Immunology 1996 **89** 289–294

Biosynthesis of human ficolin, an *Escherichia coli*-binding protein, by monocytes: comparison with the synthesis of two macrophage-specific proteins, C1q and the mannose receptor

J. LU, Y. LE, O. L. KON, J. CHAN* & S. H. LEE* *Departments of Biochemistry and *Pathology, Faculty of Medicine, National University of Singapore, Singapore*

SUMMARY

Ficolin is characterized by the presence of both collagen-like and fibrinogen-like sequences, and potentially has a similar overall structure as the complement protein C1q and the collectins. Previous studies have reported the presence of human ficolin mRNA predominantly in peripheral blood leucocytes. In the present study, the cellular origin of human ficolin was investigated in further detail. Preliminary studies using reverse transcriptase–polymerase chain reaction (RT–PCR) showed that ficolin mRNA was synthesized by U937 cells, a human monocyte cell line. This finding suggested that blood monocytes also normally synthesize human ficolin. Peripheral blood monocytes from adult human donors were harvested at serial time-points (0–20 hr) after adhesion to tissue culture plates, and total RNA was isolated and assayed for ficolin mRNA by RT–PCR. Ficolin mRNA was highly expressed in monocytes throughout the first 20 hr of adhesion. In contrast, C1q and mannose receptor mRNA were not detectable during the first 8 hr of adhesion, but were highly expressed by 20 hr. Cells were harvested at longer time intervals (1, 2, 4, 6 and 8 days) to determine whether ficolin expression was temporally regulated at later stages of monocyte differentiation. Ficolin mRNA levels decreased sharply from day 1 to day 6. In contrast, the levels of both C1q and mannose receptor mRNA showed no changing trend. These results are consistent with the absence of ficolin expression in many macrophage-rich tissues previously reported. The origin of ficolin from monocytes, together with its structural similarity to C1q and the collectins, raises the possibility that ficolin may be another plasma protein capable of binding to surface structures of micro-organisms. *Escherichia coli* was therefore incubated with human serum, and bound proteins, after elution with sugars, were analysed by Western blotting using an antiserum raised against a synthetic ficolin peptide. The antiserum identified a polypeptide of approximately 42 000 MW, which is similar in size to that of ficolin as predicted from its cDNA-derived sequence.

INTRODUCTION

Ficolin was initially identified from pig uterus as a molecule binding to transforming growth factor-β1 (TGF-β1).[1] cDNA cloning showed that it contained both collagen-like and fibrinogen-like sequences and probably had a similar overall structure to the complement protein C1q and a group of C-type lectins known as the collectins.[1–3] A single ficolin polypeptide is approximately 300 amino acid residues long and consists of three distinct regions: a short N-terminal segment, a middle collagen-like region and a C-terminal globular region. The N-terminal domain contains a cysteine residue which in C1q and the collectins forms interchain disulphide bonds in the assembly of the highly oligomerized molecules.[3] The C-terminal globular domain has homology to sequences found at the C-terminal regions of several proteins, including the fibrinogen β and γ chains and the tenascins.[1,2] It has been termed the FBG (fibrinogen beta/gamma) domain.[1,2,4] This domain is approximately 220–250 amino acid residues long and is characterized by 26 invariant and more than 40 highly conserved residues.[2] The FBG domain in tenascin has been shown to bind to heparin.[5] The functions of ficolin are still uncertain from the structural data. A putative elastin-binding protein[6] and a novel corticosteroid-binding protein,[7] both showing sequence homology to human ficolin, have been identified recently in human plasma. However, the structural similarity of ficolin to C1q and the collectins suggests that it may bind to micro-organisms via its FBG domain and could subsequently recruit certain immune mechanisms, such as phagocytosis, to its target.

Although ficolin was initially identified as a protein in pig uterus and its cDNA clones also isolated from uterus cDNA libraries, human ficolin has been found to be predominantly

Received 13 March 1996; revised 3 June 1996; accepted 9 June 1996.

