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In Vivo Biosynthesis of Endogenous and of Human C1 Inhibitor in Transgenic Mice: Tissue Distribution and Colocalization of Their Expression

Giovena Vinci, Nicholas J. Lynch, Christiane Duponchel, Thi-May Lebastard, Geneviève Milon, Cordula Stover, Wilhelm Schwaebel, and Mario Tosi

We have produced transgenic mice expressing human C1 inhibitor mRNA and protein under the control of the human promoter and regulatory elements. The transgene was generated using a minigene construct in which most of the human C1 inhibitor gene (C1NH) was replaced by C1 inhibitor cDNA. The construct retained the promoter region extending 1.18 kb upstream of the transcription start site, introns 1 and 2 as well as a stretch of 2.5 kb downstream of the polyadenylation site, and therefore carried all known elements involved in transcriptional regulation of the C1NH gene. Mice with high serum levels of human C1 inhibitor, resulting from multiple tandem integrations of the C1 inhibitor transgene, were selected. Immunohistochemistry in combination with in situ hybridization was applied to localize the sites of C1 inhibitor biosynthesis and to demonstrate its local production in brain, spleen, liver, heart, kidney, and lung. The distribution of human C1 inhibitor-expressing cells was qualitatively indistinguishable from that of its mouse counterpart, but expression levels of the transgene were significantly higher. In the spleen, production of C1 inhibitor was colocalized with that of a specific marker for white pulp follicular dendritic cells. This study demonstrates a stringently regulated expression of both the endogenous and the transgenic human C1 inhibitor gene and reveals local biosynthesis of C1 inhibitor at multiple sites in which the components of the macromolecular C1 complex are also produced. The Journal of Immunology, 2002, 169: 5948–5954.

C1 inhibitor (C1 inh) is a single-chain glycoprotein of 105 kDa that belongs to a family of serine protease inhibitors termed serpins (1–3). It is the only natural inhibitor of the serine proteases C1r and C1s of the classical activation pathway of complement. Upon binding to C1r or C1s, C1 inh dissociates the multimolecular classical pathway activation complex (C1), preventing C1s-mediated cleavage of C4 and C2 and the further downstream activation of complement (4). More recent work has provided strong evidence for a role of C1 inh in the regulation of the lectin pathway of complement activation by showing its binding to the lectin pathway-specific serine proteases mannos-binding lectin-associated serine protease-1 and mannos-binding lectin-associated serine protease-2 (5). Moreover, C1 inh recognizes and inhibits other noncomplement serine proteases such as kalirein and the coagulation factors Xla, XIIa, and plasmin (6, 7). Inherited C1 inh deficiencies lead to episodic acute s.c. or mucosal swellings, a condition termed hereditary angioedema. Acquired C1 inh deficiencies result in a clinical phenotype that is similar (reviewed in Ref. 8). New insights into the pathophysiology of increased vascular permeability are provided by a recent model of C1 inh deficiency obtained by disruption of the C1nh gene (9).

It has been suggested that hepatocytes are the major source of C1 inh biosynthesis in humans (10–12). Other studies, however, demonstrated predominant extrahepatic sources of C1 inh mainly by cells of the monocyte/macrophage lineage (13–16). In keeping with this, the human monocytic cell line U937 also synthesizes C1 inh (13). In the rat, isolated Kuffer cells have been reported to synthesize and secrete C1 inh, whereas peritoneal macrophages synthesize only trace amounts; IFN-γ-up-regulates C1 inhibitor gene expression in both cell populations (17). Fibroblasts, megakaryocytes, platelets, and endothelial cells are also able to produce C1 inh (18, 19).

Inflammation and tissue injury modulate complement expression at extrahepatic sites (20–22). Human astrocyte cell lines as well as neuroblastoma cell lines have been reported to express and secrete all the components of the alternative and of the classical pathway (23–25). In vivo results, however, demonstrated that such in vitro findings have to be considered with caution (26). C1 inh has been identified in both normal and Alzheimer’s disease brain tissue (27); its immunohistochemical detection has been reported in activated astrocytes and dystrophic neurites in plaque areas. In situ hybridization indicated that neurons might be major sites of synthesis of C1 inh in the brain (28).

