Hereditary Deficiency of the Fifth Component of Complement in Man

II. BIOLOGICAL PROPERTIES OF C5-DEFICIENT HUMAN SERUM

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ABSTRACT The first known human kindred with hereditary deficiency of the fifth component of complement (C5) was documented in the accompanying report. This study examines several biological properties of C5-deficient (C5D) human serum, particularly sera obtained from two C5D homozygotes. The proband, who has inactive systemic lupus erythematosus is completely lacking C5, while her healthy half-sister has 1–2% of normal levels. Both sera were severely impaired in their ability to generate chemotactic activity for normal human neutrophils upon incubation with aggregated human γ-globulin or *Escherichia coli* endotoxin. This function was fully restored in the sibling's serum, and substantially improved in the proband's serum, by addition of highly purified human C5 to normal serum concentrations. Sera from eight family members who were apparently heterozygous for C5 deficiency gave normal chemotactic scores. The ability of C5D serum to opsonize *Saccharomyces cerevisiae* (baker's yeast) or *Candida albicans* for ingestion by normal neutrophils was completely normal. In addition, C5D serum was capable of promoting normal phagocytosis and intracellular killing of *Staphylococcus aureus*. The proband's serum was incapable of mediating lysis of erythrocytes from a patient with paroxysmal nocturnal hemoglobinuria in both the sucrose hemolysis and acid hemolysis tests, and also lacked bactericidal activity against sensitized or unsensitized *Salmonella typhi*. The sibling's serum, containing only 1–2% of normal C5, effectively lysed *S. typhi*, but only at eightfold lower serum dilutions as compared to normals.

These findings underscore the critical role of C5 in the generation of chemotactic activity and in cytolytic reactions, as opposed to a nonobligatory or minimal role in opsonization, at least for the organisms under study.

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INTRODUCTION

Sera from humans and laboratory animals selectively lacking an individual complement (C)[1] component have assumed a prominent role in studies examining the biologic importance of particular components or reaction steps in this complex effector system (1–9). Several strains of mice lacking the fifth component of complement (C5) are well known (10, 11). C5-deficient (C5D) mouse serum demonstrates (*a*) markedly impaired capacity to generate neutrophil chemotactic activity in the presence of immune complexes or endotoxin (12, 13); (*b*) normal opsonic function for some microorganisms but not for others (14, 15); and (*c*) lack of serum microbicidal activity (16).

Hereditary deficiency of C5 has not previously been described in other species. A series of reports has appeared, however, on two human kindred with "familial C5 dysfunction" (17–21). Affected infants exhibit, among other findings, recurrent bacterial infections, mainly with gram-negative bacilli (17–19). The laboratory hallmark of this disorder is a serum opsonic defect

[1] *Abbreviations used in this paper:* C, complement; CH_{50}, hemolytic complement; C5, fifth component of complement, etc.; C5D, C5-deficient; EBSS, Earle's balanced salt solution; PMN, polymorphonuclear leukocyte.

relating to phagocytosis of baker's yeast (Saccharomyces sp.). This defect is corrected by addition of normal human C5, even though the serum C5 of affected individuals is hemolytically, immunochemically, and physicochemically normal (20). A subtle structural anomaly of the C5 molecule, which selectively impairs its opsonic function, has been proposed (20, 21).

The first recognized human family with genetically determined deficiency of C5 is reported in the accompanying paper (22). The serum of the proband (L. H.) contains no detectable C5 (< 0.01% of normal); the serum of her younger half-sister (D. H.) has a very low level of C5 activity (~ 1–2% of normal), detectable only by sensitive hemolytic assay. Described below are in vitro studies of the capacity of these C5D human sera to support several C-dependent functions related to host defense and inflammation: chemotaxis, phagocytosis and intracellular killing, and serum cytolytic reactions.

METHODS

Blood was obtained from proband L. H. during periods of full clinical remission of systemic lupus erythematosus, when C1, C4, C2, and C3 levels were normal, and activity of the properdin pathway was demonstrable by immunoelectrophoretic analysis of factor B, C3, and their activation products induced by exposure to inulin (22, 23). Blood was obtained from her healthy sibling, D. H., on three occasions. Normal sera were obtained from healthy laboratory personnel. Sera were stored at −70°C in small, sealed aliquots.