Correspondence: Dr J. Lu, Department of Biochemistry, Faculty of Medicine, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Singapore.

synthesized in peripheral blood leucocytes.[1,2] Lower levels of ficolin mRNA have also been detected in lung, spleen and thymus.[2] Previous studies did not, however, clarify the cellular origin of human ficolin. We report here that screening of a number of leucocyte cell lines by reverse transcriptase–polymerase chain reaction (RT–PCR) showed that a monocyte cell line (U937) synthesized ficolin mRNA. Since monocytes, in time, become adherent to endothelium and subsequently migrate into extravascular tissues where they differentiate and develop into macrophages, it was of interest to study ficolin expression in adherent monocytes. The expression of two known macrophage-specific proteins—C1q and the mannose receptor (MMR)[8–10]—in adherent monocytes were assayed as markers of monocyte differentiation. The present studies also provide evidence that ficolin is a human serum protein that binds to structures on the surface of *Escherichia coli*.

MATERIALS AND METHODS

The *E. coli* strain Y 1090 and a first-strand cDNA synthesis kit were purchased from Clonetech Laboratories (Palo Alto, CA). Ovalbumin, horse heart myoglobin and sugars were from Sigma Chemical Co. (St Louis, MO). 1-Ethyl-3-(3-dimethyl-amino-propyl)carbodiimide was provided by Dr H. P. Too (Department of Biochemistry, National University of Singapore) and was originally purchased from Pierce Chemical Co. (Rockford, IL). A ficolin peptide was synthesized by the Bioprocessing Technology Centre (National University of Singapore).

Cell lines

The Raji lymphoblast-like, Molt-4 acute lymphoblastic, U937 histocytic lymphoma and THP-1 human monocyte cell lines (all obtained from ATCC, Rockville, MD) were cultured at 37° in RPMI-1640 supplemented with 10% (v/v) fetal calf serum in the presence of 5% CO_2. The HepG2 hepatocellular carcinoma cell line was cultured in Dulbecco's modified essential Eagle's medium (DMEM) containing 10% (v/v) fetal calf serum under identical conditions.

Preparation of adherent human monocytes

Peripheral blood from healthy adult donors was collected into a 1/10 volume of citrate phosphate dextrose containing adenine as anticogulant. Peripheral blood mononuclear cells were isolated using Lymphoprep (Gibco, Grand Island, NY), washed twice in phosphate-buffered saline (PBS) and suspended in RPMI-1640 containing 5% (v/v) fetal calf serum. Equal aliquots of cells were then plated in multiwell tissue culture plates at a density of $1-3 \times 10^8$ cells/9·6 cm^2 well. After incubation of the plates for 1 hr at 37°, non-adherent cells remaining in suspension were removed by gentle aspiration. The plates were washed with warm RPMI-1640 and adherent cells were cultured in RPMI medium containing 10%(v/v) fetal calf serum. The medium was replaced every 3 days thereafter. In one experiment, adherent cells were harvested at seven serial time-points (0, 0·5, 1·0, 2·0, 4·0, 6·0, 8·0 and 20 hr) after adhesion for RNA isolation. In a second experiment, adherent cells were harvested at longer time intervals (1, 2, 4, 6 and 8 days) after adhesion. RNA was also isolated from cells that were non-adherent after incubation for 1 hr in tissue culture plates, and were considered to be mainly (>90%) lymphocytes.

Table 1. Oligonucleotide primers used in the RT–PCR

Primers	Sequences (5′...3′)
Ficolin	
EHFC-1	CAGTCGTGTGCGACAGGCCCA
EHFC-2	CTAGGCGGGCCGCACCTTCAT
C1q	
C1q-1	AGGACTTGTGCCGAGCA
C1q-2	CCGTGTCGAAGATGACC
MMR	
MMR-1	CTACTGGACACCAGGCAA
MMR-2	TACATGGCTTCATAACCTCT
Actin	
Actin-1	GGCGTGATGGTGGGCATG
Actin-2	GGAAGGAAGGCTGGAAGAG

