J. Membrane Biol. 173, 165–177 (2000)

The Journal of
Membrane
Biology

Topical Review

Surface Antigen CD98(4F2): Not a Single Membrane Protein, But a Family of Proteins with Multiple Functions

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Received: 10 August 1999/Revised: 28 October 1999

Introduction

CD98(4F2) belongs to a group of approximately two hundred proteins at the surface of leukocytes that are thought to be important for their function in the immune response, by specifying their migration, cell interactions and the signal transduction mechanisms that lead to lymphocyte activation. Following the discovery of CD98(4F2) as a surface antigen in lymphocytes (Haynes et al., 1981), the antigen was found to be present in all established human tissue culture cell lines tested and in most malignant human cells (Hemler & Strominger, 1982; Quackenbush et al., 1987). Although it was linked to cell proliferation and growth (Yagita et al., 1986, Gottesdiener et al., 1988) it essentially remained a protein in search of a function. Only recently, have reports linked CD98(4F2) to a number of specific cellular processes such as adhesion, fusion and amino acid transport across the cell membrane in different cell types. How these relate to its role in cell activation and proliferation, has not yet been established.

The complete molecular structure of CD98(4F2) has been resolved during the last year. Early biochemical studies showed that the 4F2 cell surface antigen (which was later designated CD98; Barclay et al., 1997) was a 120-kDa disulfide-linked heterodimer composed of two subunits, a glycosylated 80 kDa heavy chain and a 40 kDa light chain (Haynes et al., 1981); the 4F2 antibody recognized an antigenic determinant on the polypeptide backbone of the heavy chain. Later, the heavy chain was described as a type II glycoprotein of 529 amino acids (Quackenbush et al., 1987), but the nature of the associated light chain remained unknown. Recently it has been shown that "the" light chain is not a single protein, but a family of homologous proteins with similar molecular weight and membrane topology (Fig. 1). The light subunits are able to associate to the heavy chain through a disulfide bond, giving rise to different heterodimers (Mastroberardino et al., 1998); they have multiple transmembrane-spanning domains and their amino acid sequences have been shown to conform to the APC superfamily (transporters for amino acids, polyamines and choline). Consistently, the heterodimers have been shown to function as amino acid transporters.

In the context of this review, CD98 will be used to refer generically to the heterodimers (also known as 4F2) and the heavy chain and the light chains will be referred to as CD98hc or CD98lc, respectively. In those cases where information regarding the molecular nature of the light chain is available, the corresponding molecular form will be indicated following the denomination adopted by Kanai and collaborators (Kanai et al., 1998; Segawa et al., 1999) and Palacín and collaborators (Torrents et al., 1998; Pineda et al., 1999).

Although work in this area has never been more active, making this review clearly one that will quickly be overtaken by ongoing and future work, it is the very broad nature of the CD98 field, with overlap of immunology, membrane physiology, cell biology and cancer biology, that makes a current review of potential value.

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Key words: CD98 — 4F2—Amino acid transport — Cell activation — Adhesion — Proliferation

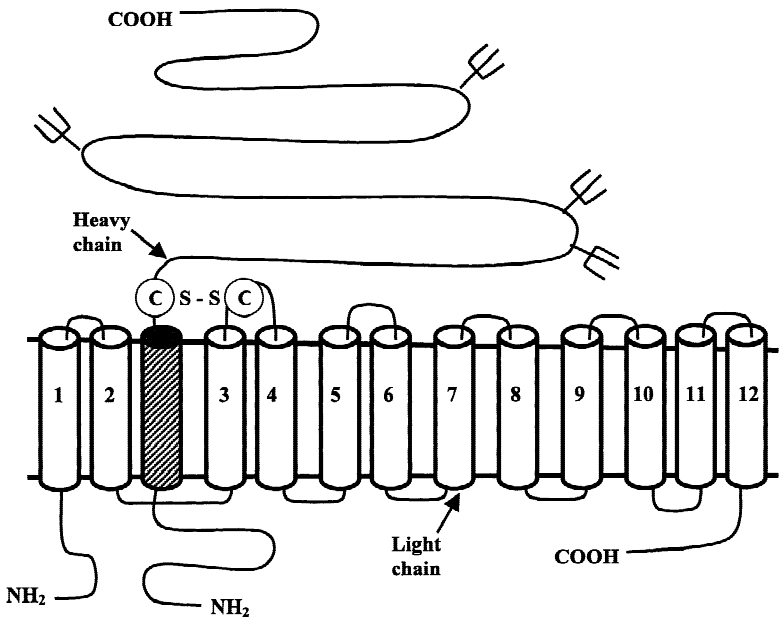

Fig. 1. Model of a member of the CD98 family of proteins. The heterodimer is formed by a glycosylated heavy chain (shaded) and a light chain with 12 membrane-spanning domains. Six different light chains have been identified (Table). The two type of subunits are linked through a disulfide bond. Redrawn from Mastroberardino et al., 1998.

Molecular Structure

Heavy Chain

The cDNA for the heavy chain of human CD98 was cloned some ten years ago (Lumadue et al., 1987; Quackenbush et al., 1987 and Texeira et al., 1987). The predicted protein encodes a glycosylated protein of 529 amino acids containing a single transmembrane segment (amino acids 83–106). The protein has an extracellular carboxyl terminus and an internal amino terminus and has been classified as a type II membrane glycoprotein (Fig. 1).

The primary sequence contains four potentially glycosylated asparagine residues which are all located in the carboxy-terminal half of the protein. Four glycan units have been reported in biochemical studies of the mouse homologue of CD98 (Luscher et al., 1985) and differences in the pattern of glycosylation account for the smaller size of the antigen in T cell than in B cell lines (Hemler & Strominger, 1982). Cys-109 has been shown to participate in disulfide bond formation with the light chain (Torrents et al., 1998; Pfeiffer et al., 1998). The heavy chain has also been sequenced from mouse (Parmacek et al., 1989), rat (Broer et al., 1997) and hamster (Fenczik et al., 1997).

