Mouse mutants lacking the cation-independent mannose 6-phosphate/insulin-like growth factor II receptor are impaired in lysosomal enzyme transport: comparison of cation-independent and cation-dependent mannose 6-phosphate receptor-deficient mice

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Two proteins have been implicated in the mannose 6-phosphate-dependent transport of lysosomal enzymes to lysosomes: the 300 kDa cation-independent and the 46 kDa cation-dependent mannose 6-phosphate receptors (CI- and CD-MPRs). The mammalian CI-MPR also mediates endocytosis and clearance of insulin-like growth factor II (IGF-II). Mutant mice that lack the CD-MPR are viable, mice that lack the CI-MPR accumulate high levels of IGF-II and usually die perinatally, whereas mice that lack both IGF-II and CI-MPR are viable. To investigate the relative roles of the MPRs in the targeting of lysosomal enzymes *in vivo*, we analysed the effect of a deficiency of either MPR on lysosomal enzyme activities in animals lacking IGF-II. In CD-MPR-deficient mice, most activities were relatively normal in solid tissues and some were marginally elevated in serum. In CI-MPR-deficient mice, some enzyme activities were moderately decreased in solid tissues and multiple enzymes were markedly elevated in serum. Finally, total levels of serum mannose 6-phosphorylated glycoproteins were ~45-fold and ~15-fold higher than wild type in CI- and CD-MPR-deficient mice respectively, and there were specific differences in the pattern of these proteins when comparing CI- and CD-MPR deficient animals. These results indicate that while lack of the CI-MPR appears to perturb lysosome function to a greater degree than lack of the CD-MPR, each MPR has distinct functions for the targeting of lysosomal enzymes *in vivo*.

INTRODUCTION

Most soluble lysosomal hydrolases are targeted to the lysosome by mannose 6-phosphate (Man-6-P) receptors (MPRs) (reviewed in [1]). The newly synthesized soluble lysosomal enzymes are post-translationally modified to contain Man-6-P residues on their N-linked oligosaccharides. This modification allows the enzymes to bind to MPRs in the *trans*-Golgi network. The MPRs and their cargo cluster into clathrin-coated transport vesicles and travel to an acidic prelysosomal compartment where the low pH triggers dissociation of the receptor–ligand complex. The free MPRs can travel to the plasma membrane or back to the Golgi to function in additional rounds of targeting.

Two distinct integral membrane proteins bind phosphorylated lysosomal enzymes: the 300 kDa cation-independent (CI) and the 46 kDa cation dependent (CD) MPRs (reviewed in [2,3]). Both are present in most mammalian cell types. The receptors differ in that while both reach the cell surface and are rapidly internalized, the CI-MPR is much more efficient in mediating endocytosis of extracellular ligands [4,5]. Also, the mammalian CI-MPR, but not the CD-MPR or amphibian and avian CI-MPRs, binds and mediates endocytosis of insulin-like growth factor II (IGF-II) [6–8], a non-phosphorylated protein that has growth-promoting activity that is important for embryonic development [9].

The relative function of the two MPRs in lysosomal enzyme transport is not fully understood. Several lines of evidence obtained from studies *in vitro* indicate that the two receptors have complementary functions. Firstly, cultured cells lacking either type of MPR alone mis-sort lysosomal enzymes and accumulate undigested storage material in lysosomes, whereas cells lacking both MPRs exhibit a more drastic phenotype [10,11]. Second, comparison of proteins secreted by single MPR-deficient fibroblasts suggests that both receptors function to target overlapping but distinct subsets of phosphorylated lysosomal enzymes [10,11]. Finally, transfection experiments indicate that overexpression of one type of MPR cannot fully compensate for the absence of the other [12,13].

Recent work using gene-targeting technology has begun to address the function of the MPRs *in vivo*. Mutant mice lacking the CD-MPR are viable and apparently have normal steady-state levels of tissue lysosomal enzymes [14,15]. However, they exhibit a partial defect in sorting, based on the presence of newly synthesized hydrolases in serum and urine [14,16]. Mutant mice lacking the CI-MPR accumulate high levels of IGF-II and usually die perinatally [17–19]. This is directly attributable to overstimulation by IGF-II, as CI-MPR-deficient mutants are completely viable in an IGF-II-deficient background, with the double mutants having a dwarf phenotype similar to that of the single IGF-II-deficient mutants. Despite the viability of the single MPR-deficient mice, the importance of the MPRs in lysosomal enzyme targeting *in vivo* was indicated by the finding

Abbreviations used: CD, cation dependent; CI, cation-independent; IGF-II, insulin-like growth factor II; Man-6-P, mannose 6-phosphate; MPR, mannose 6-phosphate receptor; AAP, alanine aminopeptidase; AMC, 7-amido-4-methylcoumarin; 4-MU, 4-methylumbelliferyl.

