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Coagulation Studies in C4-Deficient Guinea Pigs[1] (39676)

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The interactions between complement, leukocytes, platelets, and coagluation have been studied rather intensively in recent years (1). The evidence with respect to their direct physiological interaction is conflicting. Rabbits with congenital C6 deficiency have defective intrinsic coagulation (prolonged whole blood clotting times and shortened prothrombin consumption times (2)) and lower responses to injections of complement activators (3) and to slow infusion of thromboplastin (4). In contrast, a human lacking C6 did not have coagulation defects (5). *In vitro* studies of platelet-rich plasma from a patient with C2 deficiency showed less aggregation in response to ADP (6).

Earlier *in vivo* and *in vitro* studies in our laboratories (7, 8) have demonstrated multiple effects on platelet function and coagulation in guinea pigs after intraperitoneal injection of purified cobra venom factor, which is known to deplete C3. Since the link between the coagulation and complement systems requires further definition, we investigated the hemostatic mechanism of congenitally C4-deficient guinea pigs derived from the original NIH-multipurpose strain described by Frank *et al.* (9).

Methods. Sixteen guinea pigs from our randomly-bred closed colony of the NIH-multipurpose, C4-deficient strain were studied. The animals, eight of each sex, were 4–6 months old and weighed over 500 g. The control animals were 16 normal guinea pigs of our randomly-bred Hartley strain matched for age, sex, and weight. Both groups were given the same laboratory rodent food *ad lib*.

For collection of blood samples, the controls and C4-deficients were anesthetized by intraperitoneal injection of sodium pentobarbitol. Samples were taken directly from the posterior vena cava and processed for hematologic, coagulation, fibrinolytic, and complement assays as described previously (8). Coagulation and fibrinolytic assays were performed on plasma from blood anticoagulated with 0.38% trisodium citrate and by the techniques previously cited (8). Blood cell counts and total proteins were measured by standard techniques, and the distribution of serum proteins was determined by electrophoresis on cellulose acetate. Complement activities were titrated by standard cell-intermediate assays (10, 11).

Results. Table I shows the complement activities and plasma proteins in the control and C4-deficient animals. As previously reported (9), the deficient guinea pigs had undetectable C4 activity and significantly lower C1 and C2 activities than the controls. C1 levels were about one-third of normal; C2 levels were about one-half of normal. The combined activity of C3-9 was normal.

The total protein and albumin contents of plasmas from both groups were essentially identical. The proportions and amounts of α_1- and β_2-globulin were significantly lower in the C4-deficients than in the controls. In fact, β_2-globulin was undetectable.

Table II compares the hemograms of the control and C4-deficient guinea pigs. The total leukocyte and platelet counts were significantly greater, and the proportions of segmented, neutrophilic white cells and lymphocytes were significantly different in the C4-deficient group.

The results of hemostatic assays are summarized in Table III. Whole blood clotting times measured in glass and plastic tubes were not significantly longer for the C4-deficient guinea pigs, in contrast to that reported for C6-deficient rabbits (2). The clot

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TABLE I. COMPLEMENT ACTIVITIES AND PLASMA PROTEINS IN CONTROL (HARTLEY STRAIN) AND C4-DEFICIENT (NIH STRAIN) GUINEA PIGS.[a]

	Control (n = 16)	C4-Deficient (n = 16)
Complement activity (CH_{50} units/0.5 ml)		
C1	40,000 ± 13,000	13,250 ± 8200[b]
C2	8171 ± 1648	4027 ± 975[b]
C3-C9	632 ± 150	616 ± 136
C4	50,000 – 180,000	Undetectable[b] (<1)
Total protein (g/dl)	4.6 ± 0.3	4.6 ± 0.3
Albumin (% of total)	57.1 ± 3.3	56.9 ± 3.2
Globulins (% of total)		
α_1	5.3 ± 0.8	4.5 ± 0.7[c]
α_2	25.6 ± 1.9	26.8 ± 2.4
β_1	8.4 ± 3.2	9.0 ± 1.0
β_2	1.4 ± 2.4	0[d]
γ	2.2 ± 1.4	2.3 ± 1.0
A:G ratio	1.3 ± 0.2	1.4 ± 0.2

[a] Mean ± SD.
[b] $P < 0.001$.
[c] $P < 0.005$.
[d] $P < 0.025$.

TABLE II. HEMOGRAMS IN CONTROL AND C4-DEFICIENT GUINEA PIGS.[a]

	Control (n = 16)	C4-Deficient (n = 16)
Hematocrit (%)	43.9 ± 3.2	45.3 ± 2.6
Protein (g/dl)	4.6 ± 0.3	4.6 ± 0.3
White blood cells (/mm^3)	3850 ± 1068	5160 ± 1500[b]
Platelets (thousands/mm^3)	450 ± 65	636 ± 162[c]
Leukocyte differential (%)		
Eosinophils	2.8 ± 2.0	0
Segmenteds	34.2 ± 15.3	23.0 ± 7.7[d]
Lymphocytes	58.0 ± 16.5	72.2 ± 9.3[b]
Monocytes	5.0 ± 2.3	4.8 ± 3.1

[a] Mean ± SD.
[b] $P < 0.01$.
[c] $P < 0.001$.
[d] $P < 0.025$.

retraction of deficient guinea pigs was more active than that of controls, probably because of their higher platelet counts, but clot lysis times and fibrinogen and plasminogen levels were similar in both groups. Of the coagulation screening tests only the prothrombin consumption times with and without phospholipid were significantly different, being shorter in C4-deficients than in controls. Failure of phospholipid addition to correct the prothrombin consumption defect of deficient animals along with the normal Russell's viper venom time indicates normal platelet factor 3 (PF3) activity. Short prothrombin consumption times and normal PF3 activity have also been shown for C6-deficient rabbits (2).