An homologue of CD98hc, here referred to as rBAT, was identified in three separate laboratories by expression cloning from rabbit (Bertran et al., 1992*b*) and rat kidney cDNA libraries (Wells & Hediger, 1992; Tate et al., 1992). rBAT is a single polypeptide of 683 (rat) or 677 (rabbit) amino acids and has been shown to induce the Na^+-independent transport of cationic and neutral amino acids when expressed in *Xenopus laevis* oocytes. Two alternative membrane topologies have been proposed for rBAT, with one (Bertran et al., 1992*a*) or 4 (Mosckovitz et al., 1994) membrane-spanning segments. CD98 and rBAT share an overall sequence identity of 30% but there are four highly conserved motifs (10–18 amino acid residues, 67–80% identity). rBAT has been shown to be mutated in patients with Type I Cystinuria, an inherited hyperaminoaciduria of cystine and dibasic amino acids (Calonge et al., 1994).

Light Chains

So far, and within the last year, six cDNAs encoding distinct light have been identified. The light chains are markedly hydrophobic proteins of 502–535 amino acids and contain 12 putative transmembrane domains. They are linked to CD98hc through an extracellular cysteine that participates in disulfide bond formation (Pfeiffer et al., 1998). All light chains identified to date function as amino acid transporters when associated with the heavy chain and the substrate specificity of the heterodimer depends on the nature of the light chain. The molecular features, tissue distribution and functional properties of the six light chains are summarized in the Table.

The first light subunit was identified in studies of expression of early aldosterone-regulated gene products. A cDNA (ASUR-4) encoding a highly lipophilic, permease related protein (507 amino acids), with an approximate molecular weight of 40 kDa on SDS-PAGE, was cloned from *Xenopus* A6 cells (Spindler et al., 1997). When ASUR-4 was coexpressed with human CD98hc in *Xenopus* oocytes, a heterodimer was formed which localized at the cell surface and mediated the Na^+-independent transport of large neutral amino acids, as

Table. Characteristics of the heavy and light subunits of the CD98 heterodimers

Name of subunit and specificity of transport activity	Chromosomal location	Number of amino acids and cysteine residue that participates in disulfide bond	Transmembrane domains	High expression in	Not detected* or lowest expression in
Heavy chain					
CD98hc	11	529; Cys 109	1	Ubiquitous. High in proliferating cells.	–
Light chains					
LAT-1 Large neutral amino acids. Na^+ independent	16q24.3	507; Cys 164	12	Brain, placenta, skeletal muscle Spleen, testis and fetal liver (M)	Intestine* Liver (R, M)*
LAT-2 Small and large neutral amino acids. Na^+ independent	14q11.2-13	535; Cys 154	12	Kidney, placenta small intestine (R)	Lung
y^+-LAT-1 Cationic and neutral amino acids. Na^+ required for neutral amino acid binding	14q11.2	511; Cys 151	12	Kidney cortex, peripheral blood, leukocytes. Small intestine (M)	–
y^+-LAT-2 Cationic and neutral amino acids. Na^+ required for neutral amino acid binding	16	515; Cys 159	12	Not determined	Not determined
xCT Glutamic acid and cystine (anionic form)	–	502 (M); Cys 158	12	Activated macrophages, brain (M)	Lung, liver, kidney* (M)
SPRM1 Cationic and neutral amino acids. Na^+ partially required for neutral amino acid binding	–	503 (S); Cys 137	12	–	–

Unless indicated the characteristics refer to the human proteins. *Abbreviations:* H, human; M, mouse; R, rat; X, *Xenopus laevis;* S, *Schistosoma mansoni.* Alternative names given to the proteins listed in the table: CD98hc: 4F2, FRP-1, gp125; LAT-1: SCL7A5 (H), E16 (H), TA1 (M), hAmAT-L-lc (H), ASUR-4 (X), MU12 (X); LAT-2: SCL7A8 (H): y^+LAT-1: SCL7A7 (H); y^+LAT-2: HA7016 (H), KIAA0245 (H). References are given in the text.

expected for an L-type transporter (Mastroberardino et al., 1998). ASUR-4 has therefore been denominated LAT-1 (L-type amino acid transporter 1). LAT-1 was cloned independently from rat C6 glioma cells by coexpression with CD98hc in *Xenopus* oocytes (Kanai et al., 1998).

Human and rat homologues of LAT-1 had previously been cloned and designated E16 and TA1, respectively. Human E16 was first identified from peripheral blood leukocytes and associated with lymphocyte activation (Gaugitsch et al., 1992). The rat homologue (TA1) was cloned on the basis of its differential expression between hepatoma cells and normal liver and was described as a highly conserved oncofetal protein associated with liver development, carcinogenesis and cell activation (Sang et al., 1995). E16 and TA1 were later shown to be truncated products of LAT-1 (shortened by 266 amino acids) (Mastroberardino et al., 1998). The equivalence between the E16/TA1 protein and the light chain of CD98 was also demonstrated by microsequencing of the immunoprecipitated CD98 heterodimer (Mannion et al., 1998).

The complete human LAT-1 sequence (507 amino acids) was cloned and found to be 91% identical to the rat protein (Mastroberardino et al., 1998; Tsurudome et al., 1999; Prasad et al., 1999). It contains one site for N-linked glycosylation (Asn-230), which is not conserved in the rat, and multiple potential phosphorylation sites (casein kinase II, Ser-113; protein kinase C, Ser-189, Ser-346; tyrosine kinase, Tyr-119) which are also present in the rat homologue. A mouse homologue was cloned by expression using an antibody raised against the CD98 light chain (Nakamura et al., 1999).

The discovery of LAT-1 has been followed by the

identification of a number of homologous proteins in various laboratories using different strategies.

y^+LAT-1 was identified independently by Torrents et al. (1998) and Pfeiffer et al. (1999). The protein exhibits 49% identity with LAT-1 and 73% identity with the functionally uncharacterized KIAA0245 (Nagase et al., 1996) which has been designated y^+LAT-2. The heterodimer formed by CD98hc and y^+LAT-1 functions as an exchanger of neutral and cationic amino acids. Transport is Na^+ independent for cationic amino acids but Na^+ dependent for neutral amino acids as described for amino acid transport system y^+L. The gene has been mapped to chromosome 14q11.2 and shown to be responsible for the genetic disorder Lysinuric Protein Intolerance (Borsani et al., 1999; Torrents et al., 1999).

xCT was isolated by expression cloning from mouse activated macrophages (Sato et al., 1999) and shown to carry out the exchange of glutamate for the anionic form of cystine, as described for transport system x_C^- (Bannai & Kitamura, 1980). Expression is increased in macrophages after stimulation by lipopolysacharides or an electrophilic agent.