The C1q subcomponent of the C1 complex is produced by microglial cells of rats (29). It was also found in heart, kidney, lung, and brain of mice (30). In rat spleen, C1q is produced by follicular dendritic cells (FDC) and by interdigitating cells within the white
pulp as well as by monocyte/macrophage lineage cells within the red pulp (31). In line with the observed T cell deficiency in C1q-deficient mice (32), the predominant expression of C1q in the spleen and its apparent biosynthesis by FDC imply a role in triggering the components of the adaptive immune system.

We describe in this study the in vivo biosynthesis of C1 inh in transgenic mice that carry all assigned regulatory elements of the human C1NH gene (33–35).

Materials and Methods

Human C1 inh minigene

The C1 inh minigene is a construct of 7.5 kb, inserted between the XhoI and the XbaI sites of the vector pBluescript KS+ (Strategen, La Jolla, CA) and containing a shortened version of the human C1NH gene (36) (accession no. X54866), including genomic sequences up to 1.18 kb from the transcription start site and 2.5 kb of 3′ flanking sequences. This construct also includes exons 1 and 2 and the first two introns, but lacks introns 3–7 of C1NH, because the genomic stretch between the BamHI site in exon 3 and the BglII site in exon 8 was replaced with the corresponding cDNA sequence. Purification of the human C1 inh DNA minigene for embryo injection was accomplished by digesting the plasmid with XhoI and XbaI to remove vector sequences, followed by agarose gel electrophoresis and purification (Nucleotrap extraction kit; Machery-Nagel, Düren, Germany).

Generation and identification of transgenic mice

Superovulated female (C56BL/6 × DBA/2)F1 mice were mated with 10- to 20-wk-old mice of the same strain. Fertilized eggs were flushed from the oviducts, and the minigene construct was injected at 4 ng/µl into the pronuclei (11 pl = 500 copies). Viable injected oocytes were transferred to day 0.5 pseudopregnant F2 foster mothers. The integration of the transgene in the genome of founder mice was assessed by Southern blot analysis of tail DNA using a 32P-labeled peeled full-length human C1 inh cDNA probe. Each lane of the Southern blot contained 7 µg of BamHI-digested total genomic DNA. To estimate the copy number of the human C1NH minigene in the transgenic founder lines, 12 or 120 pg of the 7.5-kb XhoI-XbaI segment containing the minigene was added to 7 µg of nontransgenic mouse DNA before BamHI digestion and was electrophoresed on separate lanes of the Southern blot to mimick the presence of 1 copy and 10 copies, respectively, in a diploid mouse genome. Intensities of hybridizing fragments were quantified by phosphor imager analysis. The offspring of transgenic mice were tested routinely for presence of high plasma levels of human C1 inh (see below).

Evaluation of the plasma level of human C1 inh in transgenic mice

Blood samples were collected in 5 mM EDTA (final concentration) to prevent in vitro activation of the complement and the contact systems. Tubes were centrifuged at 1300 × g for 10 min, and plasma was aliquoted and stored at −20°C until testing. ELISA were conducted using mAb RII ° 20 g native C1 inh/ml. The mouse C1 inh template was transcribed in the opposite direction, because the genomic stretch between the BamHI site in exon 3 and the BglII site in exon 8 was replaced with the corresponding cDNA sequence. Purification of the human C1 inh DNA minigene for embryo injection was accomplished by digesting the plasmid with XhoI and XbaI to remove vector sequences, followed by agarose gel electrophoresis and purification (Nucleotrap extraction kit; Machery-Nagel, Düren, Germany).

Histology and immunohistochemical staining

Mice were sacrificed by cervical dislocation; brain, heart, lung, liver, kidney and spleen were immediately excised and fixed for 15 h in cold Kryofix (Merck, Poole, U.K.), a mixture of ethyl-alcohol and polyethylene-glycol (38). After dehydration with increasing concentrations of ethanol, tissues were embedded in paraffin at 60°C. Sections were cut on a microtome at 5 µm thickness, dewaxed, washed with PBS solution, and treated with 0.1% Triton X-100.

