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Identification of secreted CD155 isoforms☆

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Abstract

The *CD155* gene is a member of the immunoglobulin superfamily. We first demonstrate the existence of soluble CD155 (sCD155) isoforms in culture medium conditioned by CD155-expressing cells, in human serum and in cerebrospinal fluid. sCD155 concentration was measured in human serum and cerebrospinal fluid using a specific ELISA. Analysis of conditioned media indicated that sCD155 release does not require protease activity. In order to determine which tissues are responsible for sCD155 expression, we have quantified CD155 mRNAs in human normal tissues. The highest expression was observed in liver. The CD155α transcript is the most abundant and the proportion of the CD155β and CD155γ variants was similar between the tissues. Finally, serum purified sCD155 reduces poliovirus entry mediated by membrane-bound CD155. The high level of CD155 synthesis in many tissues and the presence of sCD155 in biological fluids suggest the existence of an important role for the protein in cellular function.

Keywords: CD155; Poliovirus receptor; Immunoglobulin superfamilly; Soluble forms; Splicing

The CD155 protein (or human poliovirus receptor, PVR) is a highly glycosylated ~70-kDa type Ia single pass transmembrane cell surface protein. The *CD155* gene was isolated based on its ability to mediate the attachment of poliovirus to host cells [1]. The CD155 protein is a member of the immunoglobulin superfamily. Its extracellular region contains three immunoglobulin-like domains: an outermost V-like domain followed by two C2-like domains [1]. The *CD155* gene belongs to a subgroup of the immunoglobulin superfamily sharing the general extracellular structure V-C2-C2. This subgroup includes murine and monkey homologues of *CD155* [2,3], the rat *Tage4* gene, identified as a tumor antigen overexpressed in rat colon carcinomas [4,5] and also as the rat homologue of *CD155* [6]. Four additional members first named Poliovirus Receptor Related (PRR), then nectin 1, 2, 3, and 4 [7–9], also belong to this subgroup of molecules.

Members of the immunoglobulin superfamily have a diversity of functions, but in most cases the common denominator is of a recognition role at the cell surface. Most of these proteins are involved in cell–cell interaction during normal or pathological processes. A number of these membrane proteins have soluble forms that are released into the extracellular space. Most soluble proteins are generated by proteolytic cleavage at the cell surface by "sheddases," matrix metalloproteinases or members of the ADAM (a desintegrin and metalloproteinase) family. The cleavage occurs close to the transmembrane domain releasing a nearly intact extracellular domain with binding activity. Ectodomain shedding has been reported for a number of members of the immunoglobulin superfamily including, for instance, the vascular cell membrane adhesion molecule VCAM-1/

☆ *Abbreviations*: PVR, poliovirus receptor; PRR, Poliovirus Receptor Related; ADAM, a desintegrin and metalloproteinase; VCAM-1, vascular cell membrane adhesion molecule; ICAM-1, intercellular adhesion molecule; CEA, carcinoembryonic antigen; ELISA, enzyme-linked immunosorbent assay; KIM-1, kidney injury molecule-1.

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CD106 [10], the intercellular adhesion molecule ICAM-1/CD54 [11], and nectin-1α [12]. An alternate possibility is anchoring to the plasma membrane via a glycosyl phosphatidylinositol linkage. The carcinoembryonic antigen (CEA) and the axonal glycoprotein TAG-1 share this characteristic [13,14]. These lipid-linked CAMs are released into the extracellular environment by phosphatidylinositol-specific phospholipase C. Finally, a few soluble proteins belonging to the immunoglobulin superfamily are produced by the translation of alternatively spliced transcripts. This has been shown, for instance, for the pregnancy-specific glycoproteins [15] and CEACAM1, a member of the CEA family [16].

Four isoforms of CD155 mRNA have been described that result from alternate splicing [17]. Variants CD155α and CD155δ code for membrane-bound isoforms that are both able to bind poliovirus [1]. By contrast, the exon organization of both CD155β and CD155γ predicts soluble proteins with the complete extracellular portion, lacking the transmembrane part and having C-termini identical to that of CD155α [17].

In this manuscript, we demonstrate for the first time the existence of soluble CD155 isoforms in vitro in the culture medium of cells but also in vivo in human serum and cerebrospinal fluid. These secreted isoforms were quantified by ELISA, and purified sCD155 was shown to compete with cell-bound CD155 for the binding of poliovirus to host cells.