The more recently cloned light subunit is LAT-2 (Pineda et al., 1999; Segawa et al., 1999). It has 44–50% homology with the other human light chains and, like LAT-1 it catalyzes the Na^+-independent exchange of neutral amino acids, but with broader specificity.

SPRM1, a membrane protein of the platyhelminth *S. mansoni*, is the closest nonmammalian homologue of this family of "glycoprotein-associated amino acid transporters" exhibiting 40% homology to y^+LAT-1 (Mastroberardino et al., 1998). It displays an amino acid transport activity with characteristics intermediate between systems y^+L and L (Pfeiffer et al., 1999).

These CD98 light chains are also related to the MUP1 (methionine permease) gene product of yeast (25% identity) which has a similar hydropathy profile (Isnard et al., 1992). MUP1 however does not contain the conserved cysteine residues and is not glycoprotein-associated. There is also weak homology between these light chains and the mammalian CAT family of transporters composed of several cationic-specific amino acid transporters (Closs, 1996). The level of identity of the mouse y^+LAT-1 with rat CAT-1 and human CAT-2A is respectively, 21 and 25%. Alignments show several gaps since the CAT proteins are substantially larger (624 and 657 amino acids) than y^+LAT-1. They may also have two more transmembrane domains (putatively 14 *vs.* 12).

Regulation of Gene Expression

The human CD98hc gene appears to be regulated by a number of distinct molecular mechanisms which are in turn triggered by activation of discrete intracellular pathways. The cDNA derives from a single copy gene that has been highly conserved during mammalian evolution and maps to chromosome 11 (Messer-Peters et al., 1982; Francke et al., 1983; Quackenbush et al., 1987). It is composed of 9 exons and its structure, when taken together with studies of the patterns of its transcriptional regulation, suggests a model in which both 5′ flanking sequences and exon 1 or intron 1 play a key role (Gottesdiener et al., 1988). The 5′ flanking sequences display characteristics of housekeeping genes, but also contain potential inducible promoter elements. The 5′ upstream region of the gene is G + C rich and hypomethylated in peripheral blood lymphocyte DNA and contains multiple binding sites for the ubiquitous Sp1 transcription factor, while lacking TATA and CCAAT sequences. This region of the gene also displays sequence homologies with several other inducible T-cell genes, including interleukin-2, interleukin-2 receptor (α chain), dihydrofolate reductase, thymidine kinase, and transferrin receptor genes.

Although the CD98hc gene shows many of the structural features characteristic of constitutively expressed genes, phytohemmatoaglutinin (PHA) mediated activation of resting peripheral blood T lymphocytes results in a dramatic increase in steady-state levels of CD98hc mRNA (Gottesdiener et al., 1988). PHA is known to produce the activation of a diverse set of intracellular pathways, including translocation and activation of protein kinase C and increase in intracellular Ca^{++} levels. Indeed, costimulation with phorbol ester TPA (a protein kinase C activator) and A23187 (calcium ionophore) results in strong induction of CD98hc on rat and human lymphocytes (Tanaka et al., 1988) as evidenced by an increase in the binding of monoclonal antibody; the effects of TPA and the calcium ionophore were synergistic. Addition of EGTA to the culture completely blocked the stimulatory effect suggesting that influx of extracellular calcium is obligatory for the induction of CD98hc. Since the protein appears at a very early stage of T cell activation, Tanaka et al. (1988) suggest it may have a role in subsequent cell activation mechanisms.

To dissect the different activation pathways, the effects of 4 beta-phorbol 12-myristate 13-acetate (PMA) and ionomycin (a calcium ionophore) on mRNA levels were compared in T lymphocytes (Lindsten et al., 1988). PMA produced a 20-fold increase in CD98hc mRNA, which was ascribed to the removal of a block to transcription elongation within the exon 1-intron 1 region of the gene. PMA induction of the CD98hc gene was abrogated by cycloheximide and thus requires protein synthesis. Treatment of resting T cells with PMA and ionomycin resulted in a further (60-fold) increase in CD98hc gene expression compared with treatment with PMA alone, but the ionophore had only a small effect on its own. Induction was proposed to be mediated by an in-

crease in promoter activity in addition to removal of the block of transcription elongation.

The first intron of CD98hc was shown to contain a transcriptional enhancer element which is active in a wide variety of cells including malignant human T cells. This enhancer element was mapped to a 187-base pair fragment of the first intron which contains binding sites for two nuclear proteins (NF-4FA and NF-4FB) which flank a consensus binding site for the inducible AP-1 transcription factor. Deletion analysis showed that all three sequences were necessary for full enhancer activity. These motifs show strong homology with their murine counterparts (Karpinsky et al., 1989; Leiden et al., 1989).

No equivalent studies have been performed on the genes encoding the light subunits. The finding of Nakamura et al. (1999) showing that CD98 heavy and light chains are coordinately induced in normal lymphocytes following in vitro mitogenic stimulation with concanavalin A, suggests that the regulation of the heavy and light chain may not be independent.

Tissue Expression

The individual members of the CD98 group of proteins vary in their tissue expression and, as expected from the observation that a single heavy chain associates with different light chains, the localization of the heavy chain is more widespread. The heavy chain of CD98 is expressed in most tissues and it appears to be present in all cell lines and tumor cells (Parmacek et al., 1989; Nakamura et al., 1999). A comparison of the levels of CD98hc gene expression in neonatal and adult murine tissues failed to demonstrate a consistent pattern. Thus, while expression was higher in neonatal liver than in adult liver the opposite was found for lung (Parmacek et al., 1989).

