By contrast, regular consumption of large amounts of C3 was observed. Finally, regular consumption of the later components C5–C9 was somewhat variable in degree. When antigen-antibody complexes were used to activate the complement system, minimal utilization of C4 was seen with both of the patients sera. C2 and to a greater extent C3, C6, and C7 were fixed by the brother's serum suggesting that the brother's later components can be activated by antigen-antibody complexes to some extent without the utilization of earlier components.

DISCUSSION

In this report we have analyzed in detail the complement system in all the surviving members of a family in which deficiency of C1r is associated with serious clinical disease. In the affected brother a gross deficiency or absence of hemolytic C1 and of C1r was associated with skin, renal and joint disease, and vasculitis clinically suggestive of lupus erythematosus. In the second patient the deficiency of C1r was associated with frequent infections, arthralgia, and recurrent skin lesions. Although

TABLE IV
Bactericidal Activity and Immune Adherence Function of Sera Deficient in C1r

	Bactericidal activity*	Immune adherence‡	
Patient	*E. coli*§	EA‖	EAC1¶
Brother	0.048	10	160
Sister	0.081	10	160
Normal	0.0072	320	320

* Bactericidal activity performed according to the method of Muschel and Treffers (20). Results are expressed as the amount of serum necessary to kill 50% of bacteria.
‡ Immune adherence function according to the method of Nishioka (19). Results are expressed as the reciprocal of dilutions of serum.
§ Rough strain *E. coli* (Lilly).
‖ Sheep erythrocytes sensitized with rabbit antibody.
¶ Sensitized sheep erythrocytes containing C1.

TABLE V

Alternate Pathways of The C System in C1r-Deficient Sera

	Immunodiffusion* C1q‡ (μg N/ml)	% Consumption								
		C1	C4	C2	C3	C5	C6	C7	C8	C9
Brother+CVF§	7.8	—	<10	<10	88	31	25	<10	<10	17
Sister+CVF	8.6	—	<10	<10	90	30	45	38	30	<10
Normal+CVF	8.2	<10	<10	<10	88	25	21	<10	33	<10
Brother+Endotoxin‖	11.2	—	26	35	85	26	33	42	45	25
Sister+Endotoxin	12.9	—	18	22	80	34	45	45	35	22
Normal+Endotoxin	9.2	<10	24	22	90	28	27	17	31	55
Brother+AgAb¶	2.4	—	15	16	56	13	39	44	<10	<10
Sister+AgAb	1.4	—	25	<10	17	21	12	<10	<10	15
Normal+AgAb	0.6	>90	>90	>90	>90	46	32	42	15	30

The results are expressed as the per cent inhibition of normal saline controls.

* Immunodiffusion was carried out according to the method of Mancini (15).

‡ Measurement of C1q was done with antisera prepared according to the method of Morse & Christian (10).

§ Purified cobra venom factor.

‖ Endotoxin *E. coli* (Lilly).

¶ Immune complex (BSA rabbit anti-BSA), CVF (2 μg/ml), endotoxin (15 mg/ml), and Ag-Ab (2 mg/ml) were interacted with equal volumes of serum, respectively, and incubated at 37°C for 30 min before testing. Controls consisted of respective reactants and saline.

three living siblings are normal and have been found to have hemolytically normal C1, the family history revealed that another male died in late childhood of a lupus-like syndrome similar to that of his living affected brother. Further, two siblings died in infancy probably from infection. The father and mother have normal levels of hemolytic C1. Using monospecific antiserum, C1r could not be detected in the serum of the two children even by highly sensitive Ouchterlony and radial immunodiffusion assays. It seems likely from this family study that we are dealing with a genetically determined inborn error of metabolism. Although the precise nature of the genetic fault can be established only by a more complete quantitative analysis of the C1r concentrations and by a more complete genetic analysis, the occurrence of the defect and associated serious disease in several male and female siblings of apparently healthy parents who have several healthy offspring suggests an autosomal recessive inheritance. Hemolytic C1 as titrated by this method may be normal even though a heterozygous state with partial deficiency of C1r is present. The coexistence of C1r deficiency and renal disease has previously been described by Pickering, Naff, Stroud, Good, and Gewurz (4). Thus it seems more than coincidental that serious renal-vascular disease is associated with a defect of the C1r component of the complement system. Moreover, the study in this family suggests that the error is congenital and not acquired. In the patient of Pickering et al. perturbations of the complement system in addition to the deficiency of C1 were noticed. A decreased concentration of C1s was present, C4 was elevated, and an increase in C1 esterase inhibitor was also recorded. In both of our C1r deficient patients these same perturbations of the complement system were found. The significance of the elevation of both the hemolytic C4 and C1-esterase inhibitor is not clear but C4 is a substrate for C1s and C1r may be important in the activation of C1s (25). Thus, in the absence of C1r, C4 may be protected from destruction or utilization. The C1s inhibitor fluctuates with several disease states for unknown reasons and may represent a compensatory mechanism in chronic inflammation (26). Alternatively this component may behave as an acute phase reactant. We have no proven explanation for the consistently low levels of C1s in these patients.

