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Detection of the Asialoglycoprotein Receptor on Cell Lines of Extrahepatic Origin

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The asialoglycoprotein receptor (ASGPR) is the first lectin discovered in mammals. Despite its significant biological role in binding and internalization of desialyated glycoproteins, at least in the human, little information is available regarding its tissue distribution outside of the liver. In the present study, antibodies were raised against the H1 major subunit of the human ASGPR using synthetic peptide antigens, and their binding specificity confirmed by enzyme linked immunosorbent assay. Cell surface analysis by fluorescence activated flow cytometry on various human tissue cell lines confirmed the liver parenchymal cells as the major expression site of ASGPR. Nonetheless, ASGPR was also detectable on some extrahepatic cells such as the Jurkat T-cell line. The determination of extrahepatic expression of ASGPR will have consequences in analyzing the biological role of this receptor complex as well as having implications in designing ASGPR mediated drug- or gene-delivery strategies. © 1998 Academic Press

The asialoglycoprotein receptor (ASGPR) is the first lectin discovered in humans [1]. Originally described as a hepatic lectin (HL), ASGPR was shown to bind and internalize glycoproteins with terminal galactosyl residues. However, besides this primary function, ASGPR has been also linked to other activities such serving as a putative cellular receptor for the hepatitis B [2] and Marburg virus [3] as well as mediating erythroagglutination [4] and so on. Nowadays, the ASGPR system is also considered as a novel approach for targeted gene or drug delivery into liver cells [5, 6]. Despite these important biological roles, less is known about its functions outside of the liver. Even more, only scarce information has been gathered on the tissue distribution of ASGPR, at least in humans.

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The ASGPR consists of two different subunits, which are termed H1 and H2. Transfection studies indicate that the co-expression of the two subunits, either as heterodimeric or hetrotrimeric molecules, is a prerequisite for binding and subsequent receptor-mediated endocytosis of glycoproteins such as the asialoromucoid [7]. While the H2 subunits show several alternatively spliced isoforms [8, 9], the H1 subunit is expressed as a single product encoding a 291 amino acid polypeptide with two glycosylation sites. The H1 subunit was found to be a type II membrane protein with a short cytoplasmic domain and the specific carbohydrate recognition domain (CRD) to its carboxy-terminal [10]. Since immunoelectronic studies revealed that the transfection of H1 alone shows the same plasma membrane distribution as in H1/H2 double transfected cells [7], determination of the expression of H1 is believed to be sufficient to assess the distribution of functional ASGPR in other tissues.

In the present study, antibodies were raised against the major subunit of ASGPR, the HL-H1, by immunization of mice with synthetic peptide antigens which had been designed by comparison and analysis of the human and mouse ASGPR H1 cDNA sequence. The specific reactivity of this antiserum was further confirmed by enzyme-linked immunosorbent assay (ELISA). Also, confocal laser scanning microscopy revealed the specific binding of these antibodies with the plasma membrane of the human hepatoma cell line HepG2, which is a representative cell line expressing the ASGPR protein. Using these newly generated antibodies, the tissue distribution of the H1 subunit was further analyzed on various hepatic and extrahepatic cell lines by cell surface analysis with fluorescence activated cell sorting (FACS).

MATERIALS AND METHODS

Peptide synthesis and conjugation to carrier proteins. The peptide was synthesized by the solid phase method [11] using the Fmoc-

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chemistry. Fmoc-Wang-amino acid resins (Novabiochem., San Diego, CA) were used as support. For peptide chain elongation, DCC (dicyclohexylcarbodiimide) and HOBt (N-hydroybenzotriazole) were used as coupling agent. The side chains of amino acids were protected with the following base (piperidine) stable protecting groups: Asp (OtBu), Asn (Trt), Lys (Boc), Trp (Boc), Arg (Pmc), Ser (tBu), and Tyr (tBu). The final protected peptide-resins were cleaved and protected with TFA-based reagents (88 % TFA, 5 % phenol, 5 % H_2O, 5 % thioanisole, 2.5 % 1, 2-ethandithiol and 2 % triisopropylsilane) for 2 hours, precipitated with diethylether, and dried in vacuum. The crude peptides were purified by a preparative reverse-phase (RP) HPLC on a Waters 15-μ Deltapak C18 column, 300 Å, 19 $\times$ 300 mm. Purity of the HPLC isolated peptides was checked by an analytical RP-HPLC on an Ultrasphere C_{18} column (Ultrasphere, 5 μ, Beckman, San Ramon, CA) 4.6 $\times$ 250 mm.

For immunization of the synthetic peptide, ovalbumin (OVA) was used as a carrier protein. Synthetic peptides were coupled to OVA by the N-S linking method [12] using sulfo-MBS (m-Maleimidobenzoyl-N-hydroxysulfosuccinimide ester). Two mg of OVA was dissolved in 200 μl of 50 mM sodium phosphate buffer (pH 8.0) and then mixed with 100 μl of the MBS solution (10 mg/ml). After stirring the mixture for 1 hour at room temperature, excess amounts of MBS were removed by Sephadex G-50 (15 $\times$ 150 mm) gel filtration chromatography using 100 mM sodium phosphate buffer (pH 7.0). The activated OVA (MBS-OVA) was then mixed with the peptides (2 mg) and then stirred for a further 4 hours at RT. The reaction mixture was fractionated by Sephadex G-50 gel filtration chromatography using PBS as eluent.

