Brief Definitive Report

Extrahepatic Transcription of Human C-reactive Protein

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Summary

We synthesized and cloned cDNA from human peripheral blood mononuclear cell (PBMC) transcripts that were hybrid selected by pCRP5, a liver C-reactive protein (CRP)-specific cDNA (Woo, P., J.R. Korenberg, and A.S. Whitehead. 1985. *J. Biol. Chem.* 260:13384). Three hybrid-selected cDNA clones, HScDNA1, HScDNA3, and HScDNA8, were isolated and characterized. Nucleotide sequence analysis of the 5′ end of the smaller clones, HScDNA1 and HScDNA8, demonstrated that these two PBMC clones are homologous to the 3′ and 5′ ends, respectively, of pCRP5. Our largest clone, HScDNA3, is larger than pCRP5, extending beyond both the 5′ and 3′ limits of pCRP5. Therefore, HScDNA3 was coded by human PBMC and not by the hybrid selection vehicle, pCRP5. HScDNA3 lacks the intervening sequence verifying that this clone is DNA made from a PBMC mRNA and not genomic DNA. The complete nucleotide sequence revealed that HScDNA3 is greater than 99% homologous to the CRP gene. These results demonstrate that PBMC express the CRP gene. Based on our previous report, which shows that peripheral blood cells synthesize a peptide recognized by anti-CRP (Kuta, A.E., and L.L. Baum. 1986. *J. Exp. Med.* 164:321), in conjunction with the data presented here, we conclude that human PBMC can synthesize CRP.

C-reactive protein (CRP), the major acute phase reactant in humans, is synthesized by hepatocytes and secreted into the serum (1). Our laboratory demonstrated that human PBL synthesize a peptide recognized by anti-CRP and that monocytes do not (2). The ability to eliminate NK function with anti-CRP indicates that this peptide is expressed on the surface of these cytolytic effector cells (3). Ikuta et al. (4) also demonstrated extrahepatic synthesis of a peptide recognized by anti-CRP using unstimulated human PBMC. The studies reported here were performed to determine whether the anti-CRP binding peptide synthesized by PBMC is CRP or an antigenically related peptide. CRP-related transcripts produced in PBMC were isolated and sequenced to definitively determine whether the CRP-related transcripts represent CRP, the CRP pseudogene, or another antigenically related peptide. Three PBMC hybrid-selected cDNA clones (HScDNA), HScDNA1, HScDNA3, and HScDNA8, were sequenced and determined to have identity with pCRP5. Furthermore, the complete sequence of the largest clone, HScDNA3, revealed that this clone represents a PBMC transcript specific for CRP and provides definitive proof that PBMC transcribe CRP. This finding confirms reports of extrahepatic synthesis of CRP.

Materials and Methods

Isolation of PBMC. From each of four normal healthy human individuals, 100 ml of whole blood was collected into sodium heparin, pooled, and mixed with an equal volume of Sepracell-MN (Sepratech Corp., Oklahoma City, OK). PBMC were isolated and the cell count and viability were determined by trypan blue exclusion.

Cloning of HScDNA. Acid guanidinium thiocyanate-phenol-chloroform extraction (5) was used to isolate total RNA from PBMC. The plasmid pCRP5 constructed by Dr. Alexander Whitehead (Harvard Medical School, Boston, MA) (6), was a generous gift from Dr. Harvey Colton (Washington University, St. Louis, MO). The 1.6-kb PstI insert band from pCRP5 was immobilized on nitrocellulose and used in hybrid selection as previously described (7). CRP-specific RNA was isolated from 1.7 mg of PBMC total RNA. A cDNA synthesis kit (Pharmacia Fine Chemicals, Piscataway, NJ) was used to synthesize HScDNA from the hybrid-selected PBMC RNA. The HScDNA was ligated into the EcoRI cloning site of λ ZAPII (Stratagene Cloning Systems, La Jolla, CA) and transfected into *Escherichia coli* strain XLl-Blue (Stratagene Cloning Systems).

Identification of HScDNA1, HScDNA3, and HScDNA8. An oligolabeling kit (Pharmacia Fine Chemicals) was used to radiolabel the purified 1.6-kb PstI insert band from pCRP5 with ^{32}P-dCTP. A probe with specific activity of 1.9×10^7 cpm/μg of fragment

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was obtained. Two nitrocellulose replicas were lifted from titer plates that contained 0.1, 1.0, or 10 μl of the HScDNA library. A DNA hybridization method (8) was used to screen the nitrocellulose lifts with the pCRP5 insert probe. 12 positive HScDNA clones identified from the autoradiogram of the HScDNA library screening were plaque purified, and their size was determined. The three largest clones, HScDNA1, HScDNA3, and HScDNA8, contain EcoRI inserts of 1.0, 1.6, and 0.8 kb, respectively, and were characterized further by sequence analysis.