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that triple mutants lacking the CD-MPR, CI-MPR and IGF-II are not viable [17]. However, to our knowledge, no direct measurements on lysosomal function have been conducted on CI-MPR-deficient animals.

Analysis of the function *in vivo* of the CI-MPR in lysosomal enzyme trafficking has been complicated by its role in IGF-II clearance. In this study, we have used mice with an IGF-II-deficient background to investigate the relative function of each MPR in lysosomal enzyme trafficking. We found that while deficiencies of either receptor lead to mis-sorting of lysosomal enzymes, the CI-MPR-deficient animals display a more dramatic phenotype.

EXPERIMENTAL

Tissue and serum samples

Mice were generated by mating $Igf2^{+/+}Igf2r^{+/-}M6pr^{+/-}$ females with $Igf2^{-/-}Igf2r^{+/-}M6pr^{+/-}$ or $Igf2^{-/-}Igf2r^{-/-}M6pr^{+/-}$ males. All pups inherited paternally a disrupted copy of the imprinted *Igf2* gene, which is normally not expressed from the maternal allele, and are classified as IGF-II deficient. Mice were genotyped as described previously [17] using DNA prepared from tail tips of day 7 mice. The genotype of animals killed for enzyme assays was confirmed using DNA prepared from liver. The genotypes of the experimental groups were as follows: 'wild type', $Igf2r^{+/+}M6pr^{+/+}$; CI-MPR deficient, $Igf2r^{-/-}M6pr^{+/+}$; CD-MPR deficient, $Igf2r^{+/+}M6pr^{-/-}$. All mice were $Igf2^{+/-p}$ and had a mixed C57BL/6J × 129/Sv genetic background.

At postnatal day 16, blood was collected by cardiac puncture of carbon dioxide-anaesthetized animals. Tissues were dissected, blotted on filter paper and snap-frozen in liquid nitrogen or on powdered dry-ice. The blood was allowed to clot for ~ 1 h at room temperature and serum was prepared by centrifugation at 12000 ***g*** for 15 min. Samples were stored at −80 °C until use.

Measurement of enzyme activities

Frozen mouse tissues were pulverized using a Bessman homogenizer. Samples were weighed, thawed on ice, placed in 100 volumes of homogenization solution (0.15 M NaCl/0.1 % Triton X-100) and disrupted using a Brinkmann polytron homogenizer. A soluble supernatant was prepared by centrifugation at 12000 ***g*** for 25 min at 4 °C. Assays for the proteases alanine aminopeptidase (AAP) and cathepsins B, C and L were conducted using 7-amido-4-methylcoumarin (AMC) substrates. Cathepsin D was measured using formation of trichloroacetic acid-soluble degradation products of haemoglobin substrate [20]. All other enzymes were assayed using 4-methylumbelliferyl (4-MU) substrates. Reactions were initiated by adding 40 μl of substrate solution to 10 μl of sample (supernatants diluted 2- and 4-fold into homogenization solution, each in duplicate), incubated at 37 °C and stopped by the addition of 100 μl of termination buffer (0.5 M glycine, pH 10.5, for 4-MU substrates; 0.1 M monochloroacetic acid/0.1 M acetate, pH 4.3, for AMC substrates). Samples added to substrate solutions after addition of termination buffer were used as blanks. The reaction conditions for most enzymes were as described previously [21], except incubation times were adjusted in different tissues so that typically between 1 and 5 % of the substrate was converted into product. The composition of other substrate solutions that were modified or not described previously are as follows (enzyme, substrate in 0.1 M indicated buffer): β-glucosidase, 5 mM 4-MU-β-glucoside in citrate, pH 4.5; β-hexosaminidase A, 0.5 mM 4-MU-7-(6-sulpho-2-acetamido-2-deoxy-beta-glucopyranoside) in citrate, pH 4.0; lipase, 0.2 mM 4-MU-oleate/liposome suspension [22] in acetate, pH 5.5; cathepsin C, 0.5 mM Gly-Arg-AMC in acetate, pH 5.5, containing 5 mM dithiothreitol. Substrates were purchased from Sigma and prepared freshly in reaction buffer for sulphatase and acid phosphatase assay, or diluted 100 times with the reaction buffer from a DMSO stock solution. Fluorescent reaction products were measured with a CytoFluor II (PerSeptive Biosystems, Framingham, MA, U.S.A.) plate reader using 360 nm excitation and 460 nm emission filters. Enzyme activities were linear with respect to input sample.

