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Molecular cloning and characterization of the complementary DNA coding for the B-chain of murine Clq

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cDNA clones coding for the B-chain of murine Clq were isolated from a mouse macrophage library. The characterized clones include the total coding region plus a leader sequence. High homology was found with human Clq B-chain in the coding region (81%). Northern blot analysis of total RNA from different tissues of Balb/c mice showed one band of approximately 1.2 kb. The highest signal was found in RNA preparations of thioglycolate-activated peritoneal macrophages. The probe also hybridized with mRNA from spleen, thymus and heart. Extremely weak signals were found in liver, kidney, lung and intestine tissues.

Clq; Cloning; Nucleotide sequence; mRNA

1. INTRODUCTION

Clq is a subcomponent of the first component of complement, that initiates the classical pathway by binding to the Fc-portion of immunoglobulins or to polyanions [1]. Murine Clq has been purified and characterized from serum, plasma and ascites fluid. The biochemical, physical and biological properties show high similarities to human Clq [2–5].

The site of biosynthesis of C1 and Clq in particular has been the topic of some controversy. In early biosynthetic labelling experiments and functional tests, C1 and Clq synthesis was demonstrated in epithelial cells of the small intestine of guinea pigs [6] and intestinal tissue of the piglet [7]. Since Clq is a collagen-like molecule, the synthesis by collagen-producing cells was investigated. The synthesis of Clq and C1 by human fibroblast cell lines could be demonstrated using immunoprecipitation and hemolytic assay [8,9]. In contrast, there is no evidence to indicate C1 synthesis in a fibroblast cell line [10]. Although Clq biosynthesis was found in primary cultures of guinea pig hepatocytes [11], no production could be shown in a hepatoma cell line Hep G3 [12] or in human liver tissue [13]. Human and primate peritoneal and alveolar macrophages have been reported to synthesize the 11 S globulin identified as Clq [14]. De novo synthesis of Clq by cultured mouse, guinea pig and human macrophages has been intensively studied and antigenic identities between serum Clq and macrophage-derived Clq was shown [15,16]. Production of Clq by cultured human monocyte derived macrophages has recently been described [17].

We now present the molecular cloning and sequencing of the B-chain of murine Clq and the detection of Clq in different tissues by Northern blot analysis.

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2. MATERIALS AND METHODS

2.1. *Isolation of cDNA clones*

A mouse alveolar macrophage cDNA library established in the bacteriophage λgt11 (ML 1005, Clontech Lab., Palo Alto, CA) was screened by using a human Clq B-chain cDNA probe [18]. A *PvuII/StuI* restriction fragment [19] was prepared and radioactively labeled by nick translation [20]. Recombinant phage plaques were screened in duplicate on nitrocellulose filter replicas [23]. The filters were hybridized in hybridization solution supplemented with 10% dextran sulfate plus 2×10^5 counts per minute (cpm)/ml (spec. act. 10^8 cpm/μg DNA) nick translated probe for 16 h at 42°C. Filters were finally washed in 4× standard salt concentration (SSC; 150 mM NaCl, 15 mM sodium citrate) plus 0.1% SDS for 60 min at 65°C. Air-dried filters were autoradiographed at −70°C with intensifying screens. Positive plaques were rescreened and purified. λDNA was prepared by standard techniques [21]. Two clones designated B15 and B18 were finally subcloned into the *PvuII* site of the plasmid vector pAT 153/*PvuII*/8 [22].

2.2. *Sequence analysis*

Sequencing was done in bacteriophage M13mp8 by the dideoxy chain-termination method [23], using deoxyadenosine 5′-[α-^{35}S]thiotriphosphate (Amersham, Braunschweig, FRG) and Sequenase (US Biochemical Corp., Cleveland, OH). Sequence data analysis was carried out using the PC/GENE nucleic acid and protein sequence analysis packages (Genofit, Grand-Lancy, Switzerland).

2.3. *Northern blot analysis*

Mouse peritoneal macrophages were obtained from thioglycollate stimulated male Balb/c mice [24] and purified by adhesion onto

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tissue culture plates (petri perm, Heraeus, Hanau, FRG) at 37°C for 4 h. The mouse macrophage-like cell line PU5-1.8 was grown in RPMI-medium supplemented with glutamine (2 mM), penicillin (50 IU/ml) and streptomycin (50 μg/ml). RNA was extracted from mouse tissues and cell suspensions by the guanidine thiocyanate/caesium chloride method [25].

