Modulation of complement regulatory function and measles virus receptor function by the serine-threonine-rich domains of membrane cofactor protein (CD46)

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Three major membrane cofactor protein (MCP) phenotypes with different serine-threonine (ST)-rich regions, namely ST^{C} (L-phenotype), ST^{BC} (H or U phenotype) and ST^{ABC}, and the MCP without the ST domain (ΔST) were expressed on Chinese hamster ovary (CHO) cells by transfecting the respective cDNAs. The expressed molecules migrated with a larger molecular mass on SDS/PAGE than those expected from their amino acid sequences. *O*-Glycanase digestion showed that this was due to *O*-linked sugar chains. The apparent sugar contents in each ST segment were compatible with their serine and threonine contents in the ST regions. The functional properties of these phenotypes as inhibitors of human complement (C) and receptors of measles virus (MV) were compared. The classical pathway-dependent CHO cell lysis by human C was more effectively suppressed by the expressed ΔST and ST^{C} than by the ST^{ABC} and ST^{BC} phenotypes, although the difference was not so prominent. In contrast, alternative C pathway-dependent CHO-cell lysis was most effectively suppressed by the ST^{ABC} phenotype and was only slightly blocked by the ST-deleted mutant. MV infection occurred with all of the phenotypes, but the infectious dose required to cause the same level of syncytium formation was 100-times higher in large ST (ST^{ABC} and ST^{BC}) than in small ST (ST^{C} and ΔST) phenotypes. Thus, the ST domain serves as a functional modulator in MCP: MCP with a large ST domain having high *O*-linked sugar contents is favourable to the effective suppression of both the alternative C pathway-mediated cytolysis and MV infection, whereas MCP with a small ST domain is favourable to the suppression of the classical C pathway.

INTRODUCTION

Membrane cofactor protein (MCP) is a protein of 45–70 kDa belonging to a gene family known as the regulator of complement (C) activation (RCA) (reviewed by Liszewski et al., 1991; Law and Reid, 1988). The primary role of MCP in regulating the C system is to act as a cofactor of factor I, which proteolytically inactivates C3b and C4b, thus preventing the assembly of C3/C5 convertases on autologous tissues (Seya and Atkinson, 1989; Seya et al., 1991). In addition, recently, two groups have reported that MCP also serves as a receptor for measles virus (MV) (Naniche et al., 1993a; Dorig et al., 1993). A cDNA analysis of MCP has revealed that the extracellular portion of MCP is composed of three motifs; four repeating domains termed short consensus repeats (SCR), a serine-threonine (ST)-rich domain and a 13 amino acid segment of unknown significance (Lublin et al., 1988). Each of the four SCR is composed of about 60 amino acids and SCR2, SCR3 and SCR4 are thought to be important for the cofactor function (Adams et al., 1991). No report has suggested which domain of MCP is responsible for MV binding and infection, although the SCR portions appear to be important for MV binding.

Analysis of the genomic DNA of MCP has revealed that there are three similarly sized exons each encoding an ST domain of 14–15 amino acids, namely ST^{A}, ST^{B} and ST^{C} (Lublin et al., 1988; Post et al., 1991). PCR and nucleotide sequencing analyses have suggested the expression on human blood cells and tissues of variable quantities of the three primary isoforms, ST^{ABC}, ST^{BC} and ST^{C}, which are produced by alternative splicing (Purcell et al., 1991; Russell et al., 1992; Johnstone et al., 1993). The dominant phenotypes correlate with isoforms bearing ST^{BC} and ST^{C} (Post et al., 1991; Russell et al., 1992). In addition to the structural diversity in the ST domain, the heavy *O*-glycosylation of the ST region is the cause of two broad species of 45–70 kDa, hence their initial name, gp45–70 (Cole et al., 1985). However, the relative functional potency of these three MCP phenotypes as well as the contribution of the oligosaccharides in the ST region to the cofactor activity and MV receptor function remained to be elucidated. In addition, the reason why a variety of MCPs with different ST regions needs to be expressed in a genetically controlled and organ-specific manner, requires investigation.

In the present study, we assessed the participation of the ST region in MCP functions as a regulator of C activation and a receptor of MV using Chinese hamster ovary (CHO) cells expressing the three primary MCP phenotypes, ST^{ABC}, ST^{BC} and ST^{C}, and an ST-deletion mutant. We showed that the regulatory functions of MCP in the classical and alternative C pathways are differently modulated by the ST region, which is also a critical factor for MV infection.

MATERIALS AND METHODS

Cells, proteins, antibodies and reagents

Wild type CHO cells obtained from the American Type Culture

Abbreviations used: C, complement; CHO, Chinese hamster ovary; DAF, decay accelerating factor; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; GME, green monkey erythrocytes; GVB, gelatin-veronal buffer; MCP, membrane cofactor protein; MV, measles virus; MVH, measles virus haemagglutinin; NHS, normal human serum; PFU, plaque-forming unit; SCR, short consensus repeat; ST, serine-threonine; mAb, monoclonal antibody; RT, reverse transcriptase.

