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Compound heterozygous complement C3 deficiency

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SUMMARY

Complete deficiency of the third component of the complement system is a result of defects in the two alleles of the C3 gene. In this study a family with C3 deficiency is reported; the parents expressed a distinct abnormality of the C3 gene and their two children had compound heterozygous C3 deficiency. These are the first reported cases of compound heterozygous complement deficiency. Our results indicate that the maternal abnormality leads to synthesis of an abnormal proC3 protein which is not secreted from the cells. The paternal abnormality results in ablation of synthesis of the proC3 protein.

The third component of the complement system (C3) is the most abundant complement protein in the blood serum and plays a central role in activation of the complement cascade through both the classical and alternative pathways.[1] Deficiency of C3 has been ascribed in 15 families and all affected patients suffered from recurrent pyogenic infections, autoimmune manifestations, or both. In four families, the molecular basis of the C3 deficiency was identified[2–5] and each presented with a different mutation in the C3 gene. We have recently described a C3-deficient patient, AGI, with abnormal secretion of C3 from monocytes and fibroblasts.[6] This patient expressed less than 1% normal serum levels of C3 and experienced serious meningococcal infections. The intracellular proC3 of AGI had apparently a normal molecular weight as judged by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis, but had a structural defect as identified by trypsin digestion. Cloning and sequencing of a cDNA encoded by one allele of C3 demonstrated a single amino acid substitution in the β-chain region at residue 549 (Asp to Asn), suggesting that the impairment in C3 secretion was caused by this substitution.[7] The variability in the molecular defect between the various C3 deficiencies examined raises the possibility that C3 deficiency may be a result of compound heterozygote deficiency, as reported in certain cases of thalassaemia and cystic fibrosis.[8,9] In order to examine this possibility we have analysed the synthesis of C3 in fibroblasts grown from a normal donor, the C3-deficient patient (AGI), the heterozygote mother and father of AGI (OFG and KEG, respectively) and a C3-deficient sister of AGI (AVG). The details of C3 antigenic levels and haemolytic activity in the serum of the family members are given in Table 1. Confluent fibroblast monolayers were radiolabelled with 300 μCi/ml, [^{35}S]methionine (specific activity 1000 Ci/ml; Amersham International, Amersham, UK) for 2 hr, as previously described.[6] After completion of the labelling, the medium was replaced with Dulbecco's modified Eagle's minimal essential medium (DMEM) and the cells were harvested at 0, 1, 3, 8 or 20 hr (pulse chase). C3 was immunoprecipitated from cell lysates and extracellular medium by using monospecific anti-C3 antibodies (Atlantic antibodies, Stillwater, ME) and formalin-fixed *Staphylococcus aureus* (Immunoprecipitin, BRL, Gaithersburg, MD), and analysed by SDS–PAGE. In a second set of experiments, the cell lysates were either untreated or treated with trypsin (2·5 mg/ml) for 10 min at room temperature before immunoprecipitation and analysis of C3.

Pulse chase experiments have demonstrated that in fibroblasts of the two C3-deficient patients, AGI and AVG, a considerable amount of proC3 was synthesized, but less than 10% was secreted (Fig. 1). Significant quantities of C3 were secreted from both OFG cells and KEG cells. The rate of disappearance of proC3 from fibroblasts of the mother (OFG) and the affected children was much slower than that of the father (KEG). Fifty-seven per cent proC3 was still detected inside OFG cells 3 hr after the end of the labelling. In contrast, similar to normal cells, only about 10% proC3 was present inside KEG cells 3 hr post-pulse. These findings indicated that both parents had a heterozygous C3 deficiency and that the molecular basis of the defect in their C3 was distinct. This hypothesis was confirmed in the next set of experiments, in which the metabolically labelled cell lysates, at 0 and 3 hr post-pulse, were either treated or not with trypsin prior to immunoprecipitation of the C3. As shown in Fig. 2, the proC3 in the fibroblasts of AVG and AGI had a similar trypsin digest pattern which was distinct from that of normal cells. In normal cells, and cells of KEG, trypsin treatment of proC3 yielded two