The primers were designed based on previously published sequences.[2,12–15] The ficolin primers were designed to amplify the FBG domain of human ficolin. The genomic sequence for human ficolin (J. Lu, unpublished data) showed that the FBG domain was encoded by four exons on a DNA fragment of approximately 3 kb, which is clearly distinct from the PCR product of approximately 700 bp expected from the cDNA template using the two primers. The C1q primers were designed based on the genomic sequence of the human C1q-A chain.[12] The expected PCR product was approximately 1·5 kb using the genomic template, and was only 340 bp on the cDNA templates. The MMR primers were designed based on both the cDNA and genomic sequences.[13,14] An intron would be expected in the MMR gene between the 7th and 8th nucleotide of the MMR-1 sequence, so that it would not anneal to genomic templates at stringent temperatures. The expected PCR product from cDNA templates, using MMR-1 and MMR-2, was approximately 400 bp. The β-actin primers were designed to amplify the entire exon 2 and most of exon 3, with an expected PCR product of approximately 650 bp using cDNA templates. The intron between the two exons was 441 bp; therefore the two primers would amplify a product of approximately 1·1 kb from genomic DNA as a template.

Isolation of total RNA and RT–PCR

Cells grown in suspension culture (U937, Raji, Molt-4, THP-1 and non-adherent mononuclear cells recovered from the suspension after incubation in tissue culture plates) were collected by centrifugation for 5 min at 500 *g*. Total RNA was isolated following the method of Chornazynski & Sacchi.[11] Adherent cells (HepG2 and adherent monocytes) were lysed on

tissue culture plates, after removal of medium and washing with PBS, using a guanidinium–sodium dodecyl sulphate (SDS)–phenyl-containing RNA isolation buffer.[11] Purified RNA (1·0 μg for RNA isolated from cell lines or 25% of total RNA isolated from adherent monocytes) was used for first-strand cDNA synthesis using Moloney murine leukaemia virus (MMLV) reverse transcriptase following the manufacturer's instructions (Clonetech). The reactions were incubated for 1 hr at 42° using both oligo-dT and random hexamer primers, and terminated by heating for 5 min at 95°. A 1/10 volume (2 μl) was used in each PCR (50 μl). PCR were carried out for 40 cycles with primers designed for human ficolin, C1q, the mannose receptor and, as controls, with primers to amplify the human β-actin cDNA sequence (Table 1). All primers were designed on the basis of published sequences. The primer pairs were mostly positioned on two neighbouring exons so that PCR products amplified from genomic templates would be larger than, and therefore distinct from, products amplified from cDNA sequences (Table 1). In the case of MMR, the 5′-primer (MMR-1) was designed to span an intron/exon boundary so that the primer would not anneal to genomic templates at stringent temperatures (Table 1). PCR products were analysed by electrophoresis on 1% (w/v) agarose gels containing ethidium bromide, and visualized under ultraviolet (UV) light.

Preparation of an anti-ficolin antibody

A peptide was synthesized based on the cDNA-derived ficolin sequence (NH_2-CYLMGPHESYAN-COOH), corresponding to residues 292–302.[2] The N-terminal cysteine residue of the peptide was not present in the ficolin sequence. The peptide was conjugated to ovalbumin and horse heart myoglobin following the method of Too & Maggio,[16] with slight modifications. Briefly, ovalbumin or myoglobin (1·0 g) was dissolved in distilled water (50 ml) and the pH adjusted to 7–8 with 1 M NaOH using a glass pH electrode. Solid succinic anhydride (20 g) was added in small increments, under constant stirring, and pH was maintained between 7 and 10 by addition of 8 M NaOH. At complete addition of succinic anhydride, the solution was stirred for a further 30 min and then dialysed against distilled water. The succinated proteins were lyophilized for storage and subsequent derivation with the ficolin peptide.

Succinated ovalbumin (10 mg) or myoglobin (5 mg) was dissolved in 0·1 M sodium phosphate (pH 6·0; 5 ml) and a cross-linker, 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide (100 mg), dissolved in distilled water was added with constant stirring. Ficolin peptide (2 mg), dissolved in dimethyl sulphoxide (DMSO; 10 μl), was immediately added to the solution and the mixture was stirred for a further 15 min. The conjugates were dialysed against PBS and the ovalbumin–peptide conjugate was used to raise antibodies in New Zealand White rabbits. The specificity of the antiserum for the ficolin peptide was confirmed by its reactivity with the myoglobin–peptide conjugate by Western blot analysis.