For a given tissue, all activity measurements were conducted in parallel. For practical considerations, activity measurements were initially conducted on specimens from a limited number of animals (wild type, $n = 3$; CD-MPR deficient, $n = 6$; and CI-MPR deficient, $n = 5$). Selected enzymes were reassayed in a second, independent experiment that included additional animals to increase the sample size (typically, wild type, $n = 7$; CD-MPR deficient, $n = 11$; and CI-MPR deficient, $n = 11$). The reproducibility of experimental measurements was determined by comparing the relative activities of the specimens analysed in both experiments. With the exception of three enzymes, there was excellent agreement between the two independent determinations, and most of the scatter in the data can be ascribed to animal-specific variation. The three problematic enzymes (α-fucosidase, acid lipase and β-glucosidase) showed unacceptable variation and are excluded from the analysis.

Other

Protein was measured by the Lowry method [23], modified to contain SDS and adapted to microtitre plates, using BSA as a standard. Statistics were computed using SAS version 6.12 (SAS Institute Inc, Cary, NC, U.S.A.).

RESULTS

This study was conducted to gain insight into the relative biological functions of the two MPRs in lysosomal enzyme targeting. Our simple hypothesis was that disrupted targeting of MPR ligands would cause changes in their levels in tissue and serum, which would be reflected in enzyme activity measurements. To this end, we assayed a wide range of lysosomal enzymes in wild-type, CD-MPR-deficient and CI-MPR-deficient animals (all in an IGF-II-deficient background). The significance of the changes was evaluated by analysis of variance with Dunnett's *T*-tests, comparing the CI- and CD-MPR-deficient groups with the wild-type control.

Analysis of lysosomal enzymes in solid tissues

Five different solid tissues were examined (brain, heart, kidney, liver and lung). The specific activities, both absolute and normalized to the wild-type group, are given in Tables 1–5. The activities include multiple glycosidases and proteases thought to be mannose 6-phosphorylated, as well as controls that do not utilize the Man-6-P targeting pathway. The latter group consists of acid phosphatase, a lysosomal enzyme that is synthesized as a membrane-bound precursor, and AAP, a cytosolic neutral protease activity.

CD-MPR deficiency had little effect on the steady-state activities of most lysosomal enzymes. The only exception was α-iduronidase, which exhibited a small but statistically significant decrease in kidney and brain (17 and 26 % decrease respectively). In addition, several activities were marginally (15–22 %) increased in brain (β-glucuronidase, β-hexosaminidase A + B and cathepsin C) and in lung (α-mannosidase). While these minor changes may reflect imperfect compensatory homoeostatic mech-

Table 1 Lysosomal enzyme activities in normal and MPR-deficient mouse brain

Specific activity values given ± S.D. Relative activities are normalized to the wild-type group.

	Wild type ($n = 7$)	CI-MPR deficient ($n = 10$)		CD-MPR deficient ($n = 10$)	
Enzyme	Specific activity nmol · h^{-1} · mg^{-1}	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity
β-Glucuronidase	31.3 ± 2.8	27.0 ± 4.7	0.86*	38.3 ± 3.0	1.22*
β-Hexosaminidase A + B	73.6 ± 8.9	75.3 ± 8.6	1.02	87.0 ± 9.3	1.18*
β-Hexosaminidase A	24.8 ± 2.6	16.3 ± 2.1	0.66*	25.5 ± 2.6	1.03
α-Iduronidase	3.7 ± 0.5	2.3 ± 0.5	0.60*	2.8 ± 0.6	0.74*
α-Mannosidase	3.2 ± 0.4	3.1 ± 0.5	0.99	3.5 ± 0.4	1.10
Sulphatase	998.4 ± 100.1	899.8 ± 123.7	0.90	916.7 ± 125.0	0.92
Cathepsin C	110.5 ± 10.8	60.4 ± 10.2	0.55*	127.5 ± 18.8	1.15*
Acid phosphatase	1068.4 ± 377.8	1008.3 ± 178.8	0.94	1126.4 ± 122.8	1.05

* Significant at $P < 0.05$ using Dunnett's T test for multiple comparisons against the corresponding wild-type group.