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Collection (Bethesda, MD, U.S.A.) were maintained in coated dishes (Nunc, Roskidle, Denmark) in Ham's F12 medium (Nissui Co., Tokyo) supplemented with 10% fetal calf serum (FCS). Cultures were maintained in a 5% CO_2/95% air atmosphere at 37 °C. PBS containing 0.02% (w/v) EDTA and 0.05% (w/v) trypsin was used to detach the cells. This concentration of trypsin did not affect MCP on CHO cells (Kojima et al., 1993).

Monoclonal antibodies (mAb) against MCP (M160 and M177) were produced and purified in our laboratory as described previously (Seya et al., 1990). Polyclonal antibodies against CHO cells were prepared from a rabbit immunized intravenously with CHO cells and purified with protein A-coupled Sepharose (Seya et al., 1990). Anti-ST^A and anti-ST^C peptide Abs were produced as previously reported (Hara et al., 1993). An mAb against MV haemagglutinin was prepared by Dr. S. Ueda (Osaka University, Osaka). Fluorescein isothiocyanate (FITC)-labelled anti-mouse IgG was a product of TAGO (Burlingame, CA, U.S.A.).

Normal human serum (NHS) pooled from ten healthy adults was used as a source of C. Human C3 and factor I were purified as described previously (Nagasawa and Stroud, 1977; Nagasawa et al., 1980). MV was a Nagahata and its modified strains, which underwent four passages into the hamster brain (Wong et al., 1991). Trypsin and kanamycin were purchased from Sigma (St. Louis, MO, U.S.A.). Restriction enzymes were from Takara Biomedicals (Japan).

Reverse transcriptase (RT) PCR

mRNA was isolated from 1.5×10^7 HeLa and HL-60 cells using a Quickprep mRNA purification kit (Pharmacia, Uppsala, Sweden). The ST-rich regions were reverse transcribed and PCR amplified using a Geneamp RNA PCR kit (Perkin-Elmer Cetus, Norwalk, CT, U.S.A.). The sequence of the 5′ ST-rich region-specific primer was GACAGTAACAGTACTTGG and that of the 3′ primer was TCCTTCCTCAGGTTTAGG. One microgram of mRNA was used for the RT-PCR, which was performed according to the manufacturer's protocol. The PCR products were ethanol precipitated, and analysed on a 2% agarose gel (Sambrok et al., 1988). The pUC18 fragments, digested with the *Hin*f1, were used as size markers.

Construction of plasmids

RT-PCR products obtained from the HL-60 mRNA were digested with *Bam*HI and *Eco*RV and separated by gel electrophoresis with 2% agarose. The bands corresponding to ST^{ABC} and ST^{BC} (160 bp and 115 bp, respectively) were extracted from the gel, then subcloned into the *Bam*HI/*Eco*RV sites of pCRII (Invitrogen, San Diego, CA). The nucleotide sequences of the subcloned fragments were confirmed by a gel sequencer. The pUC-MCP (full length cDNA of MCP ST^C ligated into the *Eco*RI site of pUC19) was then digested completely with *Xba*I and partially with *Eco*RV and isolated by agarose gel electrophoresis (Sambrook et al., 1988). The 360 bp fragment that contained the C-terminus of MCP was ligated into the *Eco*RV/*Xba*I sites of the $pCRST^{ABC}$ and $pCRST^{BC}$ plasmids, which were subsequently digested with *Bam*HI and *Kpn*I. These and the MCP 5′-fragment (prepared by digesting pUC-MCP completely with *Eco*RI and partially with *Bam*HI) were subcloned into the *Eco*RI/*Kpn*I sites of pUC18. Finally, these subclones and pUC-MCP were subcloned into the *Eco*RI/*Pst*I sites of pME18S, a eukaryotic expression vector (Takebe et al., 1988).

The MCP mutant with a deleted ST-rich region [amino acids 252–280 (Lublin et al., 1988)] was constructed using the T7-gen *In vitro* Mutagenesis kit (U.S.B., Cleveland, OH, U.S.A.) and the oligonucleotide, AGGTTTAGGATATCCTTTAAGACACTT-TGG which covered the region between the 3′-terminus of SCR4 and the 5′-end of the unknown region. Mutagenesis proceeded according to the manufacturer's protocol for this kit. The MCP ST^A type with deleted ST^B and ST^C portions of the ST^{ABC} phenotype was constructed using PCR. Oligonucleotides, GCT-TTGAGTCATTCAGGATATCCTAAACCTG (primer A) and CAGGTTTAGGATATCCTGAATGACTCAAAGC (primer B), 31-mers that covered between 3′-terminus of ST^A portion and 5′-terminus of the unknown region, were synthesized and used as amplification primers with the pUC-MCP ST^{ABC} (full length cDNA of MCP ST^{ABC} ligated into the *Eco*RI site of pUC19) as a template. First PCR was performed with M13 M4 primer-primer B and M13 RV primer-primer A, then the PCR products were separated by gel electrophoresis and extracted from the gel. Each PCR product was mixed and used for the second PCR template. The second PCR was performed with M13 M4-M13 RV primers, then the PCR product was digested completely with *Pst*I and partially with *Eco*RI, and isolated by gel electrophoresis. The nucleotide sequences of the products were confirmed by the autosequencer (Pharmacia Co., Sweden). The deletion mutants were subcloned into the *Eco*RI/*Pst*I sites of pME18S.