Detection of ficolin in human serum using E. coli *Y 1090 as ligand*

Escherichia coli was grown overnight in L-broth (200 ml) in a 500-ml flask, with shaking. The bacteria were harvested by centrifugation for 5 min at 500 *g*. The pelleted bacteria were washed twice with a TBS-TE buffer [50 mM Tris, 150 mM NaCl, 0·05% (v/v) Tween-20 and 10 mM EDTA, pH 7·4] and once with TBS-TCa^{2+} (TBS-TE in which EDTA was replaced by 20 mM $CaCl_2$). The washed bacteria were mixed with human serum (100 ml) overnight at 4° and, after two washes with TBS-TCa^{2+}, surface-bound proteins were eluted with a mixture of 100 mM each of mannose, *N*-acetyl-D-glucosamine and maltose dissolved in TBS-TCa^{2+}. The eluted proteins were boiled in the presence of 50 mM dithiothreitol and subjected to SDS–polyacrylamide gel electrophoresis (PAGE). The separated proteins were electroblotted onto nitrocellulose membrane, which was probed using the anti-ficolin antiserum.

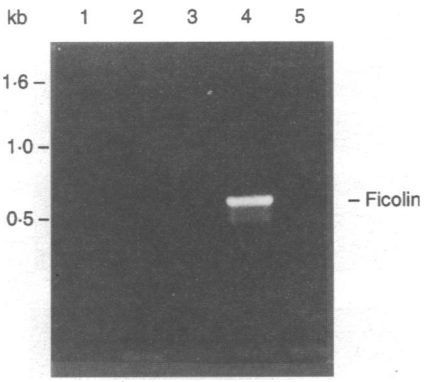

Figure 1. Detection of human ficolin sequence in cell lines. Total RNA was isolated from five different cell lines, Raji , Molt-4, THP-1, U937 and HepG2. First-strand cDNA was synthesized and subsequently used in PCR reactions with the two ficolin primers, EHFC-1 and EHFC-2. The PCR (50 μl) were carried out for 40 cycles, each consisting of 94°, 30 seconds; 67°, 30 seconds; 72°, 50 seconds. The PCR products (10 μl) were examined on a 1% (w/v) agarose gel and visualized under UV light. Lane 1, Raji; lane 2, Molt-4; lane 3, THP-1; lane 4, U937; lane 5, HepG2.

RESULTS

Identification of cell lines that synthesize human ficolin

As human ficolin mRNA has been found predominantly in peripheral blood leucocytes,[2] four common human leucocyte cell lines, Raji, Molt-4, U937 and THP-1, were examined for the presence of ficolin mRNA by RT–PCR. Two human ficolin primers were designed to amplify the ficolin cDNA sequence encoding the FBG domain (approximately 700 bp) (Table 1) in the RT–PCR assay. HepG2 was included in this experiment as a negative control. As seen in Fig. 1, a single PCR product of the expected size was strongly amplified from U937 cells (lane 4). A much weaker signal was also detected in Raji cells (Fig. 1, lane 1). In contrast, the PCR product was not amplified from Molt-4, THP-1 and HepG2 cells (Fig. 1, lanes 2, 3 and 5). Because U937 cells have characteristics of human monocytes, these results suggest that human ficolin is probably synthesized in circulating monocytes. However, this does not exclude the possibility that ficolin is also synthesized by other leucocytes (e.g. lymphocytes), especially since its mRNA was also detected weakly in the lymphoblastoid Raji cell line.