Immunization of mice. BALB/c mice (8-10 weeks old) were obtained from the Laboratory Animal Science Division of the Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea. Immunization with carrier protein conjugated peptides was performed by the following scheme: 1st immunization with 20 μg of peptide conjugates emulsified in complete Freund's adjuvant (Sigma, St. Louis, MO); after 14 days, 2nd immunization with peptide conjugates in incomplete Freund's adjuvant (Sigma); after 10 days, 3rd immunization with 10 μg peptide conjugates in incomplete Freund's adjuvant. Test bleedings for determining the antiserum titer were performed every 3 days after the 2nd and 3rd immunization. Animals showing high titer against the immunized protein were sacrificed, and the antiserum was obtained from coagulated blood by downcentrifugation of the blood clots and taking the supernatant.

Enzyme-linked immunosorbent assay (ELISA). Specificity and titer of the mouse antiserum were analyzed by ELISA. The day before analysis, 96-well flat bottom MaxiSorb ELISA plates (NUNC, Roskilde, Denmark) were coated with 1 μg of the corresponding synthetic peptides or as control with 1 μg of a control peptide (derived from the HIV-1 gp41 envelope protein) in 100 μl coating buffer (0.05 M Na-carbonate, pH 9.5) per well. The next day, coating agents were removed by extensive washing with TBS/0.05 % Tween-20, and nonspecific binding was blocked by incubation in 3 % casein (Sigma) in TBS for 1 hour at room temperature. After removal of the blocking agent, the antiserum was added into the corresponding wells in a serially diluted manner. Specific binding of antibodies was detected after serum incubation for 1 hour at room temperature by horseradish peroxidase conjugated anti-mouse IgG antibodies (Sigma). After washing the wells with TBS/0.05 % Tween-20, the reaction was developed by addition of 100 μl substrate solution (50 mM Na-phosphate-citric acid buffer, pH 5.0 containing 0.4 mg/ml o-phenyldiamine and 0.4 μl/ml 37 % H_2O_2). The optical density was measured at 492 nm by an E.max™ ELISA plate reader (Molecular Devices, Sunnyvale, CA).

Cell lines and culture. All of the cell lines used in this study were purchased from ATCC (Rockville, MA), if not else indicated. The human hepatoma cell lines HepG2 [13], PLC/PRF/5 [14] and Chang (ATCC CCL 13) cells were cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with 10 % FBS (Atlanta Biologicals, Norcross, GA). All other cell lines were maintained in RPMI-1640 (Gibco, Grand Island, NY) supplemented with 10 % FBS. The EBV-transformed B-cell line Wa [15] was a kind gift from Dr. Keisuke Sato, Asahikawa Medical College, Japan. The other cell lines used in this study comprised the Jurkat T-cell line [16], the neuronal cell line U87-MG [17], WISH amnion cells (ATCC CCL 25), the human fibroblastic cell line HeLa (ATCC CCL 2), A498 human kidney cells (ATCC HTB 44), the promyelocytic cells HL-60R [18] and the human embryonal carcinoma, Tera-1 [19]. For maintenance, cells were cultured at 37 °C in 5 % CO_2 atmosphere in a humidified incubator.

Confocal laser scanning microscopy. For cell surface staining, the day before analysis, HepG2 human hepatoma cells were trypsinzed from tissue culture flasks and seeded into 8-well Chamber Slide culture chambers (Nunc Inc., Naperville, IL) to a cell concentration of 50 % confluency. For analysis, cells were extensively washed while attached on the slides with a sufficient volume of staining buffer (PBS/0.05 % BSA/0.02 % Na-azide). The cells were then incubated with 300 μl of the anti-ASGPR H1 antibody prior diluted to 1: 500 in staining buffer. After 30 min at 4 °C, excess antibodies were removed by washing in staining buffer, and the bound mouse antibodies detected with phycoerythrin conjugated goat anti-mouse IgG antibodies (DAKO Japan Co., Kyoto, Japan). For intracellular staining and analysis, cells had to be first fixed before incubation with the anti-ASGPR H1 antibodies by incubation in 2 % paraformaldehyde in PBS for 30 min at 4 °C. The plasma membrane of the fixed cells were then permeabilized with 0.1 % saponin/ 0.1 % Na-azide/5 % FBS in PBS for 20 min at 4 °C. Further staining procedures were essentially the same as the cell surface staining as described above. Fluorescence images of the antibody treated cells were obtained by confocal laser scanning microscopy. The confocal microscope system consisted of a Leica TCS 4D connected to an Leica DAS upright microscope (Leica Lasertech GmbH, Heidelberg, Germany).