RNA samples (20 μg) were denatured and electrophoresed in a 1% agarose gel containing 0.66 M formaldehyde and blotted overnight onto nylon filters (Gene Screen, New England Nuclear, Dreieich, FRG) [26]. Single-stranded anti-sense cDNA probes of mouse C1q B-chain were radioactively labeled by primer extension of M13 subclones B15-501 and B18-532 [26] (see fig.1). Hybridization was performed in a buffer containing 10% dextran sulfate and 50% formamide at 42°C for 20 h. The nylon filters were washed twice with 2 × SSC plus 1% SDS for 30 min at 65°C and finally twice with 0.1 × SSC plus 1% SDS for 30 min at room temperature.

3. RESULTS AND DISCUSSION

3.1. *Isolation and nucleotide sequence of mouse C1q B-chain cDNA clones*

The *Pvu*II/*Stu*I restriction fragment of the cDNA clone coding for the human C1q B-chain was chosen because it spans almost the entire coding sequence of the native protein but excludes all non-coding and signal sequences.

Approximately 750000 recombinant phage plaques were screened and 24 positive signals were obtained. The insert size of the λgt11 clones that were subcloned into pAT 153/*Pvu*II/8 was estimated by *Eco*RI digestion and agarose gel electrophoresis. λB15 and λB18 had inserts of about 900 bp and 850 bp, respectively.

*Bam*HI and *Msp*I restriction fragments of plasmid clones pB15 and pB18 were subcloned in M13mp8. Fig.1 shows the sequence strategy and restriction map of mouse C1q B-chain. The sequencing was done in both orientations. In addition to deoxyadenosinetriphosphate, deoxyinosinetriphosphate was used in M13 subclones B15-410 and B15-501 to overcome the problems of band compression in G-C-rich regions [27].

The two recombinant λgt11 clones B15 and B18 cover the total coding region (684 bp) and a leader sequence (75 bp) containing an in-phase start codon. This leader sequence is identical to the N-terminal of secretory signal sequence computed by the PC/GENE program PSIGNAL [28]. The cDNA clones include a 5′-noncoding region of 11 bp and a 3′-noncoding region of 151 bp (see fig.2). The mouse C1q B-chain coding region was found to be highly homologous to that of human C1q (81% in the nucleotide sequence, 83% in the deduced amino acid sequence). The homologies of the mouse and human C1q B-chain

