Vol. 53, 1989

NEPHRON

Founded 1964 by G. Richet and G.E. Schreiner

KARGER

S. Karger · Medical and Scientific Publishers
Basel · München · Paris · London · New York · New Delhi · Bangkok · Singapore · Tokyo · Sydney

Drug Dosage

The authors and the publisher have exerted every effort to ensure that drug selection and dosage set forth in this text are in accord with current recommendations and practice at the time of publication. However, in view of ongoing research, changes in government regulations, and the constant flow of information relating to drug therapy and drug reactions, the reader is urged to check the package insert for each drug for any change in indications and dosage and for added warnings and precautions. This is particularly important when the recommended agent is a new and/or infrequently employed drug.

Printed in Switzerland by Graphische Betriebe Coop Schweiz, Basel

Contents Vol. 53, 1989

No. 1

No. 2

Original Papers

Case Report

Letters to the Editor

No. 3

Original Papers

Case Report

Letters to the Editor

No. 4

Distinguished Scientists Lecture Series

Original Papers

Case Report

Letters to the Editor

Nephron 1989;53:338–342

0028–2766/89/0534–0338$2.75/0

Biosynthesis of Complement C4 Messenger RNA in Normal Human Kidney

H.E. Feucht[a,b], *J. Zwirner*[b], *D. Bevec*[b], *Margot Lang*[b], *E. Felber*[b], *G. Riethmüller*[b], *Elisabeth H. Weiss*[b]

[a] Medizinische Klinik Innenstadt und [b] Institut für Immunologie, Universität München, BRD

Key Words. Complement C4 · Messenger RNA

Abstract. Complementary DNA (cDNA) probes were used to investigate the extrahepatic production of the major histocompatibility complex (MHC)-linked complement components C4, factor B and C2 in various normal human tissues. The presence of the corresponding messenger RNA (mRNA) was tested by Northern blot analysis. Complement C4 mRNA was found in liver, and with high intensity also in normal kidneys. In contrast, no C2 mRNA and only very low amounts of factor B mRNA could be detected in the kidney. Slot blot hybridization was performed to quantitate the amount of C4 mRNA, and the intensity of C4 mRNA hybridization in the kidney samples was about 25% compared with liver RNA. C4-specific transcripts were not present in isolated glomeruli but in the renal interstitium. Other human tissues, such as tonsil, spleen, thymus, brain, lung and peripheral mononuclear cells, contained no C4 mRNA. Low amounts of C4 mRNA were found in colon, thyroid gland, lymph node and breast carcinoma. The results obtained with lung, where C2 mRNA was found but no C4 mRNA, further indicate an independent, tissue-specific regulation of the class III gene expression. The results, showing that the complement C4 genes are transcribed very efficiently in normal human kidney, suggest a direct role of complement C4 in renal pathogenesis.

Introduction

The complement components C4, factor B and C2 are encoded on chromosome 6 between the class I and class II genes of the major histocompatibility complex (MHC) [1]. Recent studies have established the associations between alleles of the MHC-linked complement genes and various autoimmune diseases [2]. The fourth component of human complement is controlled by two highly polymorphic genes, C4A and C4B [3], and several forms of primary glomerulonephritis were found in association with certain alleles of C4 or with partial deficiency of C4 [4, 5]. This peculiar disease association raised the question, of whether the structural variants of C4 might be directly involved in the pathogenesis of renal autoimmune diseases. Using an immunoperoxidase staining technique and monoclonal antibodies against C4, we have recently demonstrated the presence of both isotypic components C4A and C4B in glomeruli of normal human kidney [6]. The question however remained of whether complement C4 is passively adsorbed, or locally produced in human kidney.

Complement components in the serum are primarily produced in the liver [7]. However, extrahepatic production has also been noted. Already in 1965, using radiolabeled amino acids and immune electrophoresis of tissue culture supernatants, Thorbecke et al. [8] have provided evidence that several normal tissues produce complement C3 and to a less extent complement C4. In rhesus monkeys e.g. C4 production was also found in normal lung, mammary gland, thyroid gland and kidney. Investigating adult human tissues, thyroidea, breast and lung were found to produce complement C4 in addition to liver [9, 10]. In these studies mononuclear phagocytes were identified as complement source, thus explaining also the local synthesis of complement components in inflamed tissues such as rheumatoid synovium [11]. The available complementary DNA (cDNA) probes specific for the MHC class III proteins C4, factor B and C2 [12–14] enabled us to readdress the question of biosynthesis of these components by Northern blot hybridization. Here we report that normal human kidney contains C4 messenger RNA (mRNA) in comparable amounts to human liver.