Chemotaxis. The basic assay utilizing peripheral blood leukocytes from normal group O human donors, a chemotaxis chamber (Bellco Glass, Inc., Vineland, N. J.), and *Escherichia coli* endotoxin or aggregated human IgG as activating agents has been described (5, 24). In the current series, 3-μm pore-size Selectron filters (Schleicher & Schuell, Keene, N. H.) were employed. Most experiments were performed according to our previously described protocol 1 (5), in which 4 vol of endotoxin (1 μg/ml) or aggregated IgG (1 mg/ml) was mixed with 1 vol of undiluted human serum, serving as C source, and allowed to stand for 20 min at room temperature before injection into the lower chamber compartment for the 3-h incubation at 37°C. Many additional experiments followed a modification of the protocol of Snyderman and Mergenhagen (protocol 2; 25), in which the mixtures of activating agent and test sera were incubated at 37°C for 30 min and then heated at 56°C for 30 min before injection into the lower compartment for the 3-h incubation at 37°C.

All test mixtures were studied in triplicate in each experiment. The number of polymorphonuclear (PMN) leukocytes in 10 high-power fields (50 mm^2) on the upper and lower surfaces of each filter were counted with a πMC electronic particle counter (Millipore Corp., Bedford, Mass.), and the results expressed as a ratio, the chemotactic index, as explained by Leddy et al. (5). Statistical analysis employed the single-tailed Wilcoxon rank sum test (26).

Correction experiments were performed on mixtures of C5D serum and highly purified human C5, prepared according to Nilsson et al. (27). For these studies our standard chambers (24), normally requiring 0.75 ml of test mixture, were fitted with wider gaskets, reducing the chamber size to 0.15 ml. C5 (~ 1/10 vol) was added to deficient sera, and the mixtures kept at 30°C for 10 min. Aliquots were removed for immediate C5 titration and in some instances hemolytic complement (CH_{50}) determination. Then 4 vol of aggregated IgG was added, and protocol 2 followed as above. Control sera received 1/10 vol buffer and were handled identically.

Opsonization of baker's yeast. Assays of the ability of sera to promote phagocytosis of *Saccharomyces cerevisiae* (baker's yeast) were based on the method of Miller and Nilsson (18), modified according to a personal communication from Dr. Ulf Nilsson, University of Pennsylvania School of Dentistry, Philadelphia, Pa. Various yeast preparations were used. Initially, dried Fleischmann's yeast purchased locally (yeast 1) was prepared by boiling a saline suspension for 1 h, filtering through gauze, and diluting to a yeast concentration of 1×10^9/ml. Aliquots were stored at −20°C. Other experiments employed a yeast preparation (yeast 2) supplied by Dr. Michael Miller, Charles R. Drew Postgraduate Medical School, Los Angeles, Calif. Because of the theoretical possibility that freshly prepared yeast suspensions might differ from those retrieved from frozen storage, yeast 2 was tested in both forms, with comparable results.

Leukocyte-rich suspensions were prepared from the heparinized blood of normal donors (blood group O) by direct centrifugation (18) or, more often, by dextran sedimentation (see below). The cells were washed five times in Earle's balanced salt solution (EBSS) and suspended at a concentration of 5×10^6 leukocytes/ml in EBSS containing 0.1 or 1% human serum albumin. By hemolytic assay, this human serum albumin preparation (Miles Laboratories, Inc., Elkhart, Ind.) contained no detectable C5 at 0.1, 1, or 5% concentrations. In one experiment a similar leukocyte suspension was obtained from the C5D proband (L. H.). Sera from patients and normal donors, diluted in EBSS, served as the source of opsonin.

Phagocytosis was tested by mixing 0.1 ml diluted serum and 0.1 ml of yeast suspension (1×10^8 or 1×10^9/ml) in capped 12 × 75-mm polypropylene tubes with constant rotation for 30 min at 37°C. Then, 0.2 ml of leukocyte suspension was added, and constant rotation at 37°C was resumed for 30 min. The preparations were centrifuged at 40 *g* for 5 min at 4°C. The sediments were smeared on glass slides and stained with Wright's stain, and each slide was assigned a code number by the technician. The number of intracellular yeast particles per 100 consecutive PMN leukocytes, as well as the percentage of PMN containing one or more yeasts, were enumerated microscopically by a single investigator without knowledge of the source of serum for each slide. Repeat counts of a single slide were within 10%. Results were analyzed by the two-tailed Wilcoxon rank sum test (26).

Using the original assay (18), we often found it difficult to distinguish intracellular yeast particles from those merely adherent to the cell surface, especially at lower serum dilutions. The following modifications were developed to facilitate reading. Heavy red cell contamination of leukocytes prepared by direct centrifugation was reduced by preliminary sedimentation of the blood (37°C, 45 min) in the presence of 3% dextran (mol wt 250,000). Adherence of leukocytes to the glass slides was enhanced by increasing the human serum albumin concentration from 0.1 to 1%, either in the initial leukocyte suspension or immediately before smears were made. Yeast: leukocyte ratios of 100: 1 impaired discrimination between intracellular and extracellular yeasts. Therefore, ratios of 10: 1 were employed for

most experiments. These modifications did not alter the outcome, although serum-free controls were lower with the lower yeast: leukocyte ratio.