Materials and methods

Cell culture. SW620 and SW48 human colon carcinoma cell lines were grown in RPMI-1640 containing L-glutamine and sodium bicarbonate, supplemented with 10% fetal calf serum, penicillin (100 U/ml), and streptomycin (0.1 mg/ml). Cells were maintained at 37 °C in humidified atmosphere of 5% carbon dioxide and passaged twice a week. The HT29-Cl.16E cells were grown in DMEM (4.5 g/l glucose) supplemented with 10% heat-inactivated fetal calf serum under standard conditions.

Western blot analysis. Proteins were precipitated with trichloroacetic acid (20% w/v) overnight at 4 °C. After centrifugation and washing with −20 °C acetone, proteins were dissolved in Laemmli's sample buffer. Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using 10% NuPAGE gels (Invitrogen, Cergy Pontoise, France). Proteins were then transferred to PVDF membranes. Filters were saturated for 30 min with non-fat dried milk (3% w/v) in Tris-buffered saline (TBS) and subsequently incubated with 5H5 hybridoma culture supernatant diluted 1:5 in TBS supplemented with 1% non-fat dried milk for 1 h under continuous stirring at room temperature. After exhaustive rinsing with TBS containing 0.5% Tween 20, membranes were incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma, St. Quentin Fallavier, France) in TBS supplemented with 1% non-fat dried milk for 1 h. After washing with TBS containing 0.5% Tween 20, the protein bands were detected using a Storm system following incubation with the ECF fluorescent substrate (Amersham Biosciences, Orsay, France). SeeBlue pre-stained molecular weight markers (Invitrogen) were used as standards.

5H5 and P44 hybridomas [17] were kindly provided by A. Nomoto (Institute of Medical Science, University of Tokyo, Japan). Antibody D171 [18] was purchased from Neomarkers (Labvision, Fremont, CA).

Purification of soluble CD155 from human serum. The soluble CD155 was purified from human plasma using Q-Sepharose Fast Flow anion exchange chromatography (Amersham) and D171-Sepharose 4B immunoaffinity chromatography. Serum (200 ml) was diluted fivefold with buffer A (phosphate buffer 10 mM, pH 6.5). The Q-Sepharose ion exchanger column (200 ml) was equilibrated in buffer A. The diluted plasma was applied to the column at a flow rate of 1.4 ml/min. The column was then washed with 600 ml buffer A. Bound proteins were then eluted with 250 ml buffer B (phosphate buffer 10 mM, NaCl 0.5 M, and EDTA 1 mM, pH 6.5). The eluate (50 ml) was applied to P44-Sepharose 4B immunoaffinity column (3 ml) equilibrated in buffer B, at a flow rate of 0.1 ml/min. The affinity column was washed with 10 ml buffer B. Immunoaffinity-purified CD155 was eluted from the column with 0.1 M glycine (pH 3.0) and extensively dialyzed against PBS (phosphate buffered saline). The protein concentration was determined by using the bicinchoninic acid protein assay (BCA) reagent (Pierce, Rockford, IL, USA). The final preparations contained ~30% of CD155 as determined by densitometric analysis of stained SDS-gels. From 200 ml serum we obtained ~3 μg of purified sCD155.

Enzyme-linked immunosorbent assay for sCD155. Except for immobilization of the first antibody to a multiwell plate, all the procedures for ELISA were carried out at room temperature. A 96-well polycarbonate plate for ELISA was coated with 100 ng of the purified anti-human CD155 monoclonal antibody D171 in 100 μl of 50 mM carbonate–bicarbonate (pH 9.6) buffer, by physical adsorption at 4 °C overnight. Potential remaining adsorption sites were blocked by incubation for 1 h with PBS containing 3% skimmed dried milk. After elimination of the blocking solution, 100 μl of calibrators or samples diluted in PBS containing 1% milk was added to each well in duplicate. The plate was incubated for 1 h to allow immunologic reaction. After washing three times with PBS containing 0.05% Tween 20, 100 μl of the P44-HRP conjugate (1 mg/ml in PBS containing 1% milk) was added and the plate was incubated further for 30 min. After washing three times with PBS containing 0.05% Tween 20, 100 μl of tetramethylbenzidine (TMB) substrate solution was added. After incubating for 10 min, the color development was stopped with 100 μl of 0.5 M sulfuric acid. The absorbance at 450 nm was measured with an EL800 microplate reader (Bio-Tek, Fisher Bioblock Scientific, Illkirch, France).

The ectodomain of CD155, produced as soluble protein in the 293-T human epithelial kidney cell line [19], was used as a standard. Serum was obtained from healthy blood donors and cerebrospinal fluids were collected in the emergency unit of our hospital.