Table 2 Lysosomal enzyme activities in normal and MPR-deficient mouse heart

Specific activity values given ± S.D. Relative activities are normalized to the wild-type group.

	Wild type ($n = 7$)	CI-MPR deficient ($n = 11$)		CD-MPR deficient ($n = 11$)	
Enzyme	Specific activity nmol · h^{-1} · mg^{-1}	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity
β-Glucuronidase	28.0 ± 6.6	31.1 ± 6.7	1.11	29.1 ± 7.6	1.04
β-Hexosaminidase A + B	5.0 ± 0.7	4.6 ± 1.2	0.92	4.9 ± 1.2	0.99
β-Hexosaminidase A	11.5 ± 3.7	9.4 ± 1.6	0.82	10.8 ± 2.8	0.94
α-Iduronidase	8.2 ± 2.3	3.9 ± 1.0	0.48*	7.6 ± 1.4	0.92
α-Mannosidase	5.1 ± 1.2	5.0 ± 1.3	0.98	5.9 ± 1.3	1.16
β-Mannosidase	10.1 ± 4.0	8.4 ± 3.4	0.83	7.8 ± 2.4	0.77
Cathepsin B	115.2 ± 22.6	104.3 ± 25.2	0.91	113.3 ± 30.5	0.98
Cathepsin C	202.7 ± 59.9	167.3 ± 37.7	0.83	231.0 ± 68.7	1.14
Cathepsin D	705.4 ± 134.0	771.1 ± 152.1	1.09	869.6 ± 235.1	1.23
Cathepsin L	212.3 ± 36.9	223.4 ± 61.6	1.05	242.8 ± 43.8	1.14
Acid phosphatase	1199.6 ± 170.2	1102.5 ± 253.2	0.92	1178.1 ± 250.4	0.98
AAP	138.7 ± 61.1	159.8 ± 53.7	1.15	204.6 ± 51.2	1.48

* Significant at $P < 0.05$ using Dunnett's T test for multiple comparisons against the corresponding wild-type group.

Table 3 Lysosomal enzyme activities in normal and MPR-deficient mouse kidney

Specific activity values given ± S.D. Relative activities are normalized to the wild-type group.

	Wild type ($n = 7$)	CI-MPR deficient ($n = 11$)		CD-MPR deficient ($n = 11$)	
Enzyme	Specific activity nmol · h^{-1} · mg^{-1}	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity
β-Galactosidase	357.6 ± 118.1	269.5 ± 83.5	0.75	332.3 ± 138.0	0.93
β-Glucuronidase	128.2 ± 25.3	149.4 ± 25.6	1.17	121.6 ± 16.1	0.95
β-Hexosaminidase A + B	62.9 ± 5.2	48.5 ± 8.5	0.77*	56.5 ± 7.4	0.90
α-Iduronidase	11.8 ± 1.7	6.6 ± 1.1	0.56*	9.7 ± 2.0	0.83*
α-Mannosidase	303.7 ± 39.2	223.2 ± 45.7	0.73*	272.7 ± 30.9	0.90
β-Mannosidase	179.6 ± 30.7	154.4 ± 32.7	0.86	170.2 ± 24.0	0.95
Cathepsin B	29.4 ± 7.7	41.3 ± 9.6	1.40*	33.5 ± 5.3	1.14
Cathepsin C	271.4 ± 81.8	415.6 ± 220.0	1.53	253.8 ± 67.8	0.93
Cathepsin L	89.9 ± 12.1	117.5 ± 22.3	1.31*	101.1 ± 19.2	1.13
Acid phosphatase	1099.72 ± 163.18	991.67 ± 113.12	0.90	1015.63 ± 163.27	0.92
AAP	636.78 ± 85.20	547.16 ± 109.89	0.86	586.73 ± 69.10	0.92

* Significant at $P < 0.05$ using Dunnett's T test for multiple comparisons against the corresponding wild-type group.