Sections were preincubated with sheep serum for 2 h to block nonspecific binding sites, and incubated for 45 min at room temperature with the primary Ab. The anti-human C1 inh rabbit polyclonal Ab OTNK 04/05 (Boehringer, Marburg, Germany) was diluted to 1/100 in 10% FCS in RPMI medium. Sections were then washed with PBS and incubated with sheep anti-rabbit second Ab for 30 min at room temperature (1/300 dilution in 1% gelatin; type B from bovine skin; Sigma-Aldrich), washed in PBS, and placed in a 1/100 dilution of HRP-rabbit peroxidase antiperoxidase (PAP) complex solution (DAKO AEC Substrate System, Carpenteria, CA) in 1% gelatin for 30 min at room temperature. Sections were then rinsed with PBS and incubated for 10 min in peroxidase substrate-chromogen solution (DAKO AEC Substrate System). Finally, sections were rinsed with water and counterstained with hematoxylin (Thermal Shandon, Pontoise, France). Negative controls included incubations of the sections without primary Ab.

Serial 5-µm-thick cryostat sections were prepared from spleens directly embedded in OCT (Tissue-Tek, Sakura Finetek, Zoeterwoude, The Netherlands), allowed to air dry for 6 h, and fixed in acetone for 10 min. The following rat anti-mouse monoclonals were used on frozen sections. The F4/80 Ab (Serotec, Oxford, U.K.) recognizes the mouse F4/80 antigen, a macrophage receptor that binds unopsonized sheep E via recognition of sialylated glycoconjugates. In the spleen, it reacts with the marginal zone macrophages at the border of the red pulp (42). SER-4 is a hybridoma supernatant directed to SER, a macrophage receptor that binds unopsonized sheep E via recognition of sialylated glycoconjugates. In the spleen, it reacts with the marginal metallophilic macrophages at the border of the marginal sinus and the white pulp (43). FDC-M1 is a hybridoma supernatant (a kind gift of M. H. Kosco-Vilbois, Serono Pharmaceutical Research Institute, Geneva, Switzerland) specific for a subpopulation of murine spleen cells morphologically related to the dendritic cells of the germinal centers (44).

Sections were preincubated for 2 h with rabbit serum to block nonspecific binding, and then incubated with the relevant mAb diluted 1/10 in 0.005% Triton, pH 7.6, 0.05% saponin. A biotinylated rabbit anti-rat second Ab (diluted 1/100 in the same buffer) was then used for 1 h. Sections were then washed and placed in avidin DH-biotinylated alkaline phosphatase H complex (1/100 dilution in buffer) for 1 h at room temperature (Vectastain ABC-AP kit, Vector Laboratories, Burlingame, CA). After three washes, the enzymatic activity of alkaline phosphatase was revealed by incubation in Fast Red substrate-chromogen solution, rinsed with water, and counterstained with hematoxylin.

In situ hybridization

Human and mouse C1 inh mRNAs were localized by in situ hybridization using 35S-labeled antisense RNA probes. In vitro transcription and in situ hybridization were performed according to the methods of Melton et al. (45) and Schaffer et al. (46), respectively. To avoid that the cRNA probe for the detection of human mRNA cross-hybridizes with murine C1 inh mRNA and vice versa, the probes were generated from areas of human and mouse C1 inh cDNA, which share no extensive homology. The human probe was transcribed from a 572-bp BglII/BamHI fragment from the 5′ end of the cDNA, which was cloned into pBSIIKS+ (str). The mouse probe was transcribed from a cDNA template comprising 222 bp between the internal EcoRI and BamHI sites, which was also cloned into pBSIIKS+. For controls, the mouse C1 inh template was transcribed in the opposite direction, generating sense cRNA probe. The 20-µm sections of mouse tissue were cut using a cryostat, mounted on polylysine microscope slides (Merck), and hybridized with the 35S-labeled probes. After hybridization and washing, the signals were detected by exposing the sections to Kodak BioMax MR x-ray film (Sigma-Aldrich) or by dipping the slides in LM-1 photographic emulsion (Amersham Biosciences).