Opsonization of Candida albicans. This assay was performed in a similar fashion to the baker's yeast assay, with certain exceptions (28). 3-day-old cultures of *C. albicans* were washed and suspended at concentrations of 1, 2, or 5 $\times 10^7$/ml in Hank's rather than Earle's balanced salt solution. Opsonization was carried out at 37°C for 30 min using 1:2–1:64 dilutions of fresh or heat-inactivated (56°C, 30 min) C5D or normal serum. Phagocytosis then took place in reaction vol of 1 ml containing 2.5×10^6 leukocytes and 2.5, 5, or 12.5×10^6 opsonized *C. albicans.* The tubes were constantly rotated at 37°C for 5 min. Phagocytosis was estimated microscopically as in the baker's yeast assay.

Opsonization and support of intracellular killing of staphylococcus. Phagocytosis and killing of *Staphylococcus aureus* 502A by PMN leukocytes from five normal individuals in the presence of C5D or normal sera were studied by a modification of previously described methods employing colony counts of viable organisms released from sonically disrupted granulocytes (29).

After Ficoll-Hypaque density gradient separation and dextran sedimentation, the granulocytes were washed twice in EBSS and adjusted to a concentration of 1×10^7 cells/ml in EBSS with 0.1% gelatin (EBSS gel). Staphylococci were prepared and quantified as previously described (29). Suspensions of 0.5 ml granulocytes, 0.1 ml EBSS containing 1×10^7 staphylococci, 0.1 ml of fresh or heat-inactivated test serum diluted to yield a final concentration of 0.5% serum, and sufficient EBSS-gel to bring the total volume to 1 ml were prepared in duplicate. Control suspensions containing cells without serum and serum without cells were included routinely. Incubation was at 37°C. After 30 min, 0.1 ml EBSS containing 1 U of lysostaphin (Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) was added to kill extracellular bacteria. Excess lysostaphin was destroyed by trypsin before leukocyte rupture (29).

For measurement of phagocytosis, intracellular killing was inhibited by prior addition of 0.1 ml of EBSS containing 2 mg of phenylbutazone (Ciba Pharmaceutical Co., Summit, N. J.). Correction for partial inhibition of phagocytosis by phenylbutazone was made as described (29). Intracellular killing was assayed by enumerating viable staphylococci recovered from suspensions exposed to lysostaphin but not to phenylbutazone. Phagocytosis and intracellular killing at 1 and 3 h were determined in five separate experiments in which both C5D sera and serum from a different normal subject were studied concurrently. Preliminary experiments demonstrated that dilution of serum to a final 0.5% concentration was needed to show a consistent and significant effect of heat inactivation of serum upon opsonization, i.e., complement dependence.

Cytolytic assays. Serum bactericidal activity for *Salmonella typhi O 901* and lysis of paroxysmal nocturnal hemoglobinuria erythrocytes in the sugar water and acid hemolysis tests were performed as previously described (5).

RESULTS

Neutrophil chemotaxis

Both C5D sera consistently displayed a markedly reduced capacity to generate chemotactic activity for normal human neutrophils in the presence of aggregated human IgG or *E. coli* endotoxin (Fig. 1). The differences between either C5D serum and normal sera are highly significant ($P \leqq 0.005$) in both experimental protocols (see Methods) and with both activating agents.

Using protocol 1, which does not involve heating of the activated sera, the serum of sibling D. H., possessing ~ 1–2% of normal C5, yielded consistently higher chemotactic scores than did the more severely deficient serum of the proband (L. H.). Using protocol 2, however, in