Real-time quantitative PCR. The amplification conditions of the CD155 template were optimized for the RotorGene 2000 instrument (Ozyme, Saint Quentin en Yvelines, F). PCR amplifications were performed using Titanium *Taq* DNA polymerase (Clontech, Palo Alto, CA, USA). The reaction mixture contained 2 μl of the supplied 10× Titanium *Taq* PCR buffer (containing magnesium chloride), 2 μl of a 1/1000 dilution of SYBR Green I (Roche Molecular Biochemicals), 1 μl of each primer (0.4 μM each), 0.4 μl of Titanium *Taq* DNA polymerase, 0.5 μl dNTPs (10 mM each), and PCR-grade water to a volume of 15 μl. Microtubes (0.2 ml) were loaded with 15 μl of this master mix and 5 μl of the template (commercially available first-strand human cDNA diluted 1/10) and the run was initiated. The cycling conditions were as follows: denaturation for 5 min at 95 °C; amplification for 35 cycles, with denaturation for 5 s at 95 °C, annealing for 15 s at 63 °C, and extension for 20 s at 72 °C. To exclude primer–dimer artifacts, fluorescence was measured at a temperature (86 °C) above the melting point of primer–dimers and below the melting point of the specific PCR product (90 °C).

Primers C1 (5′-TGGACGGCAAGAATGTGACC-3′) and C2 (5′-ATCATAGCCAGAGATGGATACC-3′) were designed from the published sequence of the human *CD155* gene [17]. They were selected for binding to separate exons (Fig. 1) to avoid false positive results arising from amplification of contaminating genomic DNA.

An external standard curve was generated with serial 10-fold dilutions of a plasmid (pGEMT easy, Promega) containing a fragment of the CD155 cDNA. The reference curve was constructed by plotting the relative amounts of these dilutions vs. the corresponding C_t (threshold cycle) values. The correlation coefficient of these curves was always greater than 0.99. The amount of CD155 transcript was calculated from these standard curves using the RotorGene software. A relative unit of 100 was assigned for the point of the standard curve containing 100 fg of plasmid DNA. Samples were tested in triplicate and the average values were used for quantification. The human cDNA preparations used have been carefully normalized to the mRNA expression levels of four housekeeping genes: α-tubulin, β-actin, G3PDH, and phospholipase A2 (Clontech).

After completion of the cycling process, samples were subjected to a temperature ramp from 68 to 99 °C, with continuous fluorescence monitoring for melting curve analysis. For each amplification a single narrow peak was obtained at the expected melting temperature (90 °C), indicating specific amplification without significant byproducts.

Qualitative analysis of CD155 expression. For analysis of CD155 splicing variants, amplifications were performed with 3 μl cDNA in a total volume of 50 μl containing 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.2 mg/ml gelatin, 200 μM of each dNTP, 100 pmol of primers CD5 (5′-TCCTGTGGACAAA CCAATCAACAC-3′) and CD15 (5′-GAGGCGCTGGCATGCTC TGT-3′), and 2.5 U *Taq* DNA polymerase (Promega). PCR amplifications were carried out for 35 cycles (30 s at 92 °C; 30 s at 60 °C; and 1 min at 72 °C) followed by a 3 min final extension at 72 °C. Amplified fragments were visualized after electrophoresis on 10% polyacrylamide gel electrophoresis and stained with Gelstar (BioWhittaker Molecular Applications). Stained gels were then scanned with a STORM 860 Fluorimager system (Amersham Pharmacia Biotech) and analyzed with the ImageQuant Image Analysis software. For each reaction, the intensity of the bands was quantified and divided by its length (bp). The relative ratio of each band was expressed as a percentage of the overall expression. All samples were analyzed at least three times.

Virus neutralization assays. These assays were performed as described previously [19]. Briefly, 140 pfu of poliovirus type I Mahoney was incubated alone or with different concentrations of recombinant sCD155 and different concentrations of sCD155 purified from serum in PBS containing 0.02% bovine calf serum for 30 min at 25 °C followed by 1 h at 37 °C. The virus titer was then determined by plaque reduction assay on HeLa cell monolayers.

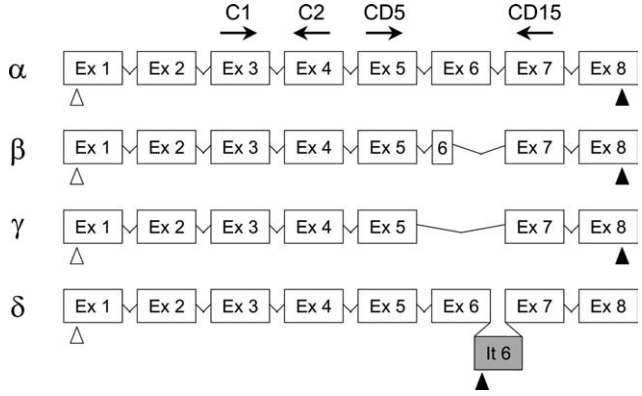

Fig. 1. Schematic representation of the different *CD155* transcript variants. Positions of the oligonucleotide primers used for RT-PCR amplifications. Oligonucleotides primers CD1 and CD2, used to quantify expression of CD155 (yielding a 116-bp fragment), are located in a region corresponding to the extracellular domain of the protein that is conserved in all splice variants. Oligonucleotide primers CD5 and CD15, used for qualitative analysis of CD155 expression, are located on each side of the different splice sites. Open triangles indicate the initiation codon. Closed triangles indicate stop codons.