Expression of cDNA

These cDNAs were transfected into CHO cells by calcium phosphate precipitation (Sambrook et al., 1988) with 20 μg cDNA and 1 μg pLTR*neo* (a neomycin-resistance gene vector). Transfected CHO cells were maintained for 24 h in Ham's F12 medium/10% FCS/0.06% kanamycin, in an atmosphere of humidified 5% CO_2/95% air at 37 °C. The cells were selected in the same medium containing 0.5 mg/ml of G418 (GIBCO, Grand Island, NY, U.S.A.). G418-resistant colonies were isolated with cloning cylinders and expanded in tissue-culture plates. Expression of MCP was confirmed by flow cytometry as described below.

Flow cytometry

Transfected cells (1×10^6) were incubated with 1 μg of murine mAb to MCP for 30 min at 4 °C. The stained cells were analysed using an EPICS-CS flow cytometer to assess the surface expression of MCP. CHO cells transfected with expression vectors pME18S and PLTR*neo* were used as controls.

Western blotting

MCP was solubilized from the transfectants as described (Seya et al., 1986), resolved by SDS/PAGE by the method of Laemmli (1970), then transblotted onto a nitrocellulose sheet (Towbin et al., 1979). MCP was detected with mAbs to MCP, M160 or M177, alkaline phosphatase-labelled second Ab and colour reagents.

Glycosidase digestion

Solubilized MCP was diluted with an equal volume of 40 mM Tris-maleate/20 mM D-galactono-γ-lactone/2 mM calcium acetate/0.2% NP-40, pH 6.0, and then incubated with 100 μunits of neuraminidase (Sigma Co., St. Louis, MO, U.S.A.) for 1 h at 37 °C. The desialylated samples were incubated with 3 munits of *O*-glycanase (Genzyme Co., MA, U.S.A.) for an additional 16 h at 37 °C (Chan and Atkinson, 1985). *O*-Glycanase-treated and untreated samples were analysed by SDS/PAGE and immuno-

blotting. Neither neuraminidase nor *O*-glycanase was added to control (designated as 'untreated') samples.

Cytotoxicity

Cytotoxicity was assayed according to the method of Lublin and Coyne (1991). The transfected cells were plated at 2×10^4 per well in 96-well trays 1 day before the assay. On the next morning, the cells were labelled with ^{51}Cr by incubating with 1 μCi per well in complete medium for 4–6 h at 37 °C. Wells were washed, then incubated with 35 μl of diluted rabbit anti-CHO antibody for 30 min at 4 °C. Then, 100 μl of 2-fold diluted gelatin-veronal buffered (GVB)-NHS (to measure activation of the classical pathway) or Mg^{2+}-EGTA-NHS (to measure activation of the alternative pathway) were added. The plates were incubated for 60 min at 37 °C and the released radioactivity was measured in a gamma-counter. Cytotoxicity was calculated as follows:

$$\text{Cytotoxicity} = \frac{(\text{sample c.p.m.}) - (\text{control c.p.m.})}{(\text{SDS c.p.m.}) - (\text{control c.p.m.})} \times 100$$

where sample c.p.m. means counts of samples, control c.p.m. means counts of the background obtained from the supernatant of the CHO cells untreated with NHS, and SDS c.p.m. is counts released upon 1 % SDS solubilization of cells. All determinations were performed in triplicate (Kojima et al., 1993, Iwata et al., 1994).

Determination of MV infectivity

Monolayered CHO cells were infected with MV at 0.001–0.5 plaque-forming units (PFU) per cell in a 24-well plate for 3 h at 37 °C, washed three times and cultured for 3 days, when the syncytia began to be visualized in this CHO system (Dorig et al., 1993). We simultaneously performed plaque-forming assays (Naniche et al., 1993a) in some experiments, and confirmed the correlation between the CHO cell syncytium and plaque formation (Giraudon and Wild, 1985). The cytopathic effect was photographed using a Nikon inverted microscope.

Production of measles virus haemagglutinin (MVH) protein was confirmed by rosette assay using green monkey erythrocytes (GME) as described previously (Giraudon and Wild, 1985).