The tissue distribution of the light chain has been studied in the case of LAT-1, LAT-2 and y^+-LAT1 (*see* Table). LAT-1 message is highly expressed in brain, spleen, placenta and only at very low levels in epithelial cells and adult liver. It was found at high levels in C6 glioma, hepatoma and hepatocarcinoma cell lines and in other tumor cell lines (Kanai et al., 1998; Prasad et al., 1999; Nakamura et al., 1999). Human LAT-2 is preferentially expressed in kidney and placenta, less strongly in liver, skeletal muscle and heart. Renal expression of LAT-2 mRNA was localized to the epithelial cells of proximal tubules (Pineda et al., 1999). In rat tissues it was also highly expressed in small intestine and brain (Segawa et al., 1999). Human y^+LAT-1 was easily detected in kidney and also found to be present at high levels in peripheral blood leukocytes and with decreasing intensity in lung, placenta, spleen and small intestine (Pffeifer et al., 1999).

Functions

Cellular Activation and Division

Three mouse monoclonal antibodies raised against human (HBJ127 and HBJ98) and rat (B3) tumor cells, and that recognize CD98hc, were shown to block the proliferation of tumor cells in vitro and to inhibit the proliferation responses of human lymphocytes following mixed lymphocyte reaction (MLR) or IL2 stimulation (Yagita et al., 1986). Since the 4F2 antibody previously raised by Haynes et al. (1981), did not inhibit these processes it was concluded that the new antibodies "appear to recognize the more biological functions of this antigen." The inhibition of rat and human tumor cell proliferation was reversed following removal of antibody and it was proposed that the effect of the antibodies is cytostatic rather than cytotoxic.

This study failed to show that the antibodies caused any phase-specific block of the cell cycle. In addition, antibody exposure did not result in downregulation of CD98 (either in activated lymphocytes or neoplastic cells) as has been observed with antibodies raised against surface receptors for growth factors. The authors point to two potential mechanisms to explain cell growth inhibition: either CD98 acts as some sort of receptor for a factor required for cell growth or the antigen acts as a regulator for cell growth by mediating a cascade reaction required for such growth. Neither speculation is irrelevant to recent studies on the role of CD98 in amino acid transport (Mastroberadino et al., 1998).

CD98, which is undetectable in peripheral blood lymphocytes, is strongly expressed following activation with concanavalin A (T-cells) or lipopolysaccharide (B cells). The kinetics of antigen appearance were shown to be rapid (12 hr for first detection and 36 hr for maximal expression). The expression of CD98 precedes the general increase in RNA content (entrance into G1) and does not require *de novo* RNA synthesis, but does require translation of mRNA encoding this antigen. Simultaneous addition of the B3 rat antibody and concanavalin A to unstimulated rat T cells blocked progression of the cell cycle at the G_o to G_1 transition and the appearance of the antigen was arrested at a moderate level (Yagita & Hashimoto, 1986). However, if the antibody was added after 24 hr of stimulation with concanavalin A it also strongly inhibited DNA synthesis, but did not arrest the cell cycle at a certain phase and did not modulate the antigen. These effects are compatible with the proposal that as well as being required for activation, the antigen is also needed for subsequent lymphocyte proliferation. Thus, initially the CD98 antigen must mediate a "competent" signal that follows the activation signal and is required before activation ensues. These results distinguish anti-CD98 blockade from that resulting from anti-

body blockade of IL2 or transferrin receptors when the "competent" signal is not abrogated by antibody (Neckers et al., 1984).

The results on the role of CD98 in T-cell "competence" need further investigation in the light of more recent work on human T cells showing that amino acid availability appears to control mRNA translation via phosphorylation-dependent signaling pathways (Iiboshi et al., 1999). It seems possible that more generally the CD98 heavy chain may play a role in delivering such signals to the cell cytoplasm thus providing a mechanism by which amino acid availability could modulate cell growth and division (*cf.* Wang et al., 1998).

Díaz et al. (1997) have reported that monocytes can suppress T-cell proliferation by a CD98–dependent mechanism that halts the progression through the cell cycle of recently activated lymphocytes. Since this represents a novel perspective on macrophage regulation of T-cell activation, the mechanism requires investigation. Similar conclusions were drawn from the findings of Stonehouse et al. (1999) that antibodies to CD98 inhibited antigen-presenting cells and thus inhibited T-cell activation. We could speculate that this behavior results because CD98, acting as an amino acid transporter in the macrophages, depletes the surrounding interstitial fluid from these solutes which are essential for the proliferation of the T cells. This proposed nonspecific mechanism is analogous to that responsible for preventing the maternal T-cell response to the implanting embryo. In this case, localized tryptophan depletion, produced by the placental enzyme Indoleamine Diamine Oxygenase (Munn et al., 1998) is sufficient to inhibit the immune attack dependent on lymphocyte activation and proliferation.

CD98hc appears to be correlated to cell proliferation in several different tissues. For example in skin, CD98 is expressed on a variety of different cell types. It is found at high levels on normal basal proliferating keratinocytes (Gottlieb et al., 1985) as well as on associated cells involved in hair keratin secretion, the cells of the outer root sheath of the hair follicle. Both these tissues are major sites of protein synthesis (Fernandez-Herrera et al., 1989). Tumors of skin melanocytes were also often strongly positive for CD98, and the extent of the overexpression is related to tumor progression (Dixon et al., 1990). Langerhans cells (involved in immune surveillance in skin) also expressed CD98 mRNA at levels that were greater in fresh than in cultured cells (Ross et al., 1998) suggesting that terminally differentiated Langerhans cells downregulate this molecule which is required for proliferation and cell survival. CD98 expression on other proliferating cells, for example, in the intestinal epithelium has also been reported as has increased expression on a variety of tumor cells (Azzarone et al., 1986).

Recently, CD98 overexpression has been shown to induce malignant transformation of NIH3T3 cells (Hara et al., 1999). Although the doubling times of the control cells and CD98-transfected clones were almost the same, the CD98-transfected clones grew to a higher saturation density than control cells. CD98-transfected clones were more efficient in the formation of colonies in soft agar than control cells and they were also able to develop tumors in athymic mice. The degree of malignancy was proportional to the expression level of CD98 in different clones. The authors thus propose that CD98 has "oncogenic potential."

