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## Research Article

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### Two Distinct Abnormalities in Patients with C8 $\alpha$ - $\gamma$ Deficiency

Low Level of C8 $\beta$  Chain and Presence of Dysfunctional C8 $\alpha$ - $\gamma$  Subunit

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#### Abstract

The sera from three  $C8\alpha$ - $\gamma$  deficient patients previously reported to have a selective  $C8\alpha$ - $\gamma$  defect were analyzed by SDS-PAGE and Western blot using two polyclonal antisera to C8 $\alpha$ - $\gamma$  and a monoclonal antibody to C8 $\alpha$ . All three sera exhibited C8 $\alpha$ - $\gamma$  bands that dissociated into  $\alpha$  and  $\gamma$  chains under reducing conditions. Quantitation of the  $\alpha$ - $\gamma$  subunit in these sera by a sensitive ELISA revealed an amount  $\sim 1\%$  of that found in normal human serum. A similar assay performed with a specific antiserum to  $C8\beta$  showed unexpectedly low levels of  $C8\beta$  in these sera, which were confirmed by hemolytic titration of C8 $\beta$ . The remarkable differences between C8 $\alpha$ - $\gamma$  and C8 $\beta$  in the C8 $\alpha$ - $\gamma$  deficient sera was that in spite of their comparable immunochemical levels,  $C8\beta$  still exhibited functional activity whereas C8 $\alpha$ - $\gamma$  was totally inactive. That the residual C8 $\alpha$ - $\gamma$ was inactive was also proved by its inability to show lytic bands in an overlay system after SDS-PAGE and subsequent removal of SDS. The implications of these findings for a novel concept of C8 deficiency are discussed. (J. Clin. Invest. 1990. 86:884-888.) Key words: complement • C8 • inherited defect • C8 subunits • combined deficiency

#### Introduction

The selective defect of the eighth complement component  $(C8)^1$  is relatively common among the inherited deficiencies of the components and regulators of the complement system (1). Patients with this defect show an increased susceptibility to meningococcal meningitis, indicating that C8 plays an important role as a component of the membrane attack complex and in the protection against neisserial infections (1, 2).

A distinctive feature of C8 deficiency is the presence of a dysfunctional C8 molecule in all the sera of C8-deficient

J. Clin. Invest.

(C8D) subjects that have been tested (2). This is rarely observed in other complement deficiencies with the exception of a small group (10–15%) of patients lacking functional C1-inhibitor (3). On the basis of reconstitution experiments and immunochemical analysis, two forms of dysfunctional C8, and consequently two groups of patients have been identified (4, 5). One form is associated with a dysfunction in the  $\alpha$ - $\gamma$ subunit and the other with a dysfunction in  $\beta$ . The absence of C8 activity in both groups is consistent with the fact that  $\alpha$ - $\gamma$ and  $\beta$  have specific functional roles and both are essential for normal C8 hemolytic activity (6). For reasons that are presently not clear, the inherited defect of C8 $\beta$  is largely predominant over that of C8 $\alpha$ - $\gamma$  and is essentially restricted to Caucasians, whereas the C8 $\alpha$ - $\gamma$  deficiency has been found exclusively in Blacks and North Africans (1, 2).

The possibility that C8 deficiencies result from the complete absence of a particular subunit is unlikely in view of recent findings by Tschopp and co-workers (7). Two C8 $\beta$ -deficient patients were found to have detectable but dysfunctional C8 $\beta$  in their serum along with normal  $\alpha$ - $\gamma$ . Partial characterization indicated that the C8 $\beta$  was structurally abnormal. Thus, at least in some patients, dysfunctional C8 $\beta$  is synthesized but in an altered form. Presumably this altered form is incapable of association with  $\alpha$ - $\gamma$  to produce functional C8.