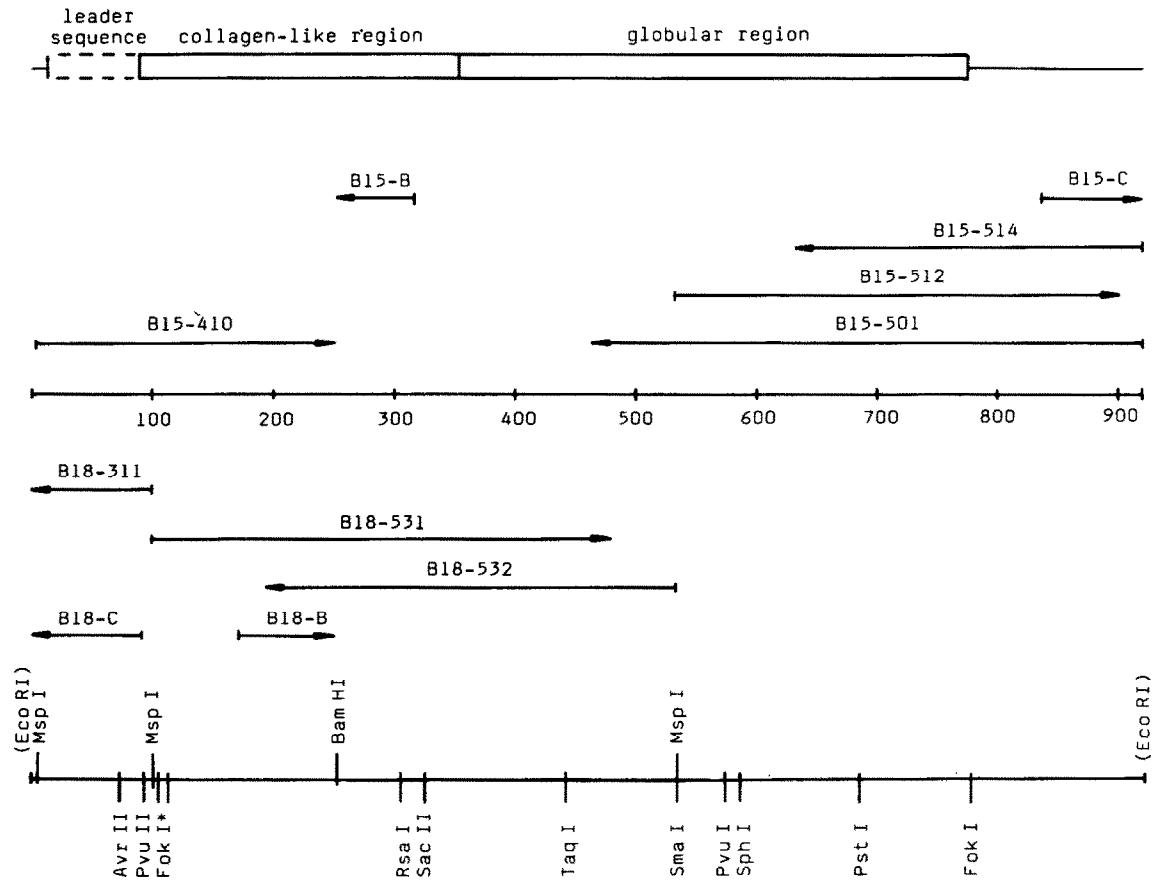

Fig.1. Cloning strategy and restriction map of murine C1q B-chain cDNA clones.

signal peptide and the 3′-untranslated region were found to be 60% and 52%, respectively.

A change in the nucleotide sequence at position 112 (C versus G) results in replacement of the Ala residue (amino acid position 9) in the human sequence with a Gly residue in the mouse. As a consequence the collagen-like region starts two Gly-Xxx-Yyy- repeats earlier in the mouse sequence. Two additional amino