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Table 1. Levels of C3 and functional complement activity in family members and normal control

	C3 (mg/dl)	CH50 (U/ml)	AP50 (U/ml)
AGI (C3 def)	UD	< 200	< 20
AVG (C3 def)	UD	< 200	< 20
ANG (sister)	165	1265	146
KEG (father)	68	976	106
OFG (mother)	47	925	60
Normal	130	1450	181
Pooled serum	140	1370	140

UD, undetectable.

major fragments of 110 000 and 68 000 MW, most probably the α' and β chains of C3b.[6] In the trypsin-cleaved proC3 profile of the two C3-deficient patients, the 68 000 MW fragments were almost completely missing (Fig. 2). Additional fragments were seen at 75 000–105 000 MW, the most prominent of which were 100 000 and 75 000 MW. The trypsin digest pattern of C3 from OFG cells at the end of the labelling (0 hr) was similar to that of C3 from normal cells with an additional two bands of 100 000 and 75 000 MW. At 3 hr post-pulse, the trypsin digest pattern of OFG C3 was almost indistinguishable from that of AGI and AVG.

The experiments described above demonstrate that the two heterozygous C3-deficient parents of AGI and AVG express distinct types of defects. The father had a lower amount of C3 than normal, but the C3 synthesized by his fibroblasts appeared normal. This suggests that the decreased amount of the father's C3 resulted from a null allele. The mother's intracellular C3 expressed two abnormalities: (1) a slow rate of disappearance from the cells and (2) an abnormal trypsin digestion pattern which was more obvious 3 hr post-pulse. Taken together, these results indicate that the cells of the mother synthesize two distinct C3 molecules. One of these is a product of a normal allele processed in a normal manner and rate and, therefore, secreted at the usual rate. This normal C3 is probably the one detected in her serum. Because it is the product of only one gene, her serum C3 level is about half the normal level. The second C3 molecule accumulates in the fibroblasts and is present even at 3 and 8 hr post-pulse. This C3 molecule is digested abnormally by trypsin. The second C3 molecule is identical to the C3 synthesized by the cells of the two C3-deficient children AGI and AVG. These findings also indicate that the abnormal structure of the C3 in AGI is the cause of the low rate of C3 secretion, as the normal C3 in the mother is processed and secreted normally whereas the abnormal C3 is retained and degraded within the cell. In parallel experiments using allele-specific polymerase chain reaction amplification we were able to demonstrate the Asp to Asn mutation in DNA extracted from the mother, but the paternal DNA did not contain this mutation.[10] The precise nature of the defect in the C3 gene in the father is a subject for further investigation.

This is the first report of a compound heterozygote abnormality resulting in a phenotypic homozygote complement protein deficiency. In two other C3-deficient families, both