Results

Soluble forms of CD155 are present in conditioned culture medium

The sequences of the splice variants CD155β and CD155γ predict soluble forms of CD155. To explore the possible existence of secreted CD155 isoforms, we first examined conditioned cell culture medium of colonic cancer cell lines. We have previously shown that the *CD155* gene is overexpressed in colorectal cancer, and that CD155β and CD155γ transcripts are present in colon cancer cell lines [20 and unpublished data]. Soluble CD155 was immunoprecipitated with Sepharose-conjugated anti-CD155 monoclonal antibody P44. Immune complexes were resolved on SDS–PAGE under reducing conditions. CD155 was visualized with monoclonal antibody 5H5.

CD155 was detected in immunoprecipitates from conditioned cell culture medium of all colonic cell lines (Fig. 2A). The soluble CD155 proteins were smaller than the cell-associated form and migrated at about 65 kDa, more slowly than the recombinant sCD155 which comprises only the extracellular domain. The diffuse aspect of the bands can be explained by the existence of the different isoforms and by their high carbohydrate content. Fresh control media did not yield signals, indicating that the detected proteins were of cellular origin and not derived from the fetal calf serum present in the media.

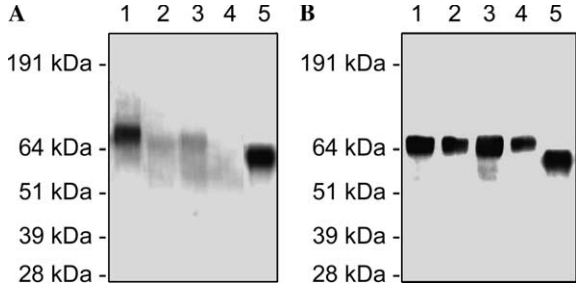

Fig. 2. Detection of soluble CD155 in vitro and in vivo. (A) Detection of soluble CD155 in cell culture supernatant. CD155 was immunoprecipitated with Sepharose-conjugated P44 from cell culture supernatant conditioned by SW620 (2) and SW48 (3) cells or from fresh medium (4). Proteins extracted from SW620 cells (1) and recombinant sCD155 (5) were used as controls for immunoblotting. Samples were immunoblotted with monoclonal antibody 5H5. (B) Detection of sCD155 in human serum. Serum was collected from healthy donors (1–4). Recombinant sCD155 was used as control (5) Samples were immunoprecipitated and immunoblotted as described in Materials and methods. Positions of molecular weight markers are indicated on the left side.

Analysis of culture medium from gastric, breast or prostate cancer cell lines, as well as melanoma cell lines, also revealed the presence of sCD155, indicating that sCD155 release is not restricted to colonic cancer cells (not shown).

Soluble forms of CD155 also exist in vivo in human biological fluids

To confirm these results in vivo, human serum was collected from healthy donors and analyzed using the same method. As shown in Fig. 2B soluble CD155 proteins were also present in human serum.

Soluble CD155 was purified from human serum and used to select monoclonal antibodies that could be used to develop an enzyme-linked immunosorbent assay. Competitive binding assays using different antibodies on purified sCD155 were realized by real-time biospecific interaction analysis based on the surface plasmon resonance technology. Pair-wise binding experiments revealed that antibodies D171 and P44 bound simultaneously to sCD155, indicating that they are directed toward independent epitopes of sCD155 (data not shown). We therefore used these two antibodies to design a sandwich ELISA for sCD155 concentration measurements. Antibody D171 was used as capture antibody and HRP-conjugated P44 as tracer antibody.