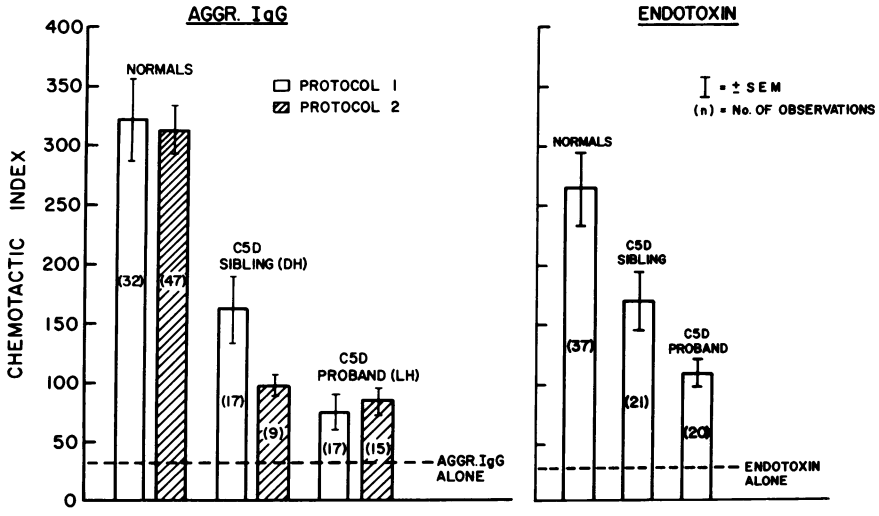

FIGURE 1 Generation of chemotactic activity for human neutrophils in C5D and normal human sera in the presence of aggregated human IgG or *E. coli* endotoxin. Values shown are means±SEM based on many observations (indicated by numbers within bars). Both experimental protocols (see Methods) were employed for studies with aggregated IgG; only protocol 1 was used for studies with endotoxin.

TABLE I

Effect of Purified Human C5 on Generation of Chemotactic Activity in C5D Sera

Assay mixtures*		Resulting complement levels‡	CH_{50}	Chemotactic index§
Serum source	C5 added			
	μg/ml serum	*Hemolytic C5 U/ml*	*U/ml*	
Experiment A				
Patient L. H.	0	0		182
	165	300,900		535
Patient D. H.	0	~3000		290
	165	307,400		883
Normal (A)	0	257,100		1008
	165	614,800		1092
Normal (B)	0	—‖		922
HBSS	165	—		200
Experiment B				
Patient L. H.	0	0	0	128
	73	170,300	120	428
	165	315,400	145	690
Patient D. H.	0	~3000	45	247
	73	167,000	122	381
	175	370,200	136	885
Normal (A)	0	315,400	148	986
	165	740,500	—	998
Normal (B)	0	—	119	944
HBSS	165	315,400	—	187

* Include aggregated human IgG, 1 mg/ml.

‡ Assays on serum:C5 mixtures or untreated sera were performed immediately after serum:C5 mixtures had been made.

§ Mean values based on scores of three chambers. Protocol 2 was followed (see Methods).

‖ Not done.

which the activated sera are heated at 56°C for 30 min before injection into the chamber, the differences between the two C5D sera are only marginally significant ($P = 0.05$). These differences are discussed below.

Reconstitution of chemotactic function in C5D serum by highly purified human C5 was investigated by protocol 2. The two experiments in which the highest serum C5 levels were attained are shown in Table I. Full correction of chemotactic function was achieved in D. H. serum by restoration of normal C5 concentration (Table I, experiments A and B). A half-normal C5 level in D. H. serum, however, was not associated with a fully normal chemotactic response (Table I, experiment B). The proband's serum appeared to be somewhat more resistant to full restoration of chemotactic function despite the achievement of normal C5 and CH_{50} titers in the reconstituted serum (Table I). This same sample of L. H. serum clearly demonstrated immunoelectrophoretic conversion of C3 and properdin factor B after incubation with the same preparations and concentrations of aggregated IgG and endotoxin employed in the chemotactic assays. Earlier studies (not shown), in which lower C5 concentrations were achieved in L. H. serum, demonstrated smaller increments in chemotactic function.

Eight heterozygous C5D sera, possessing 34–65% of the mean normal C5 level (22), were compared (in protocol 2) to the same normal control sera, using the same modified chambers. Their chemotactic scores showed a broad spread, not related to C5 level, which overlapped the normal values and were judged to be normal.

Interactions with phagocytic cells

Opsonization of baker's yeast. Shown in Fig. 2 are results of a representative experiment in which normal, C5D, and C5 dysfunction sera (the latter provided by Dr. Ulf Nilsson) are compared for their capacity to promote the phagocytosis of yeast 2 (see Methods). Each serum was tested in at least three dilutions chosen by prior experiment to include the range over which a dose response is seen. The highest and lowest dilutions used were also tested after heat inactivation (56°C, 30 min). Dose-response effects can be seen with the fresh sera and, to a lesser degree, with the heated sera, indicating the presence of heat-labile and heat-stable opsonins (Fig. 2). Agreement between the two modes of