Differentiation

CD98 has also been suggested to play a role in cell differentiation. Warren et al. (1996), using a rat monoclonal antibody (Joro 177) raised against a mouse pro-T cell clone and which recognizes CD98hc, have found that the antigen is expressed widely on hematolymphopoietic cells. In bone marrow, 75% of lymphoid and 45% of myeloid cells were positive; in thymus, virtually no small, but all large thymocytes (known to contain actively dividing cells) expressed CD98hc; in spleen, the proportion of CD98hc cells increased from 10 to 73% following mitogen stimulation with LPS (B-cell activation) and from 10 to 45% after stimulation with concanavalin A (T-cell mitogen). Early in development virtually all precursor cells of the cellular elements of blood were CD98hc positive and fetal liver hematopoietic stem cells expressed the antigen very strongly.

The authors observed that their antibody was able to aggregate lymphoid progenitor cell lines. This aggregation required protein synthesis and was prevented by co-incubation with a number of inhibitors, including blockers of tyrosine kinases or of tyrosine phosphatases. Extending this approach, the antibody itself was found to be able transiently to increase tyrosine phosphorylation of a 125 kDa cytoplasmic protein in a T-cell line (FTH5). This result shows that antibody binding to the external surface of the CD98hc molecule can transduce signals into the cell.

Functionally, exposure of hematopoietic precursor cells to the antibody was associated with selective deletion of certain pathways of differentiation, a process that was shown to result from initiation of programmed cell death of specific progenitor cells, while allowing survival of other precursor cells. Mechanisms that might account for these findings include: (a) CD98hc providing a pathway for uptake of an essential nutrient (e.g., amino acids) necessary for survival (or growth) of the cells; (b) CD98hc and a putative ligand forming a receptor ligand pair regulating cell survival. The second mechanism would predict that cell number and expansion of cell clones already present in different tissues of the devel-

oping embryo would be regulated via CD98hc acting as a ligand activated receptor.

One molecule which recently has been shown to bind to CD98hc is the lectin Galectin-3. This β galactoside-binding protein is a member of a family of molecules that have been implicated in embryogenesis and tissue formation (Hughes, 1997). Galectin-3 binding to receptors (in contrast to Selectin binding) is calcium independent. Once exported from cells that secrete Galectin-3 (macrophages, eosinophils, neutrophils, mast cells, epithelial cells of the gastrointestinal and respiratory tract) the molecule is free to combine with appropriately glycosylated proteins either at the cell surface or in the extracellular matrix. Galectin-3 participates in different processes involving cell signaling, it modulates growth and apoptosis, triggers superoxide production in neutrophils, and potentiates IL-1 release by monocytes.

Galectin-3 binds to CD98hc in both a human T-cell line (Jurkat) and in murine macrophages (WEHI-3) (Dong & Hughes, 1996), but a direct link between Galectin-3 binding to this surface antigen and its biological effect has not been demonstrated. However, considering that (a) Galectin-3 also binds to extracellular matrix components such as laminin, (b) the lectin becomes polyvalent at higher molarity (since it is then multimeric) and (c) there is polarized secretion of Galectin-3 in epithelia, it may play a role in cell-cell adhesion (Bao & Hughes, 1995).

The same authors have used a mutant epithelial cell line that is UDP-galactose transport deficient in its Golgi apparatus and that therefore cannot appropriately glycosylate (with galactoside-containing residues) any protein synthesized. Galectin-3 binding, but not the binding of other lectins tested, is thus specifically abolished. In these mutant epithelial cells, growth is markedly aberrant, suggesting that one role of the normal Galectin-3 receptor, at least in epithelia, is to transduce signals following lectin binding that act as negative regulators of cell growth thus allowing orderly elongation and branching morphogenesis of the epithelium in culture (Bao & Hughes, 1999).

Amino Acid Transport

The homology detected between CD98hc and rBAT, a protein that is able to induce a broad-scope amino acid transport activity in *Xenopus laevis* oocytes (Bertran et al., 1992*b;* Wells & Hediger, 1992) prompted the investigation of the possible involvement of the surface antigen as an amino acid transporter. Injection of human CD98hc cRNA into *Xenopus* oocytes, was indeed found to elicit amino acid transport (Bertran et al., 1992*b;* Wells et al., 1992). The activity induced by rBAT was very similar to that of system $b^{0,+}$ (van Winkle, 1988), a Na^+ independent transporter for cationic and neutral amino acids. The transport of lysine and arginine induced by CD98hc showed similar amino acid specificity to that induced by rBAT, but, as found for system y^+L (Devés et al., 1992), Na^+ independence was limited to the binding of cationic amino acids, since Na^+ was necessary for the recognition of neutral amino acids.

Several observations suggested that the functional transporter was a complex formed by CD98hc and an endogenous protein (or proteins) of the oocyte: (i) the molecular structure of CD98hc, with a single transmembrane-spanning domain, was not typical of a membrane transporter, (ii) the activity was found to saturate when the expression of CD98hc in the plasma membrane was very low and further increases in the expression of the heavy chain did not result in higher induction of system y^+L activity (Estévez et al., 1998) and (iii) subsequent studies showed that the specificity of the activity associated with CD98hc varied, depending on the cell type and the substrate used to monitor transport. Thus, when leucine was used as substrate CD98hc induced both a Na^+-dependent and a Na^+-independent transport system, but when isoleucine was used, only the Na^+-dependent system was observed. The two transport systems were similar, but not identical to systems y^+L and $b^{0,+}$ (Broer et al., 1998). In addition CD98hc was proposed to be associated to the expression of system L (a neutral amino acid transporter) in rat glioma cells (Broer et al., 1995, 1997).

The first direct evidence showing that the minimal functional unit for transport is a heterodimeric complex came from evidence obtained with cysteine mutants of CD98hc (Estévez et al., 1998). The two cysteine residues of CD98hc (C109 and C330) were mutated to serine, singly or in combination, and whereas the C330S mutation had no effect on transport, the C109S mutant exhibited only 30 to 50% transport activity compared to the wild type. Hg^{++} and the impermeant reagent p-cloromercuribenzenesulfonate (pCMBS) inhibited the transport activity of the wild type and all the mutants, the inhibition being reversed by mercaptoethanol, but notably, the C109S mutants showed a much higher sensitivity towards the sulfhydryl reagents. Increased sensitivity could also be induced in the wild type with mercaptoethanol pretreatment. It was concluded that for the expression of system y^+L transport activity, CD98hc must associate with an endogenous protein of the oocyte, and that the association involved a disulfide bond with C109. Immunoprecipitation of CD98hc under reducing or non reducing conditions, showed that the endogenous protein was similar in size to the light chain of the CD98 surface antigen.