This ELISA was first used to determine sCD155 concentration in human serum and cerebrospinal fluid (Table 1). By comparison with a standard curve generated using human recombinant sCD155, we determined sCD155 concentration in 58 samples of human serum and in 21 samples of cerebrospinal fluid. In human serum the median value of sCD155 is 65.5 ng/ml and in cerebrospinal fluid sCD155 concentration is 1.9 ng/ml. Assuming a CD155 mass weight of 65 kDa, the molar concentration of sCD155 in human serum and cerebrospinal fluid was 1.04 and 0.03 nM, respectively. Preliminary experiments also revealed the presence of sCD155 in human urine (not shown).

sCD155 is secreted by cells in culture

To further study the release of sCD155 by cells in culture, time course experiments were performed. Culture medium of HT29-Cl.16E cells was collected at different time points and the amount of sCD155 released was measured by ELISA. As shown by Fig. 3A, significant amounts of sCD155 were detected after 4 h and increased throughout the culture period (up to 5.5 ng/ml for 3×10^6 cells at 72 h).

To determine whether proteolysis is involved in the release of sCD155, we investigated its sensitivity to protease inhibitors. The effect of various protease inhibitors with multiple specificities on sCD155 release was tested. All inhibitors were used at concentrations that are non-toxic and known to inhibit the appropriate class of protease [10]. The inhibitors specific for aspartic (pepstatin A), serine (TLCK) or cysteine (antipain) proteases did not show any significant inhibitory effect at the concentrations tested whereas the serine protease inhibitor TPCK showed a slight inhibition ($14 \pm 6\%$) (Fig. 3B). By contrast, 1,10-phenanthroline, a potent inhibitor of metalloproteases, significantly inhibited the release of sCD155 ($26 \pm 9\%$ inhibition), indicating that

Table 1
sCD155 concentrations in biological fluids

	n	Means ± SD	Range	Median
Serum	58	67.9 ± 19.6 ng/ml 1.04 ± 0.30 nM[a]	10.8–113.3 ng/ml 0.16–1.74 nM	65.5 ng/ml 1.01 nM
CSF	21	1.9 ± 0.9 ng/ml 0.03 ± 0.01 nM	0.06–4.2 ng/ml 0.001–0.06 nM	1.9 ng/ml 0.03 nM

[a] Calculated assuming an apparent molecular weight of 65,000.

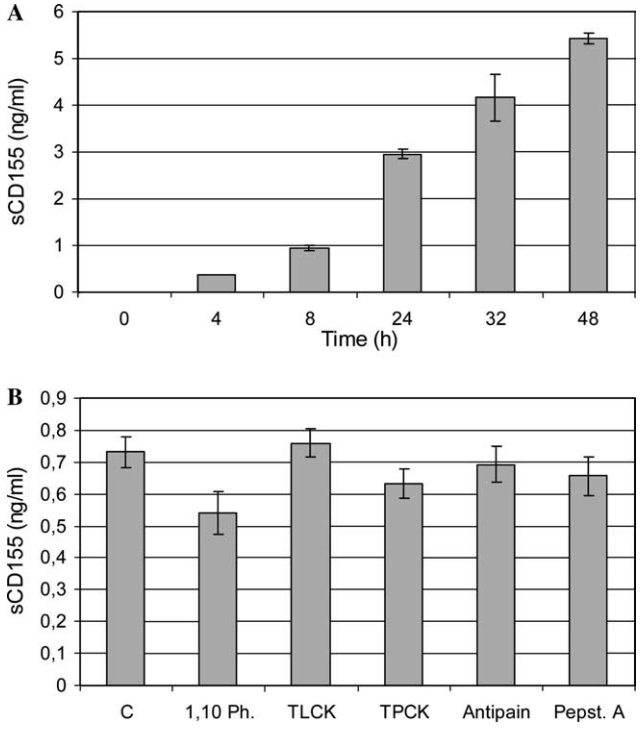

Fig. 3. Expression of sCD155 in the conditioned medium of a human colonic cell line. (A) Kinetics of sCD155 release in culture medium. HT29-Cl.16E cells were seeded at a density of 10^6 cells per well in 12-well plates and grown until post-confluency (1 week). Fresh medium (0.5 ml/well) was then added, and conditioned medium was collected at the indicated time points and processed for sCD155 measurement by ELISA as described in Materials and methods. The data are shown as means of triplicate tests from duplicate wells. (B) Effect of protease inhibitors on sCD155 release. Cells were seeded as described above. Cells were incubated for 6 h with the culture medium of control cells (c), or in this medium supplemented with 5 mM of 1,10-phenanthroline (1,10 Ph.), 100 μM TLCK (TLCK), 50 μM TPCK (TPCK), 1 mM antipain (Antipain) or 1 mM Pepstatin A (Pepst. A). The data are shown as means of triplicate tests from duplicate wells.

conversion of the membrane anchored to the soluble form of CD155 does occur in cell culture. However, its effect is limited. Therefore, we can conclude that release of the overwhelming majority of sCD155 most likely does not require protease activity.