RESULTS

Expression of MCP phenotypes in CHO cells

To obtain the cDNA encoding the ST^{ABC} and ST^{BC} regions, the ST regions in mRNA of HeLa and HL-60 were amplified by RT-PCR (Figure 1). The ST phenotypes were different in HeLa and HL-60: MCP of the ST^{C} and ST^{BC} types were dominant in HeLa and HL-60, respectively. The ST^{ABC} type was not detected in HeLa but found in HL-60 as a minor component. The cDNA of ST^{BC} and ST^{ABC} portions thus amplified were isolated and subsequentially inserted in place of the ST^{C} portion into the full length cDNA of ST^{C} phenotype. The products were all confirmed by sequence analysis.

Each of the three MCP phenotypes and the ST-deletion mutant were expressed stably in CHO cells and those transfectants expressing similar amounts of the MCP phenotypes or the ST-deletion mutant were selected by flow cytometry (Figure 2). The presence of the ST^{A} and/or ST^{C} portions in each expressed protein was confirmed using anti-ST^{A} and anti-ST^{C} peptide Abs (data not shown).

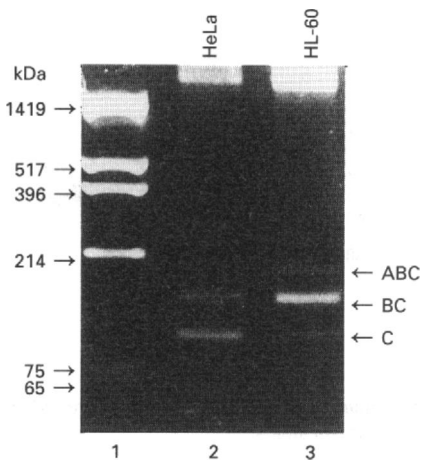

Figure 1 Amplification of MCP ST cDNA by RT-PCR of HeLa and HL-60 mRNA

Poly(A)$^+$ mRNA was isolated from HeLa (lane 2) and HL-60 (lane 3), then RT-PCR was performed using the oligonucleotide primers specific for MCP ST domain (see Materials and methods section); lane 1 shows molecular mass markers. One-third of the RT-PCR products was resolved by 2% agarose gel electrophoresis.

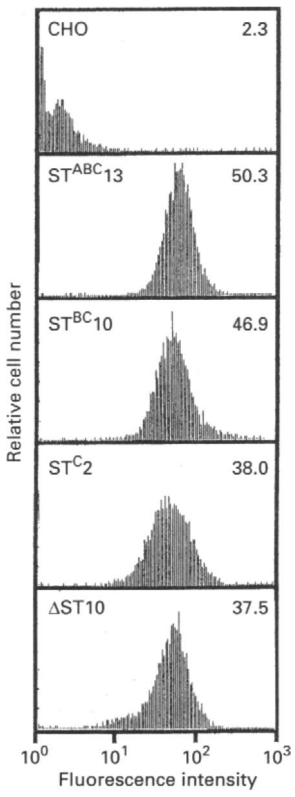

Figure 2 Flow cytometric profiles of CHO transfectants expressing three MCP phenotypes and an ST-deletion mutant

Stable ST^{ABC} 13, ST^{BC} 10, ST^{C} 2 and ΔST 10 transfectants were incubated with anti-MCP mAb M177, followed by FITC-labelled goat anti-(mouse IgG), then analysed by flow cytometry (EPICS-CS). CHO means control CHO cells transfected with the vector alone (pME18s and pLTR*neo*). Mean fluorescence shifts are indicated in each panel.

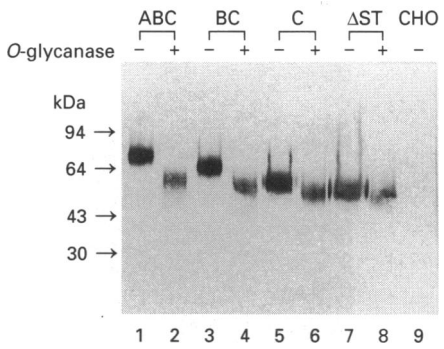

Figure 3 Immunoblots of MCP phenotypes and an ST-deletion mutant expressed on CHO transfectants

Each transfectant was solubilized by NP-40 and resolved by SDS/PAGE (10% acrylamide) under non-reducing conditions. CHO cells having no MCP were used as controls (lane 9). The proteins were transblotted onto a nitrocellulose sheet and detected with M177, alkaline phosphatase-labelled second Ab and colour reagents. Lanes 2, 4, 6 and 8, *O*-glycanase-treated samples; lanes 1, 3, 5, and 7, untreated controls; lanes 1 and 2, ST^{ABC}; lanes 3 and 4, ST^{BC}; lanes 5 and 6, ST^{C}; lanes 7 and 8, ΔST phenotypes. Bio-Rad molecular mass markers are indicated by arrows.