FACS analysis. To identify the specific binding of the anti-ASGPR H1 immune serum in a more objective and quantitative manner, cells incubated with the mouse antibodies were stained with fluorescence labeled secondary antibodies and analyzed by fluorescence activated flow cytometry. For one staining reaction, 4 $\times$ 10^5 cells of each cell lines were incubated with the mouse antiserum in a dilution of 1 to 1,000 in PBS for 30 min at 4 °C. After incubation, excessive reagents were washed out twice with staining buffer, and the specifically bound antibodies themselves were detected with FITC conjugated anti-mouse IgG antibodies (Serotec Ltd., Oxford, England). Flow cytometry was performed by a FACScan (Becton Dickinson Inc., Mountain View, CA, U.S.A) cytometer, and the obtained data were analyzed using the software program LYSYS (Becton Dickinson Inc.).

RESULTS

Generation of the ASGPR H1 Subunit Specific Mouse Antibodies

To generate antibodies against the human ASGPR in a simple and reproducible manner, a strategy was developed to use small synthetic peptides with a sequence derived from the ASGPR protein as immunizing antigens. To identify the most divergent region between the mouse and human H1 subunit, each of the H1 sequences were obtained from the GenBank and aligned to each other. Fig. 1 shows the amino acid sequences of the ASGPR H1 subunits of each species. Since the ASGPR H1 subunit is a type II membrane

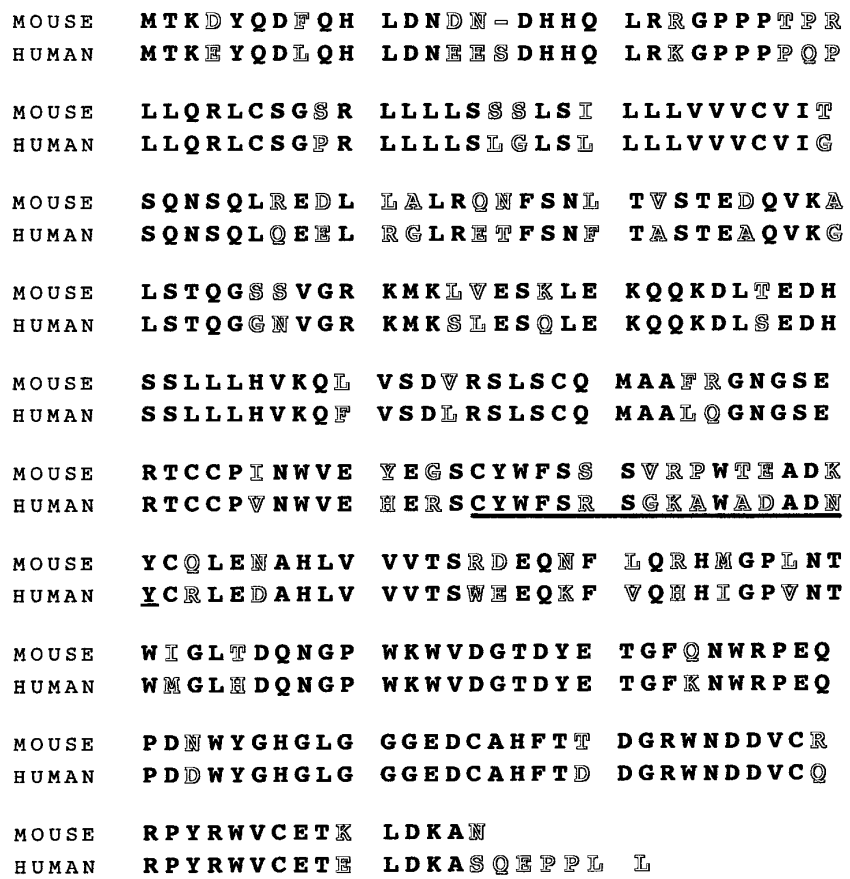

FIG. 1. Comparison of the mouse and human ASGPR H1 amino acid sequences. The ASGPR H1 subunit sequences of the mouse (GenBank accession number: g423393) and human (GenBank accession number: g71960) were aligned to each other, and the most divergent region within the extracellar domain was identified. The sequence is represented in one-letter amino acid abbreviation. Bold outlined letters indicate different amino acid residues between the two species. The underlined region shows the sequence used for peptide synthesis of the immunogen.

protein, the N-terminal sequences comprise the intracellular domain while the C-terminal region represents the extracellular part. It is obvious that antibodies should be raised against the extracellular part of the receptor to obtain reagents which will be able to identify the expression of ASGPR proteins by cell surface analysis. As shown in Fig. 1, a short fragment of 17 amino acid residues was selected from the extracellular domain for peptide synthesis and further immunization. After synthesis and purification, the peptide was conjugated by N-S linkage to a carrier protein to enhance immunogenicity, and the peptide-protein complex was used in immunization of BALB/c mice.