Results

Generation of transgenic mice

The DNA construct used to produce transgenic mice was a CINH minigene in which most exons were replaced by a cDNA fragment encoding human C1 inh. The construct retained the promoter region extending up to 1.18 kb from the transcription start site and
the integrity of introns 1 and 2 (Fig. 1). We have previously reported the mouse sequence (accession AF052039) corresponding to the region from −813 (upstream of the transcription start site) to +650 (intron 2) of the human C1NH gene, and have discussed the striking conservation of regulatory elements in the promoter region and within intron 1 (35). Functional studies also support the important role of intron 1 sequences in determining the transcriptional regulation and particularly the responses to IFNs (47) (our unpublished data).

The minigene construct was excised from the vector sequences by XhoI-Xbal digestion (Fig. 1B) and microinjected into fertilized murine oocytes. Twenty-three offspring were screened for incorporation of the transgene by BamHI digestion of their DNA, followed by Southern blot analysis (Fig. 1C). Six mice contained copies of the human C1NH gene identified as hybridizing bands of 2.4 and 5.0 kb. Two of the founder lines, F10 and F14, are shown in Fig. 1C (lanes 4 and 8, respectively). The strong 5-kb band reflects tandem integrations, because one of the two BamHI sites present in the microinjected minigene is only a few nucleotides downstream of the 5′ XhoI cloning site, and the other one is 2.4 kb downstream, whereas the 3′ end of the microinjected minigene lacks BamHI sites. The 2.4-kb band is weak in Fig. 1C because the corresponding BamHI fragment contains short exons and is only weakly recognized by the cDNA probe used. Additional hybridizing fragments indicate the presence of multiple integration sites of the transgene clusters. Transgene copy number was assessed by comparing the intensity of the 5.0-kb hybridizing band with that of the 5.0-kb band observed in lanes 1 and 2 that contained known amounts of the minigene construct corresponding to 1 and 10 copies, respectively. The 5.0-kb hybridizing fragment in these lanes contains the 3′ portion of the minigene extending from the internal BamHI site to the XhoI cloning site (Fig. 1B), whereas the fragment of the same size hybridizing in the DNA of transgenic founder lines (lanes 4 and 8) contains the same sequence, but is generated by BamHI digestion of tandemly integrated copies. Three founders in addition to F10 (lane 4 of Fig. 1C) had integrated high transgene copy numbers (~100), whereas one founder in addition to F14 (lane 8 of Fig. 1C) had a low transgene copy number (<5).

To evaluate the levels of the human C1 inh protein in transgenic mice, their plasma was analyzed by ELISA using the mAb RII. The human C1 inh protein was detected in the plasma of animals with high and low copy numbers of the human minigene construct.

**FIGURE 1.** Transgenic mice carrying multiple copies of a human C1NH minigene. A. Schematic representation of the human C1NH gene and its flanking sequence between the BamHI site at position −1182 and the BclI site, which is located 2.5 kb downstream of the polyadenylation site. B. The genomic sequences between the BamHI site in exon 3 (marked *B) and the BglII site in exon 8 (marked *BglI) were replaced with the corresponding cDNA sequence. The stars indicate that these sites were abolished in the minigene construct, without altering the C1 inh protein sequence. The minigene fragment used for microinjections is shown between the XhoI and the Xbal sites of the cloning vector. C. Copy number and structure of the human C1NH transgene estimated by Southern blot hybridization of BamHI-digested DNA. Lanes 4 and 8, Transgenic mice with high and low copy numbers (founder lines F10 and F14, respectively); lanes 5–7, nontransgenic littermates; lanes 1 and 2, contain the BamHI-digested C1NH minigene in amounts that mimic 1 and to 10 copies, respectively, per diploid mouse genome.