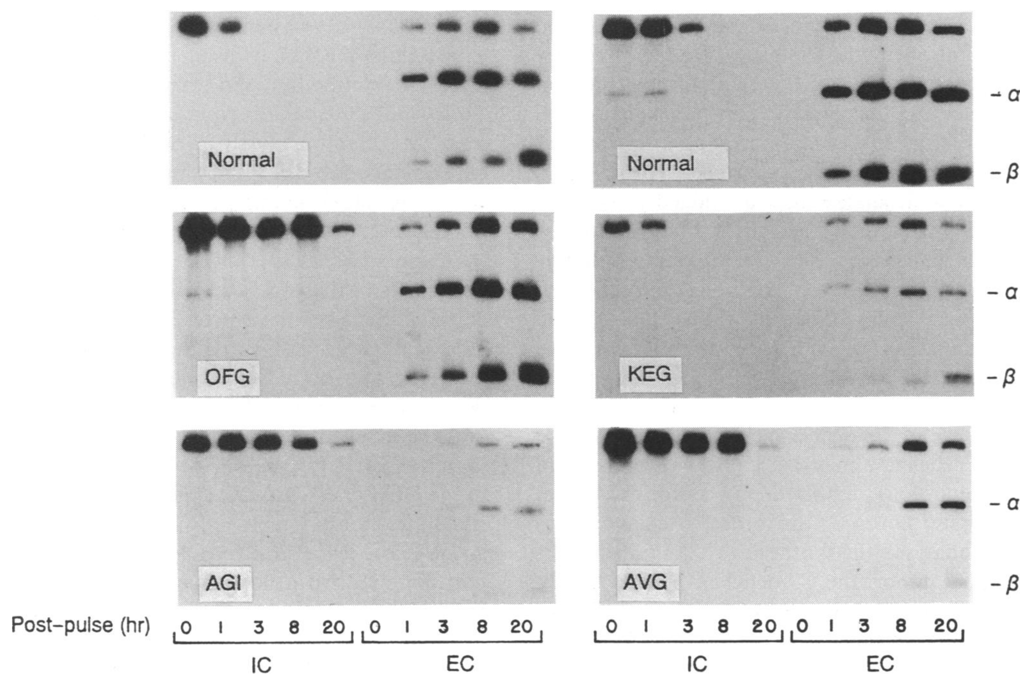

Figure 1. Rate of C3 secretion from fibroblasts of C3-deficient patients. Fibroblasts of normal individuals, father (KEG), mother (OFG), and C3-deficient patients (AGI and AVG) were metabolically labelled for 2 hr and further incubated at 37° for the times indicated. C3 was immunoprecipitated from intracellular lysates (IC) and extracellular medium (EC). Autoradiographs of the metabolically labelled and immunoprecipitated C3 analysed by SDS–PAGE are shown. The locations of the C3 α and β chains are indicated.

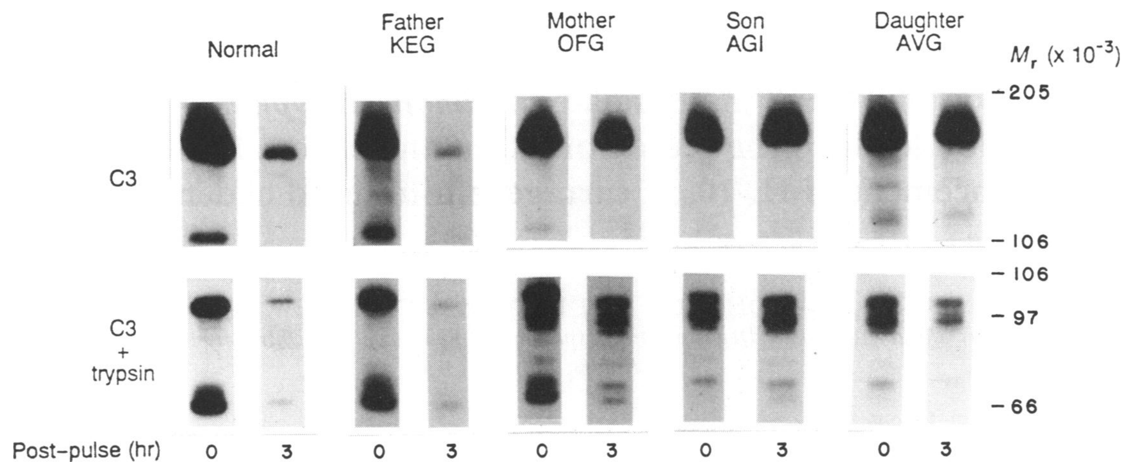

Figure 2. Trypsin digestion pattern of intracellular C3. Fibroblasts (same as in Fig. 1) were metabolically labelled for 2 hr and further incubated or not for 3 hr in medium at 37°. Cell lysates were prepared and untreated or treated with trypsin, and the digested intracellular C3 immunoprecipitated and analysed by SDS–PAGE. Autoradiographs are shown.

parents were heterozygotes and carried the same defect.[2,3] Another complement protein deficiency showing heterogeneity is C2 deficiency. Recently a C2-deficient subject in which C2 was synthesized by the cells but not secreted was described.[11] It is possible that in this kindred the C2 deficiency is also a result of a compound heterozygosity as the parents had different major histocompatibility complex haplotypes[11] but the exact molecular basis of the abnormality in the patient and the parents has not been reported.

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