Table IV shows the activities of five clotting factors by sex in the control and C4-deficient animals. There was little variation by sex; some activities appeared to be lower in females, but these differences were not statistically significant. However, when compared to the calculated average plot for the entire age- and sex-matched control group, the C4-deficient group as a whole had significantly lower factor VIII activity. Factor XI activity in this group was significantly higher, especially in females. The lower factor VIII activity of the C4-deficients may be responsible for their slightly longer partial thromboplastin times (Table III).

Discussion. The data indicate that intrinsic coagulation is slower in C4-deficient guinea pigs than in the controls. Whether their moderate C1 and C2 deficiencies contribute to the observed coagulation abnormalities remains to be determined. It may be the combined effect of all three deficiencies that interferes with generation of intrinsic thromboplastin, rather than the C4 deficiency alone.

The defects reported here bear some similarities to those of C6-deficient rabbits, but there are also important differences. The prothrombin consumption defect of C4-de-

ficient guinea pigs is less severe than that of C6-deficient rabbits. Whole blood clotting times were not prolonged in C4-deficient animals, and the lower factor VIII activity observed in this study was not reported in the C6-deficient rabbits. Although the coagulation abnormality in deficient rabbits has been attributed to defective platelet-complement interaction (2–4), neither the present study nor that of Zimmerman *et al.* (2) demonstrated abnormal PF3 activity. Further studies of the C4-deficient animals are corrected by addition of purified C4 and/or C142 and, (ii) whether C4-deficient animals reconstituted by bone marrow transplantation (11) still have the clotting defects.

TABLE III. HEMOSTATIC VALUES IN CONTROL AND C4-DEFICIENT GUINEA PIGS.[a]

	Control (n = 16)	C4-Deficient (n = 16)
Whole blood clotting time (min, n = 6)		
Glass	2.9 ± 0.4	3.0 ± 0.0
Plastic	4.7 ± 1.1	4.1 ± 0.7
Clot retraction (% at 2 hr)	78.9 ± 6.2	83.6 ± 5.6[b]
Clot lysis time (hr)	17.2 ± 8.6	20.0 ± 12.3
Fibrinogen (mg/dl)	240.0 ± 44	229.0 ± 26
Plasminogen (CTA U/ml)	2.8 ± 0.7	2.5 ± 0.8
Activated partial thromboplastin time (sec)	23.1 ± 2.3	25.1 ± 3.6
One-stage prothrombin time (sec)	35.3 ± 2.3	35.6 ± 3.4
Russell's viper venom time (sec)	10.2 ± 1.1	9.6 ± 0.8
Prothrombin consumption time (sec)	96.5 ± 28.9	61.8 ± 19.8[c]
Prothrombin consumption time with phospholipid (sec, n = 6)	79.8 ± 15.0	54.3 ± 7.4[c]

[a] Mean ± SD.
[b] $P < 0.05$.
[c] $P < 0.001$.

The significance of hemostatic abnormalities observed in various congenitally complement-deficient animal species is open to question. As Brown (1) has pointed out, complement-deficient humans do not appear to manifest these defects on *in vitro* assays. Conversely, patients with inherited coagulation disorders do not appear to have significant complement abnormalities (12), although this possibility has not been extensively studied. The clinical syndromes commonly associated with inherited complement deficiencies (infections, renal disease, lupuslike disorders) are not usually seen in patients with inherited coagulopathies. However, the failure to demonstrate such relationships in the few patients studied thus far is not conclusive. Considering the wide variability in normal ranges observed for the coagulation and complement systems, it may be impossible to demonstrate subtle

TABLE IV. COAGULATION ACTIVITIES (%) IN CONTROL AND C4-DEFICIENT MALE AND FEMALE GUINEA PIGS.[a]

Group and factor	Males (n = 8)	Females (n = 8)	Total (n = 16)
Controls[b]			
VII	116 ± 37	90 ± 25	103 ± 34
VIII	102 ± 42	112 ± 54	107 ± 47
IX	125 ± 52	97 ± 56	111 ± 54
X	118 ± 43	94 ± 26	106 ± 34
XI	104 ± 40	92 ± 25	98 ± 33
C4-Deficients[b]			
VII	103 ± 28	76 ± 21	90 ± 28
VIII	62 ± 27	58 ± 20	60 ± 23[c]
IX	121 ± 69	117 ± 44	119 ± 57
X	89 ± 25	92 ± 16	91 ± 21
XI	109 ± 36	179 ± 63	144 ± 50[c]

[a] Mean ± SD.
[b] For each factor the mean clotting time at each assay dilution for the entire control group was plotted and the plot was assigned a value of 100%. The plot for individual control and deficient animals was compared to this mean plot and the results were averaged for each group.
[c] Control vs. deficient values: $P < 0.005$.

differences without studying larger numbers of patients and/or more carefully selecting and standardizing the control population used for comparison.

Summary. C4-deficient guinea pigs (NIH-multipurpose strain) were found to have lower intrinsic coagulation activity than age- and sex-matched control guinea pigs (Hartley strain).

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