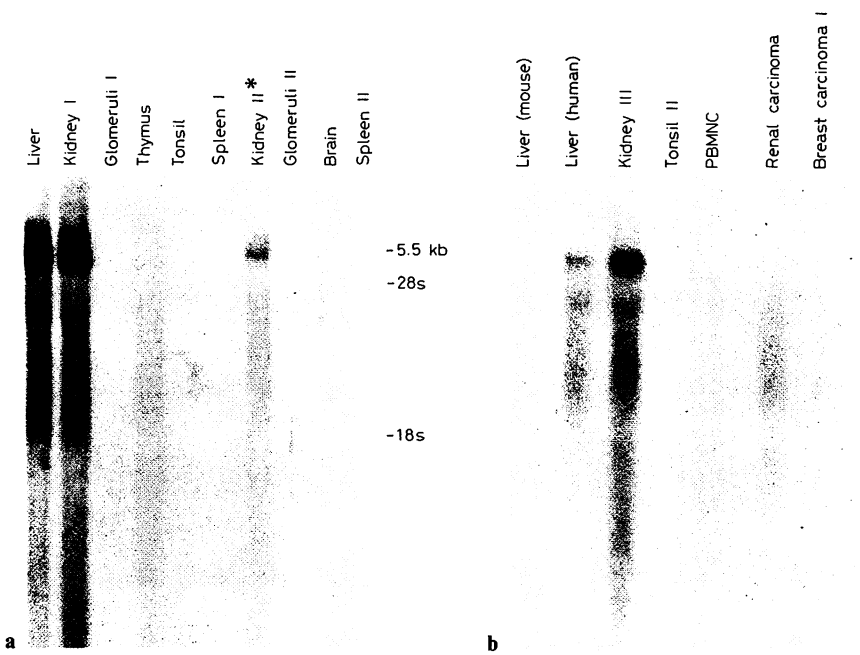

Fig. 1. Presence of C4 mRNA in human kidney and liver. Northern blot analysis of 10 μg of total RNA prepared from various human organs (except kidney II*, where 7 μg were applied) and hybridization with C4d-specific cDNA probe Alu-7 (**a**). **b** Total RNA from mouse liver is included. PBMNC = Peripheral blood mononuclear cells. 28s and 18s represent the large and small subunit of ribosomal RNA. Different numbers refer to different patients.

Methods

Tissue Samples

Human tissue specimens obtained during surgery were immediately snap frozen in liquid nitrogen and transferred to the laboratory for further processing.

Kidney tissue was obtained from 5 patients (ranging in age from 44 to 71 years) undergoing nephrectomy due to renal carcinoma. Only histologically normal, tumor-free sections, as judged by light microscopy were used for preparation of total RNA. One sample was also obtained from renal carcinoma. Whole glomeruli were isolated from renal cortex cut into small pieces. Tubular and fibrous material was removed by subsequent sieving through nylon meshes of different pore size (500 and 250 μm, respectively). Glomeruli were retained on and rinsed from a mesh with 125 μm opening size. Ice-cold media were used throughout. Tonsils were obtained from children with adenoid hyperplasia, thymus was obtained during open heart surgery. Thyroid gland was removed either totally because of carcinoma or partially due to toxic adenoma. Spleens were taken from patients after trauma. Histologically normal colon was taken from colectomies due to carcinoma. During removal of carcinoma, normal tissue specimens were obtained from lung, liver, lymph nodes, and also two samples from breast carcinoma. Peripheral blood mononuclear cells were isolated by density gradient centrifugation. Brain was obtained at autopsy.