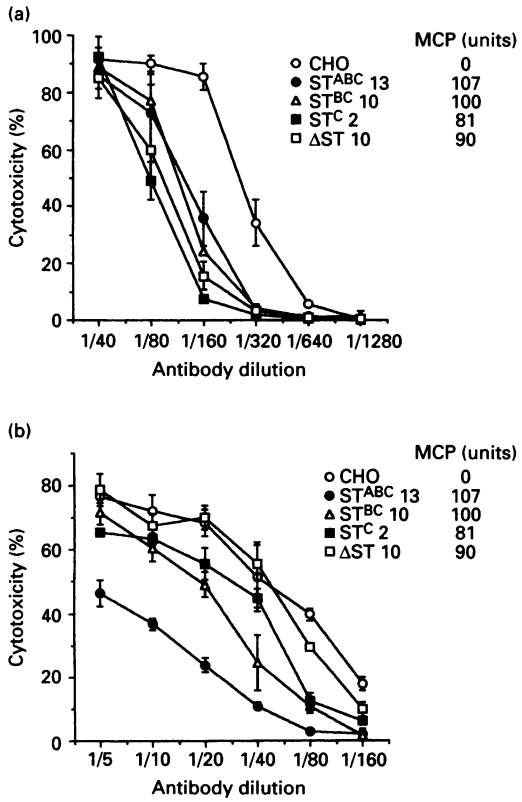

Figure 4 Human C-mediated lysis of the CHO cell clones with or without MCP

(**a**) Protection from classical pathway-dependent cytolysis of CHO cells by transfected MCP phenotypes and an ST-deletion mutant. ^{51}Cr-labelled transfectants and control CHO cells were sensitized with serially diluted anti-CHO cell antibody and incubated with 50% GVB^{2+}-NHS. The cytotoxicity was estimated from the released ^{51}Cr as described in Materials and methods. The expression levels of MCP phenotypes were estimated from flow cytometric shifts as described in the legend to Figure 2, assuming that the level of the clone ST^{BC} 10 was arbitrarily set at 100 units. (**b**) Protection from the alternative pathway-dependent cytolysis of CHO cells by transfected MCP. This assay was performed as described above, except that sensitized CHO cells were incubated with 50% EGTA-Mg^{2+} NHS. These experiments were performed three times in triplicate and similar results were obtained. The error bars represent S.D.

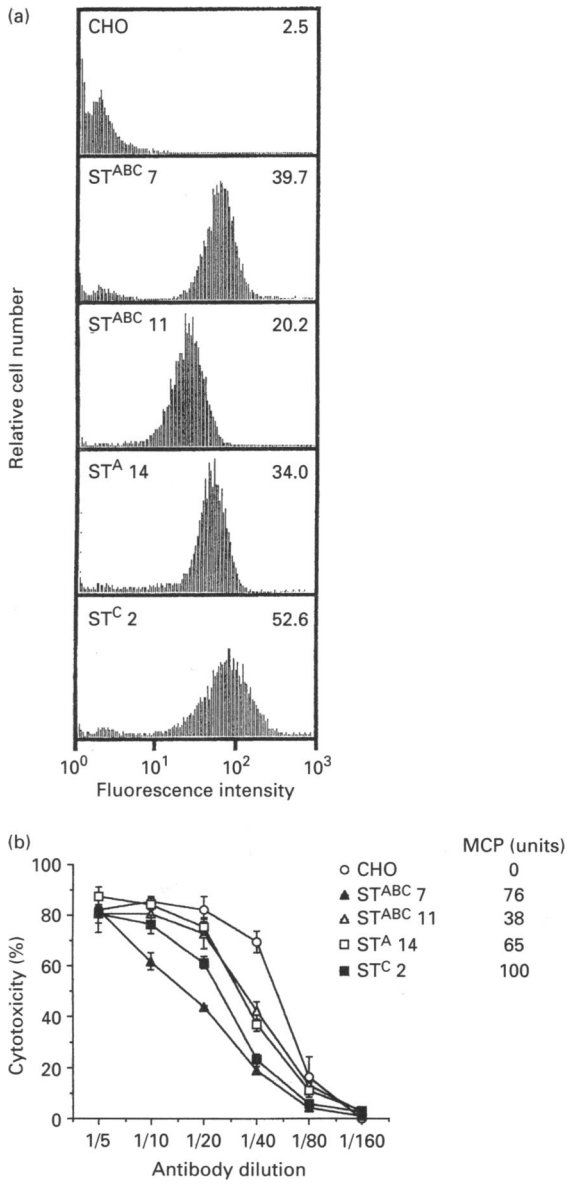

Figure 5 Protection from the alternative pathway-dependent cytolysis of CHO cells by the transfected MCP ST^{A} phenotype

(**a**) Flow cytometric profiles of CHO transfectants expressing the ST^{A}, ST^{ABC} and ST^{C} phenotypes used in this assay. The cells were treated as in the legend to Figure 2. Mean fluorescence shifts are indicated in each panel. (**b**) The regulation of alternative pathway-dependent cytolysis by each MCP phenotype. This assay was performed as described in the legend to Figure 4. The expression levels of each MCP phenotype estimated in (**a**) are expressed as relative to those of clone ST^{C} 2, whose level was arbitrarily set at 100 units. The experiments were performed three times in triplicate and similar results were obtained. The error bars represent S.D.