**FIGURE 2.** Low-resolution in situ hybridization of spleen and brain sections from transgenic mice using C1 inh probes. The 20-μm-thick cryostat sections of spleen (A and B) and forebrain (C and D) from transgenic mice were hybridized with 35S-labeled cRNA probes specific for mouse (A and C) and human (B and D) C1 inh mRNAs. The control in E shows a transgenic mouse brain hybridized with a sense RNA probe for mouse C1 inh, and the control in F shows brain from a nontransgenic mouse, hybridized with an antisense probe for human C1 inh RNA. The insets in E and F show midbrain sections obtained from transgenic mice at comparable positions to those of the nontransgenic control shown in F and hybridized with an antisense mouse cRNA probe and with an antisense human cRNA probe, respectively. All sections were exposed to x-ray films for 6 h.
at levels showing a positive correlation with the number of mini-
gene copies. There was no difference between males and females
(data not shown). Mice with high copy numbers of the human
transgene had human C1 inh plasma levels between 1 and 2 mg/ml,
and the low copy number line F14 had plasma levels of ~2.5 μg/
ml. Two founder lines (F7 and F10) with high copy numbers of the
human transgene and the low copy number line F14 were used in
all experiments, except for the in situ hybridization studies, in
which only the high copy number line F10 was used.

Distribution of mouse and human C1 inh mRNA in spleen,
brain, and liver of transgenic mice

The distribution of C1 inh synthesis was investigated in the spleen
because of the evidence of local production of complement com-
ponents, particularly C1q (31) and C3 (48), by specific cell types.
In situ hybridizations of spleen sections with human or mouse C1
inh antisense RNA probes revealed an identical distribution in the
follicles of the white pulp, in the marginal zone, and to a lesser
extent in the red pulp (Fig. 2, A and B). In keeping with the high
copy number of the human transgene, the hybridization with the
human probe was always stronger (Fig. 2B).

The observation of an identical distribution of the hybridization
signals indicates that the multiple transgene copies are expressed
faithfully, i.e., their transcription is not subject to strong position
effects resulting from the site(s) of their chromosomal integration.

The distribution of C1 inh mRNA was then examined in the
brain. Sections hybridized with mouse- or human-specific probes
revealed higher expression levels of the human RNA. However, no
qualitative difference was observed in the distribution of endoge-
 nous and of transgenic RNA (Fig. 2, C and D). Significant C1 inh
RNA expression was observed in the cortex and external capsule
of the forebrain. In the midbrain, the dentate gyrus was densely
packed with cells that express C1 inh mRNA, and the cortex was
also positive.

Control hybridizations with sense probes were always nega-
tive, as illustrated in Fig. 2E by the extremely weak signal of a
mouse sense probe hybridized on a transgenic mouse brain, which
is compared in the inset with the hybridization observed with a mouse antisense probe. Control hybridizations on spleen
or brain sections from nontransgenic mice with the human C1
inh antisense probe were also negative, as illustrated in Fig. 2F
using a human antisense C1 inh cRNA probe hybridized on a
brain section of a nontransgenic mouse, which yields a weak
and uniform background signal, compared with the specific sig-
nal observed on a similar section of a transgenic brain (shown
in the inset of F).

High power in situ hybridization on spleen, brain, and liver sec-
tions confirmed the codistribution observed for the endogenous
and the transgenic mRNA. In the liver, human C1 inh mRNA was

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**FIGURE 3.** Immunoperoxidase staining with hu-
man C1 inh Abs of sections from tissues of transgenic
mice. A, Liver, ×20 (inset, ×40); B, lung, ×10 (inset,
×20); C, kidney, ×10 (inset, ×40); D, heart, ×20; E,
brain, ×10; F, spleen, ×16. G, Lung control without
primary Ab, ×10; H, spleen control without primary
Ab, ×4. The inset in A represents a liver in situ hy-
bridization using a human C1 inh-specific RNA probe.
Note that the mRNA staining in this inset is not on hepa-
tocytes, but on cells that correspond morphologically to
the Kupffer cells revealed by immunohistochemistry.
 abundantly expressed in discrete cells that correspond in their morphology and distribution to Kupffer cells (Fig. 3, see the inset in A).

**Detection of human C1 inh protein in transgenic mouse tissues**

The observation of concordant expression, at the mRNA level, of the human transgene and of the endogenous gene in spleen and brain prompted us to study the cell type distribution of C1 inh in these and other tissues, using immunohistochemical methods. To reveal human C1 inh, we used a rabbit polyclonal Ab, which does not cross-react with mouse C1 inh (35).