Preparation of Total RNA and Hybridization with cDNA Probes

Total RNA was prepared from each tissue sample by standard procedures [15]. Ten micrograms of total RNA were separated on a formamide agarose gel and transferred to nylon membranes. cDNA probes were labeled with ^{32}P according to the method of Feinberg and Vogelstein [16]. The Northern blots were hybridized overnight at 65 °C with labeled cDNA probes at an activity of $2-5 \times 10^6$ cpm/ml in 3 × saline sodium citrate buffer (20 × SSC = 3 *M* NaCl, 0.3 *M* Na-citrate), 10 × Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), 0.1% sodium pyrophosphate, 10% dextran sulfate, 50 g/ml denatured salmon sperm DNA. Washing was performed stepwise for 30 min at 65 °C in buffers with decreasing SSC concentration, reaching 0.1% SSC, 0.1% SDS during the last step.

C4, Factor B- and C2-Specific cDNA Probes

Initially, Alu-7, a cDNA probe of 300 base pairs (BP) which is specific for the amino acid sequence of C4d of both C4 isotypes [12] was employed and later pAT-A, which contains the full-length 5.5-kb C4A transcript [17]. The 515 BP cDNA probe FB1 is specific for factor B [13] and the 900 BP cDNA probe pC2-6 is specific for C2 [14].

Bacteria containing the relevant class III cDNA clones were kindly provided by Dr. M.C. Carroll (Oxford) and Dr. P. Schneider (Mainz). The plasmids were isolated from 11 overnight cultures by the alkaline lysis method [18] and purified on a cesium-chloride gradient. Fifty micrograms of the plasmids were digested with the appropriate restriction enzymes to cut out the cDNA insert, and separated on a preparative 0.7% agarose gel. The cDNA fragments were isolated from the gel by electroelution.

Slot blot hybridization, using decreasing amounts of total RNA, was done according to the method of Kafatos et al. [19]. Control hybridization was performed with a human actin-specific cDNA probe [20].

Results

Detection of C4 mRNA in Human Kidney

Total RNA was prepared from five different kidney samples and was analyzed for the presence of C4 mRNA by Northern and slot blot hybridization with two C4 cDNA probes. Distinct hybridization with the C4d-spe-

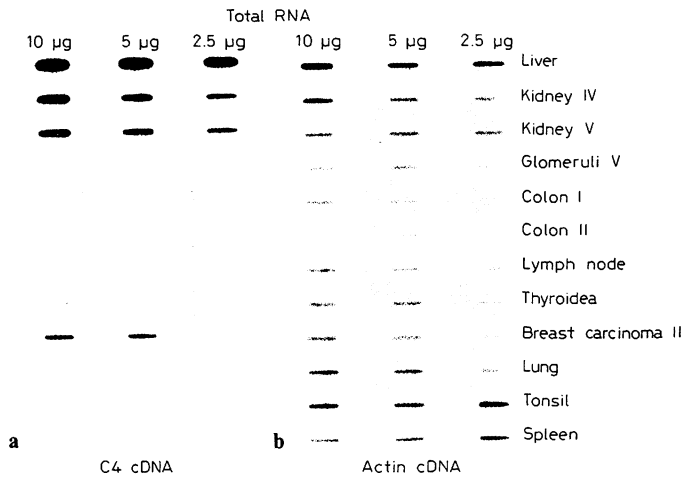

Fig. 2. Intensity of C4 mRNA biosynthesis in various human organs. Slot blot analysis of total RNA, hybridized with full length C4 cDNA probe pAT-A. Kidney IV and V and colon I and II refer to different patients. Exposure was 24 h. (**a**) Control hybridization with actin-specific cDNA probe (**b**) is shown.

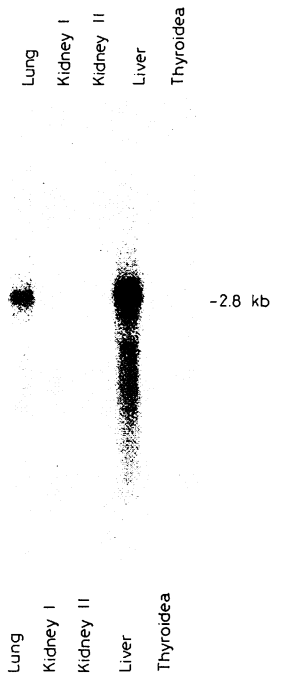

Fig. 3. Presence of C2 mRNA in human liver and lung. Northern blot analysis of total RNA and hybridization with C2-specific cDNA probe pC2-6. Different numbers refer to different patients.

