THE FIFTH COMPONENT OF COMPLEMENT (C5) IN THE MOUSE

Analysis of the Molecular Basis for Deficiency

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Genetic deficiency of the fifth component of complement (C5) (1), a serum glycoprotein of molecular weight 180,000 (2), is present in 39% of inbred strains of mice (3). Sera of deficient mice lack detectable C5 hemolytic activity and protein (3). In addition, deficient mice produce antibody to mouse C5 when injected with sera from C5 sufficient (normal) strains. Ooi and Colten (4) reported that normal mouse peritoneal cells produced hemolytically active C5 in culture and also synthesized and secreted a C5 molecule. Peritoneal macrophages from mice deficient in C5 also synthesized, but did not secrete, a C5 molecule (5). The isolation and characterization of a cDNA for mouse C5 and improved techniques for the analysis of protein synthesis by cells in culture have allowed a reexamination of the molecular basis of C5 deficiency in the mouse. We report that C5-deficient cells contain C5 protein and C5 mRNA that differ both quantitatively and qualitatively from the protein and mRNA in C5-sufficient cells.

Materials and Methods

Materials. Restriction enzymes were purchased from New England Biolabs (Beverly, MA) and Bethesda Research Laboratories (Gaithersburg, MD), α-32P-dCTP (sp act 10 μCi/16 pmol) from New England Nuclear (Boston, MA), vanadyl ribonucleoside complexes (VRC) from Bethesda Research Laboratories, and guanidinium thiocyanate from Fluka A.G. (Buchs, Switzerland). Male mice of four different strains, B10.D2/nSn (normal or C5-sufficient), B10.D2/oSn, DBA/2, and AKR (all C5 deficient), were purchased from The Jackson Laboratory (Bar Harbor, ME). Oligo(dT) cellulose (type 7) was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Mouse anti-mouse C5 was prepared as described by Cimader et al. (3); the IgG fraction of goat antiserum to human C3, which crossreacted with mouse C3, was obtained from Atlantic Antibody, Scarborough, ME.

Cell Cultures, Biosynthetic Labeling, and Immunoprecipitation. 100-mg portions of

This work was supported by grants from the National Institutes of Health (AM-26609, AI-19222 and AI-22214), and by an Allergic Diseases Academic Award (K07-AI-000545) to R. C. Strunk, and by an NIH Training Grant (HL-07195) to R. Wetzel. Address correspondence to R. C. Strunk, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206. R. Wetzel's present address is Department of Pediatrics, Washington University School of Medicine, St. Louis, MO 63178. A. Falus' present address is National Institute of Rheumatology, Orszagos Reuma-es Fisioterapias, Intezet/ORFI, 1525 Budapest, 144, Hungary.

1442 J. EXP. MED. © The Rockefeller University Press 0022-1007/87/05/1442/06 $1.00 Volume 165 May 1987 1442-1447
minced lung tissue were metabolically labeled with \(^{35}\text{S}\)methionine in methionine-free medium (6, 7). Total protein synthesis was estimated by TCA precipitation of aliquots of lysates and extracellular media (6). Specific C3 and C5 proteins were quantitated by immunoprecipitation, SDS-PAGE, fluorography, and digestion of specific gel slices, as previously described (7).

**Plasmids and Genomic DNA Preparation.** Plasmid pC5Mo has been isolated from a B10.WR mouse (C5 sufficient with DNA library and characterized by Wetsel et al. (8). A 4.2-kb insert corresponding to the murine C5 cDNA was obtained by digestion of pMoC5 with Xho I, followed by isolation on low-melting-temperature agarose. Preparation of murine liver DNA was carried out as described previously (9).

**RNA Preparation.** Total cellular RNA was prepared from frozen organ powder by lysis in guanidinium thiocyanate and cesium chloride gradient ultracentrifugation (9). Cytoplasmic RNA was extracted from dounce homogenized livers by phenol/chloroform/isooamyl alcohol extraction and ethanol precipitation (9). Nuclear RNA was prepared from livers as described by Maniatis et al. (9), except that the homogenized tissue was lysed in the presence of 32 mM sucrose in 1 mM KCl, 1 mM MgCl\(_2\), 100 mM NaOAc, pH 6.0, 0.25% Triton X-100 and 10 mM VRC; the resulting nuclear pellet was washed twice in the same solution without Triton X-100. Poly(A)\(^+\) RNA was purified from equal amounts of RNA (usually 100 \(\mu\)g) using oligo(dT)-cellulose (8) and was subjected to Northern analysis using mouse C5 cDNA, mouse C3 cDNA (the generous gift of Dr. Morinobu Takahashi, Kanazawa University, Kanazawa, Japan), and mouse factor B cDNA (the generous gift of Dr. Harvey R. Colten, Washington University, St. Louis, MO) radiolabeled with \(^{32}\text{P}\) by nick translation.