In this study, we examined in more detail the molecular abnormality of the dysfunctional C8 in three  $C8\alpha-\gamma D$  individuals and provide evidence for a further complexity of this inherited defect. Data presented indicate that  $C8\alpha-\gamma$  deficiency is a combined defect of both  $\alpha-\gamma$  and  $\beta$ , and that a small amount of dysfunctional  $C8\alpha-\gamma$  can be detected in the sera of  $C8\alpha-\gamma$  deficient patients.

#### Methods

Sera. Three unrelated  $C8\alpha$ - $\gamma D$  patients and two siblings with  $C8\beta D$ , reported in previous publications (8–11), provided serum samples for this investigation. The type of C8 deficiency was recognized by hemolytic reconstitution with the missing subunits and by identification of the subunit present in the various sera by SDS-PAGE and immunochemical procedures (4). A pool of normal human sera (NHS) was prepared by mixing equal volumes of sera obtained from 20 blood donors.

Complement reagents. Human C8 was purified following the procedure described in detail by Steckel et al. (12). The two subunits,  $C8\alpha$ - $\gamma$  and  $C8\beta$ , were dissociated from C8 in the presence of buffers of high ionic strength and further purified by gel filtration through Sephacryl S-300 (Pharmacia Fine Chemicals, Uppsala, Sweden), as reported by Rao and Sodetz (13).

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<sup>1.</sup> Abbreviations used in this paper: C8, eighth component of complement; C8D, C8 $\alpha$ - $\gamma$ D, and C8 $\beta$ D, C8-, C8 $\alpha$ - $\gamma$ -, and C8 $\beta$  deficient; EAC1-3, EAC1-7, and EAC1-8, sheep erythrocytes coated with antibody and complement components up to C3b, C7, and C8; NHS, normal human sera.

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Erythrocyte intermediates and hemolytic assays. The preparation of EAC1-3 and EAC1-7 from IgM sensitized sheep red cells using yeast-treated human serum and a C8 $\beta$ D serum has been previously described (10). The hemolytic assays for C8 $\alpha$ - $\gamma$  and C8 $\beta$  were per-

formed by incubating a mixture of 200  $\mu$ l of various sera dilutions and 50  $\mu$ l of EAC1-7 (1.5 × 10<sup>7</sup>), suspended in either C8 $\alpha$ - $\gamma$ D or C8 $\beta$ D sera, to a final dilution of 1/50 for 30 min at 37°C. Preliminary experiments proved that the concentrations of the C8D sera used for these assays were not limiting for an optimal titration of the two C8 subunits.

Antisera. A goat antiserum against whole human C8 was purchased from Atlantic Antibodies (Scarborough, ME). Antibodies to  $C8\alpha-\gamma$ were prepared in two different ways. A goat antiserum prepared against whole human C8 was passed through an agarose- $(\alpha-\gamma)$  affinity column to isolate  $(\alpha-\gamma)$ -specific Ig (14). A second antiserum with a higher avidity for  $\alpha-\gamma$  was obtained by immunizing rabbits with purified  $\alpha-\gamma$ and passing the antiserum through an agarose- $(\beta)$  column to remove traces of anti- $\beta$ . Specific antibodies against  $\beta$  were prepared similarly in rabbits using purified  $\beta$  as the immunogen. Specificity was tested by Ouchterlony and SDS-PAGE immunoblotting.

A mouse monoclonal antibody was obtained following an established procedure (15) and found to recognize the  $\alpha$  chain of C8. Ascitic fluid was produced in pristane-primed Balb/c mice and used for the experiments.

Biotin labeling of antibodies. The IgG fraction of the rabbit antisera to C8 $\alpha$ - $\gamma$  and C8 $\beta$  was obtained by affinity cromatography through protein A-agarose (Zymed Laboratories, San Francisco, CA) using 0.2 M phosphate/0.1 M citrate pH 3.5 as eluting buffer. The IgG were dialyzed against 0.2 M Tris-HCl pH 8.0 and mixed with D-biotin-*N*hydroxysuccinimide ester (Sigma Chemical Co., St. Louis, MO) (2 mg/ml in DMSO) at a ratio of 1:4 (wt/wt) for 15 min at room temperature. The excess biotin was removed by dialysis against 10 mM PBS pH 7.4 containing 0.01% NaN3.