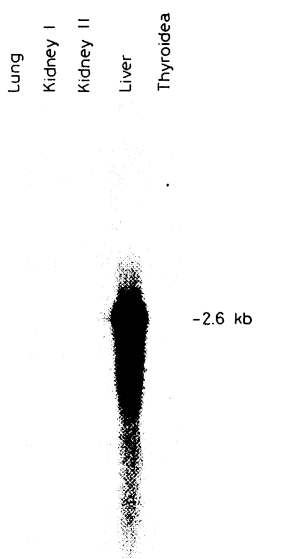

Fig. 4. Presence of factor B mRNA. Appearance of weak hybridization signals with cDNA probe FBI after prolonged exposure (48 h) in RNA preparations from lung, thyroid gland and two kidney samples. Different numbers refer to different patients.

cific cDNA probe Alu-7 and the full-length C4 cDNA probe pAT-A was obtained with each kidney sample. Figure 1 shows the comparative hybridization to RNA from various human organs, including liver, kidney, isolated glomeruli, thymus, tonsil, spleen, brain (fig. 1a), peripheral blood mononuclear cells, renal carcinoma, breast carcinoma and mouse liver (fig. 1b). The C4 cDNA probe hybridizes to RNA of approximately 5.5 kb length in the liver sample and also to kidney RNA, thus demonstrating the presence of C4-specific transcripts in the different kidneys analyzed. Interestingly, only whole renal cortex contained C4 mRNA, whereas isolated glomeruli were consistently negative. No C4 transcripts were detected with RNA preparations from thymus, tonsil, spleen, brain, peripheral blood mononuclear cells, renal carcinoma, breast carcinoma and mouse liver.

In order to quantitate the amount of C4 mRNA, slot blot hybridization was performed, using decreasing amounts of total RNA (10, 5 and 2.5 µg, respectively) per slot and the full length cDNA pAT-A as probe. A strong hybridization signal is displayed by liver RNA, whereas the intensity of C4 mRNA hybridization in two kidney samples is approximately 25% of the liver signal (fig. 2a). Again, no C4 mRNA is detected in isolated glomeruli. Very low amounts of C4 mRNA are found in RNA preparations from two colon samples, from lymph node and from thyroid gland. Weak hybridization is seen in the RNA isolated from breast carcinoma. Lung, tonsil and spleen are negative. In order to show that all RNA preparations used in this study were of equal quality and that roughly equal amounts of total RNA were tested, a control hybridization was performed with a human actin-specific probe. In all samples actin mRNA of 1.8 kb length is present and comparable signals are obtained in slot blot hybridization (fig. 2b). This result demonstrates that the failure to detect complement mRNA in the samples (e.g. in isolated glomeruli) is not due to degraded or too little RNA applied.

Detection of C2 mRNA and Factor B mRNA by Northern Blot Analysis

It was then of interest to investigate whether normal human kidney would also transcribe the class III genes coding for complement components C2 and factor B. For

this purpose, the Northern blots were hybridized with the C2-specific cDNA probe pC2-6 and the factor B-specific probe FB1, respectively. As shown in figure 3, C2 transcripts of 2.8 kb length are present in liver and in lung, whereas two kidney samples and thyroid gland are negative. In contrast, only liver RNA hybridizes strongly with the factor B-specific cDNA probe, yielding a band at 2.6 kb. After prolonged exposure, a weak signal is also observed in the lung and kidney RNA preparations (fig. 4).

Discussion

Hybridization techniques using cDNA probes have already allowed to demonstrate the renal production of proteins such as factor VIII, Tamm-Horsfall glycoprotein, α-fetoprotein and albumin [21–23]. In this study we were interested in the extrahepatic production of the MHC-linked complement components. For this purpose, we investigated various human organs for the presence of C4, factor B and C2 mRNA by Northern blot and slot blot analyses. A high amount of C4 mRNA was found in liver, and surprisingly a signal of remarkable intensity appeared also in normal human kidney. Renal carcinoma was devoid of C4 mRNA. No C2 mRNA and only very low amounts of factor B mRNA could be detected in human kidney. The presence of C4 mRNA strongly suggests that the C4 protein is locally synthesized in the kidney. These results confirm the early observations by Thorbecke et al. [8] in rhesus monkeys. Biosynthesis of C4 mRNA does, however, not take place in glomeruli but in the interstitium, as no C4-specific transcripts were present in RNA extracted from isolated glomeruli.