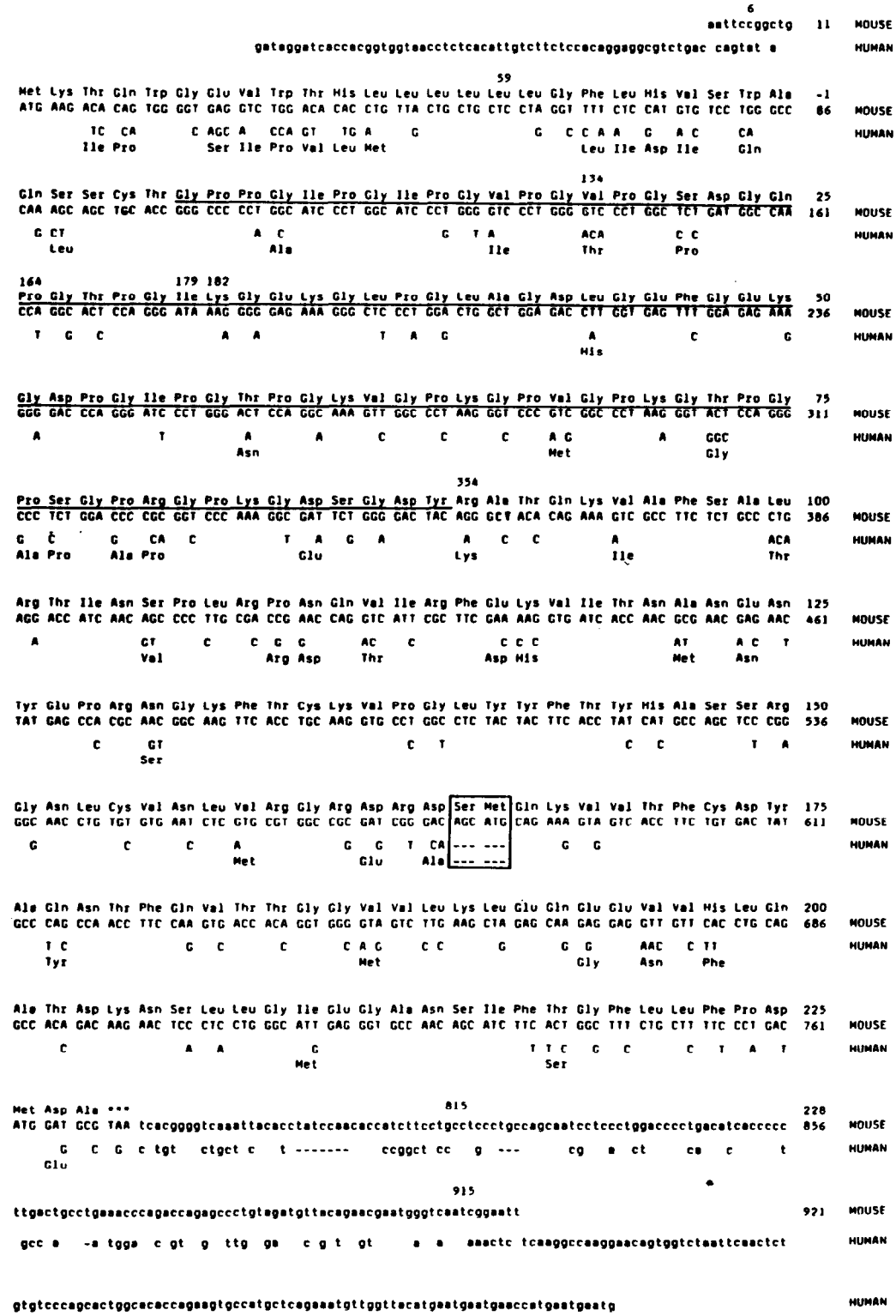

Fig.2. The cDNA sequence of the B-chain of mouse C1q. The bar represents the collagen-like region. The boxed sequence shows the positions of two additional amino acids found in the mouse sequence on comparison with the human sequence. The nucleotide and amino acid sequences of the B-chain of human C1q are given at only the position where there is a difference seen on comparison with the mouse sequences.

acids (Ser and Met at positions 165 and 166) are not present in the human sequence. The 4 Cys are completely conserved, between mouse and man, and the residues adjacent to these Cys residues are also highly conserved. While this work was in process a nucleotide sequence of the murine C1q B-chain was published [29]. The two sequences are almost identical with the following exceptions: nucleotide positions 6 and 7 in our sequence are C and G versus G and A in the sequence of Wood et al. [29]. Within the coding sequences, nucleotide positions 59, 134, 164, 179 and 182 in our sequence read C, C, A, A and G versus T, T, T, T and A in that of Wood et al. All 5 positions are last bases of a codon and do not result in a change of the deduced amino acid sequence. A discrepancy at position 354 (A versus G) leads to an Arg (AGG) residue in our sequence instead of a Gly (GGG). According to our sequence data, the C-terminus of the collagen-like region appears identical in both the mouse and human sequences.

Alignment of the mouse sequence with the human sequence shows two additional residues, Ser and Met, in the deduced amino acid sequence of the mouse at positions 165 and 166, using the genetic code matrix of the PC/GENE PALIGN-program [30]. This results in a greater homology than that produced by the insertion of Asp and Arg (positions 162 and 163) reported by Wood et al. [29].

At positions 815 and 817 we found two additional C nucleotides; as a consequence alignment of the mouse DNA sequence with the human DNA sequence results in a 52% homology instead of 47% [29]. Two nucleotides at position 915 and 916 read CG in the sequence reported here, instead of AA in that reported by Wood et al. [29].

3.2. *Biosynthesis of the B-chain of C1q by mouse tissues*

Northern blot analysis of total RNA revealed one band of approximately 1.2 kb (fig.3). This indicates that the complete mRNA of the C1q B-chain is about 0.3 kb longer than the characterized cDNA clones. The highest signal was found in RNA preparations of thioglycollate activated peritoneal macrophages. In contrast, no signal was obtained in the macrophage-like cell line PU5-1.8. The murine B-chain probes also hybridized with a 1.2 kb band of RNA from the lymphoid organs, spleen and thymus. The hybridization signal with mRNA from heart tissue is not quite understood. A pathological infiltration of macrophages into the pericard or endocard can be excluded. The signal is probably due to resident macrophage-type cells rather than cardiac muscle cells.