SDS-PAGE and immunoblotting. Normal and C8D sera were subjected to SDS-PAGE on a 10% gel under nonreducing conditions according to Laemmli (16), followed by electrophoretic transfer onto nitrocellulose (Schleicher & Schuell Inc., Dassel, FRG). After soaking the nitrocellulose sheet in 50 mM Tris-HCl pH 7.6 containing 0.5 M NaCl Tris buffered saline, 2% bovine serum albumin, and 0.5% Tween 20 for 1 h at 37°C to block the free binding sites, the C8 bands were revealed by successive incubations with the primary antibodies to C8 or C8 subunits overnight at 4°C, followed by the biotin-labeled secondary antibodies (Zymed Laboratories) for 2 h at 37°C and alkaline phosphatase conjugated to streptavidine (Jackson Immunoresearch Laboratories, West Grove, PA) for 1 h at 37°C. The enzymatic reaction was developed using nitroblue tetrazolium (0.30 mg/ml) and 5bromo-4-chloro-3-indolyl phosphate (0.15 mg/ml) (both purchased from Sigma Chemical Co.), and diluted in 0.1 M Tris-HCl pH 9.5 containing 0.15 M NaCl.

Hemolytic overlay. The functional activity of the  $C8\alpha$ - $\gamma$  subunit electrophoresed by SDS-PAGE was revealed following the procedure developed by Orren et al. (17). Briefly, after the electrophoretic run the gels were soaked in an aqueous solution of 0.25% Triton X-100 for 1 h at 4°C to remove SDS and then in H<sub>2</sub>O for 1 h at 4°C, followed by a 10-min wash with glucose veronal-buffered saline (18). The gel was covered with a mixture of 1% agarose, 0.6% EAC1-7, and 1/50 C8 $\alpha$ - $\gamma$ D serum to obtain a 1-mm thick gel. The overlay was incubated at 37°C in a humid box until the hemolytic bands developed. Further hemolysis was prevented by adding 2% glutaraldehyde in saline.

Quantitation of the C8 subunits by ELISA. The wells of microtiter plates (Dynatech, PBI, Milan, Italy) were coated with 200  $\mu$ l of the IgG fraction of either anti-C8 $\alpha$ - $\gamma$  (1.6  $\mu$ g/well) or anti-C8 $\beta$  (0.5  $\mu$ g/well) in 0.1 M sodium bicarbonate buffer pH 9.6 overnight at 4°C. After blocking the residual free sites with PBS containing 1% bovine serum albumin and 0.1% Tween 20, normal and C8D sera were added at the appropriate dilutions and incubated for 1 h at 37°C and subsequently overnight at 4°C. The binding of the C8 subunits was revealed by the addition of biotin-labeled anti-C8 $\alpha$ - $\gamma$  or anti-C8 $\beta$  antibodies. After an incubation of 2 h at 37°C, the reaction was revealed by the addition of alkaline phosphatase conjugated to streptavidine for 30 min at 37°C, and subsequently of *p*-nitrophenyl-phosphate (Merck, Bracco S. P. A., Milan, Italy) at the concentration of 1 mg/ml in 0.1 M glycine buffer pH 10.4 containing 1 mM MgCl<sub>2</sub> as substrate for the enzyme. Reading was performed in a Multiskan 340 apparatus (Flow Laboratories Inc., Milan, Italy) at 405 nm.

Depletion of C8 from human sera by affinity chromatography. Agarose- $(\alpha - \gamma)$  IgG was prepared by coupling affinity purified goat anti- $\alpha - \gamma$  IgG to agarose by standard procedures using cyanogen bromide activation (14). This resin recognizes and removes both free  $\alpha - \gamma$  and intact C8 from serum.