Ficolin expression in adherent human monocytes

A common method of monocyte isolation is based on its ability to adhere to plastic or glass surfaces of tissue culture plates

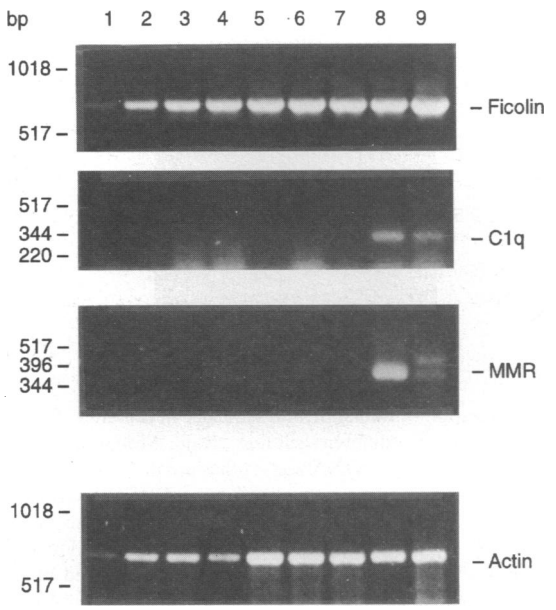

Figure 2. RT–PCR on adherent monocyte and non-adherent mononuclear cell RNA. Total RNA was isolated from adherent monocytes at serial time-points after adhesion, and from non-adherent mononuclear cells recovered after monocyte adhesion, as described in the Materials and Methods. cDNA synthesis and detection of PCR product were performed as described in Fig. 1. PCR conditions for the detection of ficolin cDNA sequence were also as in Fig. 1, but conditions for the detection of C1q, MMR and β-actin cDNA sequences were different. Primers C1q-1 and C1q-2: 94°, 30 seconds; 51°, 30 seconds and 72°, 30 seconds. Primers MMR-1 and MMR-2: 94°, 30 seconds; 53°, 30 seconds and 72°, 30 seconds. Primers actin-1 and actin-2: 94°, 30 seconds; 57°, 30 seconds and 72°, 50 seconds. The serial time-points at which adherent monocytes were harvested for RT–PCR are: lane 1, 0 hr; lane 2, 0·5 hr; lane 3, 1·0 hr; lane 4, 2 hr; lane 5, 4 hr; lane 6, 6 hr; lane 7, 8 hr; and lane 8, 20 hr. Lane 9 was from non-adherent cells in suspension after monocyte adhesion.

while other mononuclear cells (mainly lymphocytes) remain in suspension. Of additional interest is the fact that adhesion of monocytes to these surfaces also initiates the differentiation process which, with time, leads monocytes to acquire the features of macrophages.[17] We have reported that ficolin mRNA was, however, not detected in many tissues known to contain macrophages.[2] This strongly suggested that ficolin expression could be subject to regulation during the process of monocyte-to-macrophage transformation. Thus experiments were performed to examine ficolin expression not only in freshly isolated human monocytes, but also over a period of time when human monocytes, adherent to plastic surfaces, were in the process of differentiation into macrophage-like cells.

Adherent monocytes were initially harvested at serial time-points during a period of 20 hr after adhesion to plastic. RNA was isolated from the cells and assayed for human ficolin mRNA and, as markers of monocyte differentiation, C1q and the mannose receptor mRNA by RT–PCR. β-actin mRNA was also assayed in all experiments as a positive control and as a monitor of template input. Each RT–PCR amplified a predominant product of the size expected from the cDNA sequences (Fig. 2). As shown in Fig. 2, ficolin mRNA was uniformly detected at high levels in adherent monocytes within the first 20 hr of adhesion, while those C1q and MMR mRNA were not detectable up to 8 hr after adhesion but were detected at high levels by 20 hr. Thus these data define a 'window' period between 8 and 20 hr after adhesion when transcription of C1q and MMR genes began.

The results above provided clear evidence for changes in C1q and MMR gene expression during differentiation of human monocytes in the first 20 hr of adhesion, but did not show any changing trend in ficolin mRNA synthesis. To examine the possibility that ficolin gene expression was affected at later stages of monocyte differentiation, adherent monocytes were harvested at longer time intervals after adhesion. As shown in Fig. 3, while levels of C1q and MMR mRNA showed no tendency to change in monocytes from day 1 to day 6 after adhesion, there was a clear trend for ficolin mRNA levels to decrease in monocytes in the same period (lanes 3–6). The mRNA levels of ficolin, C1q and MMR on day 8 could not be properly analysed or interpreted, however, because all three species were either barely present or undetectable (Fig. 3, lane 7). It should be noted that the level of β-actin mRNA on day 8 was not, however, significantly different from samples harvested earlier (Fig. 3), suggesting that the low levels, or lack, of ficolin, C1q and MMR mRNA in monocytes 8 days after adhesion were unlikely to be due to cell death.