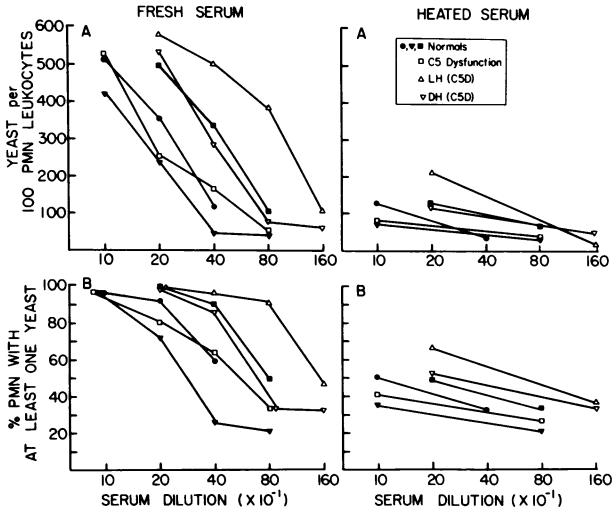

FIGURE 2 Phagocytosis of baker's yeast by human PMN leukocytes in the presence of three normal sera, two C5D sera (L. H., proband; D. H., sibling), and one C5 dysfunction serum, both fresh (left) and heat-inactivated at 56°C, 30 min (right). Shown are both (A) numbers of yeast particles/100 PMNs and (B) percent of PMNs with at least one associated (presumably ingested) yeast particle. Yeast 2 was used for this experiment.

TABLE II

Activity of Normal and C5D Sera in Opsonization of Baker's Yeast for Phagocytosis by Normal Human Leukocytes

Particle	Serum dilution ($\times 10^{-1}$)	Ratio of yeast/100 PMN leukocytes obtained with test serum to value obtained with standard normal serum*: Serum source: Normals‡	C5D proband (L. H.)	C5D sibling (D. H.)	C5 dysfunction
Yeast 1	10	1.04±0.19 (14)	0.98±0.08 (5)	ND§	0.84±0.12 (4)
	20	0.58±0.23 (14)	0.41±0.18 (5)	ND	0.47±0.18 (4)
	40	0.28±0.09 (14)	0.36±0.16 (5)	ND	0.30±0.12 (4)
	80	0.20±0.13 (3)	0.17±0.02 (2)	ND	ND
Yeast 2	10	0.98±0.03 (17)	1.16±0.12 (4)	1.22±0.38 (5)	1.09±0.05 (5)
	20	0.78±0.32 (18)	1.18±0.20 (6)	1.10±0.17 (7)	0.60±0.12 (5)
	40	0.51±0.36 (18)	0.99±0.30 (6)	0.45±0.15 (7)	0.24±0.14 (5)
	80	0.19±0.12 (12)	0.70±0.35 (4)	0.22±0.11 (5)	0.19±0.21 (5)
	160	ND	0.27±0.17 (4)	0.18±0.13 (3)	ND

* Values shown are means±1 SD (number of observations in parentheses) of data derived from six experiments with yeast 1 and seven experiments with yeast 2. These values represent the number of yeast/100 PMNs found for each serum dilution in a given experiment divided by the value obtained in that experiment for $\frac{1}{10}$ dilution of a standard reference serum (included in all experiments). Thus, the score obtained with this $\frac{1}{10}$ reference serum has a value of 1.00 in each experiment.

‡ In addition to the standard reference serum employed in all experiments, eight other sera from healthy donors were tested against yeast 1 and six, against yeast 2.

§ Not done.

scoring, i.e., yeast per 100 PMN or percent PMN with one or more yeast, was good, although the former method appeared to reflect the effects of serum dilution more sensitively. In this experiment (Fig. 2), the opsonic capacity of the two C5D sera was clearly normal or, in the case of the proband's serum (L. H.), somewhat above normal. This supranormal opsonic capacity appears to be due, in part, to the presence of heat-stable factors in L. H. serum, particularly at lower serum dilutions (Fig. 2). Both C5D sera, however, clearly possessed substantial heat-labile opsonic activity. The C5 dysfunction serum overlapped our normal controls (Fig. 2). In a single experiment (not shown), PMN from the C5D proband also showed normal phagocytosis of yeast opsonized by either normal or C5D serum.

Table II summarizes data from six experiments with yeast 1 (obtained in Rochester) and seven experiments with yeast 2 (obtained from Dr. Michael Miller). Because experiments were performed over a period of months, and with leukocytes of different normal donors, the individual values for yeasts/100 PMNs were corrected by dividing them by the score obtained for the 1/10 dilution of a normal reference serum which was included in each experiment. The actual scores for this dilution of the reference serum ranged from 183 to 717 yeasts/100 PMNs. For simplicity, the values for percent PMN with one or more yeast and for heat-inactivated sera (see Fig. 2) are omitted. L. H. C5D serum showed normal values with yeast 1. With yeast 2 all dilutions of L. H. serum and the 1/10 and 1/20 dilutions of D. H. serum gave values significantly higher than normal ($2P$ ranging from 0.05–0.01). Not shown in Table II is the observation that a major portion (ranging from 61 to 94% in various experiments) of the serum-dependent opsonic activity of the two C5D sera was found to be heat labile. The only instance in which any of the test sera gave a value significantly lower than the normals was with the 1/10 dilution of C5 dysfunction serum using yeast 1 only ($2P = 0.02$). The same dilution of the C5 dysfunction serum, however, gave significantly higher values than normal with yeast 2 ($2P = 0.05$). All other dilutions of the C5 dysfunction serum yielded values in the low range of normal.