#### Results

Detection of  $C8\alpha$ - $\gamma$  in the  $C8\alpha$ - $\gamma D$  sera. Analysis of the three  $C8\alpha-\gamma D$  sera by SDS-PAGE under nonreducing conditions followed by immunoblot revealed the presence of  $C8\alpha$ - $\gamma$  in all sera using three different sources of antibodies to  $C8\alpha - \gamma$ , one monoclonal antibody, and two polyclonal antisera. A volume of C8 $\alpha$ - $\gamma$ D sera five times higher than that of NHS was required to detect the C8 $\alpha$ - $\gamma$  in the deficient sera, suggesting that the concentration of the subunit in these sera was substantially lower than that observed in NHS. The C8 $\alpha$ - $\gamma$  of the deficient sera was more clearly recognized when the serum precipitate obtained with ammonium sulphate to 37 and 50% saturation was used instead of the whole serum (Fig. 1). This procedure allows analysis of serum samples enriched in their C8 content and that are less contaminated by other serum proteins that contribute to background staining of the nitrocellulose when high serum volumes are used. Like the  $C8\alpha$ - $\gamma$  of NHS, the subunit of the deficient sera exhibited multiple bands when examined under nonreducing conditions. Analysis of the same sera under reducing conditions (results not shown) showed dissociation of the patients  $C8\alpha$ - $\gamma$  into the two constitutive chains  $\alpha$  and  $\gamma$ , with molecular weights similar to those of the normal C8 chains.

Quantitation of  $C8\alpha$ - $\gamma$  and  $C8\beta$  by ELISA. The identification of  $C8\alpha$ - $\gamma$  in the three sera lacking hemolytically active  $C8\alpha$ - $\gamma$  prompted us to measure the level of this subunit in the same sera using a polyclonal antiserum specific for  $C8\alpha$ - $\gamma$  and a sensitive ELISA that allowed detection of limited amounts of  $C8\alpha$ - $\gamma$  in NHS. Controls for the specificity of the assay were provided by each serum depleted of  $C8\alpha$ - $\gamma$  by affinity chromatography (see Methods), that gave results essentially similar to those obtained in the absence of serum (blank). Evaluation of the  $C8\alpha$ - $\gamma$ D sera by this assay showed  $C8\alpha$ - $\gamma$  levels of  $\sim 0.5\%$ that of NHS, whereas the amount of  $C8\alpha$ - $\gamma$  in the  $C8\beta$ D sera was closer to that measured in NHS (Fig. 2 A).



Figure 1. Western blots of three  $C8\alpha-\gamma D$  sera and NHS using a polyclonal antiserum to  $C8\alpha-\gamma$ . Each serum sample was treated with ammonium sulphate to 37 and then 50% saturation, and volumes of the precipitates corresponding to 3 and 20  $\mu$ l of the original serum volume of NHS and the three  $C8\alpha-\gamma D$  sera were applied to the gel, respectively.



Figure 2. Immunoenzymatic assay of  $C8\alpha - \gamma$  (A) and  $C8\beta$  (B) in NHS ( $\blacksquare - \blacksquare$ ), two C8 $\beta$ D ( $\blacktriangle - \blacktriangle$ ), and three C8 $\alpha - \gamma$ D sera ( $\bullet - \bullet$ ). The results are expressed as ELISA index that is equal to (OD sample—OD blank)/OD blank, where blank indicates the result obtained in the absence of the serum samples.

Since  $C8\beta$  is known to be present in the  $C8\alpha - \gamma D$  sera, we wanted to quantitate the amount of  $C8\alpha - \gamma$  relative to that of  $C8\beta$  in these sera next. Employing an ELISA specific for  $C8\beta$ , the level of this subunit in the  $C8\alpha - \gamma D$  sera was found to be markedly reduced to  $\sim 1-3\%$  of that in NHS with a ratio of  $C8\alpha - \gamma$  to  $C8\beta$  varying between 0.2 and 0.5 in the three deficient sera (Fig. 2 *B*). A similar assay performed on the two  $C8\beta D$  sera revealed negligible amounts of  $C8\beta$  (results not shown).