In the liver sections (Fig. 3A), human C1 inh was abundantly detected in discrete cells that correspond to the morphology and the distribution of Kupffer cells, whereas hepatocytes as well as the vessels within the portal tract and the centrilobular veins were negative. High power in situ hybridizations with mouse C1 inh cRNA probes on liver sections (inset in Fig. 3A) revealed a similar discrete pattern, consistent with predominant expression in Kupffer cells. The sinusoids showed very weak staining; their lining cells being negative, the weak signal in the lumen was interpreted as being due to the presence of residual circulating protein.

Lung sections (Fig. 3B) revealed abundant and homogeneous human C1 inh immunostaining of the bronchiolar submucosal connective layer and of the apex of the epithelial cells. The alveolar septa stained heterogeneously: some type II pneumocytes (see inset) were positive as well as the capillaries. The connective tissue of the tunica adventitia in the vessels and the tunica adventitia of the pulmonary arterioles stained positively. These observations are consistent with our previous finding of relatively high expression of C1 inh mRNA in the lung of normal mice (35).

The renal cortex (Fig. 3C) contained abundant C1 inh-positive areas in the renal corpuscles and blood vessels, mainly localized to the lumen of the glomerular capillary network, while the capillary endothelial cells and the capillary basement membrane were negative (see inset). The periglomerular connective tissue was positive. The endothelial cells of the capillary network that surrounds the renal tubules stained positively, whereas the renal tubules were negative.

In the myocardium (Fig. 3D), the interstitial connective tissue and the tunica adventitia of the arterioles were positive.

In the brain sections (Fig. 3E), the macrophages and the endothelial cells of the choroid plexus capillaries showed strong staining for human C1 inh, whereas all other cell types were below levels of detection, in apparent contrast with the strong expression of C1 inh RNA in several areas of the brain.

In the spleen (Fig. 3F), the marginal zone was often positive. Moreover, clusters of C1 inh-positive cells were often seen in some follicles (discussed below).

Controls without the first Ab (shown for lung and spleen in G and H) were always negative.

C1 inh was localized in parallel studies performed on normal BALB/c mice using an anti-mouse C1 inh Ab that we have produced against an N-terminal peptide (aa 15–36) (35). Best results were obtained with this Ab using cryostat sections. No qualitative difference could be seen compared with the pattern of expression resulting from the human transgene (data not shown).

**Colocalization of C1 inh with a marker of FDC in the white pulp of the spleen**

To identify the C1 inh-expressing cells in the spleen, serial cryostat sections were treated with Abs that are markers of cells of the macrophage lineage or of FDC, or of marginal sinus endothelial cells, respectively (Fig. 4). Interestingly, C1 inh-expressing cells were found clustered in the follicles (Fig. 4A), with the same distribution as FDC-M1, reactive with FDC (Fig. 4B). This observation indicates that FDC express C1 inh (see, in C and D, the enlarged views of the relevant areas of the sections shown in A and B, respectively).

C1 inh-positive cells are frequently and randomly spread within the red pulp (Fig. 4E). In this area, the distribution of F4/80-positive cells of the macrophage lineage is different (Fig. 4F), suggesting that other cells, such as endothelial cells or fibroblasts, may express C1 inh. Moreover, C1 inh staining was not colocalized with either group of macrophages of the marginal zone that are recognized by the SER-4 or the MOMA-2 Abs, respectively (data not shown).

**Discussion**

In contrast to the numerous reports on C1 inh biosynthesis in cultured cells and cell lines, limited and sometimes conflicting data are available on the biosynthesis of C1 inh in vivo. Sequence comparisons of regulatory elements between the human and the mouse C1 inh genes suggest that the cell type specificity of expression and the responses to cytokines, in particular IFN-γ, should be similar (34, 35) (our unpublished data).

The human CINH minigene used in this study to produce transgenic mice (Fig. 1) contains all in vitro assigned regulatory elements in the promoter region as well as in the first intron (33, 34, 47). For this reason, 1.18 kb of upstream sequences and intron 1 were retained in the minigene, as well as intron 2 and ~2.5 kb of 3' flanking sequences. The results of immunohistochemical and in situ hybridization studies showed a remarkable fidelity of the in vivo expression from the human minigene construct, indicating that the transgene carried all essential control elements. Moreover, its expression did not appear to be affected by position effects due to different integration sites of the clusters containing multiple copies of the human minigene.
The high copy number of the transgenic lines selected for this study resulted in high mRNA and protein expression. We took advantage of these high levels of specific expression to define the distribution of C1 inh protein in several organs (i.e., liver, lung, kidney, heart, brain, and spleen). Immunohistochemical studies conducted on normal BALB/c mice using an anti-mouse C1 inh Ab raised against an N-terminal peptide (35) yielded a qualitatively similar distribution.