In one additional experiment (not shown), yeast 1 and a third yeast preparation (supplied by Dr. Ulf Nilsson) were studied in parallel with the same leukocytes, normal and deficient sera. Virtually identical results were obtained with the two yeasts.

Opsonization of C. albicans. No difference in opsonization of *C. albicans* by either C5D serum or eight normal sera could be detected. Although this opsonic activity was substantial and dose-dependent, heat in-

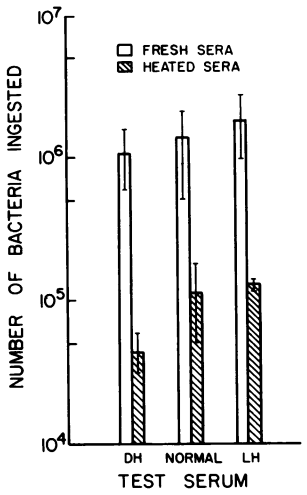

FIGURE 3 Phagocytosis of *S. aureus* in the presence of fresh and heat-inactivated sera from normal and C5D subjects (L. H., proband; D. H., sibling). Values shown are means±SEM from five experiments and represent the number of organisms ingested after 1 h of incubation (½ h after lysostaphin was added), in the presence of phenylbutazone. Values obtained after 3 h of incubation (not shown) were also comparable for C5D and normal sera.

activation (56°C, 30 min) did not significantly reduce the opsonic activity of either normal or C5D sera. In contrast, the normal opsonic capacity of sera from two untreated adult patients with agammaglobulinemia was completely heat labile. Efforts to establish C-dependent

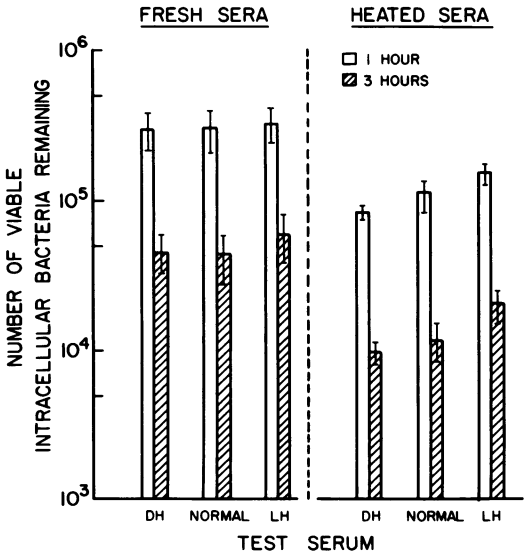

FIGURE 4 Survival of intracellular *S. aureus* incubated with fresh and heat-inactivated sera from normal and C5D subjects (L. H., proband; D. H., sibling). Values shown are means±SEM from five experiments and represent the numbers of organisms ingested and remaining viable after 1 and 3 h of incubation.

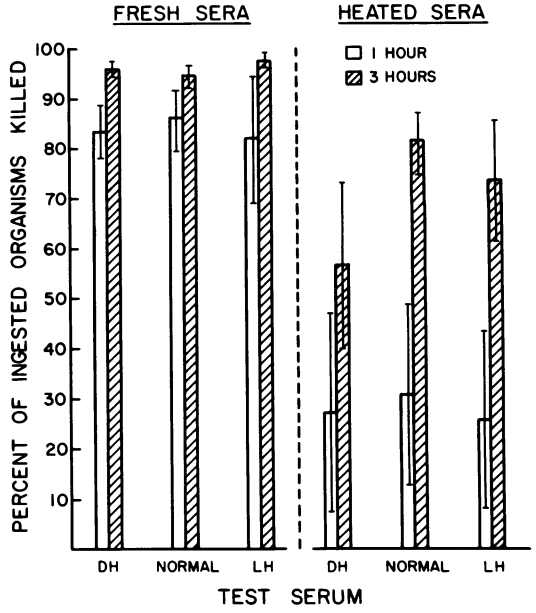

FIGURE 5 Intracellular killing of staphylococci expressed as percent of ingested organisms which were killed. Values shown are means±SEM based on the data in Figs. 4 and 5. Corrections were made for the partial inhibitory effect of phenylbutazone on phagocytosis (see Methods). Serum designations as in Fig. 3.

conditions for normal or C5D sera by varying serum concentration, yeast: leukocyte ratios, or incubation times were unsuccessful because of the dominant effect of heat-stable opsonins, presumably antibody.