Table I. Comparison of the Hemolytic Activities and the Immunochemical Levels of  $C8\alpha$ - $\gamma$  and  $C8\beta$  Subunits in NHS and  $C8\alpha$ - $\gamma D$  Sera

	Hemolytic activity (CH 50 U/ml)		Immunochemical levels (% of NHS)	
	C8α-γ	C8 <i>β</i>	C8α-γ	C8 <i>β</i>
NHS	160,000	132,000	100	100
C8α-γD1	0	1,750	0.41	2.5
C8α-γD2	0	1,200	0.56	2.1
C8α-γD3	0	1,000	0.50	0.98



Figure 3. Hemolytic titration of  $C8\alpha - \gamma$  (A) and  $C8\beta$  (B) in NHS (**a** - **b**) and three  $C8\alpha - \gamma D$  sera (**b** - **c**). The hemolytic system contained 50  $\mu$ l of EAC1-7 (1.5 × 10<sup>7</sup>) and either  $C8\alpha - \gamma D$  or  $C8\beta D$  sera to the final dilution of 1/50 in a total volume of 250  $\mu$ l.

The hemolytic activities of  $C8\alpha$ - $\gamma$  and  $C8\beta$  in the  $C8\alpha$ - $\gamma D$ sera are differentially related to their immunochemical levels. Having found remarkably low amounts of both  $C8\alpha-\gamma$  and C8 $\beta$  in the three C8 $\alpha$ - $\gamma$ D sera by ELISA, it was of importance to examine how these levels related to the functional activity of these subunits in the same sera. Previous work has clearly established that the C8 $\beta$  of the C8 $\alpha$ - $\gamma$ D patients is fully active in reconstituting the lytic activity of the C8 $\beta$ D sera (4, 5). Analysis of the C8 $\alpha$ - $\gamma$ D sera for C8 $\alpha$ - $\gamma$  (Fig. 3 A) and C8 $\beta$  (Fig. 3 B) hemolytic activities confirmed the results obtained with the immunoenzymatic assays for  $C8\beta$  but not for  $C8\alpha-\gamma$ . In particular, all three  $C8\alpha$ - $\gamma D$  sera had significant  $C8\beta$  activity, but failed to lyse EAC1-8 $\beta$ . The serum of an additional unrelated C8 $\alpha$ - $\gamma$ D patient, kindly provided by Dr. H. Jasin (Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, TX), was also found to have extremely low levels of  $C8\beta$  activity. Unfortunately, this serum was not available in sufficient amounts to allow further investigation. Comparing the results of the ELISA and of functional assays (Table I), it is apparent that the immunochemical levels of C8 $\beta$  in the C8 $\alpha$ - $\gamma$ D sera correlates with its functional activity, accounting for 1-3% of the values found in NHS. In contrast, the hemolytic activity of  $C8\alpha$ - $\gamma$  was absent in spite of a



Figure 4. Detection of functional  $C8\alpha$ - $\gamma$  subunit by hemolytic overlay. 1  $\mu$ l of NHS (lane 6) and 6  $\mu$ l of two  $C8\beta$ D sera (lanes 1 and 3) and of three  $C8\alpha$ - $\gamma$ D sera (lanes 2, 4, and 5) were electrophoresed by SDS-PAGE and the hemolytic bands were revealed as described in Methods.

detectable immunochemical level, suggesting that  $C8\alpha-\gamma$  in these sera is hemolytically inactive.