Table 4 Lysosomal enzyme activities in normal and MPR-deficient mouse liver

Specific activity values given ± S.D. Relative activities are normalized to the wild-type group.

	Wild type ($n = 7$)	CI-MPR deficient ($n = 11$)		CD-MPR deficient ($n = 11$)	
Enzyme	Specific activity nmol · h^{-1} · mg^{-1}	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity
β-Glucuronidase	230.0 ± 39.0	238.3 ± 37.0	1.04	229.2 ± 31.6	1.00
β-Hexosaminidase A + B	39.0 ± 8.7	62.4 ± 14.1	1.60*	41.1 ± 6.4	1.05
β-Hexosaminidase A	88.4 ± 24.2	132.6 ± 31.3	1.50*	91.8 ± 16.4	1.04
α-Iduronidase	9.3 ± 2.4	8.6 ± 3.4	0.92	9.9 ± 2.4	1.07
α-Mannosidase	78.5 ± 19.9	108.1 ± 35.2	1.38	84.7 ± 13.9	1.08
β-Mannosidase	51.9 ± 7.4	53.1 ± 9.4	1.02	50.0 ± 8.3	0.96
Cathepsin B	133.5 ± 21.2	153.1 ± 27.6	1.15	147.3 ± 21.4	1.10
Cathepsin C	1094.5 ± 264.2	1118.8 ± 267.6	1.02	1106.1 ± 232.4	1.01
Cathepsin D	470.2 ± 120.7	557.8 ± 134.3	1.19	547.7 ± 103.8	1.16
Cathepsin L	147.8 ± 19.5	147.8 ± 20.8	1.00	149.8 ± 14.0	1.01
Acid phosphatase	943.1 ± 140.1	885.2 ± 122.0	0.94	991.0 ± 116.1	1.05
AAP	119.8 ± 31.1	110.7 ± 26.5	0.92	121.4 ± 14.6	1.01

* Significant at $P < 0.05$ using Dunnett's T test for multiple comparisons against the corresponding wild-type group.

Table 5 Lysosomal enzyme activities in normal and MPR-deficient mouse lung

Specific activity values given ± S.D. Relative activities are normalized to the wild-type group.

	Wild type ($n = 7$)	CI-MPR deficient ($n = 11$)		CD-MPR deficient ($n = 11$)	
Enzyme	Specific activity nmol · h^{-1} · mg^{-1}	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity	Specific activity nmol · h^{-1} · mg^{-1}	Relative activity
β-Galactosidase	123.4 ± 32.7	114.3 ± 35.0	0.93	121.5 ± 42.3	0.98
β-Hexosaminidase A + B	24.0 ± 4.8	16.9 ± 2.5	0.70*	22.5 ± 6.4	0.94
α-Iduronidase	20.6 ± 4.2	11.5 ± 2.2	0.56*	18.9 ± 4.7	0.92
α-Mannosidase	13.6 ± 2.1	12.6 ± 1.8	0.92	16.6 ± 3.8	1.22*
β-Mannosidase	24.8 ± 4.6	19.4 ± 4.0	0.78	22.8 ± 6.2	0.92
Acid phosphatase	1127.7 ± 144.2	1079.9 ± 199.4	0.96	1086.2 ± 193.5	0.96
AAP	194.9 ± 15.2	180.5 ± 30.3	0.93	199.6 ± 44.4	1.02

* Significant at $P < 0.05$ using Dunnett's T test for multiple comparisons against the corresponding wild-type group.

anisms, these results are consistent with previous studies on IGF-II normal, CD-MPR-deficient mice that failed to find any biologically significant effect on tissue lysosomal enzyme activities [14,15].

In contrast, loss of the CI-MPR clearly affected steady-state activities of multiple hydrolases. The most striking effect was on α-iduronidase, which exhibited a ~ 50% decrease in brain, lung, heart and kidney. In addition, statistically significant decreases were seen in activities of β-glucuronidase (brain), β-hexosaminidase A + B (lung, kidney), β-hexosaminidase A (brain), α-mannosidase (kidney) and cathepsin C (brain). Interestingly, a few activities were significantly increased in liver (β-hexosaminidase A + B and β-hexosaminidase A) and kidney (cathepsins B and L). These increases may reflect over-compensatory mechanisms or clearance of secreted circulating enzymes (see below).