Sequence Analysis of HScDNA1, HScDNA3, and HScDNA8. Both strands of the cDNA insert fragment from HScDNA3 were subcloned into M13mp18 and M13mp19. Nested deletion subclones of both strands were constructed using the Cyclone I Biosystem (International Biotechnologies, Inc., New Haven, CT). The 5′ limit of HScDNA8 was determined by subcloning the cDNA insert into MP13mp18 and sequencing. The 5′ limit of HScDNA1 was determined by sequencing this recombinant λ ZAPII clone directly. Single-stranded DNA templates prepared from M13 subclones or directly from the recombinant λ ZAPII clone were sequenced by a dideoxy chain termination method using the Sequenase kit (United States Biochemical Corp., Cleveland, OH). A universal M13 primer (provided in the Sequenase kit) was used in the sequencing reactions of the M13 subclones. The T3 primer (Stratagene Cloning Systems) was used in the sequencing reactions of recombinant λ ZAPII clones.

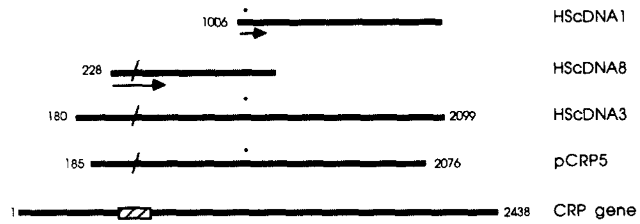

Figure 1. Alignment of HScDNA clones to pCRP5 and the CRP gene. The above map illustrates the alignment of the PBMC cDNA clones HScDNA1, HScDNA3, and HScDNA8 to the liver cDNA clone, pCRP5, and a fragment of human genomic DNA containing the functional CRP gene (6). The numbers indicate the 5′ and 3′ nucleotide limits of the cDNAs and the genomic fragment. The arrows indicate the direction and portion of HScDNA1 and HScDNA8 sequenced, whereas the entire sequence of HScDNA3 was determined. (/) The absence of the intervening sequence (▨) from the cDNA clones. (*) The presence of a single nucleotide substitution at position 1056 of the cDNA clones. The PBMC cDNA clones contain a G at this position while the liver cDNA clone contains an A.

Results and Discussion

Our earlier studies demonstrated that anti-CRP immunoprecipitates a surface peptide from IL-1– and IL-2–stimulated lymphocytes (2). This peptide is not acquired exogenously from CRP found in serum but is synthesized by the lymphocytes (2). Since the only known site of CRP synthesis is the hepatocyte, we set out to determine if the anti-CRP binding peptide produced by PBLs is CRP or an antigenically related peptide. The isolation and nucleotide sequence of pCRP5, a 1.6-kb CRP-specific cDNA synthesized from human liver RNA, was previously reported (6). RNA was isolated from unstimulated human PBMC and was hybrid selected using pCRP5. It was then converted into cDNA and cloned into the EcoRI cloning site of λ ZAPII phage to construct HScDNA clones. When the HScDNA clones were screened with radiolabeled pCRP5, we found that the liver cDNA hybridized strongly to three PBMC clones, HScDNA1, HScDNA3, and HScDNA8, which contained cDNA inserts of 1.0, 1.6, and 0.8 kb, respectively.

A partial nucleotide sequence analysis was performed on the smaller clones, HScDNA8 and HScDNA1, to determine whether these clones represent the known CRP transcript or a CRP-related transcript. We sequenced 304 nucleotides from the 5′ end of HScDNA8 and 115 nucleotides from the 5′ end of HScDNA1. When these sequences were compared to pCRP5, we found that the 0.8-kb insert of HScDNA8 is homologous to the 5′ end of pCRP5, while the 1.0-kb insert of HScDNA1 is homologous to the 3′ end of pCRP5 (Fig. 1). The CRP gene contains a 278-bp intervening sequence that is defined by nucleotides 330–607 (6). The absence of nucleotides 330–607 from HScDNA8 (Fig. 1) demonstrates that the insert contained in HScDNA8 represents a PBMC transcript and not PBMC genomic DNA, which may have contaminated our hybrid selection and cloning procedures. With one exception the nucleotides sequenced from HScDNA1 and HScDNA8 display 100% homology to pCRP5. The exception is a substitution located at position 1056. HScDNA1 contains a G at this position while pCRP5 contains an A (Fig. 2). The nucleotide homology of HScDNA1 and HScDNA8 to pCRP5 (and thus, the CRP gene), along with the absence of the intervening sequence from HScDNA8, indicate that these two HScDNA clones represent PBMC transcripts of the CRP gene.