The immune sera obtained from these mice were then tested for their specificity in an ELISA. Fig. 2A shows that the antiserum is specific for the immunized antigen but that it shows no binding activity to control peptides. The specificity of this assay was then further confirmed by an other parallel assay in which the preimmune serum from the same mouse was used for detection of the ASGPR derived peptide sequence. As shown in Fig. 2B, the pre-immune serum has no affinity to the immunized antigen.

Detection of ASGPR Expression by Confocal Microscopy and Flow Cytometric Analysis

The specificity of the anti-ASGPR serum was further confirmed by cell surface staining of HepG2 hepatoma cells. In the case of staining intact cells with the antiserum (Fig. 3A), a specific signal was observed on the cell surface indicating the presence of the antigen structure on the outer cell membrane. Since HeLa cells, which have been reported to lack ASGPR proteins, failed to be stained by the same procedure, the antigen specificity of the antibodies could be further extended onto cellular protein level. The ASGPR system is a classical model for receptor-mediated endocytosis. Regarding this fact, it was expected also to detect ASGPR proteins in endosomal compartments. For the analysis of intra-

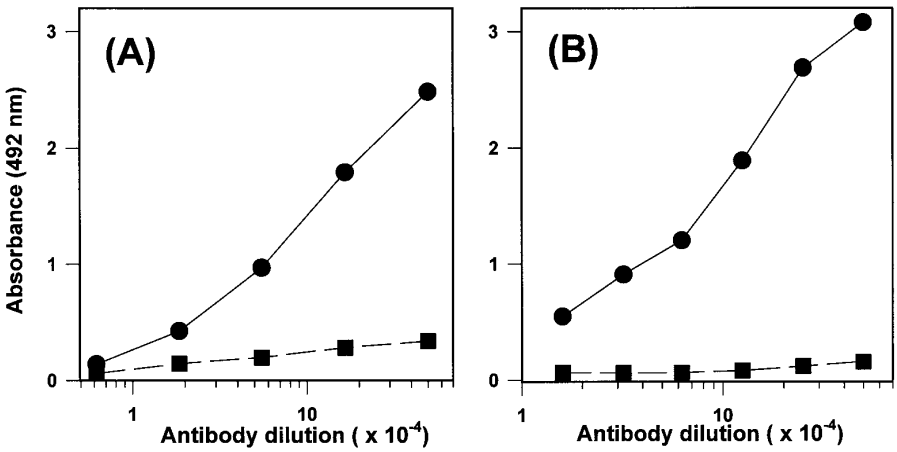

FIG. 2. Determination of antigen specificity of the newly generated immune serum by ELISA. A). Determination of antibody reactivity with the ASGPR peptide (●) and a HIV-1 gp41 envelope protein derived control peptide (■). 100 μl of serially diluted immune serum was added to each individual well of a 96-well ELISA plate prior coated with 1 μg of the ASGPR H1 derived peptides or with 1 μg of the control peptide (HIV-1 gp41 peptide - RILAVERYLKDQQLLGIWGCSGKLICTTAVPWNAS). Antibody binding was detected with 1:10,000 diluted peroxidase conjugated goat anti-mouse IgG antibodies (Sigma). B). Examination of the ELISA detection specificity. ELISA plates were coated with the ASGPR peptide and the antigen detected with either the immuneserum (●) or pre-immune serum (■) from the same mouse.

cellular distribution, HepG2 cells were first fixed with paraformaldehyde then permeabilized with saponin, and stained with the anti-ASGPR serum followed by fluorescence labeled secondary antibodies. The use of the confocal microscope system for image analysis allowed the optical dissectioning of the cell without physical damage while conserving the overall cell structure. In Fig. 3B is shown the mid-horizontal cross-section of HepG2 cells in an attached growing state. In contrast to the successful intra- and extracellular staining of HepG2 cells, no ASGPR proteins were detectable by the same method in HeLa cells (data not shown).

To expand this observation of cell line specific ASGPR expression onto cells of other tissue origin, cell lines from various human organs were obtained and used for staining with the antiserum. Since liver is regarded as the main site of ASGPR expression, firstly, cell lines from hepatic origin were screened by cell surface analysis with the antiserum by FACS. This method is preferable to confocal microscopy in that a huge number of cells can be analyzed in a relatively short time, and that the fluorescence signals indicating the expression level of the ASGPR protein can be measured and objectively compared to those of other cells. The FACS data shows that even within the hepatic cell lines the expression level of ASGPR greatly differs (Fig. 4). While the highest expression was observed on HepG2 cells, Chang liver cells do not express the ASGPR H1 subunit at all. On the other hand, PLC/PRF/5 liver cells showed an expression level somewhere inbetween that of the other two cell lines.

The ASGPR expression on cells of extrahepatic origin was also of a great variety (Fig. 5). The cell lines HeLa, HL-60R, WISH, A498, U87-MG and Wa showed no antibody binding, but on the contrary, a strong signal was observed on the T-cell line Jurkat. Also the Tera-1 cells exhibited a weak but evidently positive signal, indicating a cell line specific controlled expression of ASGPR proteins.