Recently, Ohka et al. [21] have shown that in polarized epithelial cells there was a basolateral sorting of CD155α, the most abundant membrane-associated CD155 isoform. We analyzed culture medium conditioned by two clonal derivatives from the HT29 colonic cell line, grown on porous filters and cultured until full differentiation. Measurements of sCD155 in the medium collected at the apical side and at the basal side revealed that the majority of sCD155 is released in the apical compartment (data not shown). This also argues in favor of a secretion of soluble isoforms.

CD155 transcripts in human adult tissues

To identify the tissues that are the source of sCD155 in human serum, we analyzed normal human adult tissues by real-time RT-PCR. Amplifications were performed from cDNA of human normal adult tissues with primers C1 and C2. These oligonucleotides are located in a region corresponding to the extracellular domain of the protein, which is conserved in all splice variants (Fig. 1). Quantitative real-time PCR was performed using the double-stranded DNA binding dye SYBR Green I. CD155 mRNA levels were expressed in relative units (Fig. 4A). The highest expression (~1000 U) was found in human adult liver. Tissues such as pancreas, placenta, lung, brain, heart, and kidney exhibited a significant expression (200–400 U). In all the other

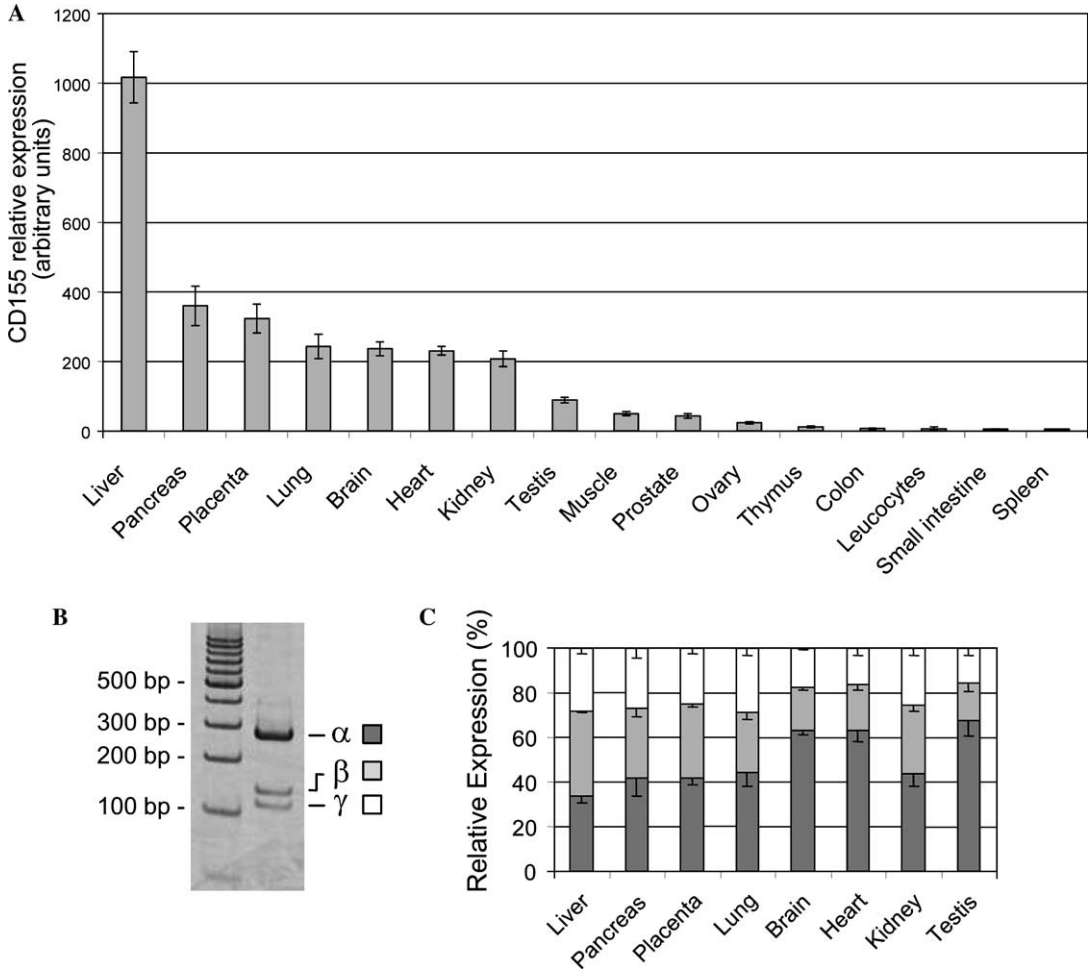

Fig. 4. RT-PCR analysis of CD155 expression in normal human tissues. (A) CD155 mRNA level was determined by real-time RT-PCR according to the calibration curve realized with a plasmid containing a part of the CD155 cDNA. A relative unit of 100 was assigned for the point of the standard curve containing 100 fg of plasmid DNA. Samples were tested in triplicate and the average values were used for quantification. (B,C) Qualitative analysis of CD155 expression. Relative expression of the CD155α, CD155β, and CD155γ variants was analyzed by qualitative RT-PCR. (B) Representative analysis showing the three major CD155 transcripts from human brain. (C) The relative ratio of the different amplicons, determined as described in Materials and methods, was evaluated for each reaction separately and expressed as a percentage of the overall expression in each sample. All reactions were performed at least three times and mean values ± SD are represented.