To obtain further evidence in favor of a dysfunctional  $C8\alpha-\gamma$  molecule being present in the  $C8\alpha-\gamma D$  sera, an additional approach was followed based on the hemolytic detection of  $C8\alpha-\gamma$  after SDS-PAGE. For this purpose, the sera were electrophoresed in duplicate on the same gel, and at the end of the run, one-half of the gel was processed for Western blotting while the second half was used for the hemolytic overlay after removal of SDS. Under these conditions, the  $C8\alpha-\gamma$  of both NHS and  $C8\beta D$  sera exhibited activity, as indicated by the appearance of lytic bands at the position of  $C8\alpha-\gamma$  migration (Fig. 4). No bands were detected in lanes loaded with the three  $C8\alpha-\gamma D$  sera, although distinct  $C8\alpha-\gamma$  bands appeared in the corresponding lanes of the gel after immunoblotting (results not shown).

#### Discussion

Patients with  $C8\alpha$ - $\gamma$  deficiency represent a minority of the whole group of C8D individuals and can be identified by the presence of hemolytically active and immunochemically recognizable  $C8\beta$  in their sera (2). The lack of functional activity in these sera has been attributed to a selective defect of  $C8\alpha$ - $\gamma$  that could not be detected either functionally or immunochemically. Although this conclusion remains basically correct, the present data indicate that the nature of the C8 defect in these sera is more complex than hitherto recognized.

An important finding of this study was the detection of  $C8\alpha$ - $\gamma$  in the deficient sera by Western blot analysis of the whole serum using a monoclonal antibody and two polyclonal antisera to  $C8\alpha$ - $\gamma$ . The higher sensitivity of both the assay system employed in this investigation and the use of antibodies specific for  $C8\alpha$ - $\gamma$ , possibly directed to additional epitopes not recognized by antisera to whole C8, may have contributed to reveal previously undetected  $C8\alpha$ - $\gamma$ . A similar situation has

been described by Tschopp et al. (7) in two C8 $\beta$ D sera in which C8 $\beta$  was detected only by antisera specific for C8 $\beta$  but not by those to whole C8. Quantitation of C8 $\alpha$ - $\gamma$  by ELISA confirmed the presence of this subunit in the C8 $\alpha$ - $\gamma$ D sera, albeit in a limited amount that did not exceed 2% of the level observed in NHS. The fact that the antiserum used for the ELISA detected only C8 $\alpha$ - $\gamma$  in NHS by Western blot proves the specificity of the assay for C8 $\alpha$ - $\gamma$ . This was further substantiated by the finding that depletion of the residual C8 $\alpha$ - $\gamma$  by affinity chromatography resulted in C8 $\alpha$ - $\gamma$ D sera values similar to those of the controls in the absence of serum.

The low levels of  $C8\alpha \cdot \gamma$  found in the  $C8\alpha \cdot \gamma D$  sera do not correlate with the lack of hemolytic activity in these sera. NHS can in fact be diluted to contain an amount of  $C8\alpha \cdot \gamma$  equal to that of the  $C8\alpha \cdot \gamma D$  sera and still induce complete lysis in a  $C8\alpha \cdot \gamma$ -dependent lytic assay. This assay is in fact exquisitely sensitive and reveals functional  $C8\alpha \cdot \gamma$  in NHS up to a dilution of 1 to 100,000. The suggestion can therefore be made that the residual  $C8\alpha \cdot \gamma$  in the deficient sera is dysfunctional. This is also proved by the inability of the subunit in the  $C8\alpha \cdot \gamma D$  sera to resume lytic activity after SDS-PAGE in an overlay system, in spite of the fact that  $C8\alpha \cdot \gamma$  can be detected immunochemically by Western blot.