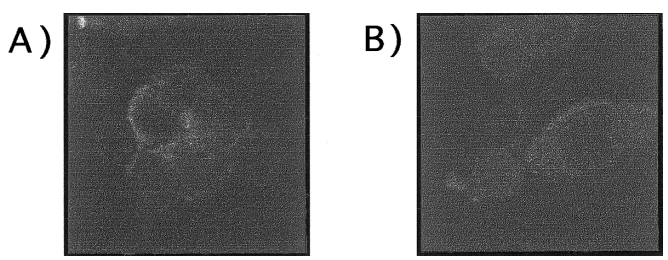

FIG. 3. Cell surface and intracellular analysis of HepG2 cells stained with the anti-ASGPR antibody by confocal laser scanning microscopy. A). Expression of the ASGPR H1 subunit as visualized by the anti-ASGPR antibodies and fluorescence labeled secondary antibodies. B). Detection of intracellular expression of ASGPR proteins by intracellular staining and confocal microscopy.

DISCUSSION

Since the first description of ASGPR, this receptor complex has been mainly regarded as a "hepatic" lectin, exclusively expressed on hepatic parenchymal cells [20]. Nevertheless, upon further investigation, it was shown that mRNA of the RHL-1 receptor, which represents the rat counterpart of the human ASGPR H1 subunit, was also detectable in the salivary glands, ileum, kidney, stomach, duodenum, jejunum, and even in the spleen of the developing rat [21]. Further studies confirmed this observation

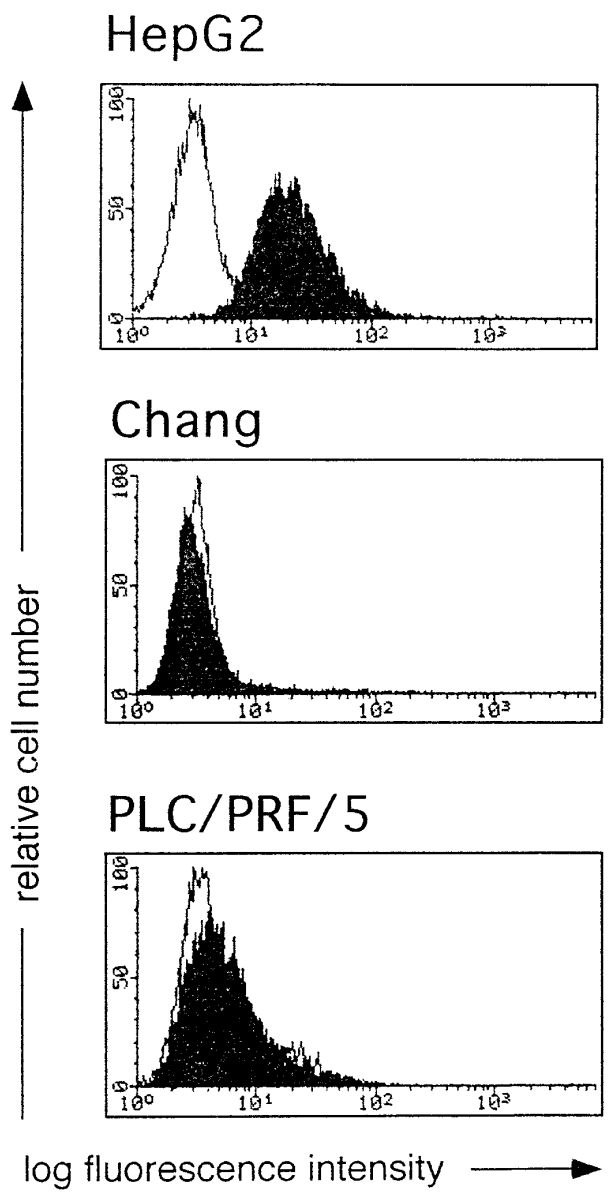

FIG. 4. Analysis of human hepatoma cell lines upon expression of ASGPR proteins by fluorescence activated flow cytometry. The liver cell lines HepG2, Chang, and PLC/PRF/5 were used in this study. 4×10^5 cells from the corresponding cell lines were stained for analysis. Filled histograms indicate cells stained with the ASGPR specific antiserum, and open histograms show the background fluorescence signal yielded by staining cells with pre-immune serum. Binding of anti-ASGPR specific antibodies were detected with phycoerythrin conjugated rabbit anti mouse IgG antibodies.

and even more extended the tissue specific detection of rat ASGPR up to thyroid and brain tissues [22]. Such reports regarding the extrahepatic expressions of the ASGPR were not restricted to the rat. Investigations about the distribution of the murine ASGPR revealed the tissue specific expression of the mouse HL-1 subunit in the liver, testis, and the epididymis [23].

Despite these rather well characterized organ specific localizations of ASGPR in rodents, in the human system, the systematic analysis of extrahepatic ASGPR expression has been lacking so far. Besides the well documented expression of ASGPR in human liver parenchymal cells, only some sporadic reports upon human ASGPR expression, like the detection of the H1 and H2 subunits in intestinal epithelial cells [24], consist the most of information available upon its extrahepatic distribution.