Within the interstitium, the tubular system is the most likely source of complement production. Macrophages, as another possible source, can be excluded, since we (own observation) and others [24] could not identify interstitial macrophages in normal human kidney with immunohistological techniques and no C4 mRNA could be detected in organs known to contain numerous tissue macrophages such as tonsil, spleen and lung. When, however, murine lupus was investigated, the increased local production of complement proteins C3, factor B, C2 and C4 was caused by macrophages infiltrating the diseased organs, primarily the kidneys [25]. Using an immunoperoxidase staining technique and several monoclonal antibodies against complement C4, we recently demonstrated the presence of C4 in all normal human glomeruli [6]. The question then arose of whether the glomeruli themselves would produce the detected C4. The present study shows that the glomeruli do not transcribe the C4 gene; the glomerular C4 protein is therefore a deposit which is passively absorbed. It should also be noted that although the renal interstitium is the obvious site of C4 production, immunoperoxidase staining with monoclonal anti-C4 reagents was consistently negative [6]. This finding is in agreement with the results obtained with sections from human liver. Despite the C4 production by hepatocytes, as confirmed by the presence of C4 mRNA, we could not detect C4 protein in liver tissue by immunoperoxidase staining. And to our knowledge the immunohistological presence of C4 in human liver has not been reported so far.

When additional normal human tissues were investigated for the presence of C4 mRNA, brain and thymus were completely negative, whereas lymph node, colon and thyroid gland contained a low amount of C4 mRNA. The results obtained with lung, where C2 mRNA was found but no C4 mRNA, indicate an independent, tissue-specific regulation of the class III gene expression.

Similar to human liver, where a genetically determined low production of C4 is predisposing to the development of autoimmune chronic active hepatitis [26], the local presence and autochthonous production of C4 might be of relevance also for the pathogenesis of renal diseases. The classical pathway of complement activation, involving the components C1, C4 and C2, resulting in the formation of the C3-convertase, is a powerful mechanism for the elimination of immune complexes [27]. It is therefore important to determine, whether the 'renal complement' enters the circulation and whether it is protective against the accumulation and deposition of immune complexes in the kidney.

With respect to the putative tubular production of C4, there is also the possibility of excretion into the tubular lumen and finally into urine. In vitro experiments have already shown production of C1 components by epithelial cells from the urogenital tract including renal pelvis [28]. We have therefore examined normal urine specimens for the presence of C4 by protein precipitation followed by immunoblotting and C4 protein could be clearly detected [data not shown]. With this approach, it is impossible, however, to determine, whether the C4 protein, although having a molecular weight of 200,000 daltons is derived from glomerular filtration, from actual tubular secretion or from both. With regard to the antibacterial and antiviral action of complement [29], another important task could therefore be the participation in the local protection against interstitial or ascending renal infection.

In conclusion, the results showing a very efficient transcription of the complement C4 genes in normal human kidney suggest a direct role of complement C4 in renal pathogenesis.

Acknowledgments

We thank Dr. G.J. O'Neill (Miami, Fla.) for continuous help and advice. Intraoperative kidney specimens were provided by Dr. M. Prosinger and Dr. R. Tauber (München). This work was supported by grants from the Deutsche Forschungsgemeinschaft, SFB 217: C8 (H.F.) and from the Genzentrum München (E.W.).