Although our results indicate that HScDNA1 and HScDNA8 represent CRP transcripts, they do not eliminate the possibility that our HScDNA clones were derived from fragments of pCRP5 that may have been eluted from the nitrocellulose during the hybrid selection step, rather than from PBMC transcripts. Since the entire CRP transcript is not represented by pCRP5, the ends of our largest clone, HScDNA3, were sequenced to determine if they contained any portions of the CRP transcript that are not present in pCRP5. Our data show that the 5′ limit of HScDNA3 extends five nucleotides beyond the 5′ limit of pCRP5 (Fig. 1). Also, HScDNA3 contains an additional 23 nucleotides on the 3′ end that are not present in pCRP5 (Fig. 1). These additional nucleotides observed in HScDNA3 are homologous to the corresponding nucleotides of the CRP gene (Fig. 2) and confirm that HScDNA3 is not derived from pCRP5. Furthermore, since HScDNA3 lacks the intervening sequence it is not derived from genomic DNA (Fig. 1), but rather from a PBMC transcript.

The human genome contains a single copy CRP gene and a pseudogene; these display 50–80% region specific identity (9). Since the complete nucleotide sequence of HScDNA3 is >99% homologous to the CRP gene with all but 20 of the 1,645 nucleotides of HScDNA3 being identical to the sequence of the CRP gene (99% identity), we conclude that these HScDNA clones are not transcripts of the pseudogene (Fig. 2). The reduced homology in the last 15 nucleotides of HScDNA3 may reflect the limitation of the polymerase used for cDNA synthesis or genetic polymorphism. Alter-

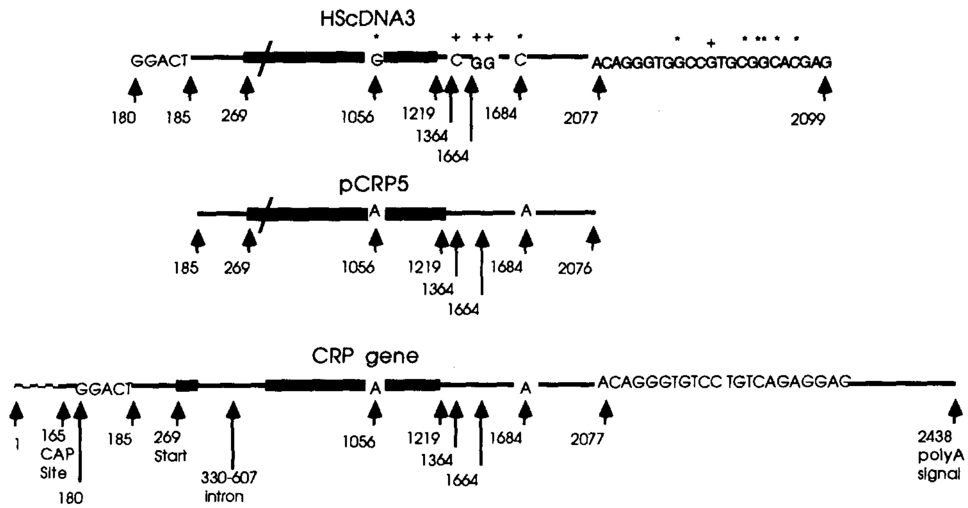

Figure 2. Comparison of HScDNA1 to pCRP5 and the genomic CRP gene. The map illustrates the alignment of our largest PBMC cDNA clone, HScDNA3, to the liver cDNA clone, pCRP5, and to a fragment of human genomic DNA containing the functional CRP gene. Only the nucleotides of HScDNA3 that differ from pCRP5 or the genomic CRP clone are shown and indicated (*) for substitutions or (+) for additions. The thick solid lines indicate the coding region, which begins with a start codon at position 269 and ends with a stop codon located at position 1219. (/) The absence of an intervening sequence defined by nucleotides 330–607 from the cDNA clones. The thin solid lines indicate untranslated regions. The dotted line indicates an untranscribed region. The complete sequence of HScDNA3 has been submitted to the EMBL Data Library under accession number X56692.

natively, HScDNA3 might have been damaged as a result of a fragmentation that may have occurred in this region during cloning. The cDNA synthesis was initiated from a poly(T) primer annealed to PBMC RNA. No poly(A) tail is observed in HScDNA3, suggesting that this cDNA did fragment. Nevertheless, the extensive nucleotide homology between HScDNA3 and the coding region of the CRP gene verifies that PBMC transcribe the CRP gene.