To generate a broader picture about the ASGPR expression sites in human, in the present study, the tissue distribution of the ASGPR was investigated using cell lines derived from various human tissues by cell surface analysis with ASGPR H1 specific antibodies. These specific antibodies were developed in the mouse by immunization with a synthetic peptide derived from heterologous regions of the human H1 unit as compared to the mouse sequence on the amino acid level. This 17-meric peptide (CYWFSRSGKAWADADNY) was synthesized after the sequence of the human ASGPR H1 subunit and comprises a part of the CRD within the extracellular domain (Fig. 1). For immunization, peptides were conjugated to carrier proteins, and injected to BALB/c mice resulting in the successful development of ASGPR H1 specific polyclonal antibodies.

Antibodies generated by use of short peptides as immunogens have the advantage of recognizing only this predefined restricted epitope within a large protein, which in turn enables the targeted production of antibodies even to structures which are closely related to the animal's own protein. The production of anti-human ASGPR antibodies in the mouse had also taken advantage of this strategy. Generally, eliciting immune response against highly conserved protein molecules in different species requires careful analysis of the whole protein. Comparison of structure and sequence homology between evolutionary conserved molecules often reveals some epitopes which are characteristic for the protein molecule but nevertheless represents a species specific motif. The evaluation of such an antigenic region leads to the convenient design of recombinant or synthetic immunogens. In the case of the ASGPR H1 subunit, high homology has been observed between mouse and human amino acid sequences [25]. This similarity has largely restricted the development of human H1 specific antibodies in the mouse so far. Although there have been reports about monoclonal antibodies directed against the rat [26] and even a single publication about the generation of human ASGPR specific mAbs [27], the unavailability of any of these or other commercial ASGPR specific antibodies has limited further investigations about the ASGPR. On the other hand, the mouse antiserum raised in this study can be easily obtained by immunization with the H1 derived peptide as described above, and the antigen peptide