Opsonization and support of intracellular killing of staphylococcus. Phagocytosis of *S. aureus* by normal PMN leukocytes in the presence of fresh C5D serum (D. H. or L. H.) was equal to that of the normal controls (Fig. 3). Substitution of heat-inactivated sera for fresh sera from both C5D siblings and from control subjects resulted in more than 90% decrease in phagocytosis of staphylococci (Fig. 3).

The C5D sera also supported normal intracellular killing of staphylococci by normal PMN leukocytes, i.e., the number of viable intracellular bacteria remaining after 1 and 3 h of incubation was essentially equal when either C5D or normal serum had been present in the original suspension (Fig. 4). By combining the data from both parts of these experiments (Figs. 3 and 4) and correcting for the partial inhibition of phagocytosis by phenylbutazone, intracellular killing as a function of the numbers of organisms ingested could be derived (Fig. 5). Again, the C5D sera were normal.

Cytolytic activity. Serum bactericidal activity of the C5D sera against *S. typhi O 901* was tested both with and without addition of an optimal quantity of specific rabbit antibody to this organism (Table III). As expected, L. H. serum was totally ineffective. D. H. serum,

TABLE III

Serum Bactericidal Activity against S. typhi O 901

Complement source	Rabbit antibody added*	Percent killing with test serum (C source) diluted				
		Undil.	½	¼	⅛	1/16
C5D proband (L. H.)	yes	0	0	0	0	0
	no	0	0	0	0	0
C5D sibling (D. H.)	yes	80	32	7	0	0
	no	80	28	2	0	0
Normals (3)‡	yes	—§	—	92±6	66±12	23±15
	no	—	—	87±10	49±10	4±4

* Specific antiserum to *S. typhi O 901*, diluted 1/6,400.
‡ Mean±SD.
§ Not tested.

which has 1–2% of normal C5, mediated significant bacterial killing when tested undiluted or at one-half dilution. Three normal sera studied concurrently killed effectively at dilutions of 1/16 or higher in the presence of rabbit antibody.

Lysis of paroxysmal nocturnal hemoglobinuria red cells in either the sucrose hemolysis or acid hemolysis systems was also totally lacking with L. H. serum.

DISCUSSION

The markedly impaired generation of C-dependent neutrophil chemotactic activity in both homozygous C5D sera, and its restoration by highly purified human C5, confirms earlier experimental work on the critical contribution of C5 to this function, either as the C5a peptide (25, 30, 31) or as part of the $C\overline{567}$ macromolecular complex (12, 32, 33). Certain aspects of these observations warrant further comment, however.

Although it was recognized that other variables were involved, the higher chemotactic scores of the half-sister's serum (see protocol 1, Fig. 1) had initially suggested that a very low serum concentration of C5 might be sufficient for generation of measurable C5-related chemotactic activity, and that small additions of C5 to the proband's serum might significantly improve her chemotactic function. This aspect did not prove to be straightforward, however. Whereas eight heterozygous C5D sera, containing as little as 34% of the mean normal C5 level, exhibited fully normal chemotactic function, neither C5D serum generated normal chemotactic activity at half-normal C5 levels, measured either as C5 protein or by hemolytic activity (Table I, experiment B). This finding was equally true when the added human C5 had been prepared by Dr. Ulf Nilsson or in our laboratory (Table I). High normal C5 concentrations finally restored fully normal chemotactic function to the sibling's serum (D. H.) and raised the proband's scores to 51–72% of the concurrent normal controls (Table I). These latter scores (for L. H.) may be within the range of normal since limitations on experimental size prevented concurrent study of a larger number of control sera. Nevertheless, the heterozygous C5D sera were compared to the same normal reference sera.

Suboptimal restoration of chemotactic function in the homozygous C5D sera could be related to elevated levels of the normal α-globulin chemotactic factor inactivator described by Ward and co-workers (34), or to some other inhibitory factor(s). Alternatively, it is conceivable that the C5 purification procedures might produce subtle changes in the C5a portion of some C5 molecules without affecting the C5b portion. This could result in a disparity between hemolytic effectiveness, associated with the C5b portion (35), and chemotactic potential, attributed mainly to the C5a peptide (25, 31). Finally, it is possible that some other C-related activity not assayed in the present study, such as the generation of alternative pathway "C5 convertase," is reduced in the proband's serum.

The two chemotactic protocols (see Methods) differed with respect to comparative activities of the two C5D sera. In protocol 1, which did not involve heat-inactivation of the chemotactic mixture, the half-sister's serum (D. H.), containing a very low C5 concentration, was consistently superior to the proband's serum, in which no C5 was measurable (Fig. 1). This difference was barely significant in protocol 2 (Fig. 1). Since Ward has reported that only the high molecular weight factor ($C\overline{567}$) is formed detectably at low concentrations of C5 (32), it is possible that protocol 2 is less favorable for the formation (perhaps due to the shorter incubation) or stability (perhaps due to heating at 56°C) of the macromolecular complex, $C\overline{567}$.