All four species of mRNA, ficolin, C1q, MMR and β-actin, were also assayed in non-adherent mononuclear cells (mainly lymphocytes), which were recovered from the suspensions after monocyte adhesion. As shown in Fig. 2 (lane 9), ficolin mRNA was also detected in significant amounts in this population of cells. This finding could be ascribed to the presence of monocytes that had not adhered to the plates after 1 hr, although the possibile origin of ficolin mRNA from lymphocytes cannot be ruled out, especially since ficolin mRNA had been detected in Raji cells, a human lymphoblastoid cell line (Fig. 1, lane 1). It was, however, surprising that C1q mRNA was also detected in significant amounts in this population of

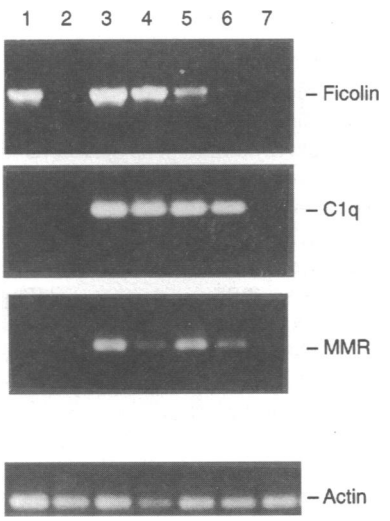

Figure 3. RT-PCR on adherent monocyte RNA. This experiment was carried out essentially as described in Figs 1 and 2. The difference was that RNA was isolated from monocytes 1 day (lane 3), 2 days (lane 4), 4 days (lane 5), 6 days (lane 6) and 8 days (lane 7) after adhesion. U937 (lane 1) and HepG2 (lane 2) cells were included as controls.

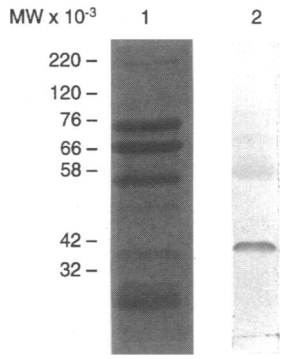

Figure 4. SDS–PAGE and Western blot analysis of human plasma proteins which bind to *E. coli*, using an antibody against a human ficolin peptide. *Escherichia coli* were mixed with human serum in the presence of Ca^{2+} and eluted with 100 mM each of mannose, *N*-acetyl-D-glucosamine and maltose. The eluted proteins were separated by SDS–PAGE, visualized by Coomassie Blue staining, and similarly separated proteins were also electroblotted and probed with a rabbit anti-ficolin peptide antibody. The blot was developed using a goat anti-rabbit IgG conjugated with alkaline phosphatase (lane 2).

non-adherent mononuclear cells (Fig. 2, lane 9). The fact that C1q mRNA was not detectable in adherent monocytes harvested immediately after the removal of these non-adherent cells from the culture plates (Fig. 2, lane 1) made it unlikely that ficolin mRNA detected in the non-adherent cell population was of monocyte origin. Lymphocytes are thus potential candidates for the cellular origin of C1q mRNA in non-adherent leucocytes, although there has not been data showing C1q synthesis in lymphocytes. The MMR primers also amplified two discrete PCR products from non-adherent leucocytes (Fig. 2, lane 9). Since the products were different in size from that predicted from the MMR cDNA sequence (Fig. 2, lane 8),[13] they were unlikely to be derived from MMR mRNA, although they could represent sequences related to MMR.