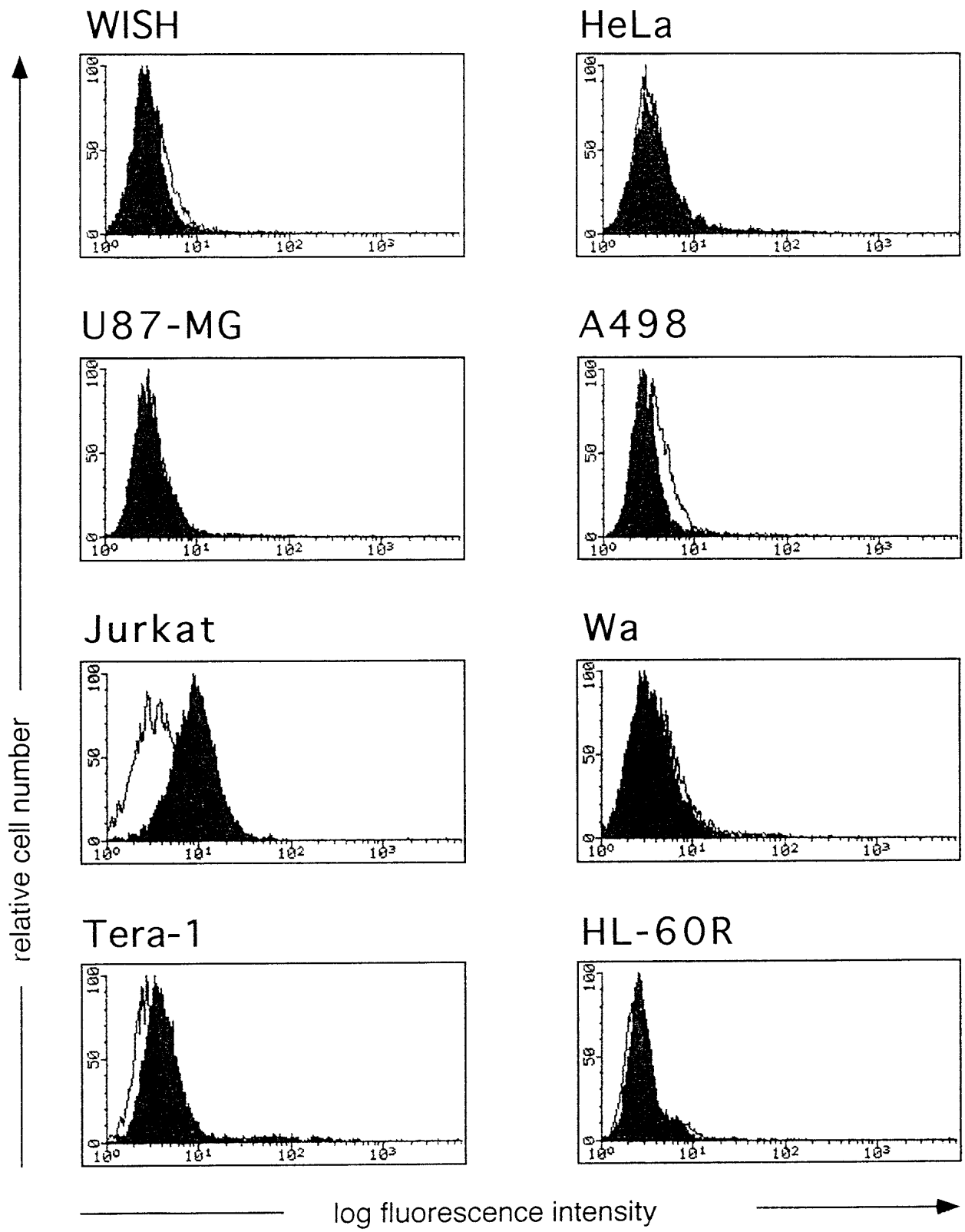

FIG. 5. Detection of ASGPR H1 receptor expression on cell lines of extrahepatic origin. For analysis, 4×10^5 cells from the corresponding cell lines were used for antibody staining. Filled histograms indicate cells stained with the anti-ASGPR H1 serum, and open histograms show the control staining with pre-immune serum from the same mouse. In the case of a ASGPR positive cell line, the fluorescence signal distribution shifts to the right along the horizontal log scale axis.

sequence as well as the simple immunization protocol will open a convenient way for the development of anti-ASGPR antibodies.

Using this specific antiserum, cells from various tissue origins were analyzed upon their expression of the H1 subunit by fluorescence activated flow cytometry. Fig. 4 and Fig. 5 summarize the cellular distribution of ASGPR expression as determined by FACS analysis in the form of histogram plots. In all the diagrams, cell surface staining with pre-immune serum (open histogram) is compared to the staining with the antiserum (closed histogram). In this way, the liver parenchymal cell lines HepG2 was confirmed again to be ASGPR positive as it has been already shown by confocal microscopy (Fig. 3). But rather unexpectedly, the Chang liver cells showed no ASGPR expression at all, and also only a low level expression was detectable on the hepatoma cell line PLC/PRF/5. Concluding from this observation, it is evident that the expression of the ASGPR protein can not be regarded as a typical characteristic for all hepatocytic cells. Rather, the expression level of the ASGPR seems to be an independently regulated event in individual cell lines. Whether this modulation happened during transformation or long-term culture is not clear and requires further investigation.

The examination of ASGPR expression was then further extended to cell lines derived from other human organs, such as the brain, amnion, kidney, and cervix as well as B- and T-lymphocytes and promyelocytic cells. Among these cell lines, the T-cell line Jurkat exhibited a strong binding signal by anti-ASGPR antibodies comparable to that of HepG2 cells (Fig. 4). Also a weak signal was detected on Tera-1 cells, which is a cell line derived from human testis tissue [19]. The observation of ASGPR expression in extrahepatic cells was not surprising for the fact itself, since in mice and rats several other tissues than the liver have been reported to harbour ASGPR positive cells [21–23]. But so far in humans, ASGPR has been only detected in hepatocytes and enterocytes. These comprised liver tissue cells and hepatoma cells like the HepG2 cells, and intestinal epithelial cell lines such as the Caco-2 [24], and as recently described, the HT-29 cells [28]. In view of these limited reports, the detection of ASGPR proteins on a established T-cell line as well as on other extrahepatic sites is of outstanding interest. While the actual biological functionality of these extrahepatic ASGPR proteins as well as the presence of the other receptor subunits has yet to be evaluated, the detection of the human ASGPR also outside of the liver is nevertheless a confirmation of the evolutionary conserved role of this system in hepatic and ectopic sites.

Expansion of the tissue distribution of ASGPR expression beyond cells of hepatic origin is of great importance not only for understanding the biological role of the ASGPR system and for the identification of the still uncleared natural ligand for this receptor, but also for further developments of its medical and biotechnological applications. Considered to be the classical model for receptor mediated endocytosis, and showing a relatively restricted tissue distribution, the ASGPR system has been regarded as an ideal model for development of gene- or drug delivery systems. Using DNA or liposomes labeled with asialoglycoproteins, strategies were developed to deliver anti-sense DNA, i.e. for the targeted inhibition of hepatitis B virus replication [29] or modulation of acute phase response [30] as well as to establish liposome based gene-delivery systems [31]. Since in all these methods, the bioactive molecule is designed to be receptor bound and internalized into the cells by the ASGPR system, the restricted tissue distribution has to be confirmed to avoid any complications or side effects by challenging ASGPR positive cells in other sites than the liver. In this aspect, the finding about ASGPR receptors on Jurkat T-cells will have consequences for designing further ASGPR mediated gene- or drug-delivery systems.

Whether the situation for the ASGPR expression *in vivo* is as the same as for *in vitro* cultured cell lines has still to be examined by analysis of their corresponding primaric counterpart cells. Especially the existence of ASGPR proteins on T-lymphocytes has to be verified, since the T-cells will have the first contact in the case of ASGP-labeled drug administration, as well as the possible biological role of the T-cells in serum clearance of asialated proteins has to be examined.