tissues analyzed, *CD155* gene was expressed to a lower degree.

The high expression of the CD155 gene in liver, lung, and kidney was confirmed by Western blot analyses and immunohistochemistry performed on frozen sections with monoclonal antibody D171 (not shown).

Tissue distribution of major CD155 transcripts

The difference of CD155 mRNA expression between the tested tissues led us to evaluate by qualitative PCR the relative expression of the three major splicing variants CD155α, CD155β, and CD155γ in the different normal adult tissues. This strategy revealed the three expected products of 247 bp (CD155α), 139 bp (CD155β), and 115 bp (CD155γ), thus distinguishing all three main transcripts within a single PCR (Fig. 4B). Variant δ was not detected in these experiments. The results indicated that all the tissues screened expressed the three mRNA isoforms. In most cases, CD155α is the most abundant transcript. Variants β and γ correspond to 40–60% of the CD155 transcripts (Fig. 4C).

Therefore, because (a) the liver is a large organ, (b) it presents the highest level of CD155 transcript, and (c) approximately 2/3 of these transcripts encode soluble proteins, we conclude that liver is probably the main source of sCD155 in human serum.

Function of sCD155

CD155 is able to mediate poliovirus binding to host cells. Therefore, we assayed sCD155 purified from serum for its ability to compete with cell-bound CD155 for the binding of poliovirus to HeLa cells. Plaque reduction assays were used to determine the efficiency of neutralization of poliovirus by sCD155. Its activity was compared to that of human recombinant sCD155 (Fig. 5). Viral infectivity was significantly reduced with 10 nM of recombinant sCD155 and this effect increased with sCD155 concentration. Similar inhibition was observed when sCD155 purified from human serum was used, indicating that purified sCD155 can compete with cell-bound CD155 and reduce poliovirus entry. These data demonstrate that serum sCD155 is functional in terms of its interaction with poliovirus.

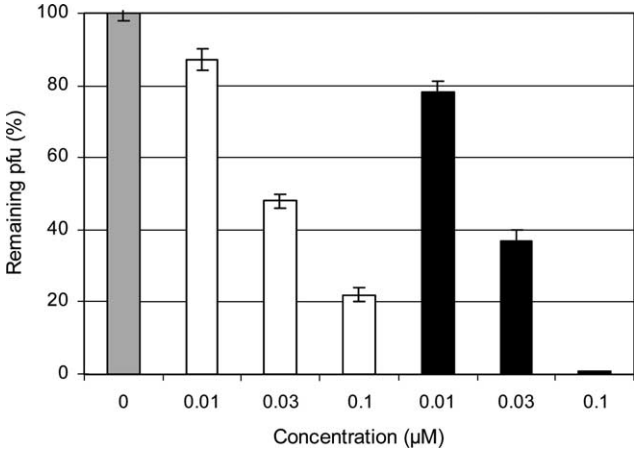

Fig. 5. Neutralization of poliovirus infectivity by recombinant sCD155. Poliovirus type 1 Mahoney (140 pfu) was incubated alone (hatched bar), with recombinant sCD155 produced in cultured cells (white bars), or with sCD155 purified from human serum (black bars) in PBS containing 0.02% bovine calf serum for 30 min at 25 °C followed by 1 h at 37 °C. The virus titer was then determined by plaque assay on HeLa cell monolayers. The experiment was performed in triplicate, and the average of the results is shown as a percentage of the results obtained in the absence of sCD155.

Discussion

Human poliovirus infections are mostly targeted to the gastrointestinal tract through binding to the transmembrane protein coded by the *CD155* gene [1]. At a low frequency, poliovirus invades the central nervous system where it destroys motor neurons thereby causing poliomyelitis. The cellular role of CD155 is unknown.