Figure 3 shows a representative immunoblotting profile of the three MCP phenotypes and the ST-deletion mutant prepared from the respective CHO transfectant cells. The differences in apparent molecular mass between ST^{ABC} and ST^{BC} phenotypes and between those of ST^{BC} and ST^{C} were 7 and 8 kDa, respectively, on SDS/PAGE. These values were far larger than the molecular masses of ST^{A} and ST^{B} domains estimated from their amino acid sequences (about 1.5 kDa), suggesting the presence of *O*-linked sugars in the ST regions of MCP in CHO cells. To estimate the apparent sizes of the *O*-linked sugar chains, the

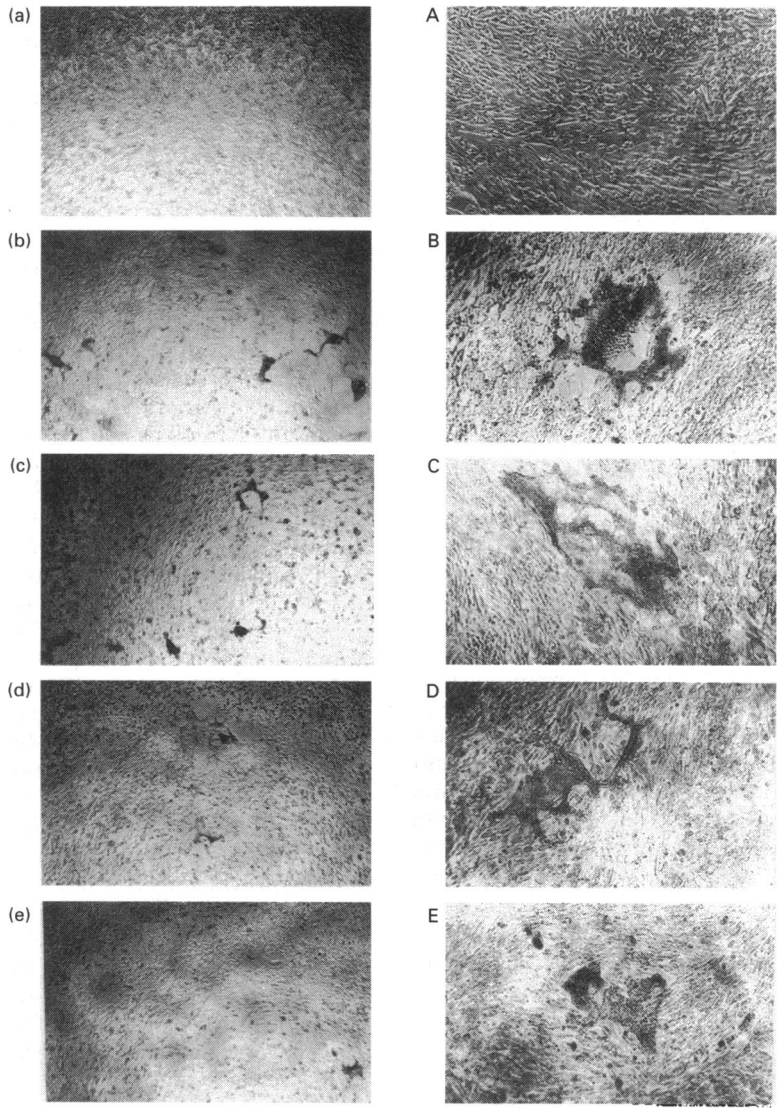

Figure 6 Infection profiles of MCP-positive CHO cell clones with MV

Native CHO and CHO cell clones of ST^{ABC} 13, ST^{BC} 10, ST^{C} 2 and ΔST 10, were inoculated with MV (0.5 PFU/cell) and viewed by phase-contrast microscopy (Nikon). Intact CHO cells (**a**), the clones ΔST (**b**), ST^{C} (**c**), ST^{BC} (**d**) and ST^{ABC} (**e**) were viewed at low magnification (× 30) 3 days after inoculation with MV. The pictures at high magnification (× 240) are also in the same order (A–E). Although not shown in the figure, no cytopathic regions were observed for the control cells without MV. The experiments were performed three times in duplicate and a representative is shown.

samples were treated with *O*-glycanase and resolved by SDS/PAGE. Although the SDS/PAGE mobility does not reflect the exact molecular mass of a sugar chain it is possible to estimate apparent size of the sugar chain. Thus, the difference in the mobilities between the *O*-glycanase-treated and the untreated samples suggested that the apparent sizes of *O*-linked sugar chains were 12 kDa for ST^{ABC}, 8 kDa for ST^{BC} and 2 kDa for ST^{C}. Based on these data, it is estimated that the ST^{A} and ST^{B} have *O*-linked sugar chains corresponding to 4 and 6 kDa, respectively.