Analysis of lysosomal enzymes in serum

We also measured activities of multiple enzymes in serum (Table 6). Multiple enzymes appeared slightly elevated in the CD-MPR-deficient group, although none reached statistical significance using Dunnett's T tests. Multiple enzymes exhibited a large (~ 2- to 6-fold), statistically significant increase in the CI-MPR deficient animals. This indicates that many, but not all, lysosomal enzymes are mistargeted into the extracellular space in the CI-MPR-deficient animals and cannot be fully cleared by CI-MPR-independent mechanisms.

As an indicator of overall accumulation of secreted, newly synthesized lysosomal enzymes, we also examined mannose 6-phosphorylated glycoproteins in serum. Samples were fractionated by SDS/PAGE, transferred to nitrocellulose membranes, and the membranes were probed with an iodinated, soluble form of the CI-MPR. All three groups were significantly different from one another, with the total mannose 6-phosphorylated serum proteins being highest in CI-MPR-deficient animals, intermediate in CD-MPR-deficient animals and lowest in the control animals (Figure 1). Comparison of the bands present in both types of MPR-deficient animal reveal that while many are common, there are clear differences, such as the ~ 26 kDa band visible only in the CD-MPR-deficient samples. This band may represent a protein that is specifically targeted by the CD-MPR.

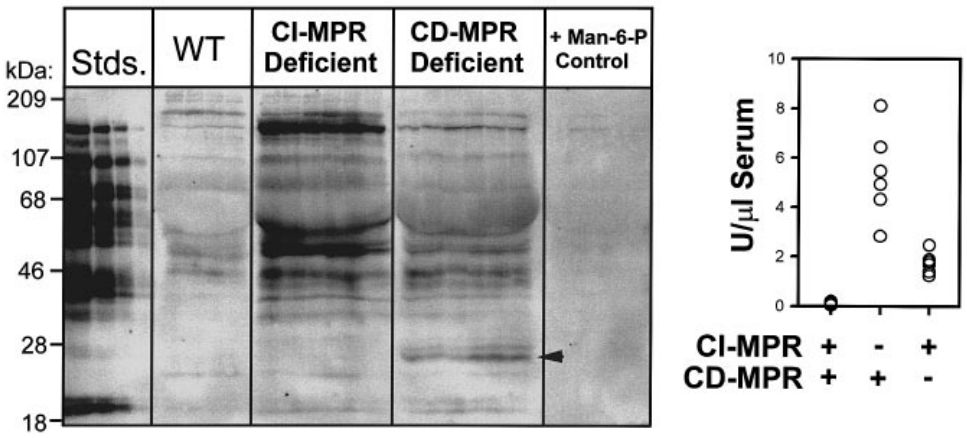

Figure 1 Mannose 6-phosphorylated glycoproteins in serum

Left panel: serum (4 μl) from different animals (normal, $n = 4$; CI-MPR deficient, $n = 6$; CD-MPR deficient, $n = 6$) was fractionated by SDS/PAGE, transferred to nitrocellulose, and glycoproteins bearing the Man-6-P modification were detected using iodinated sCI-MPR as an affinity probe as described previously [21]. The arrowhead indicates a unique prominent band present in serum from CD-MPR-deficient animals. Indicated lanes containing 150, 50, 15 and 5 μl conditioned medium from clone D9 CI-MPR deficient mouse L-cells were used as standards, with 1 μl corresponding to 1 unit. As a control, serum from one wild-type, two CI-MPR-deficient and two CD-MPR-deficient mice was probed in the presence of 5 mM Man-6-P. Right panel: levels of total mannose 6-phosphorylated serum glycoproteins in the individual animals. All three groups are statistically different from each other using the Student–Newman–Keuls test ($P < 0.05$).

DISCUSSION

The activity of lysosomal enzymes in tissues is a function of a number of processes. At a cellular level, these include the targeting of endogenous and exogenous enzymes by Man-6-P-dependent and -independent mechanisms, rates of enzyme synthesis and degradation, and, for some hydrolases, conversion of inactive proenzymes into active species. While the single MPR deficiencies are expected to directly affect Man-6-P-dependent targeting mechanisms, the net effect on tissue lysosomal enzyme activities may be moderated by compensatory changes in the remaining MPR and/or some of the other processes listed above. Indeed, Man-6-P-independent compensatory mechanisms have been noted from studies performed on patients with I-cell disease, a fatal human hereditary disorder caused by a deficiency in the key enzyme that generates the Man-6-P recognition marker. Analysis of I-cell disease specimens indicates, that while fibroblasts exhibit a profound deficiency, and serum contains elevated levels of multiple lysosomal enzymes, most lysosomal enzymes are normal or even elevated in liver, kidney, brain and spleen [24–26]. However, despite these compensatory mechanisms, our results indicate incomplete functional compensation for loss of the CI-MPR in mice and demonstrate a role *in vivo* of this receptor in targeting of lysosomal enzymes.