The results of early studies led to the prediction that the narrow tissue tropism of poliovirus would mirror the distribution of *CD155* expression in human tissues. However, Northern blot analyses have indicated that *CD155* mRNAs can be detected both in human susceptible tissues (brain, different structures of the cortex) and in non-susceptible tissues such as kidney, lung, and placenta [1,19]. Western immunoblotting analyses have also shown the presence of CD155 in non-susceptible tissues [22], but the anti-CD155 polyclonal antisera used for the cell surface detection were then found to react with CD155-negative L cells [23]. By using real-time RT-PCR, we showed an expression of CD155 mRNA in all tissues tested with a particularly high expression in liver, and also in pancreas and lung. None of these tissues are poliovirus replication sites. A similar pattern was observed in CD155 transgenic mouse in which many organs, including those that do not support poliovirus replication, produce significant levels of CD155 mRNA [24].

CD155 is the prototype of a new family of human receptors that belong to the immunoglobulin superfamily. This family includes nectins which are a growing group of adhesion molecules involved in cell-to-cell adhesion process and that serve as receptors for herpes simplex virus entry [25,26]. Nectin 1, 2, and 3 have been shown to be homotypic adhesion molecules recruited to cadherin-based cell–cell adherens junctions through their interaction with afadin, a F-actin binding protein [27,28]. The recently identified nectin 4 also interacts with afadin at cadherin-based cell–cell adherens junctions but does not act as a receptor for alpha herpes virus [8]. Because other members of the CD155-related

gene family are cell adhesion molecules, it is likely that the functions of CD155 are similar.

Several members of the immunoglobulin family can serve as pathogen receptors in addition to binding a cellular ligand. For example, ICAM-1 is the major surface receptor for rhinoviruses [29], but its native ligands are LFA-1 or Mac-1. Various pathogens like *Neisseria meningitidis* and *Haemophilus influenzae* utilize CEACAM1 (formerly biliary glycoprotein BGP) as a receptor for adherence to and invasion of host cells [30]. The physiological ligand for the extracellular domain of CEACAM1 has been proposed to be CEACAM1 itself [16]. A splicing variant of kidney injury molecule-1 (KIM-1), a type I transmembrane glycoprotein expressed on dedifferentiated renal proximal tubule epithelial cells undergoing regeneration after injury has recently been shown to be identical to the hepatitis A virus cell receptor 1. Bailly et al. [31] recently proposed that KIM-1 could bind to one or several integrins.

CAMs of the immunoglobulin superfamily act via homophilic binding, but they also bind in a heterophilic manner to other CAMs and to extracellular matrix proteins. Recently, Lange et al. [32] reported that CD155 binds specifically to vitronectin, an important component of the extracellular matrix. Vitronectin also constitutes a major serum protein and is expressed abundantly by liver cells. These data are very interesting insofar as we have shown that soluble CD155 is present in human serum and the highest level of CD155 transcription occurs in the liver. A CD155/vitronectin interaction may thus serve different functions depending on the distinct sites of their co localization.

Four CD155 isoforms have been described. The major transcripts CD155α, CD155β, and CD155γ were found to be expressed by various human tissues and no tissue-specific splicing was observed. Soluble isoforms, coded by the CD155β and CD155γ transcripts, were detected, purified, and quantitated in normal human serum by ELISA. We found that the concentration of sCD155 was about 1 nM and appeared to be functional in terms of interaction with poliovirus. Kaplan et al. [33] have shown that soluble CD155 produced in insect cells binds poliovirus and blocks infectivity. More recently, McDermott et al. [19] expressed and purified a soluble form of the poliovirus receptor from mammalian cells and showed that the soluble receptor binds poliovirus and neutralizes viral infectivity. Here we show that human sCD155, purified from human serum, is also able to prevent virions from interacting with membrane-bound CD155, reducing poliovirus entry and then its capacity to infect cells. Therefore, human sCD155 is biologically active, but its concentration in serum is too low to neutralize poliovirus. Consequently, humans are susceptible to poliovirus, despite the presence of sCD155 in serum.

Soluble receptors are part of an expanding class of regulatory molecules that are derived from the extracellular domains of integral plasma membrane proteins or generated by the secretion of alternatively spliced mRNA transcript. A function has been described for soluble isoforms of several members of the immunoglobulin family. For instance, the soluble form of ICAM-1 inhibits rhinovirus infection [34], while soluble CEACAM1 (sCEA) has been reported to induce angiogenesis [35] and to play a suppressive role in NK and TH1 cell functions in colorectal cancer [36]. Finally, during completion of this manuscript, Tanaka et al. demonstrated that the scatter factor/hepatocyte growth factor induces the ectodomain shedding of nectin-1α and proposed that this shedding is involved in the scatter factor-induced cell–cell dissociation [12].

The biological role of the different CD155 proteins remains unknown, and understanding their function and regulation are the focus of our current work.

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