Extremely weak signals were found in kidney, lung, intestine, and muscle RNA. In addition, it is of interest to note that even in the liver, the major site of biosynthesis of most complement components, only minute

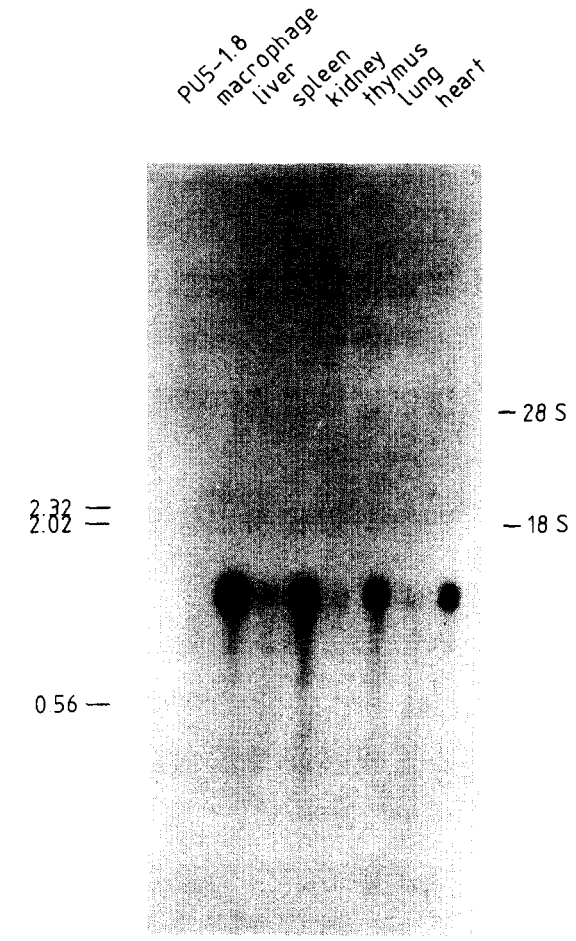

Fig.3. Northern blot analysis of total RNA from different mouse tissues. Filter hybridized with single stranded probe B15-501.

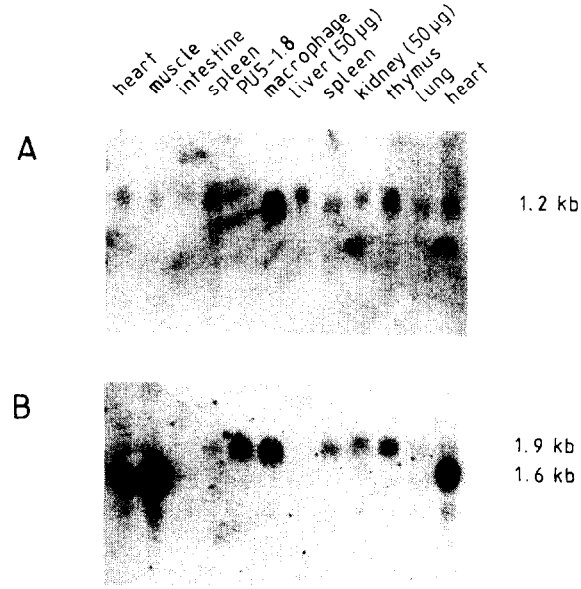

Fig.4. Northern blot analysis of total RNA from different mouse tissues. (A) Filter hybridized with single stranded probe B15-532. (B) Filter B hybridized with nick translated α-actin probe pAC 269 [31].

amounts of mRNA coding for the B-chain of C1q were detectable. These weak hybridization signals could be produced by tissue-macrophages or other macrophage-like cells, e.g. liver Kupffer cells.

The use of an actin DNA probe (fig.4A) as a control indicated that the weak signals in most of these tracks were not due to a lower loading of mRNA compared to the tracks giving a strong signal with the C1q probe. The differences in size and intensity of the signals with the α-actin probe pAC 269 [31] are due to the fact that α-actin mRNA (1.6 kb) is only found in skeletal muscle. The α-actin probe hybridized well with a mRNA from cardiac tissue of similar size. α-Actin shows 70% sequence homology with the nonmuscle β- and γ-actin sequences [31] and hybridized with a 1.9 kb mRNA.

These studies described the isolation and characterization of the cDNA coding for the B-chain of mouse C1q. Using this cDNA as a probe for the detection of the B-chain mRNA in cells and tissues, it was found that macrophages have to be considered as a major site of C1q biosynthesis.

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