Inhibition of C-mediated cytolysis of CHO cells by MCP phenotypes and the ST-deletion mutant

We used this set of CHO transfectants to evaluate the role of the ST regions in MCP as an intrinsic inhibitor of the C system.

Figure 4a shows classical pathway-dependent cytolysis of the four MCP-expressing CHO transfectants. The cells were protected from cytolysis by the three MCP phenotypes in the following order; $ST^{C} > ST^{BC} = ST^{ABC}$. The ST-deletion mutant protected CHO cells from cytolysis as effectively as the three ST phenotypes. Thus, the ability of MCP to protect cells from classical pathway-dependent cytolysis tends to decrease slightly with an increase in the size of the ST-deletion region.

Figure 4b shows alternative pathway-dependent cytolysis of the four MCP-expressing CHO transfectants. In contrast with classical pathway-dependent cytolysis, the alternative pathway was suppressed most effectively by the largest ST^{ABC} phenotype but not by the ST-deletion mutant.

We then tested whether the ST^{A} domain by itself specifically enhanced the regulatory activity of MCP against the alternative pathway. A cDNA of the ST^{A} phenotype was constructed and

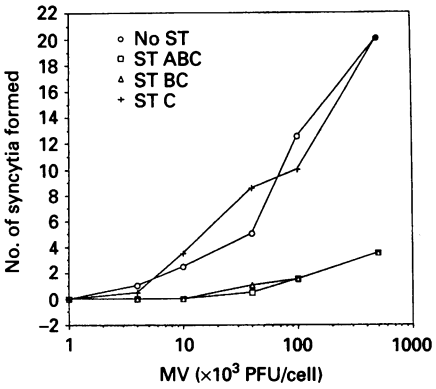

Figure 7 Sensitivity of the MCP-positive CHO cell clones to MV

The numbers of virus-dependent syncytia formed as shown in Figure 6 were calculated and plotted as a function of the MV concentration, which is indicated on the *x* axis. The experiments were performed three times and similar results were obtained. The data were confirmed using agarose medium, which prevented secondary infection (data not shown).

expressed on CHO cells. Because of the low MCP expression level of clone ST^A 14, this assay was performed using a different set of cells with MCP phenotypes as shown in Figure 4. The expression levels and regulatory activities of each MCP phenotype against the alternative pathway-dependent cytolysis are shown in Figures 5a and 5b, respectively. Clone ST^A 14 was less effective than ST^{ABC} 7, whose MCP level was similar to that of the clone ST^A 14, and as effective as ST^{ABC} 11, whose MCP level was about 60% of that of the ST^A 14 clone. Thus, the possibility was excluded that the ST^A domain itself enhances the regulatory activity of MCP against alternative pathway-dependent cytolysis.

Effect of the ST-rich domain on MV infectivity

CHO cells expressing similar amounts of ST^{ABC}, ST^{BC}, ST^C, or the ΔST mutant were incubated with MV at 2 PFU per cell for 3 h at 37 °C. The cells were washed, then reacted with anti-MVH mAb and FITC-labelled second Ab. All of these cells were stained with fluorescence, while untransfected and decay accelerating factor (DAF)-transfected CHO cells were not stained (data not shown).

We treated these transfectants with a serially diluted MV source, and after washing, incubated them in Ham's medium containing FCS for 3 days at 37 °C. These conditions were established after many trials to evaluate the MV infectivity in the CHO transfectants. In some experiments, the medium was solidified with agarose to prohibit secondary spreading of the virus. The typical syncytia formed in the CHO cell transfectants are shown in Figure 6. The microscopic features of the syncytia were similar among the CHO cell clones with various mutant MCP. However, the numbers of MV-mediated syncytia were different for each clone: MV infectivity was relatively high in the ST^C and ΔST phenotypes compared with those of ST^{ABC} and ST^{BC} (Figure 7). The MVH protein synthesis assayed by GME rosette formation also reflected this tendency (data not shown).

DISCUSSION

There is a group of membrane proteins that possess an *O*-glycosylated ST-rich region near the functional domains. These include thrombomodulin (Suzuki et al., 1987), DAF (Lublin and Atkinson, 1989), MCP (Lublin et al., 1988), and the low density lipoprotein-receptor (Yamamoto et al., 1984). Of these proteins, DAF has the greatest similarity to MCP in both structural and functional aspects. The ST region of DAF is proposed to simply fulfil a spacer role (Coyne et al., 1992), presumably projecting the functional SCR units further from the plasma membrane. Regarding other ST-containing proteins, the participation of the ST region as well as the *O*-linked sugars in their functions remains largely unknown. The aim of this study was to focus upon the variation of the ST domains and its contribution to the function of MCP, namely the protection of host cells from C-dependent cytolysis and MV infection.