Subsequently, CD98hc has been shown to form heterodimers with six different light chains, as described in a previous section: LAT-1 (Mastroberardino et al., 1998), LAT-2 (Pineda et al., 1999; Segawa et al., 1999),

y^+LAT-1, y^+LAT-2, (Torrents et al., 1998), SPRM1 (Matroberardino et al., 1998) and xCT (Sato et al., 1999). The resulting dimers are the minimal functional units for transport activity. All the CD98hc linked transporters (except rat LAT-2 which is able to perform net transport) appear to function as highly coupled amino acid exchangers, but the specificity depends on the associated light chain.

LAT-1 transports medium-size and large neutral amino acids and does not show Na^+ dependence. The transport characteristics and the tissue distribution correspond to subtype L1 (substrate affinity in the micromolar range, low expression in the liver). The transporter shows preference for leucine and tryptophan. The values of the half-saturation constants are: K_{mLEU}, 32 μM (*Xenopus*), 18 μM (rat) and 16 μM (human); K_{mTRP}, 15 μM (human) (Mastroberardino et al., 1998; Kanai et al., 1998; Prasad et al., 1999).

LAT-2 is also a Na^+ independent transporter for zwitterionic amino acids, but unlike LAT-1 its specificity is not restricted to medium-size and large neutral amino acids; LAT-2 is also able to interact with small L-neutral amino. The apparent K_m of human LAT-2 for L-leucine is 221 μM and for L-alanine 978 μM (Pineda et al., 1999). Rat LAT-2 shows a Km of 30–50 μM for leucine, tyrosine, phenylalanine, tryptophan, serine, valine, glutamine, isoleucine, threonine, asparagine and a K_m of 180–300 μM for histidine, alanine, methionine and glycine (Segawa et al., 1999). Therefore, LAT-2 is a broad specificity variant of LAT-1. As mentioned by Pineda et al., (1999) a system similar to LAT-2 had been described in the basolateral membrane of intestinal enterocytes (Mircheff et al., 1980; Lash et al., 1984) and placental syncytiotrophoblast (Hoeltzli et al., 1989); this is in agreement with the tissue distribution of LAT-2 which is mainly epithelial (Table). Interestingly, human and rat LAT-2 showed different exchange properties. Whereas the human protein functioned as an obligatory exchanger, the rat protein did not exhibit accelerated exchange since leucine exit was the same in the absence or presence of 0.1 or 1 mM unlabeled leucine in the external medium.

y^+LAT-1 transports cationic and neutral amino with a peculiar Na^+-dependence since Na^+ is required for the binding of neutral amino acids, but not of cationic amino acids (Torrents et al., 1998; Pfeiffer et al., 1999). This behavior is characteristic of system y^+L first described in human erythrocytes (Devés et al., 1992; 1993). Like system y^+L of erythrocytes (Angelo et al., 1994), y^+LAT-1 is an efficient amino acid exchanger, but the amino acid specificity is not identical. The major difference resides in the apparent affinity for arginine which in y^+LAT-1 is considerably lower than that reported in erythrocytes. The reported values of K_{mARG} for y^+LAT-1 are 341 μM (Pfeiffer et al., 1999) and 55 μM (Torrents et al., 1998) and for y^+L, 3 μM (Forray et al., 1995). The K_m for leucine is similar in the two cases (10–20 μM).

Recently, it has been established that mutations in the gene (SLC7A7) encoding y^+LAT-1 are responsible for the genetic disorder Lysinuric Protein Intolerance (LPI) (Borsani et al., 1999; Torrents et al., 1999). This is a rare, recessive disorder with a worldwide distribution, but with a higher prevalence in the Finnish population (Simell, 1995). On a normal diet, patients present with poor feeding, vomiting, diarrhea, episodes of hyperammoniemic coma and failure to thrive. Growth retardation, muscle hypotonia, life-threatening pulmonary involvement and hepatosplenomegaly are also seen. A defect in the plasma membrane transport of dibasic amino acids has been demonstrated at the basolateral membrane of epithelial cells in small intestine and renal tubules of patients (Desjeux et al., 1980; Rajantie et al., 1980, 1981) and in the plasma membrane of cultured skin fibroblasts from LPI patients (Smith et al., 1987). The gene causing LPI has been assigned by linkage analysis to chromosome 14q11-13 (Lauteala et al., 1997).

The evidence that SLC7A7 is the LPI gene confirms the suggested role for system y^+L in intestinal absorption and renal reabsorption of cationic amino acids (Eleno et al., 1994; Torrents et al., 1999). The characterization of system y^+L as a transporter that was able to exchange cationic and neutral amino acids in the presence of sodium, had led to the suggestion that it should play an important role in the exit of cationic amino acids across the basolateral membrane in exchange for leucine plus Na^+. Leucine had been shown to stimulate lysine absorption from the lumen when present at the basolateral side (Cheeseman, 1983, 1992).

y^+LAT-2, the other light chain showing y^+L-type activity, has not been fully characterized. It shows 72% identity with y^+LAT-1 and is able to transport lysine and arginine in the absence of Na^+, but Na^+ is required for the binding of leucine (Torrents et al., 1999).

Light chain xCT (Sato et al., 1999) encodes for a transporter (system x_c^-) that is physiologically involved in the obligatory exchange of extracellular cystine for intracellular glutamate. The system is almost ubiquitous in cell lines (Ishii et al., 1992; Bannai, 1986). Northern blot analysis showed three xCT transcripts (12, 3.5 and 2.5 kb) in macrophages cultured for 8 hr with LPS and or diethylmaleate. xCT-specific signals were not visible in RNA from freshly prepared macrophages.

An heterodimeric structure had also been proposed for the transport inducing protein rBAT which is homologous to CD98. Wang and Tate (1995) showed that in the rat kidney and jejunal brush border membrane, rBAT is found in association with a 50 kDa protein and the association has been shown to involve one or more interprotein disulfide bonds. A similar finding was reported by Palacín and collaborators (Palacín et al., 1996).