Unexpectedly, we have been unable to demonstrate an opsonic defect in the serum of either C5D subject using the baker's yeast assay (18). Our studies, summarized in Table II, employed a locally purchased batch of yeast, one supplied by Dr. Michael Miller, and a third batch from Dr. Ulf Nilsson. Drs. Miller and Nilsson have confirmed in their own laboratories that L. H. serum is opsonically normal, or even supranormal as we found with yeast 2. The only factual discrepancy between our laboratories is that the adult C5 dysfunction serum, kindly sent to us by Dr. Nilsson, produced low normal results in our hands but has been consistently deficient in his. We feel that this is a very difficult assay, principally because of the subjective problem of confidently discriminating between intra- and extracellular yeast particles. For this reason all of our assays were read in blind fashion (see Methods).

The two C5D sera also supported normal phagocytosis of *C. albicans* by PMN leukocytes, although these ef-

fects were attributable to high levels of heat-stable opsonins in both C5D and all normal sera tested. The proband has had Candida infections on multiple occasions, but these always remained limited to mouth and vagina (22).

In any event, it seems clear that C5 is not an absolute requirement for ingestion of either baker's yeast or Candida. On the other hand, there is persuasive evidence for a relative contribution of C5 to optimal opsonization of some organisms (14, 15), and this function may be particularly relevant in a nonimmune host dependent on the natural heat-labile opsonic system. Thus, it is conceivable that in our C5D subjects a high level of heat-stable opsonin, e.g., antibody, may be offsetting the C defect.[2]

Because of our desire to study another clinically relevant organism and the availability of an objective, quantitative assay (29), the capacity of C5D serum to support phagocytosis and intracellular killing of *S. aureus* by normal human PMN leukocytes was evaluated (Figs. 3–5). No differences were observed between normal and C5D sera in either of these functions. With this organism, heat-stable opsonin clearly was not above normal in the C5D sera and accounted for only 5–10% of overall phagocytosis (Fig. 3). In the studies on normal sera, the indication of less effective killing of ingested organisms, which had been exposed to heat-inactivated rather than fresh serum (Fig. 5), is in keeping with other observations (36) and is the subject of continuing investigation.[3]

The proband's serum, as expected demonstrated a total lack of cytolytic activity against sheep red cells, human paroxysmal nocturnal hemoglobinuria red cells, and *S. typhi O 901*. The half-sister's serum, on the other hand, with only 1–2% of normal C5, had exhibited a CH_{50} titer of 45 U/ml (normals 80–160) (22) and, in the present study, killed 80% of the bacterial inoculum mixed with her undiluted serum. These observations emphasize the relatively great cytolytic efficiency of the C5 step in the C cascade (37).

From our studies to date, the principal defects in host defense mechanisms which, aside from the underlying SLE and corticosteroid therapy, we can associate with the proband's remarkable susceptibility to bacterial infection (22) are the chemotactic defect and, possibly in part, the serum bactericidal defect. It must be acknowledged that the half-sister (D. H.), who has a similar but less profound chemotactic deficiency (Fig. 1), has so far had much less difficulty with infections (22). However, she does not carry the added burden of steroid therapy or SLE, and does possess serum bactericidal activity (Table III).

Our finding that some exudates from the proband's abscesses or skin ulcers contained large numbers of neutrophils (22) does not necessarily negate these in vitro studies of chemotaxis because it says nothing about the *rate* of leukocyte accumulation in vivo. Moreover, in bacterial infections the neutrophil response is probably triggered by bacterial (38) as well as C-derived (and possibly other) chemotactic substances. Studies on C5D mice have shown that the chemotactic defect demonstrable in Boyden chambers *is* correlated with impaired leukocyte exudation in vivo (13). Concerning the serum bactericidal defect, our previously reported C6D proband also totally lacked this function but was healthy except for two episodes of gonococcal arthritis after appropriate exposure (5). C5D mice are healthy under routine laboratory conditions but are modestly hypersusceptible to in vivo challenges with certain infectious agents (14, 39, 40). Considering all of these points, we would suggest that the absence of C5-related defense mechanisms may prove to carry only a relative risk to the host, depending upon the nature of the microbial challenge and/or the presence of other compromises in host defense.

[2] If this is the case, however, one must ask why the patients with C5 dysfunction do not offset their defect by acquisition of antibodies. Even adults with this disorder had persistent opsonic dysfunction in vitro (18, 19), despite cessation or absence of abnormal susceptibility to infection.

[3] Steigbigel, R. T., S. I. Rosenfeld, and J. P. Leddy. To be published.

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