**Results**

**Tissue Localization of C5 mRNA.** Total cellular RNA was obtained from the liver, lung, heart, spleen, intestine, and kidney of a B10.D2/nSn (C5-sufficient mouse). Poly(A)\(^+\) mRNA was extracted from 100 \(\mu\)g total cellular RNA with oligo(dT) to increase the sensitivity of detection of specific mRNA signals. Northern analysis indicated that the liver and lung were the only organs that contained C5 mRNA (Fig. 1). In contrast, all six organs contained C3 mRNA and all organs except the heart contained factor B mRNA. A doublet of the factor B mRNA was observed in the intestine and kidney.

**Synthesis of C5 Protein in Lung Tissue from C5-sufficient and C5-deficient Mice.** For the C5-sufficient lung tissue, a single-chain C5 molecule (~177 kD) (pro-C5) was present in the intracellular lysate within as early as 15 min of a continuous pulse with \(^{35}\text{S}\)methionine (data not shown). By 30 min, the amount of the pro-C5 had increased and \(\alpha\) (~120 kD) and \(\beta\) (~78 kD) chains were also present in the cell lysate. After 24 h the \(\alpha\) and \(\beta\) chains were prominent in the extracellular medium; there was also a small amount of pro-C5 in the medium. The C5-deficient tissue had a protein (~187 kD) in the cell lysate at 60 min, but no protein was visible in the extracellular medium, even at 24 h. The amount of pro-C5 protein in the C5-deficient tissue after the 60-min pulse was only 5% of the amount in the C5-sufficient tissue, as determined by soft-laser densitometry of autoradiographs. In contrast, the amounts of C3 protein synthesized and secreted by the C5-sufficient and C5-deficient lung tissue were similar (data not shown).

**Characterization of C5 mRNA from C5-sufficient and C5-deficient Mice.** C5-sufficient cells contained a single species of C5 mRNA, with a size of ~6.0 kb; this result was observed for both total cellular RNA (data not shown) and cytoplasmic RNA (Fig. 2). C5-deficient cells contained this 6.0-kb species, but
also contained a second and higher molecular mass form of C5 mRNA (~6.5 kb). This result was observed for both total cellular and cytoplasmic RNA in all three deficient strains; cytoplasmic RNA from one deficient strain, B10.D2/oSn, is shown in Fig. 2. In addition to the qualitative differences, between the normal and deficient RNAs, the C5 mRNA from the deficient mice was decreased in quantity when compared with the amounts in the normal mice. In five separate experiments, amounts of C5 mRNA in the deficient RNA were only 5% of the amounts in the normal RNA, as determined by soft-laser densitometry of the radioautographs. Factor B mRNA content of the same blots was similar (data not shown), indicating that the reduction of C5 mRNA content in the C5 deficient strains was not due to variations in amounts of total poly(A)^+ RNA analyzed per lane. The nuclear fractions from both normal and deficient animals contained only a single species of RNA that was the same size as the larger molecular mass form present in the cytoplasm of the deficient animal (Fig. 2). The amount of poly(A)^+ C5 RNA in the nucleus of the C5-deficient mouse was reduced in quantity compared with that in normal mice.

Southern Analysis of Normal and C5-deficient DNA. For digestion with Hind III and Pvu II, the patterns for the C5 DNA from the three C5-deficient strains were identical and distinct from the C5-sufficient pattern (Fig. 3). For Hind III, the deficient DNA contained an additional band with a size of ~6.0 kb and
FIGURE 2. Analysis of C5 mRNA in cytoplasmic and nuclear compartments in C5-sufficient and C5-deficient mice. Poly(A)* RNA was extracted using oligo(dT)-cellulose from equal amounts of cytoplasmic and nuclear RNA (100 μg) from C5-sufficient (N) and C5-deficient (O) mice and subjected to Northern analysis using a 32P nick-translated mouse C5 cDNA. The band labeled HMW is the 6.5-kb mRNA species found in the nucleus of both animals yet found also in the cytoplasm of the C5-deficient mouse. The lower band, labeled LMW, is the mRNA species found alone in the cytoplasm of the C5-sufficient mouse and present with the HMW in the cytoplasm of the C5-deficient mouse.