Discovery of a combined deficiency of  $\alpha$ - $\gamma$  and  $\beta$  in C8 $\alpha$ - $\gamma$  patients is unexpected in view of the fact that the amount of C8 $\beta$  in these sera is sufficient to reconstitute the lytic activity of C8 $\beta$ D sera (4), and also that in C8 $\beta$ D sera  $\alpha$ - $\gamma$  is frequently present at near normal levels. If inherited C8 deficiency manifests itself as a defect in either  $\alpha$ - $\gamma$  or  $\beta$ , then one would also expect to find near normal levels of  $\beta$  in C8 $\alpha$ - $\gamma$ D. Results of this study clearly indicate that this is not the case. One explanation may be that in C8 $\alpha$ - $\gamma$ D sera there are molecular defects in both  $\alpha$ - $\gamma$  and  $\beta$  such that levels of each are reduced. This seems unlikely however, because only  $\alpha$ - $\gamma$  is functionally abnormal. An alternative explanation is that  $\beta$  depends on association with normal  $\alpha$ - $\gamma$  for proper biosynthesis and secretion

or for stability in the circulation. Synthesis of an abnormal  $\alpha$ - $\gamma$  would then produce a corresponding decrease in  $\beta$ . This would contrast with C8 $\beta$  deficiency, where neither the level nor the functional state of  $\beta$  have a major impact on levels of  $\alpha$ - $\gamma$ . Stability of free  $\alpha$ - $\gamma$  in the bloodstream has been suggested from biosynthetic studies showing that hepatocytes secrete excess  $\alpha$ - $\gamma$  relative to  $\beta$  (14), and from studies that found significant levels of free  $\alpha$ - $\gamma$  along with C8 in NHS (19, 20). Thus, it may be that serum levels of  $\beta$  are dependent on  $\alpha$ - $\gamma$  but the converse is not necessarily true.

We originally proposed that the inherited deficiency of C8 be divided into two groups on the basis of immunochemical analysis and reconstitution experiments (4). The present findings of combined deficiency of  $C8\alpha-\gamma$  and  $C8\beta$  subunits in patients with inherited defect of  $C8\alpha-\gamma$  do not contradict our previous observation. The genetic and molecular defect responsible for each type of C8 deficiency may still be restricted to either  $\alpha$ - $\gamma$  or  $\beta$ , but the consequences of these deficiencies differ with regard to the opposite subunit. In the combined deficiency for instance, a defect in  $\alpha$ - $\gamma$  would have an adverse effect on serum levels of  $\beta$ . At present, the nature of the molecular defects responsible for C8 deficiency is unknown. A major deletion of the  $\beta$  gene has been excluded based on studies of genomic DNA from patients with  $C8\beta$  deficiency (21), while for the combined deficiency the defect may be in  $\alpha$  or  $\gamma$ . A major gene deletion in  $\alpha$  seems unlikely in view of the close physical linkage between  $\alpha$  and  $\beta$  on chromosome 1 and the expression of functional  $\beta$  in the C8 $\alpha$ - $\gamma$ D (22). The exact nature of the  $\alpha$ - $\gamma$  defect must await further characterization of  $\alpha$ and  $\gamma$  genomic structures.

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#### References

1. Ross, S. C., and P. Densen. 1984. Complement deficiency states and infection: epidemiology, pathogenesis and consequences of Neisserial and other infections in an immune deficiency. *Medicine (Baltimore)*. 63:243-273.

2. Tedesco, F. 1986. Complement deficiencies.8. The eighth component. *In* Hereditary and Acquired Complement Deficiencies in Animals and Man. K. Rother and U. Rother, editors. *Progr. Allergy.* 39:295–306.

3. Agostoni, A. 1989. Inherited C1 inhibitor deficiency. *Complement Inflammation*. 6:112–118.

4. Tedesco, F., P. Densen, M. A. Villa, B. H. Petersen, and G. Sirchia. 1983. Two types of dysfunctional eighth component of complement (C8) molecules in C8 deficiency in man. Reconstitution of normal C8 from the mixture of two abnormal C8 molecules. J. Clin. Invest. 71:183–191.

5. Alper, C. A., D. Marcus, D. Raum, B. H. Petersen, and T. J. Spira. 1983. Genetic polymorphism in  $C8\beta$ -chains. Evidence for two unlinked genetic loci for the eighth component of human complement (C8). J. Clin. Invest. 72:1526–1531.