Ficolin is an *E. coli*-binding protein

The monocytic origin of human ficolin and its structural similarity to C1q and the collectins suggested that it may be a plasma protein. As both C1q and the collectins are capable of binding to, and subsequently mediating the killing of, micro-organisms, the possibility that ficolin may have similar functions was considered. To examine simultaneously the presence of ficolin in human plasma and its capacity for binding Gram-negative bacteria, a washed culture of *E. coli* Y1090 was mixed with fresh human serum overnight. The most abundant structures on Gram-negative bacteria are oligosaccharides, to which both C1q and collectins bind directly or indirectly (via anti-carbohydrate antibodies). Therefore, we eluted *E. coli*-bound serum proteins with a mixture of sugars, mannose, *N*-acetyl-D-glucosamine and maltose. The eluted proteins were separated by SDS–PAGE and analysed by Western blotting using an antiserum raised against a ficolin peptide (Fig. 4). A polypeptide of approximately 42 000 MW was recognized by the antiserum (Fig. 4, lane 2), which was similar to the size of human ficolin polypeptide predicted from its cDNA sequence (approximately 300 amino acids long), taking into account post-translational modifications.[2] Therefore, human ficolin is probably a plasma protein that, directly or indirectly, binds to sugar residues on *E. coli* Y1090. As controls, similar blots were probed with antisera against human mannan-binding protein (MBP) and lung surfactant protein D(SP-D),[18] respectively. MBP, but not SP-D, was detected on the blots (data not shown). However, neither antiserum reacted with a 42 000 MW polypeptide (data not shown), showing the specificity of the anti-ficolin peptide antiserum. While the functional significance of ficolin binding to *E. coli* Y1090 is not clear, it could lead conceivably to the killing and clearance of target microbes via mechanisms similar to those invoked by C1q and the collectins.[18]

DISCUSSION

Ficolin or ficolin-like proteins have been identified in several studies to bind to apparently unrelated proteins, e.g. TGF-β_1[1] and elastin[6], as well as to a corticosteroid.[7] The binding capacity of human ficolin for *E. coli* Y1090 shown in the present study adds further to the diversity of structures to which ficolin binds. Although no precise function can yet be definitely ascribed to ficolin based on these findings, its ability to bind *E. coli* together with its structural similarity to C1q and the collectins, raises the distinct possiblity that ficolin participates in immune defence against micro-organisms. The sugar-dependent binding of ficolin to *E. coli* implies that it may also be a lectin and thus could be purified from human serum by affinity chromatography on saccharide-derived matrices.

Circulating monocytes are known to be precursors of tissue macrophages. After a period of residence in the circulation, monocytes adhere to the vascular endothelium and migrate into extravascular tissues where they differentiate into tissue macrophages.[17] The differentiation process involves a major functional transformation of monocytes which consequently acquire the properties of macrophages, and is associated by developmentally regulated expression of certain genes during monocyte differentiation. For example, C1q and MMR are synthesized in tissue macrophages but not in circulating monocytes.[8–10] The patterns described in the present study of C1q and MMR expression during the course of monocyte differentiation *in vitro* on tissue culture plates appears to have revealed a 'window' of the process during which transformation of adherent monocytes into macrophages commences at the transcriptional level by the activation of macrophage-specific genes. This 'window' occurs between 8 and 20 hr after adhesion. Results from the current study do not, however, indicate whether the biosynthesis of monocyte-specific proteins also terminates during this 'window' period. It is possible that the significant decrease of ficolin mRNA in adherent monocytes, which was evident 2 days after adhesion, may reflect accelerated ficolin mRNA degradation rather than silencing of ficolin gene transcription which must, however, have occurred earlier. Nevertheless, the decrease and eventual termination of ficolin gene expression could not have been the result of loss of cell viability or of any experimental artefact, since the differentiation process of the adherent monocytes was clearly in progress in these cells, as judged by the regulated expression of the C1q and MMR genes assayed at the same time. The results of the present study help to explain why some tissues containing macrophages do not synthesize ficolin,[2] but do not

definitively identify the cell of origin of ficolin mRNA in extravascular tissues. Ficolin mRNA in extravascular tissues could either be derived from non-mononuclear cells or from macrophages which differentiate in possibly a tissue-specific manner in the heterogeneous microenvironment of diverse tissues. These conditions are unlikely to have been replicated fully in the experimental system used in these studies in which monocyte differentiation occurred on plastic surfaces.