FIGURE 3. Southern analysis of C5-sufficient and C5-deficient DNA. Liver DNA from the C5-sufficient and three C5-deficient strains were digested using restriction endonucleases Hind III and Pvu II and subjected to Southern analysis using a 32P nick-translated mouse C5 cDNA. Genomic DNA was isolated from a C5-sufficient (B10.D2/nSn) mouse (A) and three C5-deficient mice: B10.D2/nSn (B), AKR/J (C), and DBA/2 (D). For both Pvu II and Hind III, all three C5-deficient mice demonstrated the same restriction pattern that was different when compared with the C5-sufficient mouse.

lacked a band of ~3.0 kb. For Pvu II, the deficient DNA contained an additional band with a size of ~4.3 kb. For digestions with Pst I and Msp I, the patterns for DNA from the the C5-deficient strains were not different from the patterns for the C5-sufficient DNA (data not shown). When the same Southern blots were
probed with \( ^{32}P \)-factor B cDNA, no differences were observed between the C5-deficient and C5-sufficient DNA (data not shown).

**Discussion**

We report that mice deficient in serum C5 (C5-deficient or C5D) differ from normal mice (C5-sufficient or C5S) both quantitatively and qualitatively in the C5 protein synthesized within the cell, the C5 mRNA present in the cytoplasm and nucleus, and the structure of the C5 gene. The presence in the C5-deficient cells of a single-chain C5 precursor that is not processed further is consistent with the previous findings of Ooi and Colten (5). The comparable decreases in C5 protein and C5 mRNA in the C5-deficient cells compared with C5-sufficient cells suggest that C5-deficient mRNA is translated with normal efficiency, but that it codes for an abnormal protein.

In addition to not being processed into a two-chain molecule, the protein synthesized by the C5-deficient cells also had a larger molecular mass than the precursor protein in the normal cells. Further studies will be required to determine if the increased mass of the deficient molecule is due to increased numbers of amino acids or an increase in the degree of glycosylation of the molecule or both. The defect(s) in the C5D pro-C5 molecule that prevent(s) its processing and eventual secretion and the relationship of the defect(s) to the abnormalities in RNA and DNA have not been addressed in this study. Possible abnormalities include inappropriate levels of glycosylation (10) or an abnormality in the \( \text{NH}_2 \)-terminal sequence of the protein necessary for protein translocation across and integration into the endoplasmic reticulum membrane (11).

One of the most striking findings in the C5-deficient cells is the presence of the two forms of cytoplasmic C5 mRNA. The larger form of mRNA present in the cytoplasm could be explained by an abnormality(ies) in the primary transcript that retards the processing of the C5 RNA (e.g., due to inefficient splicing). The decreased C5 mRNA content in the C5-deficient nuclear and cytoplasmic RNA would be the result of turnover of this defective and inefficiently processed C5 RNA in the nucleus. The concept of unspliced RNA being transported to the cytoplasm is consistent with the findings of Green et al. (12). The decreased levels of nuclear and cytoplasmic mRNA in the C5-deficient cells also could be due to a defect in either polyadenylation or capping of the C5 RNA precursor and subsequent instability in the transcript. However these seem unlikely as primary explanations because the nuclear form of the C5 mRNA in the C5-deficient cells is polyadenylated and the C5-deficient mRNA has approximately the expected translation efficiency. The C5 deficiency provides another model with which to study the relationship between RNA structure and splicing efficiency and accuracy.

**Summary**

C5-deficient mice differed from C5-sufficient mice both quantitatively and qualitatively in C5 protein, C5 mRNA, and the C5 gene. C5-deficient protein was present as decreased amounts of an unprocessed, single-chain precursor. C5-deficient mRNA was decreased in amount and present in two forms, the smaller
of which was the same as the single form in normal cells. Nuclei from both normal and deficient cells contained the larger form of C5 mRNA, and C5-deficient DNA demonstrated differences from the normal pattern on Southern analysis for two restriction enzymes. These data suggest that the primary transcript of the C5-deficient gene is abnormal, retarding the processing of the C5 mRNA, and that the C5-deficient mRNA codes for an abnormal protein.

We thank Denise Eidlen for excellent technical assistance and Jane Watkins for preparation of the manuscript.

Received for publication 13 November 1986 and in revised form 26 February 1987.

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