While hepatocytes express secreted CRP, we provided evidence in a previous report that shows that CRP expressed by peripheral blood cells is a membrane protein that does not appear to be secreted (2, 3). Therefore, we examined the deduced amino acid sequence of HScDNA3 to determine if it has an alternate or additional transmembrane region that pCRP5 does not have or any other differences that may account for the expression of a membrane peptide by peripheral blood cells and a secreted peptide by liver cells from the same CRP gene. The only difference found near the 3′ end of the translated region is located at position 1056 (Fig. 2). HScDNA3, as well as HScDNA1, have a G substituted for the A observed at this position in pCRP5. However, this is a neutral substitution that does not change the amino acid coded for by the affected codon. It is, therefore, unlikely that this nucleotide difference allows the expression of membrane CRP by lymphocytes. Other sequence differences were observed in the 3′ untranslated region of HScDNA3 as compared with pCRP5. These include the addition of a single C after nucleotide 1364, the addition of two Gs after nucleotide 1654, and the substitution of a C for an A at position 1684 (Fig. 2). Since nucleotides 1364, 1654, and 1684 are located in the 3′ untranslated region of the CRP transcript, these differences are unlikely to account for the expression of variant forms of the CRP peptide by different cell types. Therefore, the nucleotide sequence of HScDNA3 suggests that the CRP peptide expressed by peripheral blood cells has a primary structure identical to the pre-CRP peptide expressed by liver cells. Although PBMC do not appear to add a transmembrane region or alter the COOH terminus of the CRP transcript, other mechanisms, such as usage of a phosphatidylinositol glycan anchor, could account for its presence as a membrane protein. Clearly, variations in the processing and assembly of CRP subunits, variations in other membrane proteins, or variations in the signals received by cells could also account for the expression of a membrane protein by peripheral blood cells and a secreted protein by liver cells from a single CRP gene.

While it is unlikely that the few nucleotide differences observed between our HScDNA clones and pCRP5 or the CRP gene play a role in modifying the form of the CRP peptide expressed in PBMC, they may represent genetic variations within the CRP gene. This is supported by the finding that when we compared the nucleotide sequence of our HScDNA clones with the CRP gene sequence reported by Goldman and coworkers (9, 10), rather than to the sequence of pCRP5 and the CRP gene reported by Woo et al. (6), we found that our sequence is identical to the CRP gene at positions 1056 and 1684. There appear to be several genetic variations within the CRP gene as demonstrated by sequence discrepancies reported by various investigators at positions 1056, 1444, and 1684 of the CRP gene and CRP-specific cDNA clones. Goldman et al. (9) previously suggested this when he reported variations in the length of the polyGT stretch located within the intervening sequence of the CRP gene.

Although PBLs and liver cells may synthesize identical pre-CRP peptides, the CRP molecule expressed by peripheral blood cells may differ in tertiary structure from the mature CRP molecule secreted by the liver. Immunofluorescent studies showed that a CRP epitope that is present on unprocessed CRP and not on serum CRP is preferentially expressed on lymphocytes (11). Perhaps the pre-CRP peptide synthesized by PBMC is incompletely processed or reacts with cellular factors that result in the expression of a conformationally altered molecule that contains a region that is cryptic in the pentameric form of CRP found in the serum. The newly exposed region may function as a membrane anchor, associate with a phosphatidylinositol glycan anchor, or associate with some other protein present in the membrane.

Many of the activities attributed to CRP involve the immune response. It seems logical that hepatocytes might synthesize and release pentameric CRP, which is protected from denaturation and remains inactive until it reaches the site of inflammation, while lymphocytes at the site of inflammation or malignancy might synthesize and express the conformationally active form. Along with demonstrating PBMC expression of CRP, these data support studies describing a role for cell surface CRP in cell-mediated immunity.

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