The identification of a light chain associated with rBAT has been reported recently. The transporter has been designated BAT1 ($b^{0,+}$-type amino acid transporter 1) and has been found to be related to the light chains that are linked to CD98. The light chain (BAT-1) and heavy chain (rBAT) were shown to be colocalized in the apical membrane of the renal proximal tubules. When expressed in COS-7 cells it induces the Na^+ independent transport of neutral and cationic amino acids and cystine (Chairoungdua et al., 1999).

The role that the heavy chain plays to render the light chain a functional transporter is not known, but appears, at least in part, to be related to guiding the light subunit to the membrane surface. Nakamura et al. (1999) have demonstrated that CD98hc on its own can be expressed efficiently as a monomer on the surface of COS cells. In contrast, the light chain LAT-1 is, in the absence of the heavy chain, expressed minimally at the plasma membrane, remaining in the Golgi. Thus, LAT-1 appears to require the heavy chain to be sorted to the surface. The "guidance" effect has been shown to be independent of disulfide linkage. A C103S mutant of mouse CD98hc that cannot form the light-heavy chain S-S bond is capable of inducing cell surface expression of mouse LAT-1 as efficiently as wild type CD98hc in Hela cells.

The role of the S-S bond in determining the cell localization of the two protein components of the heterodimer was also tested by analyzing the effect of mutations on the human CD98hc (C109S) and the *Schistosoma mansoni* light chain SPRM1 (C137S) on the cellular distribution of these proteins. As found for LAT-1, cell surface expression of the light chain was maintained provided that the two subunits (wild or mutated) were co-injected (Pfeiffer et al., 1998) showing that there is noncovalent steric association.

A similar type of interaction occurs in P-ATPases, such as the Na^+ pump and the H^+, K^+ pump, where a β subunit (which differs in the two cases) guides the catalytic subunit to the membrane surface (Geering et al., 1989; reviewed in Moller et al., 1996). Amino acid permeases in yeast are also regulated in their cell trafficking. In this case, an ER-resident protein has been described, without which transport of the permease to the cell surface is impaired selectively; the other protein, however, remains in the intravesicular membranes (Ljungdahl et al., 1992). It is also relevant that mutants of rBAT which are responsible for the genetic defect Cystinuria Type I display an intracellular trafficking defect that impairs their transport to the oocyte surface thus confining them to an intracellular location (Chillarón et al., 1997).

In epithelial cells, CD98hc appears to play a role in the localization of light chains in a given domain of the plasma membrane. Nakamura et al., 1999 have shown that in OTF9 embryonic carcinoma cells, CD98hc is located selectively at the cell-cell adhesion sites with distribution similar to E cadherin. In fact, the polarity of surface expression appears to be regulated by cadherins; in L cells stably transfected with E- or N-cadherin cDNA the CD98hc chain is expressed at sites of cell-cell adhesion, whereas without cadherin it is expressed diffusely on the surface of the L cell.

Adhesion

Recently, CD98 has been shown to associate with functional integrins and to regulate integrin activation (Fenczik et al., 1997). Integrins are heterodimeric proteins composed of an α and β subunit, each with a large extracellular, a single transmembrane and a short cytoplasmic domain, involved in cell growth, migration and tumor metastasis. They adhere to various extracellular matrix proteins and their affinity for these ligands is altered in response to intracellular signals. Integrin activation appears to be due to a conformational transition since it induces the appearance of novel epitopes that can be detected with specific antibodies (Lasky, 1997).

CD98hc was shown to rescue a dominant suppression of integrin function which results from overexpression of a fragment of the β subunit. The experimental strategy used a Chinese hamster ovary (CHO) cell line that expresses a chimeric integrin which is constitutively active. The chimera contains an extracellular and transmembrane domains of αIIbβ3 integrin fused to the cytoplasmic domains of α6Aβ1 integrin; it thus has the ligand binding properties of αIIbβ3 and is activated through α6Aβ1 cytoplasmic binding domain. Overexpression of the β1 cytoplasmic domain in the form of a Tac-chimera resulted in suppression of integrin signaling. Suppression was interpreted as resulting from the titration by the overexpressed β1 subunit of proteins forming an "integrin activation complex." Consistently, expression of Tac-β1 in fibroblast cell lines interfered with cell spreading, migration, fibronectin matrix assembly and integrin activation.

Cotransfection of the cells containing the chimeric integrin with a CHO cell cDNA expression library and Tac-β1 showed that CD98hc was able to complement the suppression and suggested that CD98 is a regulator of integrin function. As pointed out by the authors, the mechanism by which CD98 was able to complement suppression may have involved clustering of the overexpressed antigen because antibody mediated crosslinking of CD98hc was found to stimulate β1 integrin dependent cell adhesion of H345 cells (a small cell lung-cancer cell line) to both laminin and fibronectin. Monovalent Fab fragments did not enhance cell adhesion, although they blocked enhanced adhesion caused by the intact antibody. However, CD98hc lacking a cytoplasmic domain failed to complement dominant suppression indicating

that the association between integrins and CD98hc may be mediated by the cytoplasmic domain of both proteins.

Additional functional linkage between CD98 and integrins is suggested by the effect of Pentoxifylline (PTX) on integrin-mediated adhesion (Gonzalez-Amaro et al., 1998). PTX is a methylxanthine that inhibits adhesion and activation of human T lymphocytes by interfering with intracellular signaling. PTX was also shown to have a significant effect on the expression of antigens CD25 (IL-2Rα- chain), CD69 (activation inducer molecule) and CD98hc induced by PHA and it has been suggested that the downregulation of the expression of these activation antigens by PTX contributes to the immunomodulatory effect of the drug.

It has also been reported that the pro-adhesive and chemotactic activities of the extracellular matrix protein, thrombospondin-1 (TSP1), for breast carcinoma cells are mediated by α3β1 integrin and regulated by CD98hc and insulin-growth factor (Chandrasekaran et al., 1999). Integrin α3β1 is maintained in an inactive or partially active state in these cell lines, but can be activated by exogenous stimuli including serum, insulin, IGF1 and ligation of CD98hc by divalent antibodies. The effect of CD98hc clustering on integrin activation may be due to clustering of associated integrin or may involve specific signal transduction from CD98hc.