The ability of one type of MPR to compensate for the lack of the other depends on both the amount of the remaining receptor present and its intrinsic functional properties. The two receptors are expressed at different levels in different tissues during mouse development [27], so, quite possibly, capacity considerations are important. In addition, the more pronounced effect of the CI- compared with CD-MPR-deficiency is consistent with the known functional properties of the receptors. Firstly, binding studies *in vitro* indicate that, in general, the CI-MPR has a higher affinity for and recognizes a broader spectrum of individual lysosomal enzyme isoforms than does the CD-MPR [28,29]. Second, in cells cultured under physiological conditions, the CI-MPR mediates efficient endocytosis of extracellular phosphorylated lysosomal enzymes, whereas the CD-MPR does not [4,5]. Taken together,

Table 6 Lysosomal enzyme activities in normal and MPR-deficient mouse serum

Specific activity values given ± S.D. Relative activities are normalized to the wild-type group.

	Wild type ($n = 5$)	CI-MPR deficient ($n = 9$)		CD-MPR deficient ($n = 10$)	
Enzyme	Activity nmol · h^{-1} · mg^{-1}	Activity nmol · h^{-1} · mg^{-1}	Relative activity	Activity nmol · h^{-1} · mg^{-1}	Relative activity
β-Galactosidase	134.9 ± 45.6	217.9 ± 91.3	1.62	199.9 ± 66.1	1.48
β-Glucuronidase	116.7 ± 18.0	274.0 ± 53.9	2.35*	186.7 ± 73.3	1.60
β-Hexosaminidase A + B	126.5 ± 9.9	241.5 ± 39.5	1.91*	159.8 ± 26.6	1.26
α-Mannosidase	426.0 ± 78.5	2485.3 ± 323.1	5.83*	457.7 ± 57.9	1.07
β-Mannosidase	41.9 ± 16.5	127.0 ± 27.8	3.03*	46.7 ± 9.9	1.12
Sulphatase	215.8 ± 33.5	496.4 ± 79.3	2.30*	311.6 ± 140.8	1.44
Acid phosphatase	1011.8 ± 70.5	1402.5 ± 507.5	1.39	1264.7 ± 502.9	1.25
AAP	425.4 ± 39.4	496.8 ± 98.9	1.17	447.2 ± 124.6	1.05

* Significant at $P < 0.05$ using Dunnett's T test for multiple comparisons against the corresponding wild-type group.

this could explain the decreased tissue and increased serum activities of the CI-MPR-deficient animals compared with both the wild-type and CD-MPR-deficient animals. This is consistent with studies on cultured cells [5,11,13] where a CI-MPR deficiency led to more pronounced mistargeting than a CD-MPR deficiency.

This and other studies indicate that both MPRs together are required to prevent hypersecretion of lysosomal enzymes. It is likely that sugar or protein determinants on different lysosomal enzymes, and on different isoforms of a given lysosomal enzyme, influence their affinity for each receptor [11,12,28–30]. Thus, the two receptors together may be required to bind the complete spectrum of mannose 6-phosphorylated proteins synthesized by the cell. In addition, it has been proposed that the CD-MPR and CI-MPR differentially target lysosomal enzymes to early and late endosomes respectively [13]. If so, variation in the relative levels of the MPRs may provide a mechanism for different cell types to vary the populations of lysosomal enzymes in these different subcellular compartments. This hypothesis could be tested by monitoring the intracellular routing of lysosomal enzymes that are targeted exclusively by one type of receptor, but this has been complicated by lack of such receptor-specific ligands. One such ligand may be represented by a 26 kDa protein that appears to arise exclusively from the CD-MPR deficiency (see Results section and Figure 1). Thus, identification and characterization of this protein may be instrumental in elucidating the relative function of the two MPRs.

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