6. Sodetz, J. M. 1988. Structure and function of C8 in the membrane attack sequence of complement. *In* Current Topics in Microbiology and Immunology. E. R. Podack, editor. Springer-Verlag, Berlin. 140:19-31.

7. Tschopp, J., F. Penea, J. Schifferli, and P. Spath. 1986. Dysfunctional C8 $\beta$  chain in patients with C8 deficiency. *Scand. J. Immunol.* 24:715-720.

8. Petersen, B. H., J. A. Graham, and G. F. Brooks. 1976. Human deficiency of the eighth component of complement. The requirement of C8 for serum Neisseria gonorrhoeae bactericidal activity. J. Clin. Invest. 57:283–290.

9. Agnello, V., K. M. Pariser, and D. Babott. 1978. Association of C8 deficiency with sickle-thalassemia hemoglobinopathy. J. Immunol. 120:1761a. (Abstr.)

10. Tedesco, F., M. Bardare, A. M. Giovanetti, and G. Sirchia. 1980. A familial dysfunction of the eighth component of complement (C8). *Clin. Immunol. Immunopathol.* 16:180-191.

11. Zimran, A., B. Rudensky, M. R. Kramer, F. Tedesco, M. Ehrenfeld, R. Raz, Z. Greif, M. Gelber, M. Lishner, E. Golan, and C. Hershko. 1987. Hereditary complement deficiency in survivors of meningococcal disease: high prevalence of C7/C8 deficiency in Sephardic (Moroccan) Jews. *Q. J. Med.* 240:349–358.

12. Steckel, E. W., R. G. York, J. B. Monahan, and J. M. Sodetz. 1980. The eighth component of human complement: purification and physicochemical characterization of its unusual subunit structure. *J. Biol. Chem.* 255:11997-12005.

13. Rao, A. G., and J. M. Sodetz. 1984. Purification of functional subunits of the eighth component of human complement (C8) under nondenaturing conditions. *Complement*. 1:182–186.

14. Ng, S. C., and J. M. Sodetz. 1987. Biosynthesis of C8 by hepatocytes. Differential expression and intracellular association of the  $\alpha$ - $\gamma$ and  $\beta$  subunits. J. Immunol. 139:3021–3027.

15. Galfré, G., S. C. Howe, C. Milstein, G. W. Butcher, and J. C. Howard. 1977. Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature (Lond.).* 266:550–552.

16. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. 227:680-685.

17. Orren, A., W. H. Lerch, and E. B. Dowdle. 1983. Functional identification of serum complement components following electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate. J. Immunol. Methods. 59:63-71.

18. Mayer, M. M. 1971. Complement and complement fixation. *In* Experimental Immunochemistry. E. A. Kabat and M. M. Mayer, editors. Charles C. Thomas, Springfield, IL. 133–240.

19. Densen, P., and C. M. McRill. 1988. Differential functional expression of the C8 subunits: primary role of C8 $\beta$  in assembly of intact C8. J. Immunol. 141:2674–2679.

20. Doglio, L. T., M. S. Gawryl, and T. F. Lint. 1988. Analysis of human C8 with monoclonal antibodies: characterization of a monoclonal antibody that recognizes free  $C8\alpha-\gamma$  subunit. J. Immunol. 141:2079-2083.

21. Schneider, P. M., B. Dittmer, F. Tedesco, P. J. Spath, C. Rittner, and J. M. Sodetz. 1987. Genomic structure of the complement C8 $\beta$  locus in individuals with inherited C8 $\beta$  deficiency. *Complement.* 4:221*a.* (Abstr.)

22. Kaufman, K. M., J. V. Snider, N. K. Spurr, C. E. Schwartz, and J. M. Sodetz. 1989. Chromosomal assignment of genes encoding the  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits of human complement protein C8: identification of a close physical linkage between the  $\alpha$  and the  $\beta$  loci. *Genomics.* 5:475–480.