Deficient hemolytic activity of the complement system attributable to a defect of C1 was reported by Pondman, Stoop, Cormane, and Hannema (5), This defect correctable by addition of purified C1s was also associated with a lupus-like syndrome. In our patients, on the other hand, hemolytic activity was restored in both patients by addition of purified C1r to the serum. Quantitatively when equal amounts of C1r were added the restoration was greater in the male than in the female patient. The difference in restoration provided by C1r in this experiment is probably attributable to differences in the concentration of the C1-esterase inhibitor. The effected sister had higher concentration of the inhibitor than did the male patient. Ratnoff, Pensky, Ogston, and Naff (27) have previously shown that this inhibitor blocks the activity of C1r as well as that of C1-esterase.

Of further interest is the observation that levels of all of the terminal complement components C3–C9 were elevated in the serum of the male patient while some of the terminal components C3, C5, and C9 were elevated in the serum of his sister. Although the significance of these increases must await further analysis it is provocative that our family study revealed that the healthy family members all showed high total serum complement hemolytic activity, increases of some of the later components, and consistent elevations of C8. Perhaps further genetic analysis will reveal the meaning of these associations. Little is known of the basis for increases in terminal complement component concentrations, but elevations of total complement and complement components on a familial basis might reflect increased stimulation by the more frequent infections which feature the clinical disease. Further studies of the family from this point of view seem desirable.

We found further that using bacterial endotoxin and cobra venom, activators of the alternative pathways (22-24, 28, 29), the complement system could be engaged in the absence of C1r. The absence of C1r did not completely prevent activation of the complement system even by antigen-antibody complexes. Of the later components especially C3, C6, and C7 were utilized upon activation of the system with Ag-Ab complexes. This observation supports the finding of Sandberg, Osler, Shin, and Oliveira (21) that Fab_2 fragments can engage an alternate pathway into the complement system. It is remotely possible that very small amounts of C1r undetectable by the sensitive methods used in this study are sufficient to permit some activation of the C system by the conventional pathway and it is difficult to be certain that serum preparations are completely free of endotoxin. However, both saline controls and BSA controls failed to show activation of the terminal components of the C system in these sera. Surprisingly the sera of the two C1r-deficient siblings behaved differently in the experiment in which Ag-Ab complexes were used to activate the C system. In the male patient clear evidence of activation of the terminal components was observed while in the female relatively little evidence for activation was found. Perhaps the difference may be due to a more efficient alternative pathway in the male than in the female. Both sera on the other hand permitted activation of terminal components with endotoxin and CVF.

Studies of the C1r-deficient serum showed deficient bactericidal activity as well as deficiencies in development of immune adherence and facilitation of phagocytosis.[3] The deficiency in bactericidal activity was to be anticipated since Goldman, Ruddy, Austen, and Feingold have previously shown that intact C1 is essential for bactericidal function (30).

The central question posed by the study of our patients with C1r deficiency as well as of the patient studied by Pickering et al. (4) is: How does the isolated absence of C1r predispose to the associated clinical and pathological disorders that have been observed? Perhaps the deficiency of C1r favors selective use of alternative pathways into the complement system, that are more likely to be associated with destructive inflammatory or vascular reactivity than is the conventional pathway utilizing the intact first component of the complement cascade. It could be that the deficiency of C1r and C1r-dependent actions of the complement system increases susceptibility to infection, as was especially clear in the clinical history of the sister, and that this in some way leads to the vasculitis. Utilization of active C1s has not been ruled out but high levels of C4 and normal levels of C2 are against this possibility. The medical history of the several siblings in this family, indicating that the absence of C1r is associated with an increased frequency of infection, suggests the possibility that C1r or the intact complement cascade has a major function in host defense and that the absence of this component causes a susceptibility to infection with microorganisms capable of producing the lesions observed.

[3] Biggar, W. D., N. K. B. Day, D. Windhorst, and R. A. Good. Impaired phagocytosis in C1r-deficient sera. Submitted for publication.

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