It is expected that the newly generated anti-ASGPR antibodies from the present study will serve as a valuable tool in evaluation of the role of the ASGPR in humans in further studies.

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REFERENCES

1. Baenzigesr, J. U., and Maynard, Y. (1980) *J. Biol. Chem.* **255,** 4607–4613.
2. Treichel, U., Meyer zum Buschenfelde, K.-H., Stockert, R. J., Poralla, T., and Gerken, G. (1994) *J. Gen. Virol.* **75,** 3021–3029.
3. Becker, S., Spiess, M., and Klenk, H.-D. (1995) *J. Gen. Virol.* **76,** 393–399.
4. Stockert, R. J., Morell, A. G., and Scheinberg, I. (1974) *Science* **186,** 365–366.
5. Yamamoto, M., Hayashi, N., Miyamoto, Y., Takehara, T., Mita, E., Seki, M., Fusamoto, H., and Kamada, T. (1995) *Hepatology* **22,** 847–855.
6. Wu, G. Y., and Wu, C. H. (1988) *Biochemistry* **27,** 887–892.
7. Henis, Y. I., Katzit, I. Z., Shia, M. A., and Lodish, H. F. (1990) *J. Cell. Biol.* **111,** 1409–1419.
8. Lee, D. G., Lee, S. G., Kim, K. L., and Hahm, K. S. (1997) *J. Biochem. Mol. Biol.* **30,** 299–301.

9. Paietta, E., Stockert, R. J., and Racevskis, J. (1992) *Hepatology* **15,** 395–402.
10. Iobst, S. T., and Drickamer, K. (1994) *J. Biol. Chem.* **269,** 15512–15519.
11. Merrifield, R. B. (1986) *Science* **232,** 341–347.
12. Kitagawa, T., and Aikawa, T. (1976) *J. Biochem.* **79,** 223–236.
13. Aden, D. P., Fogel, A., Plotkin, S., Damjanov, I., and Knowles, B. B. (1979) *Nature* **282,** 615–616.
14. MacNab, G. M., Alexander, J. J., Lecatsas, G., Bey, E. M., and Urbaowicz, J. M. (1976) *Br. J. Cancer* **34,** 509–515.
15. Choi, E. A., Park, J. H., Cho, E. W., Hahm, K. S., and Kim, K. L. (1996) *Mol. Cells* **6,** 622–627.
16. Weiss, A., Wiskocil, R. L., and Stobo, J. D. (1984) *J. Immunol.* **133,** 123–128.
17. Beckman, G., Beckman, L., Ponten, J., and Westermark, B. (1971) *Hum. Hered.* **21,** 238–241.
18. Park, J. H., Cho, E. W., Lee, Y. J., Hahm, K. S., and Kim, K. L. (1997) *Hybridoma* **16,** 551–556.
19. Fogh, J. (1978) *Natl. Cancer. Inst. Monogr.* **49,** 5–9.
20. Spiess, M., Schwartz, A. L., and Lodish, H. F. (1985) *J. Biol. Chem.* **260,** 1979–1982.
21. Mu, J. Z., Tang, L. H., and Alpers, D. H. (1993) *Am. J. Physiol.* G752–G762.
22. Pacifico, F., Laviola, L., Ulianich, L., Porcellini, A., Ventra, C., Consiglio, E., and Avvedimento, V. E. (1995) *Biochem. Biophys. Res. Comm.* **210,** 138–144.
23. Monroe, R. S., and Huber, B. E. (1994) *Gene* **148,** 237–244.
24. Mu, J. Z., Fallon, R. J., Swanson, P. E., Carrol, S. B., Danaher, M., and Alpers, D. H. (1994) *Biochim. Biophys. Acta.* **1222,** 483–491.
25. Sanford, J. P., and Doyle, D. (1990) *Biochim. Biophys. Acta.* **1087,** 259–261.
26. Harford, J., Lowe, M., Tsunoo, H., and Ashwell, G. (1982) *J. Biol. Chem.* **257,** 12685–12690.
27. Kohgo, Y., Kato, J., Nakaya, R., Mogi, Y., Yago, H., Sakai, Y., Matsushita, H., and Niitsu, Y. (1993) *Hybridoma* **12,** 591–598.
28. Mu, J. Z., Gordon, M., Shao, J. S., and Alpers, D. H. (1997) *Gastroenterology* **113,** 1501–1509.
29. Nakazono, K., Ito, Y., Wu, C. H., and Wu, G. Y. (1996) *Hepatology* **23,** 1297–1303.
30. Rajur, S. B., Roth, C. M., Morgan, J. R., and Yarmush, M. L. (1997) *Bioconjug. Chem.* **8,** 935–940.
31. Hara, T., Kuwasawa, H., Arakami, Y., Takada, S., Koike, K., Ishidate, K., Kato, H., and Tsuchiya, S. (1996) *Biochim. Biophys. Acta* **1278,** 51–58.