ACKNOWLEDGMENTS

The authors thank Professor Ken Reid of the MRC Immunochemistry Unit, University of Oxford, and Dr H. P. Too of the Department of Biochemistry, National University of Singapore for advice; Dasilah Dolmanan for technical assistance; and Josephine Howe for photography. This project is funded by National University of Singapore research grant no. RP940311 and J. Lu is also in receipt of the Lee Kuan Yew Research Fellowship.

REFERENCES

1. Ichijo H., Helman U., Wernstedt C. *et al.* (1993) Molecular cloning and characterization of ficolin, a multimeric protein with fibrinogen-like and collagen-like domains. *J Biol Chem* **268,** 14505.
2. Lu J., Tay P.N., Kon O.L. & Reid K.B.M. (1996) Human ficolin: cDNA cloning, demonstration of peripheral blood leucocytes as the major site of synthesis and assignment of the gene to chromosome 9. *Biochem J* **313,** 473.
3. Lu J., Wiedemann H., Timpl R. & Reid K.B.M. (1993) Structural similarity between C1q and the collectins as judged by electron microscopy. *Behring Inst Mitt* **93,** 6.
4. Bork P. & Bairoch A. (1995) Extracellular protein modules. *Trends Biochem Sci* **20,** Appendix.
5. Fisher D., Chiquet-Ehrismann R., Bernasconi C. & Chiquet M. (1995) A heparin binding region within the fibrinogen-like domain in chick tenascin-C. *J Biol Chem* **270,** 3378.
6. Harumiya S., Omori A., Sugiura T., Fukumoto Y., Tachkawa H. & Fujimoto D. (1995) EBP-37, a new elastin-binding protein in human plasma: structural similarity to ficolins, transforming growth factor-β1-binding proteins. *J Biochem (Tokyo)* **117,** 1029.
7. Edgar P.F. (1996) Hucolin, a new corticosteroid-binding protein from human plasma with structural similarities to ficolin, transforming growth factor-β1-binding proteins. *FEBS Lett* **375,** 159.
8. Bensa J.C., Reboul A. & Colomb M.G. (1983) Biosynthesis in vitro of complement subcomponent C1q, C1 s and C1-inhibitor by resting and stimulated human monocytes. *Biochem J* **216,** 385.
9. Tenner A.J. & Volkin D.B. (1996) Complement subcomponent C1q secreted by cultured human monocytes has a subunit structure identical with that of serum C1q. *Biochem J* **233,** 451.
10. Stahl P.D., Wilemann T.E., Diment S. & Shepherd V.L. (1984) Mannose-specific oligosaccharide recognition by mononuclear phagocytes. *Biol Cell* **51,** 215.
11. Chornazynski P. & Sacchi N. (1987) A single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162,** 156.
12. Sellar G.C., Blake D.J. & Reid K.B.M. (1991) Characterization and organization of the genes encoding the A-, B- and C-chains of human complement subunit C1q. *Biochem J* **274,** 481.
13. Taylor M.E., Conary J.T., Lennartz M.R., Stahl P.D. & Drickamer K. (1990) Primary structure of the mannose receptor contains multiple motifs resembling carbohydrate-recognition domains. *J Biol Chem* **265,** 12156.
14. Kim S.J., Ruiz N., Bezouska K. & Drickamer K. (1992) Organization of the gene encoding the human macrophage mannose receptor (MRC1). *Genomics* **14,** 721.
15. Nakajima-Iijima S., Hamada H., Reddy P. & Kakunaga T. (1985) Molecular structure of the human cytoplasmic beta-actin gene: interspecies homology of sequences in the introns. *Proc Natl Acad Sci USA* **82,** 6133.
16. Too H.P. & Maggio J.E. (1991) Radioimmunoassay of tachykinins. In: *Method in Neurosciences* (ed. P. M. Conn), Vol. 6, p. 232. Academic Press, Inc. San Diego, California.
17. Gordon S. (1996) The macrophage. *BioEssays* **17,** 977.
18. Holmskov U., Malhotra R., Sim R.B. & Jensenius J.-C. (1994) Collectins: collagen-like lectins of the innate immune system. *Immunol Today* **15,** 67.