Finally, the linkage between CD98 and integrins explains why CD98-mediated membrane fusion of cells transfected with HIV gp 160 is integrin dependent (Ohta et al., 1994; Ohgimoto et al., 1995) and also why when T cells are activated, CD98 expression increases before the rise in β 1 integrin mediated adhesion (*see below*).

Fusion

CD98hc has also been shown to be involved in cell fusion. Ohgimoto et al. (1996) demonstrated, from amino acid sequence data, that CD98hc and the Fusion Regulatory Protein (FRP-1) are the same molecule. FRP-1 had been described as a glycosylated 80-kDa protein expressed at the surface of most human cell lines (Ito et al., 1992; Ohta et al., 1994). Antibodies raised against this protein enhanced cell fusion, particularly that induced by viruses such as measles, HIV and Newcastle Disease Virus (Tabata et al., 1994). Moreover, these antibodies were also (in the absence of virus) able to fuse blood monocytes and the resulting multinucleate cells express structural and functional properties of osteoclasts (Higuchi et al., 1998). These polykaryote cells are required for normal bone growth and remodeling.

FRP-1 (or CD98hc) was found to be selectively expressed on a subpopulation of human peripheral blood mononuclear cells, but to be absent from granulocytes. Macrophages also expressed CD98hc as did activated cultured monocytes. Antibodies to CD98hc produced cell aggregation and multinucleate giant cells and this effect was blocked by β2 integrin (Ohta et al., 1994). It was proposed that the effect of antibody might be the result of integrin activation; this observation is in agreement with the role of CD98hc on integrin activation proposed by Fenzsik et al. (1997). Suga et al. (1995) described a number of molecules associated with CD98hc delivery to the cell surface in HeLa cells. These proteins include heat shock protein 70, actomyosin and vimentin.

More recently Okamoto et al. (1997) have shown that anti-CD98hc antibody (HBJ127) is able to inhibit fusion induced by some viruses (e.g., human parainfluenza type-2) while enhancing fusion induced by certain other viruses, and from this they concluded that the antibody's target (i.e., CD98hc) is 'multifunctional.' Subsequently the same authors used CD98hc constructs to investigate this; expression of one such chimeric protein (with a substituted non-CD98hc intracellular domain) caused dominant negative suppression of CD98hc induced fusion. This experiment shows that intact CD98hc is indeed required for viral-mediated cell fusion. The authors speculate that the dominant negative suppression observed may result from the mutated molecule binding to, and hence sequestering, the CD98 light chain. Similarly, expression of a CD98hc protein carrying a point mutation (C330A) which abolishes covalent bond formation between the heavy and light chains also inhibits viral induction of fusion (Okamoto et al., 1997).

Recently the same group has shown that an antibody against CD98hc has effects on protein phosphorylation (Tabata et al., 1997). Following the finding that fusion was blocked with protein kinase inhibitors such as genistein, experiments were performed to investigate if the anti-CD98hc antibody also affected cell protein phosphorylation. Tyrosine-phosphorylation of multiple cellular proteins was observed within one minute of exposure and the effect was suppressed by genistein and herbimycin A. Cytoplasmic protein pp130 was tyrosine-phosphorylated rapidly after antibody blockade of CD98hc. This suggests that protein kinases are linked to CD98hc and phosphorylate protein substrates during cell fusion.

A Possible Role in Nutritional Control of Cell Activation

It is clear that CD98 can activate intracellular signaling through a pathway (at present unknown) involving protein phosphorylation. It is also clear the CD98 heterodimers are amino acid transporters for a wide range of amino acid substrates. Is there any plausible connection between these two apparently discrete properties? A number of recent experimental studies suggest that they could be related. There is a growing literature showing that amino acid depletion may prevent, and conversely

that the return of amino acids may activate, intracellular target protein phosphorylation in many mammalian cells. Thus Fox et al. (1998) have shown that in adipocytes, of all amino acids added to the medium, L-leucine is specifically required for phosphorylation of a transcriptional regulator (4E-BP1). D-leucine is markedly less effective (by a factor of about 50) than L-leucine, and the activation constant (K_a) for the latter is about 0.4 mM. Similarly, Hara et al. (1998) have shown that p70 S6 kinase activity in CHO cells depends on the presence of amino acids in extracellular medium; while removal of any single amino acid reduced phosphorylation, this inhibition was greatest following removal of either leucine or arginine. While, in the absence of all other amino acids, the addition of either amino acid on its own was ineffectual, the addition of both in combination very substantially restored significant intracellular phosphorylation of the target proteins assayed (both of which participate in the regulation of translation). The authors comment on this unexpected synergistic and exceptionally strong effect between leucine and arginine (prototypic substrates for amino acid transport systems L and y^+L). Both authors suggest that some sort of receptor is likely to be needed to explain the effect of amino acid depletion from the medium on downregulating protein synthesis, although the nature and location of such a receptor is unknown. Similar observations concerning the dominant role of branched chain amino acids such as leucine on p70 S6 activations have been made by Xu G et al. (1998) and Shigemitsu et al. (1999). It seems at least reasonable to speculate that one class of such receptor, able to monitor the extracellular amino acid environment and to generate an intracellular phosphorylation cascade, are the CD98 heterodimeric proteins. This hypothesis is now open to experimental investigation.

Conclusion

CD98 must now be seen as an oligomeric and multifunctional protein. Although in neither respect it is unique, these and other interesting properties pose particularly challenging questions. One is related to the fact that the heavy chain forms covalent bonds to a substantial family of different light chains. At present little is known of what factors determine the proportion of the individual light chain/heavy chain heterodimers, a proportion that obviously will determine the transport phenotype of an individual cell. In epithelial cells, it appears that the heavy chain of CD98 determines basal membrane targeting of specific amino acid transporters and at this face of epithelial cells there is also the potential to interact with the basal lamina of the extracellular matrix. In fact CD98hc has been shown to bind Galectin-3, a lectin that is involved in integrin-mediated cell adhesion. This together with the observation that external stimuli working via CD98hc can generate intracellular phosphorylation-mediated signals, make the biology of these interactions likely to be a particularly interesting area of future study.

This work has been supported in part by a FONDECYT-Chile (1980716) grant.

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