The results on the functional properties of the ST region of MCP can be summarized as follows. Classical pathway-dependent cytolysis of CHO cells was suppressed most effectively by the ST^C phenotype and the ST-deletion mutant. This implies that the *O*-linked sugar chains as well as the space between the plasma membrane and the SCR are not important for MCP to regulate the classical pathway. On the contrary, the alternative pathway-dependent cytolysis was regulated most effectively by MCP with the largest and heavily glycosylated ST phenotype (ST^{ABC}). The ST-deletion mutant hardly suppressed alternative pathway-dependent cytolysis. These findings may be associated with the fact that the binding sites for C3b and C4b on MCP are different (Oglesby et al., 1993). Thus, we speculate that the spacer-like ST domain is not required for interaction between surface bound C4b and the C4b-binding site of MCP, unlike DAF, but is critical for interaction between surface bound C3b and C3b-binding site of MCP. The alternative possibility that the ST^A domain is responsible for the enhanced interaction between MCP and surface bound C3b is excluded because the MCP phenotype with only ST^A had similar regulatory activity as that with ST^C.

MCP phenotype with a small ST domain (ST^C) and the ST-deletion mutant are 100 times more effective as a receptor for MV than those with large ST domains (ST^{ABC} and ST^{BC}). MV infection is accomplished via MV fusion to host cells, and a speculative molecular mechanism for fusion is that there is an H–F protein complex on MV: the MVH protein serves as a ligand for MCP engaging cell–viral interaction (Naniche et al., 1993b), then the F protein inserts into the host cell membrane (Naniche et al., 1993b; Wild et al., 1991). The MV binding site is not located within the ST-rich domains but within the SCR of MCP (K. Iwata, T. Seya, Y. Yanagi, S. Ueda, H. Ariga and S. Nagasawa, unpublished observation). Hence, the ST domain could modulate both C inhibitory activity and MV infectivity. The functional participation of the ST domain in MCP does not support the results on the ST domain of DAF (Coyne et al., 1992).

Cell- and organ-specific variations of MCP are mostly due to the ST region, which is generated by the alternative splicing of exons 7, 8 and 9, each encoding three distinct ST domains, ST^A, ST^B and ST^C, respectively (Post et al., 1991; Purcell et al., 1991; Russell et al., 1992). The ST domain is composed of the amino acid stretch (Lublin et al., 1988; Post et al., 1991) and *O*-linked sugars (Ballard et al., 1987; Matsumoto et al., 1992). It is important to determine which of the two moieties is a critical factor for functional modulation. To answer this issue would thus far be impossible, since the total sugar content and the properties of the sugars in each ST domain are variable among tissues and have not been determined even in human cells, so that the differences in the *O*-linked sugars between a typical human MCP (assuming that it were present) and the MCP expressed by CHO cell cannot be verified. Thus, we currently hold that the ST domain modulates the MCP functions in two ways: controlling

the distance between the ligand binding-site and the cell membrane or shielding the cell membrane by the bulky and negatively-charged *O*-linked sugars.

Based on the differences in molecular size between these *O*-glycanase-treated and -untreated MCP phenotypes (ST^{ABC}, ST^{BC} and ST^{C}), the apparent molecular sizes of the *O*-linked sugar chains in the ST^{A}, ST^{B} and ST^{C} were estimated to be 4, 6 and 2 kDa, respectively. These sugar values in the three ST domains appear to be compatible with their Ser and Thr contents and consistent with those obtained from human cells and cell lines (Ballard et al., 1987; Matsumoto et al., 1992). Further accurate analysis using mutant cells incapable of *O*-linked sugar synthesis is in progress to clarify the above issue.

The physiological role of the ST domain can be interpreted as follows based upon our present results. Most individuals are genetically classified into H (ST^{BC})-dominant, L (ST^{C})-dominant and HL equivalent (Ballard et al., 1987; Seya et al., 1988), and malignant cells often express ST^{ABC} (Johnstone et al., 1993; McNearney et al., 1989). These three groups and ST^{ABC}-dominant malignant cells should respond differently to MV and C activation. If the alternative C pathway is mainly activated in body fluids, malignant cells would be protected most effectively under pre-immune stages from C-mediated cell damage. Under a pathological state such that the immune complex activates the classical pathway, C-regulatory potency would be minimal on malignant cells. In addition, malignant cells may be relatively unsusceptible to MV. These tendencies may be adapted in principal to H phenotype-dominant individuals and the reverse is true of those who are L phenotype-dominant. Moreover, MCP phenotypes are differently distributed in organs in addition to the genetic differences (Johnstone et al., 1993). Above all, the brain predominantly expresses the ST^{C} phenotype even in individuals classified as being H phenotype-dominant (Johnstone et al., 1993). Based on evidence that ST^{C} phenotype is the most susceptible to MV of the three common ST phenotypes, the hypothesis could be proposed that the predominant expression of ST^{C} in the brain is somewhat related to the high sensitivity of the brain to MV and to MV-mediated encephalitis including subacute sclerosing panencephalitis (Ter Meulen et al., 1985). Studies of the infectivity of MV in other organs and the relationship to each MCP phenotype will be important to further clarify the physiological role of MCP and its ST-variable phenotypes.

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