References

1 Carroll MC, Campbell RD, Bentley DR, et al: A molecular map of the human major histocompatibility complex class III region linking complement genes C4, C2 and factor B. Nature 1984; 307:237–241.
2 Rittner C, Bertrams J: The significance of C2, C4 and factor B polymorphism in disease. Hum Genet 1982;56:235–247.
3 O'Neill GJ, Yang SJ, Dupont B: Two HLA-linked loci controlling the fourth component of human complement. Proc Natl Acad Sci USA 1978;75:5154–5169.
4 Wank R, Schendel DJ, O'Neill GJ, et al: Rare variant of complement C4 is seen in high frequency in patients with primary glomerulonephritis. Lancet 1984;i:872–874.
5 Welch TR, Beischel L, Balakrishnan K, et al: Major-histocompatibility-complex extended haplotypes in membranoproliferative glomerulonephritis. N Engl J Med 1986;314:1476–1481.
6 Feucht HE, Jung C-M, Gokel MJ, et al: Detection of both isotypes of complement C4, C4A and C4B, in normal human glomeruli. Kidney Int 1986;30:932–936.
7 Fey G, Colten HR: Biosynthesis of complement components. Fed Proc 1981;40:2099–2104.
8 Thorbecke GJ, Hochwald GM, van Furth R, et al: Problems in determining the sites of synthesis of complement components; in Wolstenholme GEW, Knight J (eds): Ciba Symposium 'Complement' London, Churchill, 1965, pp 99–119.
9 Cole FS, Schneeberger EE, Lichtenberg NA, et al: Complement biosynthesis in human breast milk macrophages and blood monocytes. Immunology 1982;46:429–441.
10 Cole FS, Matthews WJ, Rossing TH, et al: Complement biosynthesis by human bronchoalveolar macrophages. Clin Immunol Immunopathol 1983;27:153–158.
11 Ruddy S, Colten HR: Rheumatoid arthritis: Biosynthesis of complement proteins by synovial tissues. N Engl J Med 1974; 290:1284–1288.
12 Carroll MC, Porter RR: Cloning of a human complement C4 gene. Proc Natl Acad Sci USA 1983;80:264–267.
13 Campbell RD, Porter RR: Molecular cloning and characterization of the gene coding for human complement protein factor B. Proc Natl Acad Sci USA 1983;80:4464–4468.
14 Woods DE, Edge MD, Colten HR: Isolation of a complementary DNA clone for the human complement protein C2 and its use in the identification of a restriction fragment length polymorphism. J Clin Invest 1984;74:634–638.
15 Chirgwin JM, Przybyla AE, Mac Donald RJ, et al: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979;18:5294–5299.
16 Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1984;137:266–267.
17 Belt KT, Carroll MC, Porter RR: The structural basis of the multiple forms of human complement component C4. Cell 1984;36:907–914.
18 Birnboim HC: A rapid alkaline extraction method for the isolation of plasmid DNA. Methods Enzymol 1983;100:243–255.
19 Kafatos FC, Jones WC, Efstratiadis A: Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. Nucleic Acids Res 1979;7:1541–1552.
20 Weiss EH, Cheah KSE, Grosveld FG, et al: Isolation and characterization of a human collagen I (I)-like gene from a cosmid library. Nucleic Acids Res 1982;10:1981–1994.
21 Nahon J-L, Gal A, Erdos T, et al: Differential DNase I sensitivity of the albumin and α-fetoprotein genes in chromatin from rat tissue and cell lines. Proc Natl Acad Sci USA 1984;81: 5031–5035.
22 Pennica D, Kohr WJ, Kuang W-J, et al: Identification of human uromodulin as the Tamm-Horsfall urinary glycoprotein. Science 1987;236:83–88.
23 Wion KL, Kelly D, Summerfield JA, et al: Distribution of factor VIII mRNA and antigen in human liver and other tissues. Nature 1985;317:726–729.
24 Boucher A, Droz D, Adafer E, et al: Characterization of mononuclear cell subsets in renal cellular interstitial infiltrates. Kidney Int 1986;29:1043–1049.
25 Passwell J, Schreiner GF, Nonaka M, et al: Local extrahepatic expression of complement genes C3, factor B, C2 and C4 is increased in murine lupus nephritis. J Clin Invest 1988;82: 1676–1684.
26 Vergani D, Wells L, Larcher VF, et al: Genetically determined low C4: A predisposing factor to autoimmune chronic active hepatitis. Lancet 1985;ii:294–298.
27 Schifferli JA, Ng YC, Peters DK: The role of complement and its receptor in the elimination of immune complexes. N Engl J Med 1986;315:488–495.
28 Morris KM, Colten HR, Bing DH: The first component of complement. A quantitative comparison of its biosynthesis in culture by human epithelial and mesenchymal cells. J Exp Med 1978;148:1007–1019.
29 Joiner KA, Frank MM: Molecular mechanisms in the antibacterial action of complement; in Cinader B, Miller RG (eds): Progress in Immunology. Part VI. Orlando, Academic Press, 1986, pp 282–290.

Accepted: March 17, 1989

Dr. Helmut E. Feucht
Medizinische Klinik Innenstadt
Universität München
Ziemssenstrasse 1
D-8000 München 2 (FRG)