The concordant expression of the human transgene and of the endogenous C1inh gene probably reflects the high level of conservation of promoter and regulatory sequences in their 5′ regions. Although the available mouse sequence (accession AF052039) extends only up to −750 bp from the transcription start site (35), thus preventing an estimate of the extent of overall sequence conservation in the more remote 5′ flanking regions, a stretch of 147 nt of the human sequence starting at position −141 and overlapping the transcription initiation site contains several key regulatory elements (33) and reveals a very high degree of sequence conservation. Specifically: 1) the IFN-γ-activated sequence mapped at position −126 to −118 in the human gene (34, 35) is conserved; 2) the sequence CGGAATTCGCTTGGCAGTG between −94 and −73, which contains an hepatocyte nuclear factor-1 binding site and a stimulating factor (Sp-1) site (33), has only two nucleotide differences (positions underlined); 3) the CAAT box at position −62 to −59 and the initiator site at position −3 to +5 are both conserved; and 4) a shorter version of the polyurine poly-pyrimidine segment at position −48 to −17 (33) is found in the mouse sequence. Moreover, most of intron 1 reveals a high degree of sequence conservation, particularly the stretch between positions 355 and 410 of the human sequence, which contains two intrinsic IFN-γ-activated sequence elements (35) that we have characterized functionally (our unpublished data). The inclusion of all the above elements in the construct used in this study and the presence of rather long 5′ and 3′ flanking sequences of the human C1INH gene (1.18 and 2.5 kb, respectively) are probably the key elements that explain the high specificity of expression of the human transgene.

The results of these studies confirm the previously reported strong expression of C1 inh by Kupffer cells in the liver of rodents (17). Combined evidence, based on in situ hybridization and immunohistochemistry, indicates that also in normal mice C1 inh expression in hepatocytes is below level of detection. Our immunohistochemical data are consistent with the previously observed high levels of mRNA expression in lung, kidney, and spleen (35). In contrast, the expression of the C1 inh protein in the brain does not seem to follow the relatively strong expression observed at the RNA level. Although the reasons for this discrepancy remain to be elucidated, this observation suggests that C1 inh expression may be regulated also at a posttranscriptional level in several areas of the brain.

Human C1 inh was detected abundantly in the red pulp of the spleen, but did not seem to colocalize completely in this area with the distribution of F4/80-positive macrophages. C1 inh was not expressed by two populations of macrophages of the marginal zone, defined by the MOMA-2 and by the SER-4 Abs. Interestingly, distinct C1 inh staining was observed in patches of the white zone that contain cells recognized by the FDC-M1 Ab, which is a marker commonly used for FDC (32, 44). These data indicate that FDC (or at least a subpopulation thereof) are able to produce not only C1q (31), but also C1 inh. Additional in situ hybridization studies using C1r- and C1s-specific probes also suggest colocalization of the biosynthesis of all components of the macromolecular C1 complex in several tissues, including the spleen (data not shown). Local activation of the classical pathway of complement, under the control of C1 inh, may play an important role in the maintenance of Ag on FDC or its presentation to primed B lymphocytes, or in triggering the subsequent steps of adaptive immunity. It remains to be seen which of these functions require full activation of the classical pathway of complement.

The broad distribution of tissues and cell types expressing C1 inh probably reflects the important role of local activation of the classical pathway of complement and of its control. The transgenic mice described in this work provide a model system for specific expression at high levels of human C1 inh in vivo and should be particularly useful to study the evolution of local inflammatory responses. Moreover, similar constructs, carrying the promoter and regulatory sequences described in this work, may be used to produce transgenic mice for faithful expression in vivo of any C1 inh variant or to reproduce for any protein of interest the specificity in time and space of C1 inh expression.

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